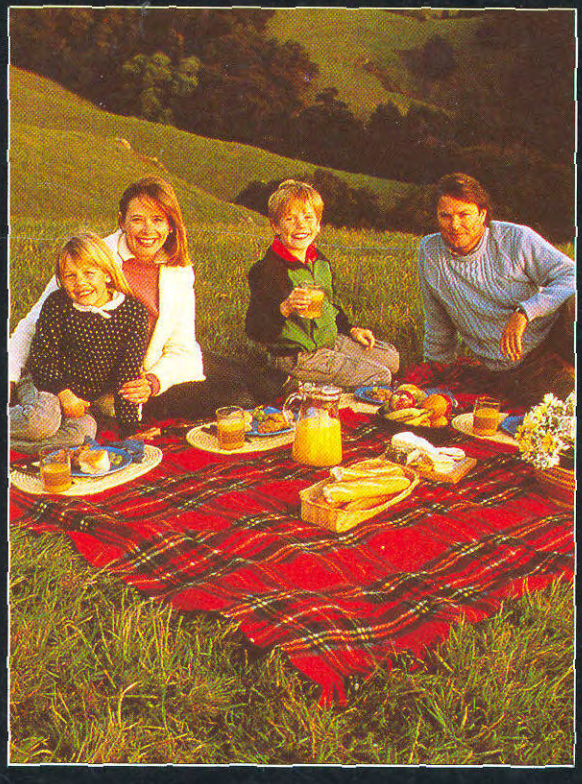
Baker Medical Research Institute

Annual Report 1989/90



Heart Research - The Quest for Life

Annual Report 1989/90
Baker Medical Research Institute

Affiliated with Alfred Hospital
and Monash University

**Tenth Annual Report of the
Baker Medical Research Institute**

**Forty-first Annual Report of
the Alfred Baker Medical Unit,**
(formerly Clinical Research Unit)
Alfred Hospital

How to support medical research at the Baker Medical Research Institute

The Baker Medical Research Institute is funded from many sources including Federal and State Governments, the Baker Benefactions, investment income, research grants, corporate sponsorship and private gifts.

The field of research in which the Institute is engaged touches the lives of the entire Australian community. If research into heart disease is to succeed it will not be because of the combined effort of scientists, governments, companies, individuals, and all Australians, in the alleviation of a common problem and the achievement of a common goal.

The Institute needs your help to finance its research.

There are many ways in which you can help. These include making annual or more frequent gifts; placing capital in our account on which the Institute receives interest (but not capital); making provision in your will; making a donation in lieu of a birthday gift or in memory of a loved one.

Donations are tax deductible

For further information contact:-
Public Relations Officer
Baker Medical Research Institute
P.O. Box 348
Prahran Victoria, 3181
Telephone (03) 522 4333

Persons contemplating bequests are most welcome to visit the Institute by appointment. Advice from an Accountant or a Solicitor is always advisable, but the following is a suggested form of a bequest in Wills.

"I ..... Bequeath to the Baker Medical Research Institute, Commercial Road, Prahran, in the State of Victoria, to advance the work of the Institute the sum of $ ..... / ..... per cent of my estate/the residue of my estate, free of all duties for which the written acknowledgement of the Associate Director shall be sufficient discharge."

Our Target

In Australia 50% of all deaths and serious illness are due to diseases of the heart and circulation.

Most of them are due to hypertension (high blood pressure) and atherosclerosis (clogging up of arteries with fatty cholesterol-laden plaques) which cause stroke, heart attack, heart failure and kidney failure.

Aims of our Research

To increase understanding of the basic causes of hypertension and atherosclerosis.

To use this knowledge to improve medical and surgical treatment.

To prevent heart and vascular disease in the community particularly in the younger age groups.

History

The Baker Medical Research Institute was founded 64 years ago, in 1926, from funds provided by Thomas Baker, his wife Alice and his sister-in-law, Eleanor Shaw. Thomas Baker was born in England in 1854 and emigrated to Australia in 1865. He became one of the pioneers in the production of photographic materials, founding a firm which eventually became Kodak (Australasia) Pty. Ltd.

From its beginning in 1926 until 1980 the Institute was managed by a Board of Trustees. In 1980, the Victorian Parliament passed the Baker Medical Research Institute Act, 1980, which provided the Institute with a new constitution and an enlarged Board of Management.

In 1965, the Institute became formally affiliated with Monash University.

The Baker Institute was initially established to provide laboratory biochemical and clinical pathology services to the Alfred Hospital. However, in 1946, the clinical laboratory services moved into the hospital leaving the Institute to devote its entire effort to research. Over the next few years the Institute slowly expanded and evolved into what is now recognized as a major medical research facility. Until 1974, the research encompassed many fields of medicine, but from 1975 onwards, it has become much more focussed and now concentrates its entire research effort into studying the heart and blood vessels.

Since 1983, the Institute has been the recipient of a Block Institute Grant from the National Health and Medical Research Council of Australia.

In 1990, the Institute was recognized by the World Health Organisation as a Collaborating Centre for Research and Training in Cardiovascular Diseases.

Contents

Heart Research - The Quest for Life

The Baker Institute at a Glance

1. Founded in 1926, through funds provided by Thomas Baker, Alice Baker and Eleanor Shaw.

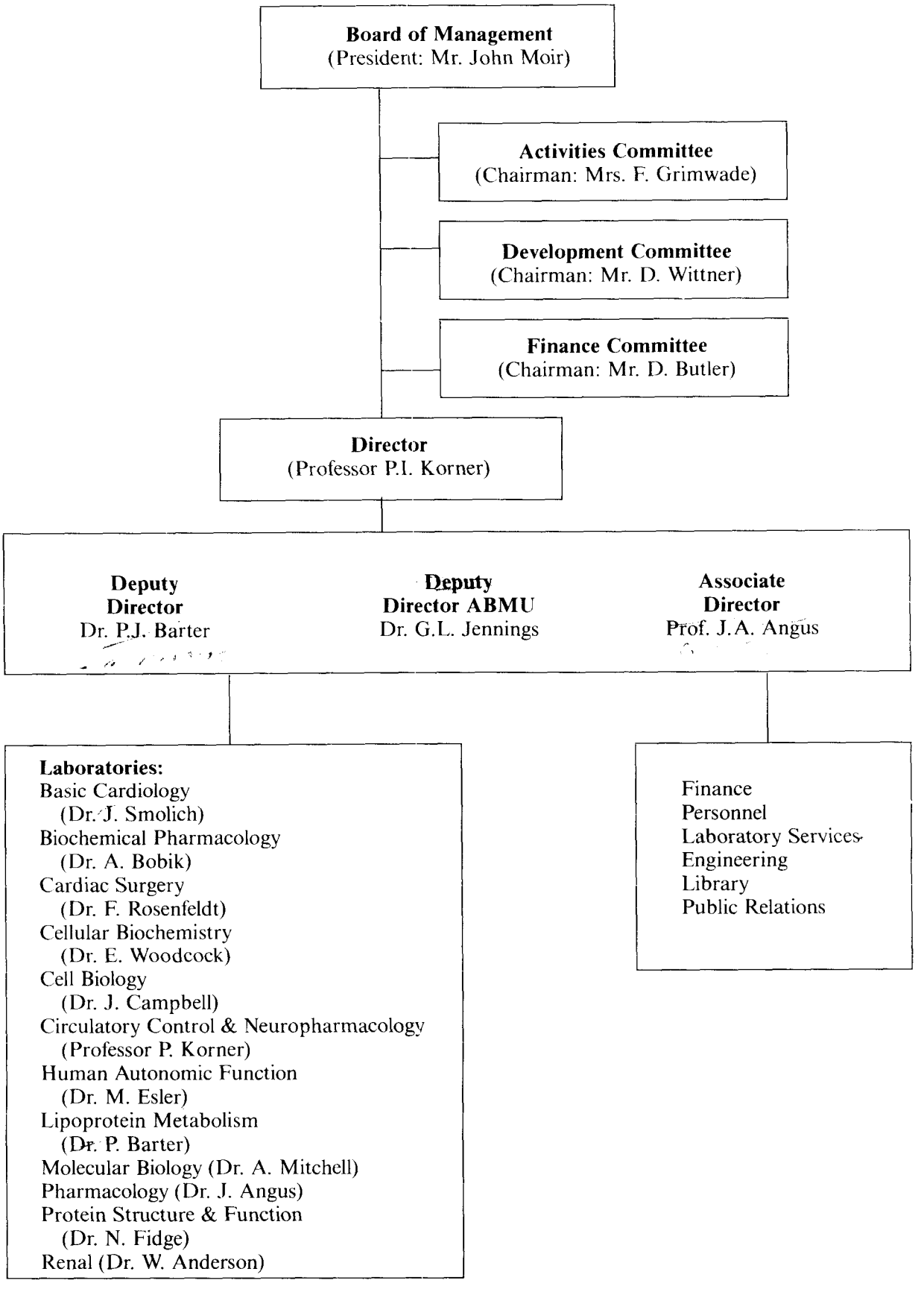
2. It is Australia's chief research centre working on diseases of the heart and blood vessels.

3. It is an independent research institute with its affairs run by a Board of Management. It is affiliated with Alfred Hospital and Monash University.

4. It is a recipient of a Block Institute Grant from the National Health & Medical Research Council of Australia.

5. It has a staff of 143 people. The research is carried out in 12 laboratories and in the Alfred Baker Medical Unit of the Alfred Hospital.

Organisation Chart



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W.D. McPHERSON, A.O., A.A.S.A.
W.G. PHILIP, A.M., B.Comm., F.C.A.
D. WITTNER

Mr. J. D. Moir President, is a Consultant to the legal firm Minter Ellison.

Mr. D. F. Hogarth Vice-President, was Chairman of Directors of Kodak (Australasia) Pty. Ltd.

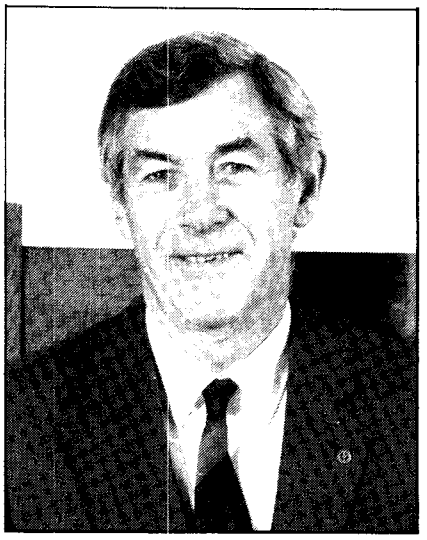
Mr. W.A. Kricker Secretary to the Board, is Chief Executive Officer of the Alfred Group of Hospitals.

Mr. D. J. Butler Honorary Treasurer, is Group Executive, Finance, ANZ Banking Group.

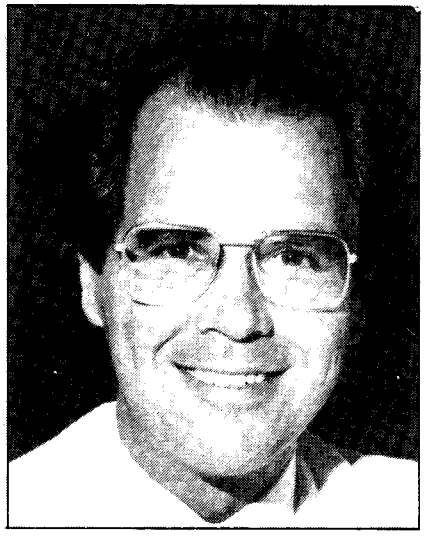
Mr. R.J. Barcham is a Company Director.

Professor P.I. Korner is Director of the Baker Medical Research Institute.

Mr. W.D. McPherson *is Director of BHP Ltd. and Tubemakers of Australia Ltd.*



Mr. W.G. Philip *is partner in Price Waterhouse and Treasurer of the Alfred Group of Hospitals.*



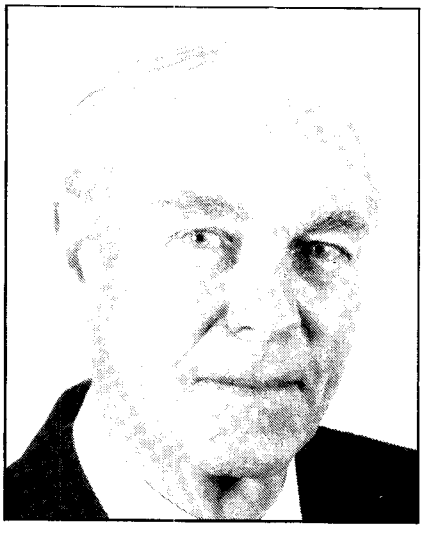
Professor G.B. Ryan *is Dean of the Faculty of Medicine, University of Melbourne.*

Professor R. Porter *is Dean of the Faculty of Medicine of Monash University*

Mr. D. Wittner *Chairman and Managing Director, Wittners Australia Ltd.*

Professor John Funder *is Deputy Director, Medical Research Centre, Prince Henry's Hospital.*

Mrs. F.S. (Joan) Grimwade *O.B.E.*



Mr. J.C. Habersberger *is a Past President of the Institute and of the Alfred Hospital. Until his retirement in 1976, he was Managing Director of Kodak (Australasia) Pty. Ltd.*

Sir Laurence Muir *is Patron of the Institute. He is a member of the Board of Australian and International Companies.*

Staff List

Director
Professor P.I. Korner, A.O., M.D., B.S., M.Sc.(Syd), Hon.D.Sc. (NSW), Hon.D.Med.(Melb), F.R.A.C.P., F.A.A.
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Dr. P.J. Barter, M.B., B.S., Ph.D.(ANU), F.R.A.C.P.
Associate Director
Dr. J.A. Angus, B.Sc., Ph.D.(Syd)
Associate Director

Dr. G.L. Jennings, M.D., M.B., B.S., F.R.C.P., F.R.A.C.P.
Associate Director (Management)
S.C. Price, B.Comm., A.A.S.A.

Basic Cardiology Laboratory

Scientific Staff
Dr. J. Smolich, M.B., B.S., B.Med.Sc., Ph.D.
Technical Staff
Ms. Y. DeNeef, Cert. App. Sci. (till December 1989)

Cardiac Surgical Research Unit

Scientific Staff
Dr. F.L. Rosenfeldt, M.B., B.S., M.D.(Adel), F.R.C.S.E., F.R.A.C.S., Senior Research Fellow
Technical Staff
Ms. C. Boyes, Cert. Animal Technology
Mrs. L. Langley, B.Appl.Sci.
Research Students
Dr. A. Cochrane, M.B., B.S., F.R.A.C.S.
Dr. L. Chi, M.B., B.S.
Collaborating Scientists
Prof. J.F. Williams, D.Sc., Ph.D., Department of Biochemistry, Australian National University
Dr. R.A.J. Conyers, M.B., B.S., D.Phil., F.R.C.A.A., Department of Biochemistry, Alfred Hospital

Cell Biology Laboratory

Scientific Staff

Dr. J.H. Campbell, B.Sc.(NSW), Ph.D.(Melb), Principal Research Fellow
Dr. G.R. Campbell, B.Sc., Ph.D., Senior Research Associate
Technical Staff
Ms. J.D. Rogers
Ms. E. Spanidis, B.Sc.
Ms. S. Kalevitch, B.Sc.
Research Students
Ms. M.J. Black, B.Sc.(Hons)
Ms. S. Horrigan, B.Sc.(Hons)
Ms. G. Cockerill, B.Sc.(Hons)

Cellular Biochemistry Laboratory

Scientific Staff
Dr. E.A. Woodcock, B.Sc.(Hons), Ph.D.
Research Students
Ms. I. Kuraja, B.Sc.(Hons)(Argentina)
Ms. J. Tanner, B.Sc.
Ms. L. Caroccia, B.Sc. (till December 1989)

Circulatory Control and Neuropharmacology Laboratory

Scientific Staff
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Technical Staff
Ms. S. Godwin
Research Student
Ms. B.A. Kingwell, B.Sc.(Hons)(Melb)
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Dr. C.A. Courneya, B.Sc.(Guelph), M.Sc.(UWO), Ph.D.(UBC)
Dr. N. Minami, M.D.(Tohoku)
Dr. T. Saigusa, M.D. (Japan)

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Professor G. Wallin, M.D., Ph.D.
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Dr F. Dudley, F.R.A.C.P., Director, Gastroenterology Service, Alfred Hospital
Dr. M. Horne, F.R.A.C.P., Director, Clinical Neurophysiology, Alfred Hospital

Lipoprotein Structure and Function Laboratory and Molecular Biology Laboratory

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Mr. J. Morrison, B.Sc.(Hons)
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Scientific Staff
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Dr. G. McPherson, B.Pharm., Ph.D.(Melb), Senior Research Officer
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Prof. G. Ryan, University of Melbourne

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Sr. S. Kay, S.R.N.
Sr. L. Watson, S.R.N.
Sr. M. Tress, S.R.N.
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Ms. C. Voight
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Ms. T. Novak, Dip.Comm.Studies (from September 1989)

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The Rouse Family Library
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Ms. J. Pritchard

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Ms. S. Morley

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Mr. I. Johnston
Ms. K. Walls

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Mr. F. Hannemann, B.E., M.I.R.E.E., Officer in Charge
Mr. C.G. Lawson
Mr. J. Kirkas
Mr. D. Lee

Alfred Baker Medical Unit

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Dr. A.M. Dart, B.A.(Physiol), D.Phil., B.M., B.Ch., M.R.C.P.(UK), Assistant Physician
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Dr. J. Thompson, M.B., B.S.

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Dr. J. Burnes, M.B., B.S.
Dr. S. Goldfeld, M.B., B.S.
Dr. N. Houseman, M.B., B.S.
Dr. D. Mann-Segel, M.B., B.S.
Dr. T. Rajam, M.B., B.S.

Biochemical Pharmacology Laboratory
Dr. A. Bobik, Ph.D.(Syd), M.Sc., B.Pharm., Associate Director (Laboratories)
Dr. P.J. Little, Ph.D.(Syd), M.Sc., B.Pharm.
Dr. J. Saltis, B.Sc., Ph.D.(Mon)
Dr. A. Agrotis, B.Sc., Ph.D.(Mon) (from January 1990)
Dr. G.J. Jackman, B.Sc., Ph.D.(Lond), A.R.A.C.I., A.R.C.S. (till August 1989)

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Ms. R. Walker, B.Sc., Grad.Dip.Diet.

Clinical Chemist
Ms. C.J. Oddie, M.Sc.

Nursing, Scientific and Technical Staff
Ms. E. Dewar, B.Sc.
Mrs. S.A. Grinpukel, M.Sc. (till November 1989)
Ms. A.N. Grooms, B.Sc.
Mr. P. Kanellakis, B.Sc.
Ms. M. Larsen, Vet. Nursing Certificate
Mr. G. Mill, B.Sc.(Hons)
Ms L. Moeller, B.Sc.(Hons) (from November 1989)
Ms. L. Nelson, B.Sc.
Sr. A. Bruce, S.R.N.
Sr. L. Johnston, S.R.N.
Sr. J. King, S.R.N.
Sr. S. Free, S.R.N.

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Dr. C. Ferrier, M.D.
Dr. F. Lacombe, M.D.
Dr. J.A. Millar, D.Phil.(Glasgow), B.M., B.Ch., B.Sc.
Prof. V.A. Tkachuk, D.Sc., Ph.D. (Moscow)
Dr. W.C. Zeng, M.Med.

Secretary
Miss N. Scott

Recent Achievements

Hypertension

1. That early genetically-determined development of structural changes in small arteries is the cause of primary (essential) hypertension. The critical change is a narrowing of the average vascular diameter.
2. Growth factors, such as angiotensin II and platelet-derived growth factor B-B chain, play a role in these changes.

Exercise

1. Regular exercise is one of the most reliable life-style methods for lowering blood pressure in sedentary individuals.
2. The initial reason for the fall in blood pressure is the opening up of new vessels during training. Later on, reduction in sympathetic nerve activity helps to maintain blood pressure.

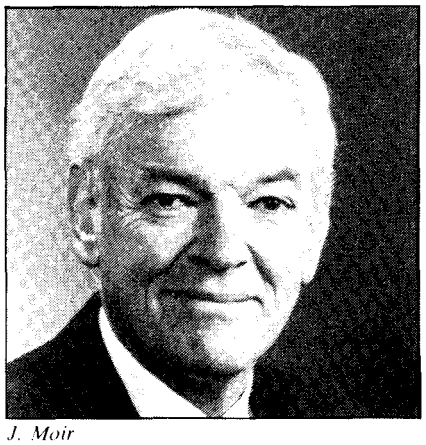
Atherosclerosis

1. Cholesteryl ester transfer protein (CETP) determines the proportion of cholesteryl esters carried between 'protective' high density lipoproteins (HDL); and 'atherogenic' low density lipoproteins (LDL). Unesterified fatty acids (as in obesity and smoking) disrupt this equilibrium, so that more cholesterol is carried by LDL lipoproteins, leading to increased risk for developing heart attack.
2. Resistance of the arterial wall to atherosclerosis is reduced after injury, owing to degradation by scavenger macrophages of heparin-like material in the artery wall. Reduction of this material changes muscle cells from their normal contractile state to one where they divide and accumulate fatty material.

Natural Substances

1. Discovery of the circumstances of release of the hormone, medullipin, when pressure increases in the kidney. The hormone is transformed by the liver and lowers blood pressure.
2. A natural substance. ω-conotoxin, derived from sea-snails, blocks calcium entry in sympathetic nerve endings, resulting in specific reduction in noradrenaline release.

President's Report



J. Moir

The year again marks a period of excellent scientific progress by the staff of the Institute. Our accomplishments are all the more creditable since they have been performed against a background of general difficulties in the Australian economy which have left their mark on the Baker Institute.

One highlight of the year was the official designation of the Baker Institute by the World Health Organisation (WHO) as a Collaborating Centre for Research and Training in Cardiovascular Diseases. We are the first Australian institution to be designated in this way. Dr. Sang Tae Han, the World Health Organisation Regional Director for the Western Pacific Region, together with senior officials from the Commonwealth and Victorian Health Departments, was with us on 4th April, 1990 to inaugurate this change in our status. He emphasized the international nature of research into the causes of high blood pressure and atherosclerosis. He commended our basic research and our most recent venture into the community health field. WHO obviously regards the Institute as an important resource and was confident that we would contribute to a number of global programmes aimed at the prevention of heart and vascular disease.

The linchpin of our community health effort is of course the Heart Risk Reduction Clinic, which was built through the herculean efforts of the Rotary Club of Melbourne. It has added a wonderful string to our bow, but has given us some headaches to find new sources for financing its ongoing operation. I am happy to report that the National Mutual Life Association has decided to help support the Clinic and has provided us with a grant of $250,000 over a period of five years to help defray its running expenses. We are particularly grateful to Mr. Gil Hoskins, the National Mutual Life's Managing Director, for his interest and support. The public health research program of the Clinic has received much needed support from the Victorian Health Promotion Foundation, who have provided a grant of $1 million, over a period of 3 years. The grant is large, but it is needed to run a very substantial novel research programme, for which it will be fully used.

I have much pleasure in acknowledging our many continuing supporters. First among these is of course the Commonwealth Government, who supports us through a Block Institute Grant from the National Health and Medical Research Council. Next

WORLD HEALTH ORGANIZATION — ORGANISATION MONDIALE DE LA SANTÉ

REGIONAL OFFICE FOR THE WESTERN PACIFIC
BUREAU RÉGIONAL DU PACIFIQUE OCCIDENTAL

Professor P.I. Korner
Director
Baker Medical Research Institute
Commercial Road, Prahran
P.O. Box 348
Prahran, Victoria
Australia 3181

14 June 1989

Dear Professor Korner,

I have pleasure in informing you that the World Health Organization, after consultation with your Government, has designated the Baker Medical Research Institute, Melbourne, Australia as a WHO Collaborating Centre for Research and Training in Cardiovascular Diseases. As previously agreed, you will be the head of the Centre.

The terms of reference of the Centre will be as follows:

(1) To carry out research and training in the field of cardiovascular diseases with special reference to the primary prevention and control of hypertension and atherosclerosis;

(2) To collect, evaluate and disseminate information on cardiovascular disease research;

(3) To advise WHO and provide information to other collaborating centres in the field of primary prevention and treatment of hypertension and atherosclerosis.

The designation of the Baker Medical Research Institute as a WHO Collaborating Centre for Research and Training in Cardiovascular Diseases will be effective for a period of four years as from the date of this letter. Either party may however revoke the designation in any year by giving notice of its intention to do so three months before the end of that year. Unless redesignation procedures have commenced prior to May 1993, the designation of your Institute as a WHO collaborating centre will automatically lapse.

Looking forward to our successful cooperation, I remain,

Yours sincerely,

S.T. Han, MD, Ph.D.
Regional Director

Official letter from Dr. Han of the World Health Organisation.

Mr. Ken Widdle, presenting the National Mutual donation to Professor Korner.

come the Baker Benefactions, who have given us over $1 million this calendar year. We are deeply grateful to the Victorian Government who have significantly increased their overall support to medical research in this state. They have increased their contribution to our maintenance by doubling it from $300,000 to $600,000 p.a. and I believe this is a prudent and far-sighted investment in preventive medicine. The Glaxo Programme for pharmacological research has been expanded further and I express the Board's sincere gratitude to Mr. Ken Windle, Managing Director of Glaxo (Australia), who has been extraordinarily helpful in facilitating our interaction with the international company. We are also grateful to the many contributors, both new and long-standing whose support is so critical for our research.

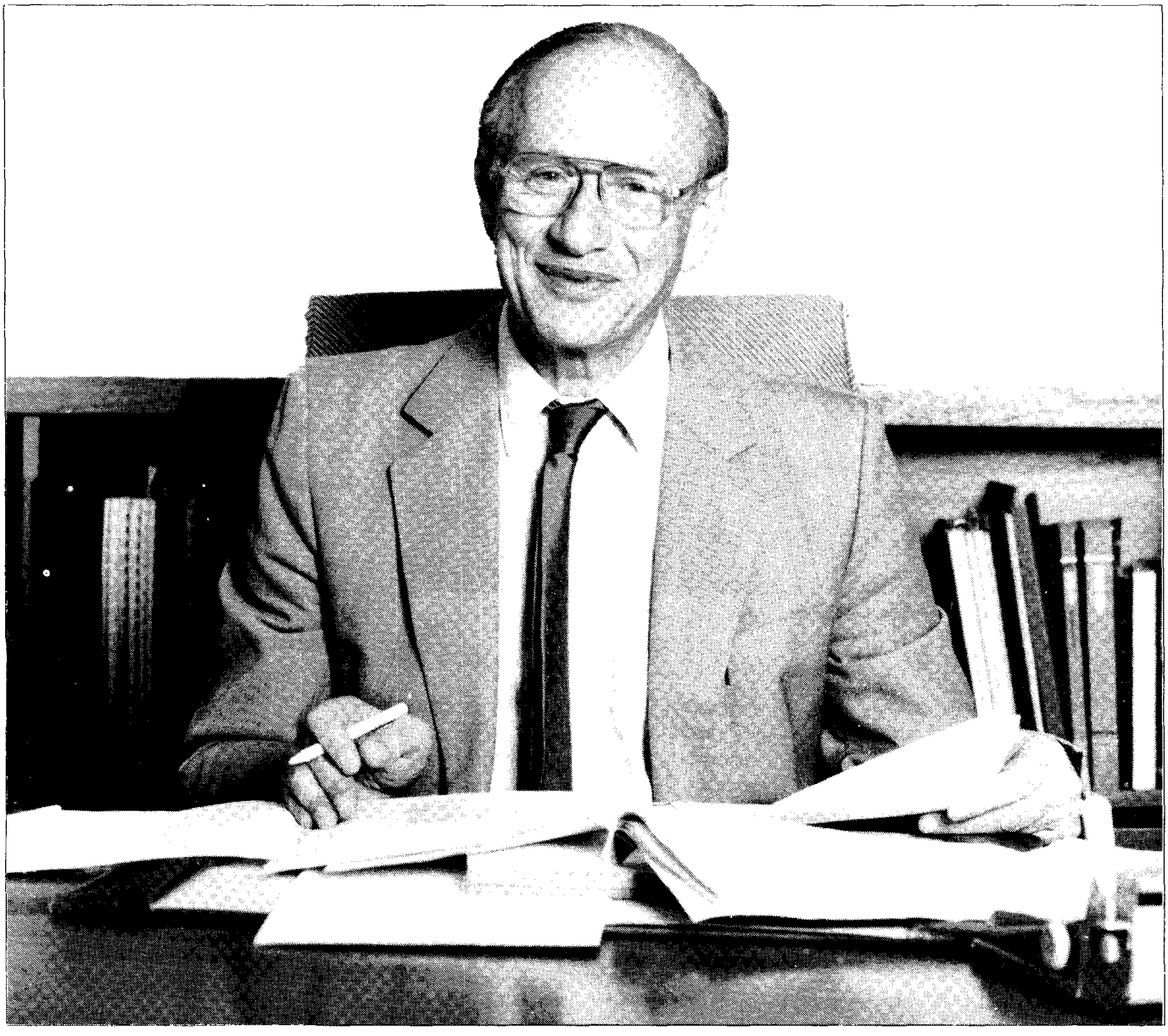
Despite this excellent support, the Institute has had an exceptionally difficult financial year, where some belt-tightening has been necessary. I record the Board's appreciation to our Director and his senior colleagues for their skill in steering us through the difficult times. Our income from both government and private sector needs to increase further to keep up with the rising costs of medical research.

This year marks the end of Professor Paul Korner's term of nearly 16 years as Director of the Baker Institute. The Institute's standing in Australia and overseas owes much to his leadership and to his talents in attracting a staff of excellent scientists. His contributions to medical research were recognised in this year's Australia Day Honours list, where he became an Officer inthe Order of Australia; shortly before that, he was awarded an HonoraryDoctorate in Medicine by the University of Melbourne. We will miss him, particularly as he and his dear wife, Jennifer, have decided to return to their home in Sydney to be closer to their family. Paul is not the retiring type and has indicated that he is keen to continue a scientific association with the Institute on a part-time basis. He has left the place in good order for his successor, Professor John Funder.

John Funder comes to us with an excellent track-record in medical research. From discussions with him, I can say that the focus of the Institute's research will remain in the cardiovascular research area, with all our areas of strength likely to continue. Professor Funder will bring a group of neuro-endocrinologists with him, which will increase the range of basic science disciplines in the Institute.

I want to conclude by thanking all my colleagues on the Board of Management for their support and the staff for their dedicated effort. I am particularly grateful to Joan Grimwade, David Wittner and David Butler for their work on the Ladies', Development and Finance Committees. The Board has every confidence that, given the excellence of our scientific staff, the Institute faces an excellent scientific future. With patience, perseverance and continuing public support, our financial problems will be overcome.

Director's Report and Scientific Highlights



Professor Korner

This is my last report as Director of the Baker Medical Research Institute. The period of nearly 16 years since I arrived here from Sydney in January 1971 has passed all too quickly. It has been a most creative and wonderful period. I came accompanied by five young and hopeful researchers - Jim Angus, Warwick Anderson, Peter Fletcher, Peter Blombery and my longstanding assistant, Judy Oliver. We marvelled at the Institute's excellent physical facilities (built by my predecessor, Tom Lowe) and wondered how we would ever fill them. We need not have fretted ourselves, at least on that score, as the place is now bulging with scientists and space is at a premium.

The close links between the Institute and the Alfred Hospital provided an unparalleled opportunity of linking laboratory and clinical science and I believe we have made good use of it. The Institute's affiliation with Monash University was important, since it has allowed us to play a part in training Honours, Ph.D. and Masters-students.

I was extraordinarily fortunate that my arrival in Melbourne helped to attract some very bright scientists, who shared my vision of a national cardiovascular research institute. I have already mentioned Jim Angus and Warwick Anderson, who subsequently became the respective heads of the Pharmacology and Renal Laboratories. Both have given me tremendous support. Jim has also assisted with the Institute's administration since his appointment as Associate Director from January, 1990. Alex Bobik had come from Sydney a year before me and, through my entire period, has been

a tower of strength in our work on biochemical aspects of hypertension. Garry Jennings came soon after my arrival and has been responsible for the outstanding success of our basic clinical research in hypertension. Murray Esler has pioneered new methods for measuring sympathetic nervous system activity, which are now well recognized throughout the world. For this he received the 1989 Wellcome Australia Medal and Award. My own group, including Geoff Head and Pat Dorward, has worked on a range of topics related to neuro-hormonal control of the circulation. Most importantly, Paul Nestel joined the Institute in 1977 and, through his work in atherosclerosis, ensured that the Institute's horizons extended beyond hypertension research. He brought a strong group from Canberra, which included Noel Fidge, who all provided outstanding expertise in lipoprotein biochemistry. Noel Fidge has since extended his activities to the protein chemistry and molecular biology of atherosclerosis. To have an outstanding researcher like Paul Nestel as a leading member of our team was a great boost and the Institute is greatly in his debt. Not long afterwards, Kerin O'Dea joined the team as an expert in nutritional biochemistry. In another area, Gordon and Julie Campbell brought a fresh and original approach with their work on the cell biology of atherosclerosis. Frank Rosenfeldt developed an experimental cardiac surgery laboratory, with the goal of making open heart surgery better and safer, which has come into its own in relation to the Alfred Hospital's heart transplantation programme. Arch Broughton and Joe Smolich added to our knowledge of cardiac enlargement. John Ludbrook joined us from Adelaide and Elspeth McLachlan from Monash and their work greatly increased the range of our research effort in different areas of circulatory control by the autonomic nervous system.

This group was responsible for the rapid recognition of the Institute as a major force in the Australian research scene, culminating in theaward to us in 1982 of a Block Institute Grant by the National Health and Medical Research Council (NHMRC). Over the past 16 years, we have, of course, had numerous staff changes and several of our swans have taken up important positions elsewhere. The new arrivals, including Philip Barter, Alana Mitchell, Tom Cocks, Peter Little, Grant McPherson, Robyn Woods, Elizabeth Woodcock, Tony Dart, Graeme Eisenhofer, Rod Dilley and several others, have ensured the maintenance of our high scientific standing.

Many of our present and former senior staff are now international figures, well known in their own right. This has certainly given me great satisfaction. Their work has has helped attract an increasing number of visiting scientists from all over the world. Over the last 16 years, we have had almost 100 post-doctoral scientists and a number of distinguished scientists, including Jim Black, Bjorn Folkow and Irwin Kopin. Their presence has enriched us greatly and I believe they, in turn, have learned something from our particular approach to problem solving. We have had the benefit of advice from our international scientific advisors, including Dick Havel, Cliff Barger, Alberto Zanchetti and Yukio Yamori. This has been of the utmost value at a number of critical phases of our development.

You have heard from our President that we have become a World Health Organisation Collaborating Centre for Research and Training in Cardiovascular Diseases. The Baker Institute has also been the chief co-ordinating centre for closer links with the USSR in the cardiovascular field. We are currently working on closer ties with France. Scientific problem solving is an international affair and it is important to be part of the network.

Rapid problem solving is what institutions such as ours are about. Having different basic disciplines under one roof has helped to increase the breadth and boldness of our biological thinking. Few scientists who have not worked in this kind of atmosphere, realise just how exciting and intellectually stimulating is the ability to complement the capacities of one's scientific colleagues in solving important biomedical problems. Research is often about seizing opportunities at the right time and the structure of a research institute enhances the probability of this occurrence. What makes the Baker Institute unique has been the very close integration of laboratory and clinical research. Our clinical research focuses on basic human physiology and pathophysiology and the hypotheses arising from this are often pursued at several analytical levels in animal and cell culture experiments. This strong interactive setting between Institute and Clinical Research Unit (recently and very appropriately renamed Alfred-Baker Medical Unit) is something that should be cherished.

Our longstanding affiliation with the Alfred Hospital has been placed on an even firmer footing through the recent re-negotiation of a new affiliation agreement. One of my somewhat rash decisions about 3 years ago was to accept the chairmanship of the Board of Management of the Alfred Group of Hospitals. The role of the Hospital President is like the old-fashioned definition of 'neutral' as someone with whom all sides are at war! However, working on the Board of the Hospital makes it instantly obvious just how important it is to greatly increase the amount of research performed in major teaching hospitals. This is the only way to solve the very real problems that face the health care system in an age when public demand for services is in excess of available resources. Against a national health care expenditure of $20 billion p.a., the overall Australian research expenditure of about $150 million p.a, is derisory.

The Baker Institute has suffered from underfunding by the Federal Government for most of the time we have been here. In terms of scientific

productivity, we compare favorably with the other great Australian medical research institutes, but this is not reflected in relative NHMRC funding. Our Block Institute Grant from the NHMRC covers only 38% of our expenditure. This is too low – it should be closer to 50% and it must be increased if our financial difficulties in 1989 are not to recur. The Victorian Government has recently recognised the importance of the State's research institutes and has doubled its contribution to our maintenance grant. This owes much to the vision of Peter Sheehan, the former Director of the Department of Management and Budget, and to David White and Caroline Hogg, the past and present Ministers for Health. In addition, the Victorian Health Promotion Foundation, a product of the tax against tobacco sales, has financed our first major public health research project on improvements in early prediction of risk from cardiovascular disease and on the development of optimum strategies for reversal of risk. A lot is said these days by government about commercialisation of research. We, of course, do some basic drug research in conjuction with the pharmaceutical industry, but our research is predominantly in the 'public interest' arena. This, too, brings financial benefit of national value. We must recognise that any significant measure that prevents the development of cardiovascular disease will result in as great financial savings as those associated with the development of most marketable drugs or devices. In the containment of health costs, prevention is the name of the game!

I have spoken of my indebtedness to my scientific colleagues and, indeed to all the staff of the Institute, including my secretary, Judy Segal, and, of course, Judy Oliver. I want to add how deeply grateful I am for their undeviating support to the three Presidents, under whom I have served – John Habersberger, Sir Laurence Muir and John Moir – and to all members of our Board of Management. They have had enormous faith in the Institute's scientific goals and have mostly found the means by which we could achieve them. Last, but not least, we learned a lot about fundraising from Mike Downes during the seventies and the tradition has continued culminating in the Rotary appeal under Simon Price.

I must pay public tribute to my wife, Jennifer. She has been a tower of strength throughout my career and, in the early days of our stay in Melbourne, acted as 'mother' to the young group of researchers at the Institute. She is held in affectionate regard by all.

I want to conclude with my very best wishes for the future all members of the staff. Our affiliation with the hospital will ensure strong clinical focus in our basic research. This is a good way of keeping our feet on the ground rather than in the clouds. The affiliation with Monash University requires updating. Our staff deserve better recognition by the University and our research training resources could be better used. I have every confidence this will eventually come about, since the Dean, Professor Robert Porter, has been most supportive of these goals.

I feel confident that my successor John Funder will bring the Institute a whole range of new concepts on endocrine aspects of circulatory regulation and will increase the Institute's capacities in cellular and molecular biology. These, added to the existing strengths, will make the Institute's scientific future bright indeed.

Professor Korner with his wife Jennifer, at the conferring of his honorary Degree of Doctor of Medicine, University of Melbourne.

Scientific Highlights

This year I have concentrated on a small number of topics instead of a global overview. A more detailed account is given in the individual laboratory reports.

Hypertension

One feature of the circulation in hypertension is the occurrence of structural changes in the small arteries, with enlargement of their smooth muscle coat. Hypertension means elevation of blood pressure and the above changes occur in patients with either primary (i.e. of unknown causes) or secondary hypertension (i.e. of with known pathology, e.g. renal stenosis). They also occur in animals with primary hypertension (e.g. spontaneously hypertensive rat, SHR) or with secondary hypertension Some of our recent work has highlighted the critical features of the structural changes that are important in hypertension (Angus, Korner). The most important property is the narrowing of the average internal diameter of the small arteries, due to enlargement of the muscle cells and/or due to stiffening of the wall

of the artery. The narrower artery ensures that during all kinds of stimuli, there is 'amplification' of the changes in resistance to blood flow. For a given degree of muscle shortening, there is a greater narrowing of the vessel than normal, resulting in greater changes in blood pressure. We were the first to show that, in chronic hypertension, the amplifier properties are responsible for more than 70% of the maintenance of blood pressure at a high level (Anderson, Woods, Korner). This has application to the treatment of human hypertension: the aim of drug treatment must be, not only to lower blood presssure but to cause reversal of structural changes. This varies substantially with different drugs (Jennings, Esler, Korner). Those that produce substantial reversal of the structural changes cause a longterm effect once treatment is stopped, with a much slower rise in blood pressure to its previous high levels. Under these conditions, it is easier to maintain blood pressure at normal levels by 'life style measures, such as diet, weight reduction and exercise. In hypertension, veins too undergo structural changes and are less distensible than normal (Sudhir, Angus, Korner).

The structural changes in the small arteries have traditionally been considered to be the direct result of having to constrict against an increased pressure load. However, we have recently found that in the SHR, the amplifier properties develop before the rise in blood pressure (Adams, Bobik, Korner). This suggests that the underlying structural changes are the cause of primary hypertension. In addition, we showed that brief periods of treatment of SHR before the age of 14 weeks with ACE inhibitors (drugs that prevent the production of the pressor hormone angiotensin II, Ang II), produce permanent attenuation of hypertension, by preventing the development of the amplifier properties of the small arteries at a critical time in post-natal life (Bobik, Adams, Korner). Circumstantial evidence suggests that the action of ACE inhibitors on this type of longterm attenuation of arterial structure is through Ang II, located in the wall of the blood vessels.

This has led to the hypothesis that the structural changes occur as a variant of normal development. The vessels need to be only 5 - 10% thicker than normal vessels to lead to the development of hypertension. Currently, we are focusing on a number of likely 'factors' that may play a role (Bobik, Grinpukel, Grooms, Saltis). Ang II appears to act on muscle growth in conjunction with platelet-derived growth factor B-B chain. The sympathetic transmitter, noradrenaline, may play a role, as well as growth factors released from the cells of the endothelium that line the blood vessels. In tissue culture, vascular smooth muscle cells from SHR grow more rapidly than cells from normotensive animals. This is due to both an increased sensitivity to specific growth factors and to reduced susceptibility to a number of other factors that inhibit replication.

How does all this relate to hypertension in patients? We are, at present, trying to improve current methods available for earlier recognition of abnormal structure in individuals destined to develop hypertension (Jennings, Dart, Laufer, Sudhir). The methods include discriminant function analysis to permit early recognition of abnormalities in (i) structural changes of the small arteries in the retina; (ii) structure and function of the left ventricular muscle; and (iii) increased stiffness in veins.

Atherosclerosis

Our research has concentrated on the question of why individuals with increased plasma concentrations of high density lipoprotein (HDL) are less susceptible to atherosclerosis and associated heart attacks. HDL plays a role in removing cholesterol out of cells and one area of research has examined the 'receptor' by which HDL attaches itself to cells. This has involved purification of the HDL receptor and analysis of structure (Fidge, Mitchell).

One area of research has focused on the properties of an important protein carrier of cholesterol, the cholesteryl ester transfer protein (CETP). This determines the proportion of cholesteryl esters carried by the different lipoprotein fractions, including HDL and low density lipoproteins (LDL) (Barter, Rye). The former 'protect' against heart attacks, whilst the LDL lipoprotein are much more atherogenic (Barter, Rye). One important finding has been that the capacity of HDL to accomodate cholesterol is markedly reduced by the interaction between CETP and unesterified fatty acids, resulting in mass transfer of cholesterol to LDL lipoproteins. The fatty acids increase in obesity, smoking and diabetes, which are all factors that increase the risk of developing heart attacks.

Good progress has been made on the cell biology of atherosclerosis. Smooth muscle cells are of critical importance in the formation of cholesterol-containing plaques in large arteries. The cells are not normal contractile muscle cells, but become transformed into cells that accumulate fatty particles and are highly sensitive to factors which promote cell division (Campbell and Campbell). Whether or not smooth muscle is in the 'contractile' or 'synthetic' state is determined by the matrix material surrounding the muscle cells, which includes compounds called glycosaminoglycans. One of these, heparan sulphate, is probably the most important from the viewpoint of the atherosclerosis. It has long been thought that the starting point of the atherosclerotic process is injury of the arterial wall. Our work has indicated that, after injury, macrophages (scavenger white cells) enter the wall and degrade the heparan sulphate in the matrix, thereby increasing the proportion of smooth muscle cells in the non-contractile state (Campbell).

Clinical Physiology of Exercise

Several years ago, we were the first to show that regular exercise was a most effective way of lowering blood pressure, both in subjects with high and with normal blood

pressures (Jennings, Esler, Korner). To help work out the underlying mechanisms, we examined in detail the time course of the changes in blood pressure, vascular resistance, heart rate and autonomic activity (Meredith, Jennings, Esler, Korner). We found that the fall in blood pressure was well established very early by about third bout of exercise, but lowering of resting heart rate and sympathetic activity took longer and was established only after about 2 weeks from the start of training. It is therefore unlikely that they are responsible for the early fall in blood pressure, which is probably due to remodelling of the vascular bed of the active skeletal muscle vessels as a result of training. However, reduction in sympathetic activity helps to maintain blood pressure at low levels from about 2 weeks after the start of training. We found that when training stopped, both sympathetic activity and blood pressure remained low, so that the sympathetic changes appear longterm ones. The take-home message for regular joggers is that one need not be too compulsive and worry about missing the occasional bout of exercise. The reduction of sympathetic nerve activity during chronic exercise affects the renal sympathetic nerves very selectively (Meredith, Esler, Jennings) and is probably due to signals from the remodelled heart associated with training (Dart). Exercise also produces longterm changes in baroreceptor-heart rate reflex properties and in 'intrinsic' heart rate (after autonomic blockade) (Kingwell, Dart, Jennings). In some ways it is extraordinary that with something that is as common as exercise, it is only now that information about these important physiological mechanisms has come to light.

Circulatory Control

This encompasses a large range of topics, including regulation by the brain, autonomic nervous system and various local and circulating hormones and the role of the kidney.

In the brain, we have concentrated in recent years on the noradrenergic system of nerve cells in the hindbrain (Head, Korner). These neurons appear to be a major inhibitory system on sympathetic constrictor function and also have a cardio-depressor effect on vagus and cardiac sympathetic. They are critical to the central action of well-known anti-hypertensive drugs, including α-methyldopa and clonidine.

Over many years, we have studied circulatory reflexes in the intact organism, which are compound reflexes evoked in response to a number of inputs from different parts of the body. In chronic hypertension, depression of constrictor and heart rate reflexes is common. We have recently found that depression occurs in response to pressure-sensitive signals from the heart and lungs (Korner, Oliver, Head, Dorward). This mechanism has advantages to the organism in (i) placing a brake on the vascular amplifier properties, thus limiting further rises in blood pressure; (ii) limiting the degree of dilatation of the enlarged and stiff hypertensive heart.

Other work has dealt with the question of the relative importance of sympathetic nerves and hormones in circulatory regulation in moderate hemorrhage (Korner, Courneya, Oliver, Woods). This work has indicated how much more effectively the autonomic nervous system acts to maintain blood pressure during moderate hemorrhage than do the major pressor hormones. The autonomic nervous system is finely tuned by signals arising from both arterial and cardiac pressure sensitive receptors and stimulate both heart and kidney.

The human autonomic function laboratory has developed a range of new methods for quantifying regional sympathetic activity (Esler, Eisenhofer). One important recent finding has been the selective increase in cardiac sympathetic activity during laboratory-induced mental stress.

The Pharmacology Laboratory has been working with a toxin secreted by a rather beautiful sea-snail (Angus, Pruneau). The toxin, ω-conotoxin, blocks special 'channels' important in calcium movement into sympathetic nerve endings, which regulate release of transmitter. This channel may provide a target for a new type of drug. Other work relates to the regulation of production of the peptide endothelin (Cocks, Angus), which may play a role in the production of vasopressin. Major focus has been on factors that determine reactivity of blood vessels in hypertension and heart failure and in collateral coronary vessels (Angus, Sudhir, Ward, McPherson, Cocks, Broughton), and thanks to co-operation with the Alfred Hospital, we have had the opportunity of studying, for the first time, material from small human coronary arteries.

The Renal Laboratory has been studying the role of a hormone, medullipin, produced by the kidney (Anderson, Woods, Denton, Christy, Oliver). Intensive work began in this field following Bjorn Folkow's visit here last year. The hormone is produced in response to rises in blood pressure and one question is whether it plays a role during small changes in pressure or whether it comes into play during huge pressure rises, as an emergency mechanism.

Experimental Heart Surgery

During 1989/90, Frank Rosenfeldt's laboratory served as a significant resource for the transplantation programme under Mr. Don Esmore, head of the transplantation program. This has been one of the Alfred Hospital's major recent success stories and the results obtained have been better than the world's best. Dr. Rosenfeldt's work on myocardial preservation, patiently perfected over the last 15 years, has also come into its own and has allowed us to bring donor hearts to the Alfred Hospital from far afield. The record has been a donor heart from North Queensland, which was preserved over 5 hours 10 minutes and doing very well in its new recipient.

Paul Ivan Korner – An Appreciation

Paul Korner will retire as Director of the Baker Medical Research Institute in September 1990, 16 years after taking up his appointment in 1975. He inherited an Institute which had over almost 50 years established a reputation in general medical research. Paul saw the need for the Institute to concentrate on one principal area of medical research, and he has built the Institute into a thriving centre of research into cardiovascular disease.

In building a centre of international repute, Paul first established his own group working on high blood pressure and the regulation of blood pressure. He then added other complementary groups. These groups were usually based around a promising young scientist, such as Julie Campbell in smooth muscle cell biology, Jim Angus in cardiovascular pharmacology, Murray Esler in human autonomic function, Garry Jennings in cardiology, Warwick Anderson in physiology, Alex Bobik in biochemical pharmacology, and so on. The appointment of Paul Nestel, with protein chemist Noel Fidge, and other first rate scientists, to establish a strong research effort in atherosclerosis was also crucial to the development of the Institute as a broadly based cardiovascular centre, as was the appointment of Philip Barter following Dr. Nestel's appointment to the Head of the CSIRO Division of Human Nutrition.

Perhaps the most important part of the development of the Institute as a comprehensive cardiovascular centre has been Paul's fostering of close links between clinical and basic research, between the clinical research unit and the more basic laboratories at the Institute. Garry Jennings's energy and skill in building the Clinical Research Unit under Paul's guidance has been essential to the integrated research approach of the Institute.

Dr. P.I. Korner

Paul's contribution can also be measured in more concrete ways. For example, in 1989 the Institute staff published 99 papers versus 15 papers in 1974. In 1989, the Institute had almost 40 senior scientific staff, in 1974 it had less than 10. In 1989, the Institute has 178 total staff, in 1974, 39. There are now 21 postgraduate students. The income to the Institute in 1989 was $5.5 million, in 1974 it was just less than $1 million (in 1989 dollars). In 1989, income from peer-review granting bodies was about $2.75 million, in 1974 it was about $100,000 (1989 dollars).

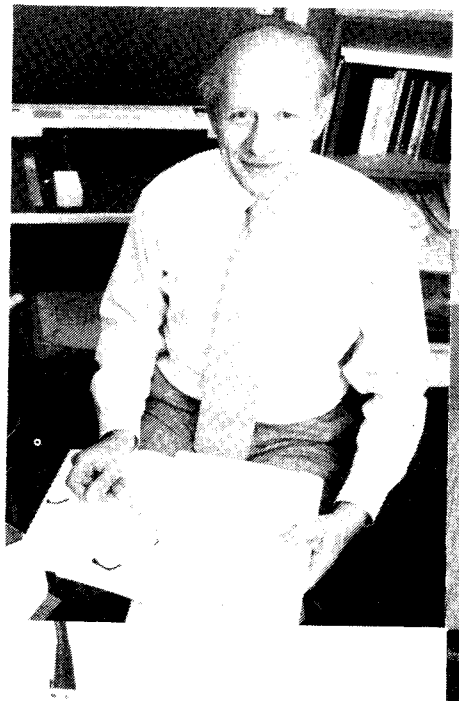
All of us who have worked with Paul would agree that they have benefited greatly from their association with him. Paul's enthusiasm and energy affects everyone, whether the research collaboration is close, as in the cardiovascular control research areas, or more at arms length, such as in atherosclerosis research. Working near Paul, one cannot but help sharing his commitment to the scientific analysis of important problems (and his eschewment of ephemeral fashionable issues), to being exact and careful in the laboratory, to an open-minded view on his own work and others and a readiness to re-examine its interpretation if necessary, to looking behind the obvious and to never accepting second-rate work.

Some of these qualities have given Paul a somewhat daunting reputation. Inside and outside the Institute, many have been the recipients of Paul's loud insistence that we were in error in our interpretation of presented results. If we were wrong on scientific grounds, Paul has never minced words in pointing this out. And he was never euphemistic to those daring to present trivial work pompously, or careless work, or poorly analysed work.

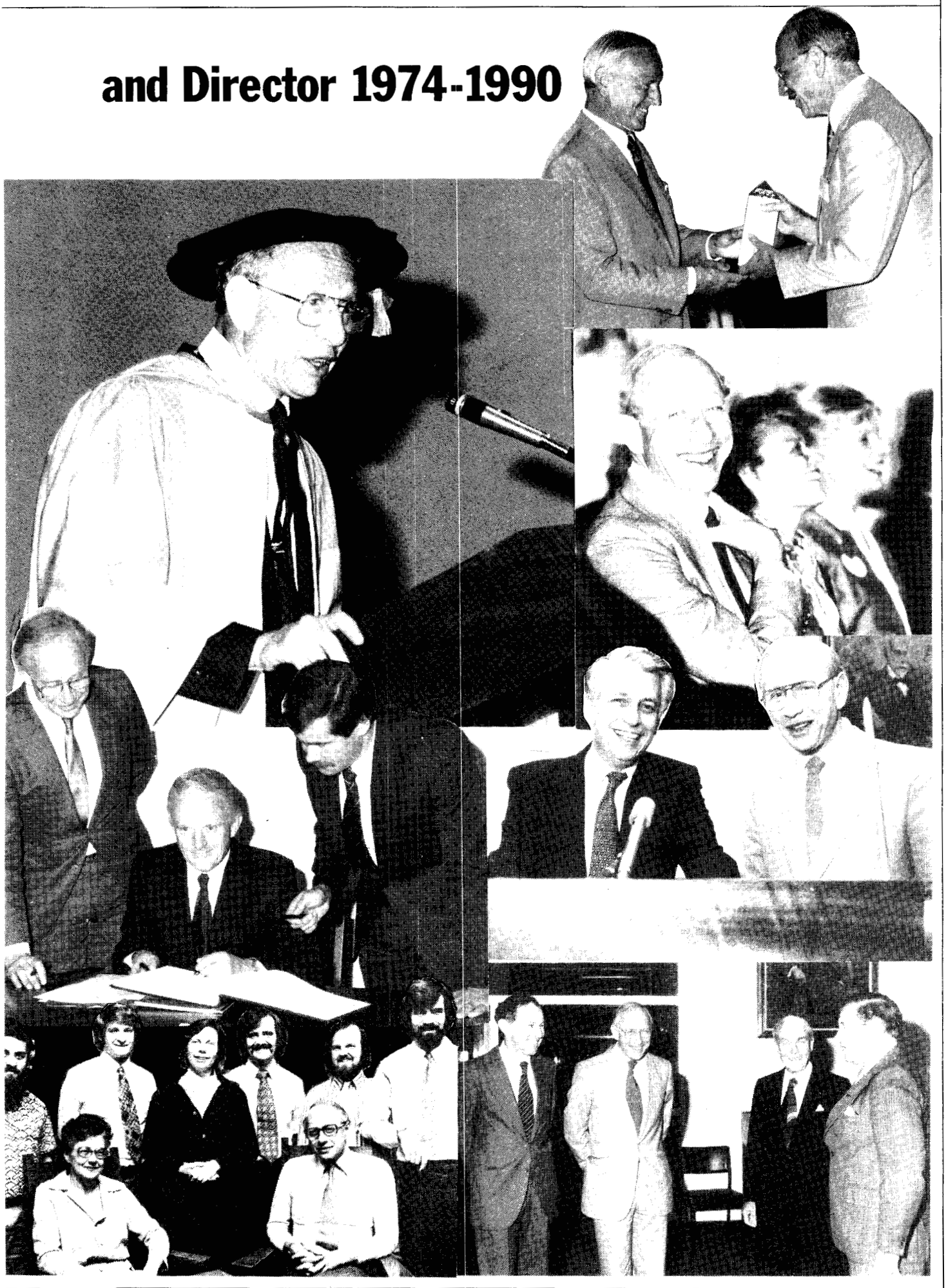
It comes a surprise to many therefore to find out that, behind this exterior is a kind, thoughtful and indeed a somewhat shy man. He was always the first person to visit sick members of staff, to enquire about sick members of families and to visit them, to write thoughtful notes to bereaved family members, to care about the work load of the most junior staff, and so on. The contrast between his public reputation for fearlessness, and his kindness in private, are remarkable.

The opportunity to direct a research Institute is a privilege and a responsibility. In good hands this brings out the best in talented scientific staff; if mishandled, staff become frustrated, lose enthusiasm, and the Institute ceases to function as an integrated whole. The success of the Baker Institute over the last 15 years is a testimonial to Paul's qualities which combine skill, persuasive powers, personal strength

Prof. Paul Korner – Research Scientist



and Director 1974-1990



in never flinching from making difficult decisions, a recognition of the power of new techniques in science, and a relentless commitment to the Institute.

Paul has been particularly skillful in using the scientific advantages of an independent research institute. He has been able to have a much more direct effect on the work, and to be flexible in his approach to problems. His ability to utilise this flexibility successfully (and unobtrusively) has been a hallmark of his Directorship. A scan of the Annual Reports since 1975 will quickly show that the research effort of the Institute has broadened and changed from its initial focus of blood pressure control to now include a range of attacks on the broad spectrum of cardiovascular disease. The mechanisms of attack include molecular and cell biology through to research into techniques of delivery of the research findings to the community.

Paul made single personal contributions to several areas of medical research. These have especially included the control of the heart and blood vessels by the autonomic nervous system, and the causes of hypertension. In both, his distinctive ability has been to find order in disorder, to build an integrated picture, to collaborate across scientific disciplines, and to combine both laboratory and clinical research.

His work on the control of the circulation by the central nervous system is without peer internationally. During his time at the Institute, Paul extended and expanded his studies in this area, and built a comprehensive, integrated and dynamic picture of how the cardiovascular system is regulated; the properties of the afferent inputs, the complex interplay and modulation of these inputs within the central nervous system, and the nature and relative importance of the neural and hormonal outputs in countering disturbances.

His research on hypertension, where his concept that the initiating or primary cause of hypertension is small and is amplified by secondary factors, particularly cardiovascular hypertrophy, has had an important impact on the whole direction of hypertension research. This idea sprang from studies of experimental hypertension in animals and was confirmed, in elegant experiments with Garry Jennings and Murray Esler, in patients. A practical outcome has been a change to the way in which hypertensive patients are treated.

Paul has continued active laboratory work throughout his term of Director of the Institute. This has had two benefits. It has been good for him, because of his enthusiasm for seeing how biology works. It has also been good for the Institute, because it has kept him in direct contact with the day-to-day problems of the Institute, and it has acted as an example to other staff.

In fact, Paul's success as a Director lies to a large extent in his skill and reputation as a scientist. He will leave the Institute with the valuable ethos that perhaps may be summarised as - "Is it important? Is it solvable? How can I best study it? Who can help?"

Paul came to the Institute after periods as Foundation Professor at the University of New South Wales, and Scandrett Professor of Cardiology at the Hallstrom Institute, University of Sydney. He plans to move back to Sydney to live but will keep links with members of the Institute. He was awarded the Order of Australia this year, is a Fellow of the Australian Academy of Science, has held many distinguished positions in national and international physiology and hypertension research, and been the keynote speaker at many international conferences.

In 1990, the Institute will move into the new era under its new Director as a very productive entity with a strong co-operative spirit and a high international reputation. Its distinctive feature is its interdisciplinary effort, involving the various branches of medical science and basic and clinical investigators. This is the scientific legacy that Paul Korner has created. The beneficiaries are the Baker Institute, in the narrower sense, and the Australia community in a wider sense, through better understanding, prevention and treatment of cardiovascular disease.

The Glaxo Australia – Pharmacology Laboratory Research Programme

Dr. James Angus and Dr. Ernest Mario discuss progress on the work of the Pharmacology Laboratory supported by Glaxo (Australia)

April 1989 was the beginning of the second year of an initial five year agreement between Glaxo Australia and the Baker Medical Research Institute to financially support in part, the Pharmacology Laboratory. Progress has been particularly pleasing in the three areas of research covered by the agreement including human coronary pharmacology, collateral blood vessel reactivity and endothelium-derived vasoactive factors. In the second year we have increased the areas of collaboration following a substantial increase in support from Glaxo Australia. This has enabled us to support our first Ph.D. graduate from the Pharmacology Laboratory, Dr. Michael Lew, on his return from a postdoctoral post in the United States. The collaboration with Glaxo has opened up exciting areas of new drug development and a dialogue over possible new targets for drug discovery. We were delighted to welcome Dr. Pat Humphrey, Head of the Pharmacology Division, Glaxo, Ware, England, to the Institute on 20th October where we discussed the projects and he gave a seminar on the development of the novel antimigraine drug sumatriptan. We were honoured to have Dr. Ernest Mario, Vice President of Glaxo Holdings Inc. visit us briefly on 17th November. He was able to assess first hand the facilities of the Institute and commented very favourably on the value of the collaboration. He also gave a radio interview from the Pharmacology Laboratory with Mr. John Jost (3LO). As part of the restructuring of Glaxo Research, cardiovascular research will be mainly controlled from Glaxo Research Laboratories, Research Triangle Park, U.S.A. In preparation for this change, Dr. Angus reported on the research at the Baker Institute to Dr. Jeff Leighton, Vice President, Pharmacology in December 1989. We look forward to the scientific interchange between Glaxo and Baker scientists and the excellent opportunities that this agreement allows between the pharmaceutical industry and basic research.

The Edgar Rouse Travelling Fellow

Professor Bjorn Folkow, Emeritus Professor of Physiology, University of Goteborg, was the Edgar Rouse Travelling Fellow in 1989. Professor Folkow is co-author of the best known student textbook on the circulation. He is a man of immense charm, enthusiasm (of the understated Swedish variety), humour, and knowledge of things cardiovascular, physiological, and cultural. Professor Folkow and our Director, Professor Paul Korner,

Edgar Rouse Travelling Fellow, Professor B. Folkow (centre), with Dr. Warwick Anderson (left), and Professor Korner.

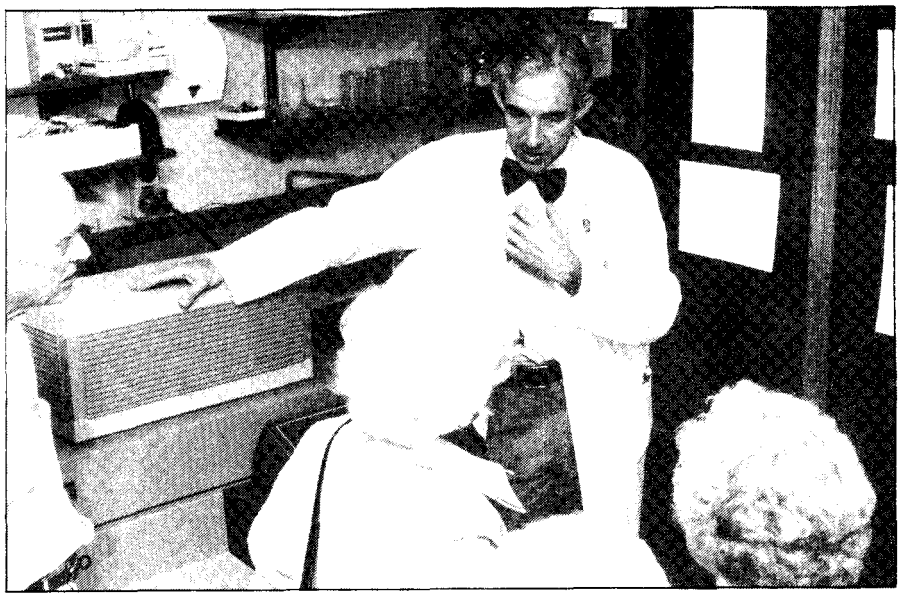
have long held complementary views on the pathogenesis of hypertension. Professor Folkow's time at the Institute facilitated the discussion on future directions of the Institute's work on the role of the hypertrophy of the heart and blood vessels as causes of high blood pressure. Professor Folkow worked with Drs Bobik, Jennings, Esler, Angus and Anderson, as well as Professor Korner on this topic while he was in the Institute.

Professor Folkow also infected the Renal Laboratory in the Institute with his enthusiasm for the question of whether the kidney possesses a vasodepressor substance. While he was here, Drs Anderson, Woods, Christy, Denton and others began a number of experiments to test whether such a vasodepressor substance is released from the kidney under physiological conditions in two species; how the substance lowers blood pressure, and what is its nature.

Tin Rattle at the MCG

On 13th May, 1989, the Institute conducted a 'Tin Rattle' outside the Melbourne Cricket Ground to raise funds for research.

Open Day at the Baker



On Sunday, 15th October, 1989, the Institute opened its doors to its donors by way of a special 'invitation only' display. About 300 donors attended, and viewed selected displays in all laboratories.

Victorian Ladies' Bowling Association

In August, 1989, Professor Paul Korner, Director of the Institute accepted a cheque for $80,000 raised by the Victorian Ladies' Bowling Association for Heart Research at the Institute.

Mrs. Joan Norton, President of the Victorian Ladies Bowling Association, presents the Association's donation to the Institute's Director and President.

The $80,000 represents the combined takings from many charity days held by individual Clubs throughout Victoria. Many of the 38,000 members of over 550 bowling clubs contributed to this magnificent fundraising effort.

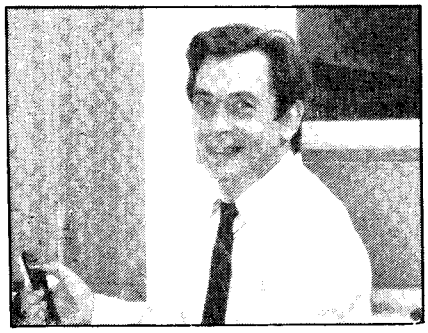
The Victorian Ladies' Bowling Association has been raising money for charities since 1952. They selected the Baker Institute for their charity of the year because the bowlers wanted to do something about heart disease, which is recognised as the World's No. 1 killer causing half of all deaths in Australia each year.

The Institute was extremely honoured to be the beneficiary and the money was used to purchase a major item of equipment.

Visiting Scientists

Dr. Didier Pruneau from Fournier Laboratories, Dijon, France, arrived in March 1989 to spend one year in the Pharmacology Laboratory. He has been most productive working on the role of N-type voltage operated calcium channels in the cardiovascular system. His return to France is also rumoured to give the trout a break from his skillful control of his favourite lure, a MEPS No. 2.

Dr J. Alasdair Millar from the Department of Pharmacology, University of Otago, Dunedin, New Zealand departed in April, 1989 after spending 6 months of his sabbatical leave working with Dr. Bobik on intracellular pH control in endothelial cells and oncogene regulation in vascular smooth muscle.



Prof. S. Tkackuk, visiting scientist from Moscow.

In November, 1989 Professor Seva Tkachuk from the Institute of Experimental Cardiology, Moscow, USSR spent 5 weeks in Dr. Bobik's laboratory investigating the mechanisms responsible for releasing calcium from intracellular stores in vascular smooth muscle and the activation of receptor operated calcium channels.

Professor Gunnar Wallin, from the Sahlgren Hospital, University of Gothenburg performed sympathetic nerve recording studies in the Human Autonomic Function Laboratory during November-December 1989, as part of a collaborative study on the effects of stress on the nervous system.

Dr Takeshi Saigusa from the Yamanashi Medical College in Yamanashi, Japan, arrived at the Institute in August 1989. He will spend two years in the Circulatory Control/ Neuropharmacology Laboratory, working on the involvement of central adrenergic pathways in baroreflex control of renal sympathetic nerve and heart rate.

Dr Naoyoshi Minami from the Department of Internal Medicine, Tohoku University School of Medicine in Sendai, Japan, is currently spending two years in the Circulatory Control and Neuropharmacology Laboratory with Dr Geoffrey Head examining the effects of anti-hypertensive treatment on the cardiac baroreflex. Dr Minami is interested in how the central nervous system regulates the heart and circulation and how this may change with the presence of high blood pressure. He is accompanied by his wife and small son.

Dr Chi Lei from the Cardiac Surgery Department at Fu Wai Hospital, Beijing, China, is currently working with Dr Frank Rosenfeldt in the Cardiac Surgical Research Laboratory, on laser angioplasty research using the excimer (cold) laser. Dr Zhang Fu Min, a cardiologist from Nanjing, China, is assisting Dr Rosenfeldt with the study of orotic acid after heart attacks. She is also attached to the Cardiology Department at the Monash Medical Centre.

Recently from the University of Wisconsin in the United States, Dr. David Ebert will spend three years in the Molecular Biology Laboratory studying the cholesteryl ester transfer protein gene in relation to both its structure and expression.

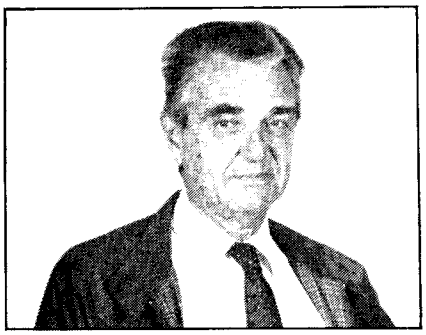
Dr Cetti Alafaci, a neurosurgeon and research scientist in the Department of Neurosurgery at the University of Messine, Italy, visited the Institute in November for one month. She divided her time working in collaboration with Susan Luff and Dr James Angus.

Dr Yi Gao is a Postdoctoral Fellow in the Renal Laboratory. She is a medical graduate of the University of Heidelberg, West Germany, and has also completed postgraduate work on the topic of atrial natriuretic factor in salt hypertension. Her work in the Institute centres on cardiovascular hypertrophy in renal hypertension.

Dr. Hiroya Hidaka has taken up a position as Visiting Scientist in the Lipoprotein Structure and Function Laboratory and comes from Shinshu University in Matsumota City, Japan. He is a biochemist and has been working on new methods of isolating the putative HDL receptor and has contributed significantly to our understanding of the glycoprotein nature of membrane HDL binding proteins. The Institute appreciates the voluntary assistance provided by his wife, Eiko Hidaka, a science graduate, to the Electronmicroscopy/ Morphology Laboratory.

Dr. Claudia Ferrier joined us in April 1989, from the Department of Nephrology and Hypertension Research at the University of Berne. She has been working in the Alfred and Baker Medical Unit with Drs. Jennings, Esler and Dart. Her projects have included participation in studies of the central nervous system release of noradrenaline as a possible test for sympathetic function. She has also been studying changes in microvessels in patients with heart failure and has become involved in our retinal artery project.

Dr. Florence Lacombe joined us in May 1989. She is a Cardiologist from Paris who specialises in Echocardiography. During her time here she has been involved in studies of aortic stiffness in patients with hypercholesterolaemia, coronary disease and anormals. It is hoped that these studies will lead to a relatively simple test which helps distinguish patients with high levels of risk factors who are at particular risk of developing coronary heart disease.



Edgar Rouse Fellow Professor Bjorn Folkow.

Laboratory Reports

Alfred-Baker Medical Unit

Director: Professor P.I. Korner
Deputy Director: Dr. G.L. Jennings

Projects

Effects of antihypertensive drug therapy on structural changes and overall cardiovascular risk in hypertension.

Structure and function of human resistance vessels in hypertension.

Non-invasive quantification of micro vascular structure changes by computer analysis of retinal images.

Relationship between the elastic properties of the aorta and hypercholesterolaemia.

Venous changes in hypertension.

Relationship between ambulatory BP and cardiovascular structural changes in hypertension.

Effects of exercise training on the arterial baroreflex.

Effects of exercise training on cardiac and renal sympathetic activity measured by noradrenaline spillover.

Exercise training and cardiac remodelling.

Effects of high and low intensity (walking) programme on cardiovascular risk factors.

Interactions between the effects of sodium restriction and exercise training on renal sympathetic activity.

Antihypertensive effects of weight loss and exercise training.

Dopamine release into the cerebrovascular circulation.

A comparison of microneurographic and regional noradrenaline spillover changes during laboratory stress, isometric exercise and desipramine infusion.

Effects of alterations in cardiac preload on the arterial baroreflex.

Effects of aging and of impaired ventricular function on cardiac sympathetic activity.

Pathophysiological studies in autonomic insufficiency.

The effects of congestive heart failure on structure and function of resistance vessels.

Sympathetic activity in heart failure.

Long term studies of the efficacy of simvastatin in hyperlipidaemia.

Effects of acipimox in patients with mixed hyperlipidaemia.

Accuracy and precision of blood cholesterol measurement.

Cardiac transplantation related hyperlipidaemia and hypertension.

Assessment of the value of self-referred screening for cardiovascular risk factors.

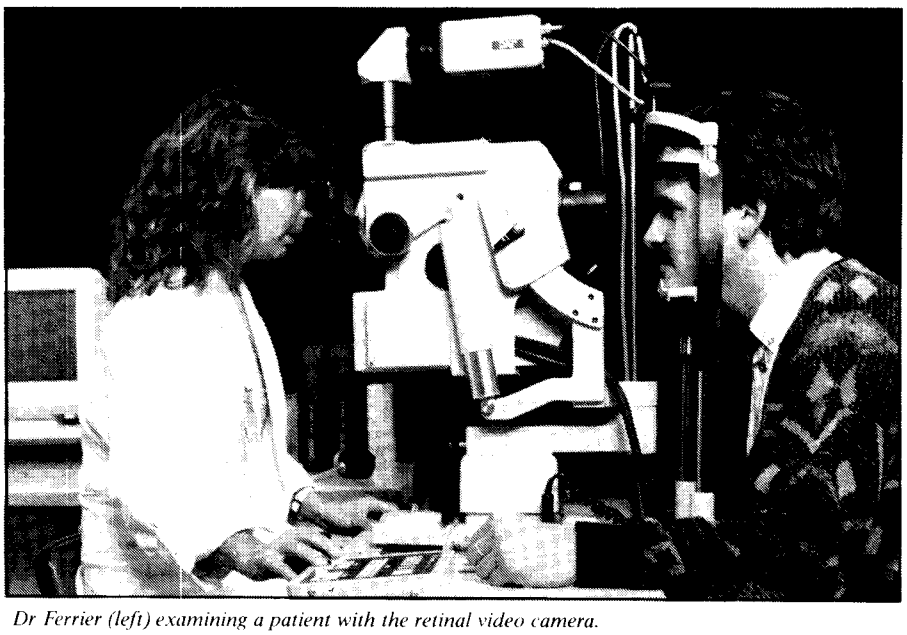
Effects of vitamin C administration on maximum aerobic capacity at various ages.

Human pharmacology of nebivolol.

Summary

Since the last Annual Report, the "Clinical Research Unit" has changed its name to the "Alfred and Baker Medical Unit". This took place during a general reorganisation of medical departments in the Alfred Hospital. The previous name has served us well over the past 40 years and is an excellent description of part of the work of the department but it was not so suitable for use in our clinical service role. The "Alfred and Baker Medical Unit" is a name which emphasises the links the Unit has with both hospital and research institute. It also has flexibility in the event that progress in the cardiovascular field or other factors might change our role in the future. The major purpose of the medical reorganisation in the Hospital, was to alter the balance between medical and specialist units. Because of the unique position of the Alfred and Baker Medical Unit in offering both general medical and specialist cardiovascular services, the day to day patient care activities of the Unit have changed little. However we greatly look forward to the eventual benefits which will accrue from the general strengthening of medical services as a result of the reorganisation.

The integration of patient care and research activities has always been an important priority. Being active in studying the causes and treatment of cardiovascular disease undoubtedly helps our physicians in maintaining a higher level of patient care. On the other hand, basic research activity is likely to be more relevant and opportunistically applied in the setting of an active clinical unit. This integration has



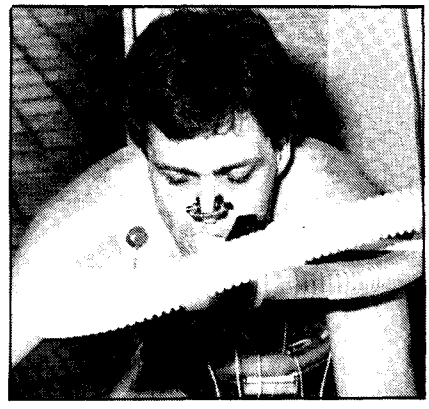
Dr Ferrier (left) examining a patient with the retinal video camera.

been greatly facilitated this year by the development of a computer network and patient database. Our patient databases have been evolving over several years now, initially with the help of Ann Tremayne and later by Chris Reid and Mark Sapper. We now have a most impressive programme which allows us to keep a record of all consultations, investigations and procedures, generate reports and audit our activities. We are also able to rapidly assess the success we have in reaching the various therapeutic targets. Although we developed this programme for our own use, it has generated tremendous interest amongst Australia's largest companies and many such as BHP, BP Australia, National Mutual and Woodside Petroleum, as well as the Victoria Police are using the system for their own preventive health programme. In this way the programme has become a vehicle by which the expertise in prevention of heart disease of our small research-based group reaches directly many thousands of people.

The upgrading of our equipment which took place as a result of the Rotary Club of Melbourne's establishment of our Heart Risk Reduction Clinic has also had a significant impact on our research work. The list of projects shown above includes a very broad range of clinical investigations. These include substantial programmes in hypertension, exercise, the sympathetic nervous system and atherosclerosis. In hypertension we have new techniques for focusing on the early structural changes in resistance vessels. Animal work performed in the Institute suggests that this may be a fundamental abnormality leading to high blood pressure. We are able to study these small vessels by isolating them from a small skin biopsy (see Pharmacology Laboratory report). We also hope to be able to follow the changes non-invasively using computer enhanced images from a retinal video camera so that the size of the small blood vessels in the eye can be measured.

In another field, the cardiovascular benefits of exercise, we have come a long way since our initial finding that training three times per week lowered blood pressure and improved other risk factors. Our more recent studies have focused on the time course, the amount of exercise required to achieve benefit, the changes in "target" organs such as the heart and the mechanisms involved. Our present hypothesis is that the initial changes which lower blood pressure during an exercise programme involve opening up of the blood vessels of the exercising skeletal muscle. Later a more complex interaction between structural changes, the autonomic nervous and hormonal systems helps to maintain the lower blood pressure.

Amongst the comings and goings of the last year, there has been a particular influx from Europe. Dr. Tony Dart has joined us as Senior Research Fellow, an NHMRC position in the Baker Institute. Dr. Dart is an experienced cardiologist and medical researcher who has already added much to our activities. He will be particularly involved in the development of a clinical atherosclerosis programme. Dr. Ferrier from Switzerland and Dr. Lacombe from France have also joined us for shorter periods. Clare Harwood departed after 10 years of devoted secretarial service and Drs. Meredith and Sudhir have completed work towards their Ph.D. and will be moving on in 1990. We have had help from virtually every other Hospital and University department and are extremely grateful for their collaboration and forbearance.



A volunteer during the bicycle exercise programme.

The effect of exercise training on cardiac dimensions, sympathetic activity and the arterial baroreflex

G. Jennings, I. Meredith, B. Kingwell, M. Esler, A. Dart, P.I. Korner

Regular exercise lowers resting blood pressure, heart rate and total norepinephrine (NE) spillover. We examined the extent to which the cardiac sympathetic is involved, both at rest and during activation of the cardiac baroreflex, in 19 subjects who performed one month each of sedentary activities and of exercise training 3/weekly for 30 min in a randomised crossover design. All measurements were performed 48 h after the last exercise bout.

We found that training reduced resting MAP by 5-7 mmHg, resting HR by 6 beats/min, and increased maximum oxygen consumption by 13% compared to sedentary values (all $P<0.05$). LV internal diameter (echo) increased by 4% ($P<0.05$). Despite a 24% reduction in resting total NE spillover, measured by a tracer technique in 9 subjects, cardiac spillover was unchanged and 2/3 of the total fall was due to reduction in renal spillover. In contrast baroreflex curves obtained by the 'steady-state' method using vasoactive drugs (n=11) showed attenuation of the sympathetic responses to BP reduction by training without change in the vagal components. The difference in the curves was abolished by i.v. propranolol (0.2 mg/kg). The resting bradycardia of training persisted after total cardiac autonomic blockade (102 to 95 ±1.5 beats/min, n=11, $P<0.05$).

These results suggest that training induces a major fall in resting renal sympathetic activity but no change in cardiac spillover. The resting bradycardia primarily reflects a fall in intrinsic rate. However the reflex cardiac sympathetic response to acute BP lowering is attenuated by training. These changes may reflect altered CNS afferent input

due to cardiac remodelling and increased central blood volume.

Structural changes of retinal arteries in primary hypertension

C. Reid, C. Ferrier, A. Dart, G. Jennings

In hypertension one of the most important aims is to normalize blood pressure. Many drugs have been developed which lower blood pressure in hypertension. However, factors other than the blood pressure value per se may be important indicators for long term morbidity and mortality. Studies in vitro have shown that hypertension leads to changes in the micro vascular structure and function. However, a non-invasive methodology for studying small arteries in vivo has not yet been developed. We hope to detect structural changes using retinal photography in small arteries of hypertensive patients and quantitate their response to antihypertensive therapy. Retinal video images are enhanced and quantitated using an image analysis system. Current projects include assessment of structural changes in relatives of hypertensives, and the reversibility of blood vessel alterations with antihypertensive treatment.

Impaired contraction and relaxation in resistance arteries from patients with congestive heart failure

J. Angus, G. Jennings, C. Ferrier, A. Dart

We have used the double myograph technique to measure the reactivity to a wide range of agents of microvessels in patients with congestive cardiac failure. The microvessels were small arteries obtained from a skin biopsy. The results in cardiac failure patients were then compared to those in age-matched healthy volunteers. Vessels from patients with cardiac failure had less than half the maximum response to the administration of vasoconstrictors. Interestingly, there was little difference in the reactivity of the vessels to noradrenaline, angiotensin I or angiotensin II between vessels from patients previously treated or not with ACE inhibitors. The vasodilators C-GRP and acetylcholine relaxed precontracted vessels by 74-90% in healthy volunteers, but by only 40-53% in cardiac failure patients. These findings indicate that congestive cardiac failure is accompanied by severe loss of reactivity to vasoconstrictor and vasodilator stimulu in skin resistance arteries.

Relationship between elastic properties of the aorta and hypercholesterolaemia

F. Lacombe, A. Dart, E. Laufer, G. Jennings

The elastic properties of the aorta are impaired physiologically with age and pathologically with atherosclerosis. It has been suggested that early functional evaluation of atheroma could be obtained from the assessment of these mechanical aortic properties. However few non invasive studies have already been performed in man, and there is no consensus on the best index to use.

In our study, we used echo-Doppler to assess aortic distensibility from suprasternal views of the aortic arch and determined functional stiffness indices. To establish the validity of the method, we studied a sample of healthy volunteers without cardiovascular risk factors and assessed the normal sensitivity to age of the major indices. The application of the method to hypercholesterolaemic subjects, with or without signs of atheroma, is now in progress. Preliminary indications suggest that these methods may provide early evaluation of the deleterious effects of disease before clinical manifestations, discrimination between subjects at higher risk of stroke and follow-up of the effects of drug treatment.

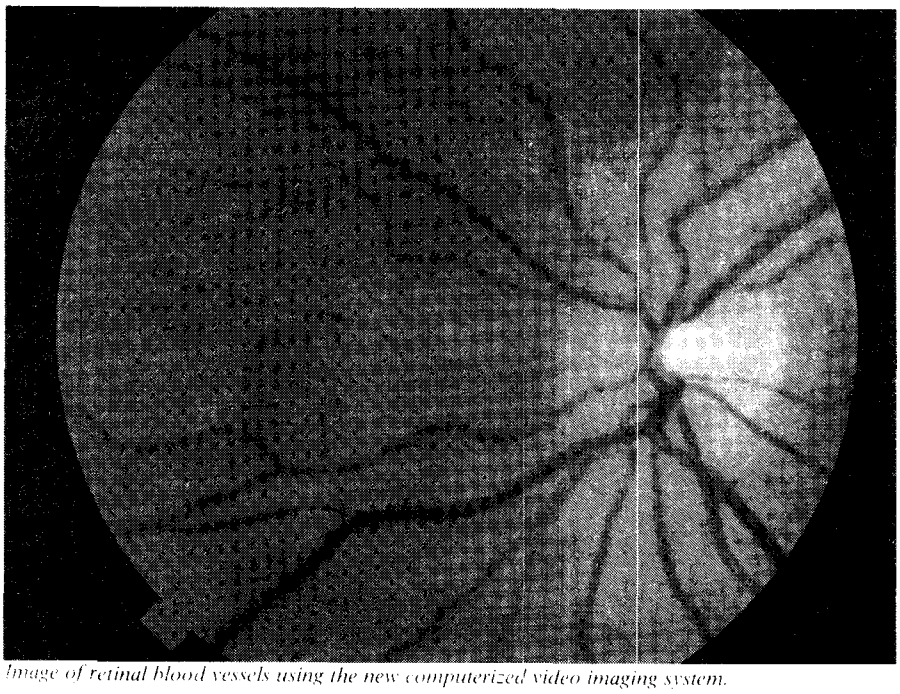
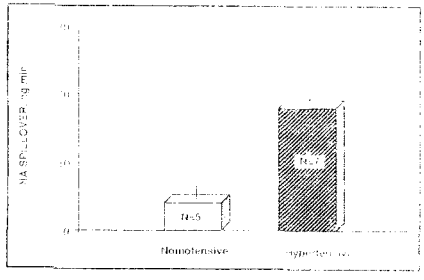


Image of retinal blood vessels using the new computerized video imaging system.



Cerebral noradrenaline spillover in hypertensive and normal subjects. Higher values in the former could reflect brain central nervous system turnover of noradrenaline in hypertension and may provide a new method of measuring the link between the nervous system and high blood pressure.

Biochemical Pharmacology Laboratory

Head: Dr. A. Bobik

Projects

Calcium ions and oncogene regulation.

Receptor operated calcium channels.

Effects of angiotensin II and noradrenaline on platelet derived growth factor receptors.

Growth characteristics of vascular smooth muscle from spontaneously hypertensive rats.

Interactions between transforming growth factor beta 1 and growth factors for vascular smooth muscle.

Development of cardiovascular hypertrophy in stroke prone spontaneously hypertensive rats.

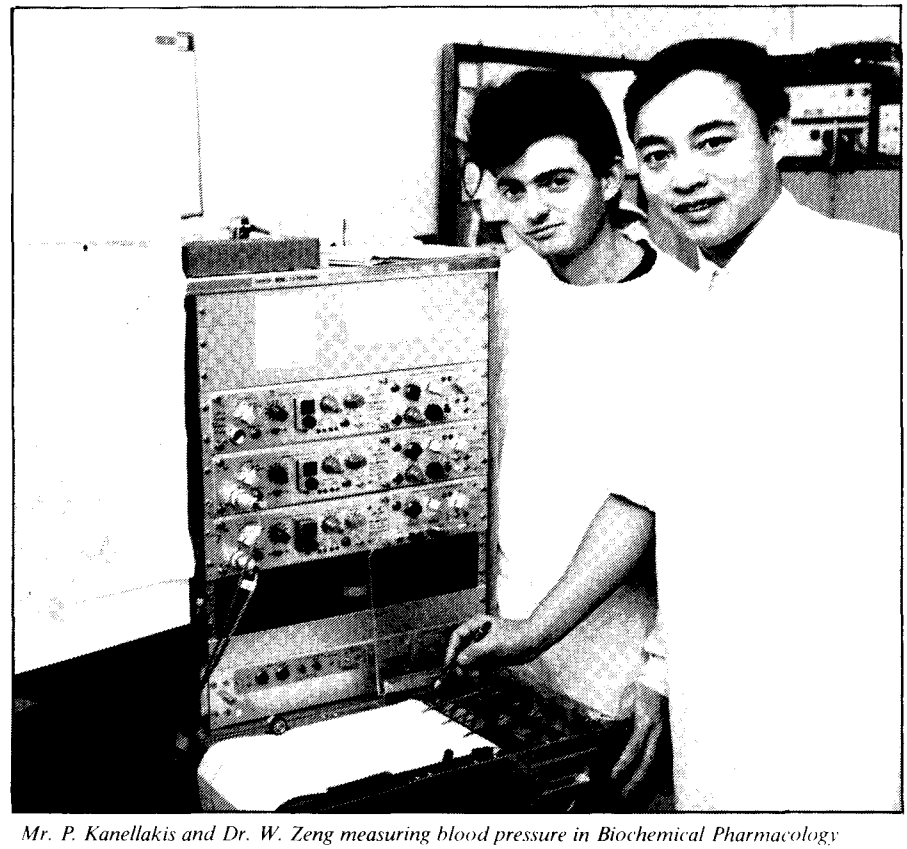
Role of catecholamines in cardiovascular hypertrophy in spontaneously hypertensive rats.

Summary

During the past year we have continued to focus on understanding the process responsible for the transport of sodium and calcium ions in vascular smooth muscle. These ions have the potential to markedly affect the development of cardiovascular hypertrophy (thickening of the walls of blood vessels and an increase in muscle mass in the left ventricle of the heart) in hypertension. We have also extended our studies to define the role of growth factors in the development of cardiovascular hypertrophy. Previously we suggested that cardiovascular hypertrophy may be the cause of the elevated blood pressure in animals with genetically inherited hypertension. In other types of hypertension, where hypertrophy is not causative, there is no doubt that it is a major contributor to the elevated blood pressure once the hypertension has become established.

We have demonstrated previously that the activity of sodium-hydrogen exchange, a membrane transport system for sodium and hydrogen ions which can influence cell growth, is markedly dependent on elevations in calcium levels in vascular smooth muscle. This year we have examined whether calcium ions influence the activation of two oncogenes involved in cell growth, c-fos and c-myc. Calcium levels are rapidly elevated when vascular smooth muscle are exposed to growth factors or constrictor agents. This elevation in calcium is accompanied by the activation of the c-fos and c-myc genes. When calcium influx into these cells is prevented, gene activation is abolished. Direct evidence for the activation of the genes by calcium was obtained by elevating intracellular calcium with agents which carry calcium across cell membranes (ionophores). These studies have enabled us to conclude that an increase in intracellular calcium may be a signal which activates these two growth genes. Our finding that both growth factors and constrictor agents are capable of activating c-fos and c-myc strongly suggest that both types of agents have the ability to initiate vascular hypertrophy. In these studies we have also examined how the calcium signal is subsequently processed within the cell. The elevation in calcium activates the phosphorylating enzyme system protein kinase C. This enzyme has recently been shown to be also present in the nucleus and it activates the c-fos gene in vascular smooth muscle. Neither protein kinase C nor calmodulin dependent systems were involved in processing the calcium signal for activating the c-myc gene.

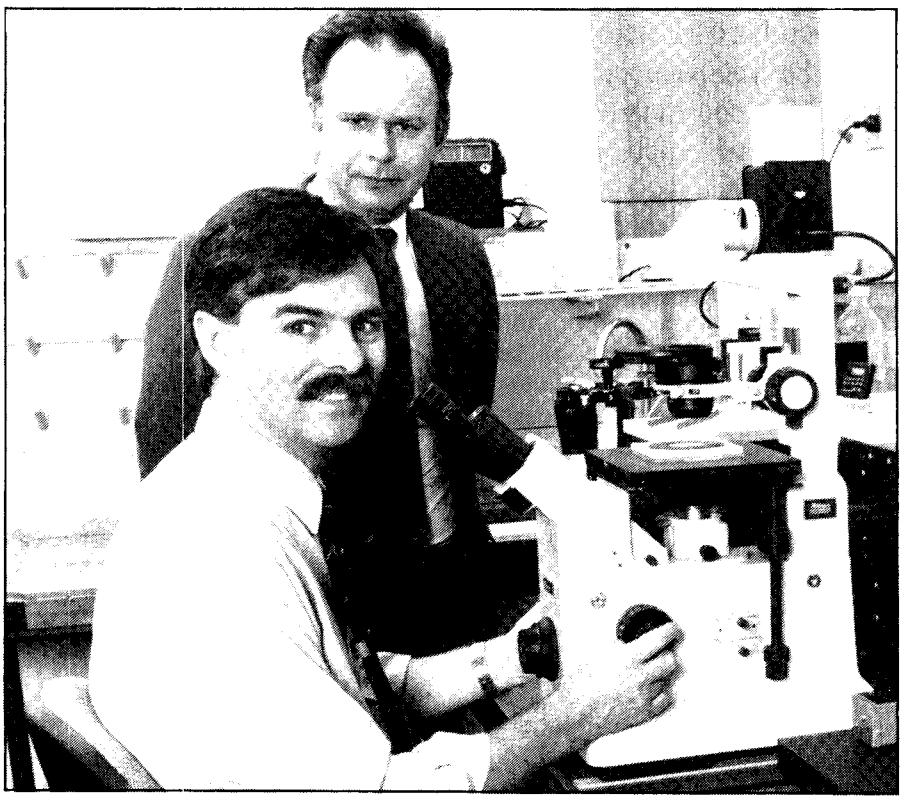
Because calcium ions play such an important role in regulating vascular smooth muscle function, our studies have also concentrated on understanding more fully the mechanisms responsible for elevating intracellular calcium concentrations when smooth muscle is exposed to growth factors or constrictor agents. Under these conditions the elevation in the free cellular calcium



Mr. P. Kanellakis and Dr. W. Zeng measuring blood pressure in Biochemical Pharmacology Laboratory.

concentration is due to the release of calcium from intracellular stores and/or an influx of calcium into the cells. An elevation in the concentration of the intracellular messenger inositol trisphosphate has been suggested to be responsible for releasing the calcium from intracellular stores. Our studies with two endothelial cell derived factors, endothelin-1 and endothelin-3, indicate that two processes may be responsible for releasing intracellular calcium stores; an inositol trisphosphate dependent and -independent mechanism. In other studies we have demonstrated that vascular smooth muscle cells possess a number of different receptor operated channels through which calcium may enter.

This year our studies on growth factors for vascular smooth muscle have concentrated on examining (i) how the constrictor agents angiotensin II and noradrenaline increase the sensitivity of smooth muscle to the growth factor PDGF-BB and (ii) the ability of type 1 transforming growth factor to influence vascular smooth muscle cell replication. We found that angiotensin II and noradrenaline increased the number of receptors on vascular smooth muscle for PDGF-BB. This effect was specific to this subtype of receptor for platelet derived growth factor (PDGF) and accounted for virtually all the increase in the sensitivity of the smooth muscle to the mitogenic effects of PDGF-BB. Our studies with type 1 transforming growth factor beta (TGFβ-1) indicate that this agent, which alone has no effect on smooth muscle replication, can markedly increase the potency of growth factors which activate tyrosine kinase. These include, for example, basic fibroblast growth factor (bFGF) and the platelet derived growth factors - AB and - BB. TGFβ-1 has little effect on the increases in cell growth induced by factors such as endothelin-1 and α-thrombin whose stimulatory properties appear dependent on activating the enzyme phospholipase C. Taken together these latter studies indicate that endothelial cells which are capable of secreting TGFβ-1, bFGF and the PDGF dimers can control the potency of its growth promoting factors via its secretion of TGFβ-1.



Dr. Peter Little (foreground) and Dr. Alex Bobik.

One of the characteristics of vascular smooth muscle isolated from spontaneously hypertensive rats (SHR) is its ability to grow more rapidly in tissue culture than smooth muscle from normotensive animals. Our studies on the growth properties of vascular smooth muscle from these hypertensive animals indicate that these cells also grow to a higher cell density in culture. Not only do these cells replicate more rapidly when exposed to growth factors, they are also less susceptible to substances which inhibit cell replication. One very interesting finding was that vascular smooth muscle from normotensive WKY rats took on the properties of smooth muscle from the hypertensive animals after multiple subculture. These studies indicate that the smooth muscle from SHR possess a defective growth regulatory mechanism which may contribute to the vascular hypertrophy in these animals. Our studies on genetically inherited hypertension have focused on (i) determining the precise contribution which catecholamines released either from sympathetic nerves and/or adrenal glands make to the cardiovascular hypertrophy which develops in spontaneously hypertensive rats [SHR] prior to any abnormal elevation in blood pressure and (ii) whether in spontaneously hypertensive rats highly susceptible to stroke [SHR(SP)] cardiovascular hypertrophy is the sole determinant of their blood pressure. We found that simultaneous removal of sympathetic nerves from SHR together with blockade of circulating catecholamines with the alphal adrenoceptor antagonist prazosin 'normalised' the cardiac hypertrophy in four week old pre-hypertensive SHR. The small effect on vascular hypertrophy is consistent with our other studies indicating that additional mechanisms, involving growth factors also contribute to the vascular hypertrophy in these animals. Our findings in SHR(SP) rats indicate that other factors in addition to cardiovascular hypertrophy contribute to their elevated blood pressure. The extent to which vascular hypertrophy had developed in these animals was identical to that previously observed in SHR, the blood pressure was elevated to a greater extent than in SHR.

Calcium influx into vascular smooth muscle: a signal for the expression of c-fos and c-myc mRNA

A. Bobik, A. Grooms, P. Little, A. Mitchell, J.A. Millar.

To gain an insight into the cellular mechanisms responsible for an increased ability of vascular smooth muscle to replicate, as has been observed in genetic hypertension, we investigated the influence of growth factor initiated elevations in intracellular calcium and increases in protein kinase C activity on the activation of two primary response growth genes c-fos and c-myc. Confluent cultures of rat aortic smooth muscle made quesient by 24-48h exposure to defined serum-free medium were exposed to platelet derived growth factor (PGDF-BB), α-thrombin and endothelin -1. In all instances c-fos mRNA was elevated from undetectable to peak levels (15 fold) within 30 min and completely gone by 1h. Peak c-myc mRNA levels occurred after 2h and were maintained through to 6h. The calcium ionophores A23187 and ionomycin also elevated c-fos (5 fold) and c-myc mRNA (2 fold) in these cells. When the extracellular calcium was reduced below 100nM both the growth factor and ionophore induced calcium influx into smooth muscle were abolished as were the elevations in c-fos mRNA and c-myc mRNA. Similarly protein kinase C depletion attenuated both the growth factor and ionophore induced elevations in c-fos mRNA by 50 to 69%. Neither the elevations in c-myc mRNA nor calcium influx into the smooth muscle were affected by protein kinase C depletion or dependent on calcium/calmodulin dependent processes. We conclude that an increase in cytoplasmic calcium is an early regulatory signal for the activation of c-fos and c-myc genes. Subsequent signal transmission for c-fos activation occurs in part via protein kinase C dependent mechanisms whilst for c-myc it is completely independent of protein kinase C. In neither case were calcium/calmodulin dependent mechanisms involved.

Receptor operated calcium channels in vascular smooth muscle

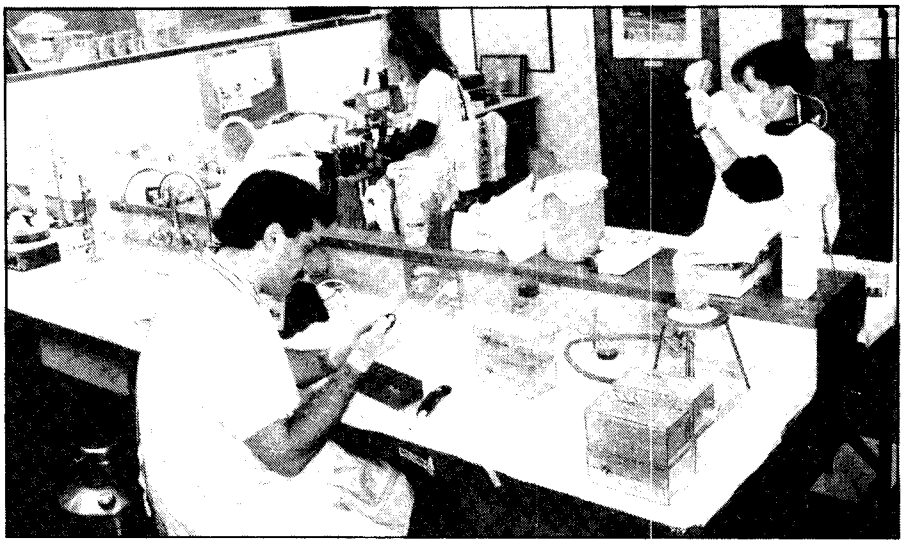
P. Little, V.A. Tkachuk, A. Bobik.

It is generally accepted that many growth factors and a variety of constrictor agents elevate intracellular calcium ion [Ca^{2+}] in vascular smooth muscle by initiating either the release of calcium from intracellular stores and/or an influx of extracellular calcium via the activation of specific receptor operated channels. At present little is known about the mechanisms responsible for these elevations in [Ca^{2+}]. Receptor mediated increases in inositol trisphosphate via phospholipase C activation are thought to be responsible for the release of calcium from intracellular stores. We investigated this hypothesis by examining the ability of endothelin-1 and endothelin-3 to elevate [^{3}H] inositol trisphosphate levels in relation to their ability to release intracellular calcium in cultural rat vascular smooth muscle loaded with the calcium sensitive dye Fura-2. Endothelin -1 markedly elevated [^{3}H] inositol trisphosphate in smooth muscle and this was associated with marked calcium release from intracellular stores. In contrast endothelin-3 did not significantly affect [^{3}H] inositol trisphosphate levels but did induce intracellular calcium release. Thus receptor mediated intracellular calcium release can also be induced via inositol trisphosphate independent mechanisms. To further investigate whether a single receptor can release intracellular calcium stores via both mechanisms - inositol trisphosphate dependent and -independent processes, vascular smooth muscle cells were pre-exposed for varying periods of time to the vasodilator sodium nitroprusside. This agent induced a marked (75%), time dependent (maximum at 1h) inhibition of the endothelin -1 induced elevation in [^{3}H] inositol trisphosphate, presumably by inhibiting the activation of phospholipase C, since both [^{3}H] inositol mono and bisphosphate levels are also reduced. Under these conditions intracellular calcium release was reduced by only 19%. We conclude that two mechanisms may be responsible for receptor mediated release of calcium from intracellular stores in vascular smooth muscle an inositol trisphosphate dependent and -independent mechanism. The relative importance of the two mechanisms is dependent on the receptor system being activated.

Angiotensin II and noradrenaline potentiate PDGF-BB induced DNA synthesis via an increase in PDGF-BB receptors

S. Grinpukel, G. Jackman, P. Little, A. Grooms, A. Bobik.

Our previous studies into the mechanisms by which angiotensin II and noradrenaline could affect the development of vascular hypertrophy in hypertension demonstrated that both agents increased the sensitivity of vascular smooth muscle in culture to sub-maximal mitogenic concentrations of platelet derived growth factor BB chain (PDGF-BB) but not platelet derived growth factor AB chain (PDGF-AB). We have now demonstrated that the different responses to the two growth factors could be accounted for by their interaction with two different membrane receptor systems. Over the concentration range 8 to 200 nM, PDGF-BB but not PDGF-AB displaced [^{125}I]PDGF-BB specific binding to smooth muscle in a concentration dependent manner. We also observed that 24h exposure of the smooth muscle to angiotensin II or noradrenaline increased the number of PDGF-BB receptors without affecting their affinity for the growth factor. The increases, which averaged 23% and 20% after incubating the cells with angiotensin II and noradrenaline respectively,



Smooth muscle cells studies (from left) are Alex Agrotis, Annette Grooms and Ingrid Moeller.

could account for all the increase in mitogenic activity of PDGF-BB. We conclude that both angiotensin II and noradrenaline can increase the sensitivity of vascular smooth muscle to the mitogenic effects of PDGF-BB by specifically increasing the number of membrane receptors. Further studies will be required to determine whether this increase in PDGF-BB receptor numbers is the consequence of an increase in their rate of biosynthesis or degradation.

Growth characteristics of vascular smooth muscle from spontaneously hypertensive rats

J. Saltis, P. Little, A. Bobik.

It has recently been suggested that an enhanced proliferative responsiveness of vascular smooth muscle from spontaneously hypertensive rats (SHR) to growth factors may

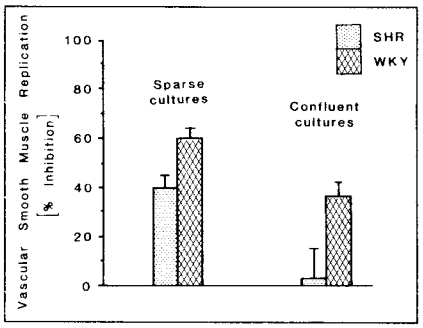


Fig. 1 Differential inhibitory effects of type 1 transforming growth factor beta (TGFβ-1) on the replication of aortic smooth muscle from SHR and WKY rats. In these experiments 10% serum was used as the growth stimulant.

be a contributing factor to the early increase in vascular wall thickening which occurs in these animals before they develop hypertension. To further define the growth characteristics of vascular smooth muscle from these animals we compared their ability to replicate in serum containing medium with the smooth muscle obtained from normotensive Wistar Kyoto (WKY)

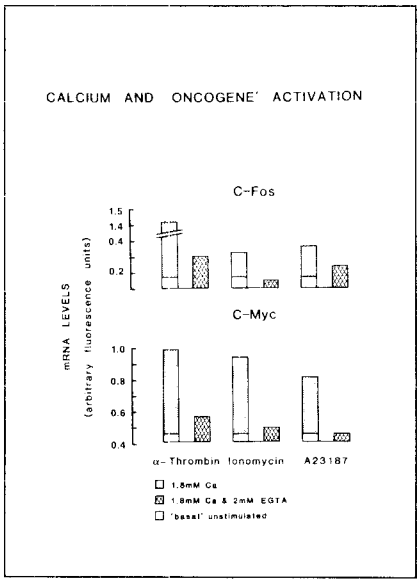


Fig. 2 Dependency of c-fos and c-myc gene activation on calcium influx into vascular smooth muscle.

rats. We examined cells placed into primary culture and also after both early and late passages of subculture. Vascular smooth muscle from SHR grown in primary culture arrested after reaching multi-layer confluency at a higher cell density than did smooth muscle from WKY. Early passages of subcultured cells behaved in an essentially similar fashion. Subcultured smooth muscle from SHR replicated more rapidly than those from WKY independently of the initial seeding density. In late passages the growth characteristics of the smooth muscle from the two strains of rats became essentially indistinguishable as smooth muscle from WKY took on the growth characteristics of smooth muscle from SHR.

Since vascular smooth muscle growth in vivo is also under the influence of inhibitory growth factors, we also examined the responsiveness of the smooth muscle

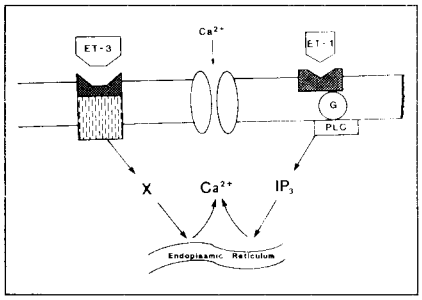


Fig. 3 Schematic diagram depicting the mechanisms by which calcium ions can be released from intracellular stores of vascular smooth muscle. Endothelin -1 (ET-1) stimulates the production of inositol trisphosphate (IP3) which releases calcium stored in the endoplasmic reticulum. In contrast endothelin-3 (ET-3) releases calcium from the same endoplasmic stores by novel mechanisms independent of IP3.

from the two strains of rats to type 1 transforming growth factor beta (TGFβ-1). Smooth muscle from SHR replicating in serum containing medium was less susceptible to the inhibitory effects of TGFβ-1. The growth rate of early passaged subcultured smooth muscle from WKY rats was reduced by approximately 35% whilst those from SHR were reduced by less than 5%. This difference in sensitivity of the smooth muscle from the two strains of rats was not a consequence of differences in the IC50 for TGFβ-1 which in both instances averaged 0.08 g/ml.

We conclude that vascular smooth muscle from SHR is not only more responsive to stimulatory

growth factors present in serum but also less responsive to the inhibitory effects of TGFβ-1.

Type 1-transforming growth factor beta enhances the effects of tyrosine kinase activating growth factors on vascular smooth muscle replication

J. Saltis, P. Little, A. Bobik.

Type 1 transforming growth factor beta (TGFβ-1) has the unusual ability to both stimulate and inhibit the replication of cells in culture. Because endothelial cells have the capability to produce and secrete this peptide, we examined its effects on replicating rat aortic smooth muscle. In tissue culture TGFβ-1 consistently inhibited the replication of smooth muscle cultured in serum containing medium. Similarly in chemically defined culture medium containing transferrin and insulin, TGFβ-1 attenuated the small growth stimulatory effects of endothelin -1 and α-thrombin. In contrast TGFβ-1 potentiated the effects of tyrosine kinase activating growth factors on smooth muscle replication, approximately doubling their replication rate in the presence of platelet derived growth factor - BB or AB, epidermal growth factor or basic fibroblast growth factor. Alone, this concentration (1ng/ml) of TGFβ-1 had no significant effect on vascular smooth muscle replication. Taken together our observations indicate that TGFβ-1 can markedly enhance the effects of local growth factors produced within the walls of blood vessels. The mechanisms responsible for this enhancement are currently under investigation.

Vascular amplifier properties in stroke prone spontaneously hypertensive rats

K. Oddie, P. Scott, A. Bobik, P. Korner.

Since blood pressure is elevated to a greater extent in stroke prone spontaneously hypertensive rats [SHR(SP)] than in SHR, we have investigated whether further increases in vascular hypertrophy above those seen in SHR could account for the higher blood pressure [SHR(SP)]. We examined the time course of development of vascular hypertrophy in these animals and compared it with those from SHR and normotensive WKY rats. Vascular hypertrophy was assessed indirectly from changes in the perfusion pressure following perfusion of the animals' hindquarters under conditions of constant flow. We found the relationship between the perfusion pressure during maximum constriction of the vasculature and body weight to be identical in the SHR(SP) and SHR between 4-20 weeks of age. Furthermore, at every age, the perfusion pressures observed under these conditions were higher than those observed in the normotensive animals. In young (4 to 9 weeks old) SHR(SP) cardiac hypertrophy was similar to that observed in SHR; however in the older animals the hypertrophy developed more rapidly and to a greater extent, reflecting the more rapid development of blood pressure in this strain. Our observations indicate that other factors in addition to cardiovascular hypertrophy such as, for example, an increase in sympathetic nervous activity, are also important contributors to blood pressure elevations in SHR(SP).

Effects of combined sympathectomy and alpha1-adrenoceptor blockade on pre-hypertensive cardio-vascular hypertrophy in genetic hypertension

A. Bobik, K. Oddie, P. Friberg, P. Korner.

Cardiovascular hypertrophy is a major contributor to the elevated blood pressure in spontaneously hypertensive rats (SHR). Both the vascular and, to some extent, the cardiac hypertrophy develops very early in life, before there is any abnormal rise in blood pressure. It has been suggested that this hypertrophy plays a critical role in the development of the hypertension in this rat strain and may even be responsible for the hypertension. The mechanisms by which cardiovascular hypertrophy develops are not known. We have investigated in very young pre-hypertensive SHR and normotensive WKY rats the effects of combining peripheral sympathectomy with alpha1 adrenoceptor blockade on the development of hypertrophy. Alpha1 adrenoceptor blockade was added to the sympathectomy protocol to abolish the effects of overactivity of adrenal glands following sympathectomy. Two day old male SHR and normotensive WKY rats were sympathectomised by injecting antibodies to nerve growth factor and large doses of guanethidine. Prazosin was also administered to ensure that catecholamines released from the adrenal glands would not activate alpha1 adrenoceptors in the cardiovascular system. When the animals reached 4 weeks of age, the effects of this procedure on vascular hypertrophy were assessed haemodynamically by comparing the resistance of the hindquarter vasculature under conditions of constant flow with control, untreated animals. Cardiac hypertrophy was assessed from left ventricular: body weight ratios. The sympathectomy protocol abolished differences in left ventricular: body weight ratios between the hypertensive and normotensive strain of rats. Combined sympathectomy plus alpha1 adrenoceptor blockade also appeared to reduce the severity of the vascular hypertrophy in SHR, with differences in perfusion pressures during maximal constriction of the vasculature between the two strains being reduced by approximately 15%. We conclude that the sympathetic nervous system can influence the development of the early pre-hypertensive cardio-vascular hypertrophy in SHR, but it is not the sole cause of the vascular hypertrophy.

Cardiac Surgical Research Laboratory

Head: Dr. F.L. Rosenfeldt

Projects

Clinical study of preservation of the donor heart.

Study of cardiac preservation using the isolated rat heart.

Effect of orotic acid and of ribose in the rat heart with a recent myocardial infarct.

Comparison of excimer laser and balloon angioplasty in the atherosclerotic rabbit.

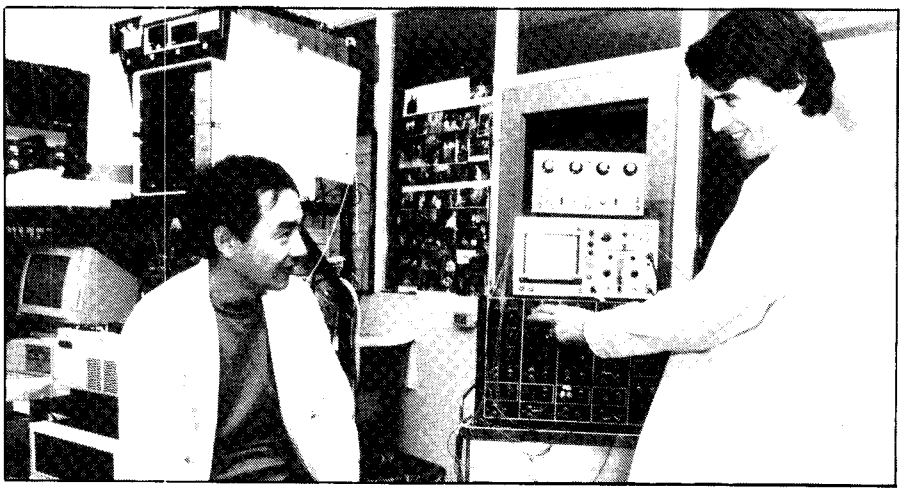
Summary

The last year has been an historic one both for the Alfred Hospital Cardiac Surgery Unit and the Baker Cardiac Surgical Research Unit, with the establishment of Cardiac Transplantation at the Alfred Hospital. In the first twelve months, 30 patients were transplanted without mortality and with excellent overall results. Throughout this time, the head of the Transplant Unit, Mr. Don Esmore and his clinical team at the Alfred worked closely with the staff of the Cardiac Surgical Research Unit at the Baker Institute.

For the first 18 transplants, the hearts were obtained within the distance covered by one hour's flying time by chartered jet. For case 19, the transplant team was presented with a challenge. A donor heart was offered from Townsville, Queensland which would involve a transfer time from donor to recipient of approximately 5 hours. Generally speaking 4 hours has been regarded as the upper limit for safe transfer time for hearts, even under ideal conditions. The previous longest transfer time in Australia of 4 hours and 20 minutes involved a donor flight by the Sydney St. Vincent's team to Alice Springs and back. On that occasion the donor heart was large and in good condition. For the proposed Alfred Hospital long distance tranfer, the heart was relatively small for the recipient and had already sustained some strain in the donor. Clearly conditions were not ideal and special techniques would be required to make safe transfer possible. Utilizing the combined expertise of the Baker Institute and the Alfred Transplant team, a new strategy of preservation was put together and a decision made to use the heart. Several techniques borrowed from cardiac surgical practice and the research laboratory were combined. Oxygen was bubbled into the preservation and storage solutions. An "intracellular" formulation including a very high potassium concentration was used for the storage solution. On arrival back at the Alfred Hospital, additional doses of cold and then warm, blood-containing cardioplegic solutions were given during the implantation procedure. To our great satisfaction, the "five hour heart", showed excellent function and the patient recovered rapidly from surgery. Soon afterwards, another heart was offered from North Queensland, this time from Cairns. This was also transplanted successfully using the new preservation strategy and involved a transfer time of 5 hours and 10 minutes.

Although the new preservation strategy appeared to be effective in practice, it was put together on a rather empirical basis. What we are involved in now is a systematic investigation in the laboratory of the various techniques already used and some more innovative techniques, with the aim of extending preservation of the donor heart to 8 hours or more. Increased confidence in long term safe preservation of the heart will have additional benefits, such as rendering unnecessary, haste in transporting the heart between the airport and the hospital, with the attendant dangers. These dangers were vividly exemplified in the vehicle accident involving the transplant team during 1989.

The late 1970's and the 1980's witnessed an explosive expansion of interventional radiology and cardiology. This is typified by the widespread use of balloon dilatation (angioplasty) of narrowed coronary arteries. The first coronary angioplasty was carried out in 1967. In Australia last year, over 3,000 such procedures were performed. However, this technique simply stretches the arterial wall as a whole, and usually splits the inner layers at the site of the narrowing but leaves the deposit of atheroma behind. The use of laser techniques to unblock arteries has the potential to provide better results because the laser can actually remove atheroma by vaporizing it. We have been collaborating with a Californian-based company to evaluate the ability of the ultraviolet (excimer) laser to ablate atheroma in rabbits and to compare the success rate with that achieved by balloon angioplasty. The results of the study encourage us to plan a trial in patients at the Alfred



Dr Chi Lei (left) and Dr Andrew Cochrane study ways of saving heart muscle function in the Cardiac Surgery Laboratory

Hospital which we hope will commence in 1990.

We have continued our studies on orotic acid as a metabolic supplement for the heart under stress. We have previously shown in the dog and the rat that a heart attack before operation impairs the ability of the heart to withstand cardiac surgery. We have now begun to investigate the biochemical mechanism of this effect in rats by analyzing the levels of pyrimidine nucleotides in the heart muscle.

Preservation of donor hearts for cardiac transplantation

F.L. Rosenfeldt, L. Langley, A. Cochrane, R. Conyers

The first step in improving the state of the donor heart at the time of implantation was to look for a numerical measure of the quality of preservation in clinical practice. Drill biopsies were taken from the donor heart at the end of the ischaemic period just prior to reperfusion in the recipient. These biopsies were assayed for myocardial levels of ATP, creatine phosphate and lactate. We found a correlation between ischaemic time on one hand and the rise in lactate and decline in creatine phosphate on the other. ATP levels were at the lower limit of normal in most hearts but changed little with time. We are now making the same measurements in the hearts perserved using new techniques to detect any improvements in myocardial metabolism.

In parallel with these clinical studies, we have been performing laboratory studies in a rat model of donor heart preservation. Using the isolated rat heart apparatus, hearts are made ischaemic for 4 hours at 4°C. After preservation using a variety of techniques, cardiac pump function is measured. Our first step was to test the efficacy of oxygenation of the initial infusate. We found that a single infusion of oxygenated solution at the onset of ischaemia was not sufficient to improve recovery. Presumably the oxygen delivered was rapidly used up and to confer a measurable benefit, the infusion would have to be repeated at intervals during the ischaemic period.

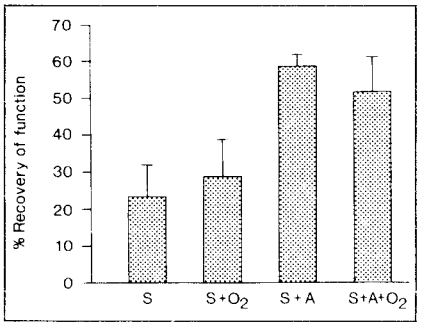


Fig.1 Recovery of rat hearts (6-8 per group) after 4 hours preservation under transplant conditions at 4°C. S = cardioplegic solution; O_2 = oxygen; A = aspartate. S vs SA: $p < 0.02$; SO_2 vs SAO_2: $p < 0.05$.

Research scientists at the University of Auckland had shown in the rat heart that preservation of hearts could be improved if oxygen and aspartate were added to the cardioplegic solution. We confirmed their observations and showed that the protective action was entirely due to the aspartate and not to the oxygen (Fig. 1). We now plan to add aspartate to our clinical transplant preservation solution.

Excimer laser vs balloon angioplasty in atherosclerotic rabbit iliac arteries

F.L. Rosenfeldt, Che Lei, S. Black, J. Waugh

Thermal laser angioplasty has received wide clinical application in femoral arteries and to a lesser extent in coronary arteries. However, it is generally necessary to contain the laser light in a metal tip to reduce the incidence of perforation and also to supplement the laser angioplasty with a subsequent balloon angioplasty. These techniques can achieve 70-80% success in widening stenotic femoral arteries. However in the coronary circulation, the results of thermal laser angioplasty have been less encouraging and heat-induced coronary spasm appears to be a major complicating factor. The excimer (ultraviolet) laser has the potential to remove tissue without heating by a process known as photoablation and hence should be much safer than thermal lasers in the coronary circulation. In rabbits we aimed to study the acute and medium term results of excimer laser angioplasty in atherosclerotic iliac arteries and to compare the results with those of balloon angioplasty.

Morphological assessment was made of the cross-sectional area of the lumen and of the severity of stenosis due to atheroma by planimetric measurement of histological sections. Sections were obtained from normal arteries and from atherosclerotic arteries removed before, immediately after and one month after laser or balloon angioplasty. Also assessment of the diameter of the lumen of the arteries at the same time intervals was made using conventional angiographic techniques. The laser treated arteries showed a reduction in the amount of atheroma in the artery immediately and at one month after angioplasty. In contrast the balloon treated arteries showed no reduction in the amount of atheroma immediately after angioplasty and a tendency for an increase in atheroma after one month. The final result was

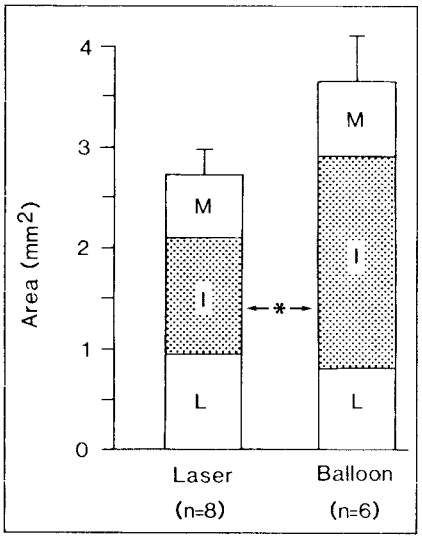


Fig.2 Cross-sectional area of arteries four weeks after treatment by laser or balloon angioplasty. The area occupied by atheroma in the balloon group (2.1 ± 0.5 mm^2) was significantly greater than in the laser group (1.2 ± 0.2 mm^2) ($p < 0.05$).

that there was 75% more atheroma remaining in the arteries treated by balloon angioplasty than in those treated by laser angioplasty (Fig. 2). The percentage stenosis by area in the laser group one month after angioplasty was 52 ± 8% which was significantly less than in the balloon group, 74 ± 6% ($P < 0.05$). Also microscopic examination of arteries treated by excimer laser showed no evidence of thermal damage. Radiological assessment of the diameter of the treated arteries supported the morphological findings. There was a greater tendency to restenosis in the balloon treated than in the laser treated arteries.

We concluded that excimer laser angioplasty ablated atheroma, whereas balloon angioplasty simply stretched the atheroma and the arterial wall. Balloon treated arteries still contained atheroma and were likely to undergo later restenosis. In contrast arteries treated by excimer laser demonstrated a reduction in the bulk of atheroma and tended to maintain patency over time.

Metabolic protection for the heart after myocardial infarction and during cardiac surgery using orotic acid and ribose

F.L. Rosenfeldt, C. Munsch, M. Newman, J.F. Williams, R. Conyers

Patients with recent myocardial infarction have high mortality and morbidity rates if they undergo urgent heart surgery. We hypothesised that this is due in part to changes in the surviving, non-infarcted myocardium. This region of the myocardium, remote from the infarct is known to be stressed by the infarct and ultimately to undergo hypertrophy. We hypothesised that if the process of hypertrophy could be accelerated then the response to surgery might be improved.

Orotic acid is a non-toxic precursor of nucleic acids which has been shown to stimulate protein synthesis in hypertrophying myocardium. In dogs we have previously studied the response of the heart with a recent infarction to induced cardiac arrest (cardioplegia) during stimulated cardiac surgery and also the effect of treatment with orotic acid. We found that orotic acid therapy improved the function of the heart after myocardial infarction. We also found that recent infarction decreased the tolerance of the heart to cardiac surgery using hypothermic cardioplegia, but that this tolerance was markedly increased by treatment of orotic acid. We then set out to confirm these effects in rats, to test the effect of orotic acid in non-stressed hearts and also to investigate the effect of another metabolic supplement, the sugar ribose.

We induced myocardial infarction in rats by coronary ligation and two days later used an isolated working rat heart preparation to measure cardiac function. We tested the effect of orotic acid and ribose, singly, and in combination, in normal hearts, in hearts with myocardial infarction and in hearts subjected to cardioplegic arrest, as in cardiac surgery.

We found that orotic acid had no effect on normal hearts. Oral orotic acid treatment for two days after infarction produced only a small increase in baseline cardiac function. However, infarcted hearts subjected to cardioplegic arrest recovered 83% of their pre-arrest function if treated with orotic acid, whereas untreated, infarcted hearts recovered only 49%. Ribose had no beneficial effect on normal or infarcted hearts and did not augment the effects of orotic acid.

Orotic acid is used in the synthesis of pyrimidines, which in turn are used as building blocks for ribonucleic acid (RNA) which is required in increased quantities for protein synthesis in stressed hearts. To study the mechanism of action of orotic acid at a biochemical level, we assayed pyrimidine nucleotides in the myocardium using high performance liquid chromatography and conventional enzymatic methods. We found that infarction produced a 33% increase in uridine nucleotides in the remote myocardium, consistent with mild hypertrophy and a surprising 88% increase in uridine nucleotides in the infarct itself, presumably due to the processes of repair and fibrosis. Orotic acid therapy after infarction tended to produce a further increase in levels of uridine nucleotides in both zones.

Currently we are continuing these studies in two areas. Firstly, in dogs we are attempting to localize the area of myocardium where orotic acid produces its beneficial effect after infarction: is it the infarct itself or the remote myocardium? Secondly, we aim to test the effect of orotic acid therapy in a clinical trial in patients undergoing urgent cardiac surgery after infarction.

Dr. Frank Rosenfeldt (left) and his team work to improve techniques in open heart surgery and heart transplantation.

Cell Biology Laboratory

Head: Dr. J.H. Campbell

Projects

Extracellular matrix components influence the phenotype of vascular smooth muscle cells.

Macrophages enhance uptake of β-very low density lipoprotein by smooth muscle cells in vitro.

The effect of intravenously injected β-very low density lipoprotein on small and large arterial injuries.

Does angiotensin II affect the incidence of vascular smooth muscle polyploidy in chronic hypertension?

Collagen synthesis by cultured rabbit aortic smooth muscle cells - alteration with phenotype.

Summary

There is now a large body of evidence indicating that smooth muscle cells in the intima of human arteries involved in atherogenesis are phenotypically different from those of the underlying media. These differences include changes in volume fraction of myofilaments (Vvmyo), cell shape, actin isoforms, myosin isoform, tropomyosin content, intermediate filaments, caldesmon, meta-vinculin, cyclic nucleotides, PDGF expression, fibronectin, and expression of major histocompatibility complexes. Some of these changes may play a crucial role in development or initiation of the disease.

In primary cell culture, medial smooth muscle cells which have been enzyme-dispersed and seeded at densities below confluence undergo similar changes in phenotypic expression during the first five days after isolation. This is accompanied by distinct alterations in biology. Prior to change in phenotype, while the cells have a high Vvmyo as in the normal vessel wall, the cells are contractile, do not proliferate logarithmically in response to mitogens from serum, synthesise minimal collagen, and accumulate little lipid even after exposure to high concentrations of β-very low density lipoprotein (β-VLDL) for several days. In contrast, smooth muscle cells of low Vvmyo are unable to contract, but proliferate logarithmically in response to mitogens from a variety of sources, synthesise 26-45 fold the amount of collagen (particularly type I) as cells with a high Vvmyo, and accumulate 7 fold the amount of lipid on exposure to β-VLDL. Since a low Vvmyo, proliferation, synthesis of extracellular matrix (particularly collagen type I) and accumulation of lipid are all characteristic features of smooth muscle cells in atheroma, change in phenotype of smooth muscle cells may be an important initial event in the development of this disease. This raises the question, what determines the phenotypic expression of smooth muscle cells? In particular, what determines whether a smooth muscle cell is contractile (high Vvmyo) and unresponsive to atherogenic stimuli such as mitogens and certain lipoproteins, or whether it has a low Vvmyo and is responsive to these agents? Studies in our laboratory implicate cell-matrix interactions in control of smooth muscle phenotype. In primary cell culture, a crude extract of glycosaminoglycans from the aortic intima plus inner media maintains sparsely-seeded smooth muscle with a high Vvmyo. Treatment of this aortic extract with heparinase from Flavobacterium heparinum destroys the active factor, indicating that glycosaminoglycans of the heparan sulphate species are responsible; and indeed, addition of the closely related glycosaminoglycan heparin maintains sparsely-seeded smooth muscle with a high Vvmyo. This has recently been confirmed by others who also found a similar effect with pentosan polysulphate, a semi-synthetic sulphated polysaccharide. Smooth muscle cells in primary culture can also be maintained in the contractile state by seeding the freshly-dispersed cells at confluent density, or by placing sparsely-seeded cells with a spatially separated feeder layer of confluent contractile smooth muscle cells or endothelial cells which are known to produce large amounts of an antiproliferative heparan sulphate species which is readily incorporated into their basal lamina. It thus appears that the presence of heparin-like glycosaminoglycans is an important determinant of the phenotype that smooth muscle expresses, and that high levels of this glycosaminoglycan species maintain the cells in a contractile (high Vvmyo) state. Any factor which removes this substance (e.g. enzyme dispersion and dilution through sparse-seeding), may therefore induce the cells to undergo a change in phenotype to a low Vvmyo. Smooth muscle cells which have been seeded sparsely on to a layer of type IV collagen or basement membrane Matrigel (a solubilised extract of EHS tumour basement membrane containing collagen type IV, laminin, heparan sulphate proteoglycan and entactin) do not undergo a change in phenotype, nor do isolated smooth muscle cells which have been completely embedded in a gel of collagen type I. Thus replacement of certain components of the basal lamina, or providing a microenvironment in which the basal lamina can be rapidly reconstituted, encourages maintenance of the contractile state.

Peritoneal macrophages grown in co-culture with a confluent monolayer of high Vvmyo smooth muscle cells induce a significant decrease in Vvmyo after three days, compared with both the freshly isolated smooth muscle cells and those grown for three days in the absence of macrophages. Since invasion of the artery wall by monocytes (which subsequently become macrophages) is one of the earliest cellular events in experimental atherogenesis, it may be through the influence of these cells that smooth muscle phenotypic change is induced in the initial stages of the disease. Indeed, we have recently demonstrated that macrophages completely degrade heparan sulphate-rich matrix to low molecular weight

fragments, and that the subcellular site of enzyme activity is the lysosome. Macrophage lysosomes by themselves were shown to be sufficient to induce a change in smooth muscle phenotypic expression, and their effect was mimicked by a commercial preparation of heparinase. We have suggested that proteases released from macrophages (or those on macrophage plasma membranes), cleave the protein core of proteoglycans which are then internalised and the heparan sulphate side-chains completely degraded in the macrophage lysosomes. We further proposed that removal of heparan sulphate from the basal lamina of smooth muscle cells by macrophages is the trigger that initiates smooth muscle phenotypic change and atherogenesis.

Macrophages degrade heparan sulphate proteoglycans and initiate atherogenesis

J.H. Campbell, S. Kalevitch, G.R. Campbell

We have previously shown that macrophages in co-culture with rabbit aortic smooth muscle cells induce a decrease in volume fraction of myofilaments (Vvmyo) of the smooth muscle. The aim of the present study was to determine whether this induction of phenotypic modulation could be correlated with degradation of heparan sulphate proteoglycans. Rabbit aortic smooth muscle cells were seeded at confluent density in primary culture, and after 8 days the cells (which had a high Vvmyo) were exposed to macrophage lysosomal lysate, heparinase (10 units/ml), chondroitin ABC lyase (10 units/ml) or trypsin (10 μg/ml). Only the macrophage lysosomal lysate and heparinase induced a decrease in the Vvmyo of the smooth muscle cells, similar to that previously observed. Living macrophages completely degraded the ^{35}S-labelled components of endothelial cell-produced extracellular matrix into small molecular fragments which eluted with a K_{av} of 0.84 on Sepharose 6B, similar to that of free $[^{35}S]O_4^{2-}$ (K_{av} = 0.86). A lysate of whole macrophages produced degradation peaks at K_{av} 0.60 (Mr 10,000 daltons) and 0.84. Both macrophage lysosomal lysate at neutral pH and heparinase (10 units/ml) degraded the ^{35}S-labelled matrix components into fragments which eluted at K_{av} 0.63, and which were identified as heparan sulphate chains by their complete degradation in the presence of low pH nitrous acid and resistance to chondroitin ABC lyase. At acid pH, the macrophage lysosomal lysate produce a second peak eluting at K_{av} 0.84, indicating that the enzymes responsible for the complete degradation of the heparan sulphate chains were present in the lysosomes and only active at acid pH. Only a small amount of heparan sulphate degrading enzyme was released into the incubation medium by living macrophages, and there was no activity on isolated plasma membranes of macrophages, although proteolytic enzymes were evident in both instances. The results suggest that removal of heparan sulphate from the surface of smooth muscle cells and its degradation may be the trigger for smooth muscle phenotypic modulation.

Does angiotensin II affect the incidence of vascular smooth muscle polyploidy in chronic hypertension?

M.J. Black, J.H. Campbell, G.R. Campbell

In chronic models of hypertension, smooth muscle cells of large arteries undergo hypertrophy and in some instances an increase in ploidy; i.e. the cells replicate their DNA but do not divide. The incidence of polyploid cells increases with age and duration of hypertension. Angiotensin II has been implicated in the development of artery wall hypertrophy and there is mounting evidence for increased activity of a local vascular renin-angiotensin system during hypertension. The effect of angiotensin II on the incidence of vascular smooth muscle polyploidy was investigated in this study using both in vivo and in vitro methods.

The angiotensin converting enzyme inhibitor enalapril was administered in the drinking water of spontaneously hypertensive rats (SHR) to block the production of angiotensin II in vivo from 4 to 14 weeks of age (Group I) to inhibit development of hypertension, and from 14 to 20 weeks of age (Group R) to reverse established hypertension. The incidence of polyploid aortic smooth muscle cells, as determined by flow cytometric DNA analysis, was significantly attenuated compared to the age matched untreated SHR during both time courses of enalapril treatment. At 14 weeks in Group I the incidence of polyploid cells was 6.13 $\pm$ 0.15% compared to 13.38 $\pm$ 0.15% in the untreated SHR. Likewise, in Group R the incidence of polyploid cells was 4.37 $\pm$ 0.04% at 20 weeks compared to 14.21 $\pm$ 0.16% in the untreated SHR. Addition of enalapril to primary cultures of smooth muscle had no direct effect on incidence of polyploidy. However, when angiotensin II at a concentration of 1 μM was added to confluent cultures of human vascular smooth muscle cells, the cells increased in size, incorporated 3H-thymidine, but did not divide.

These results suggest that angiotensin II induces hypertrophy of smooth muscle cells which in turn may lead to polyploidy induction. Angiotensin II is thus implicated in the development of vascular smooth muscle polyploidy in vivo, either by a direct effect on the cells or indirectly by elevating blood pressure.

Macrophages enhance uptake of β-VLDL by smooth muscle cells in vitro

R.E. Rennick, J.H. Campbell, G.R. Campbell

The presence of lipid-laden foam cells is a characteristic feature of the early stages of atherosclerosis in cholesterol-fed animals. These cells

Dr. Julie Campbell (centre), and her group, study the biology of smooth muscle cells.

are mainly of monocyte/macrophage origin, but may also be derived from smooth muscle cells. In previous studies, we have shown that macrophages stimulate phenotypic modulation of smooth muscle cells and their subsequent proliferation. The present studies investigate the effect of macrophages on uptake of the atherogenic lipoprotein β-VLDL by smooth muscle cells.

When mouse macrophages were co-cultured with smooth muscle cells, binding of ^{125}I-β-VLDL and subsequent accumulation of intracellular lipid was increased when compared with binding in smooth muscle cells cultured alone. This increase in binding was due to modification of the lipoprotein moiety since binding to smooth muscle cells of ^{125}I-β-VLDL preincubated with macrophages was 12.5 fold that of fresh ^{125}I-β-VLDL. Subsequent investigation of the effect of the macrophages on lipoprotein composition showed no significant alteration. When macrophages were incubated with β-VLDL in the presence of probucol (200 μg/ml), binding of the conditioned lipoprotein (239.6 ± 23.7 ng/mg cell protein) approached that of controls with fresh lipoprotein (217.2 ± 77.8 ng/mg cell protein), whereas smooth muscle cells bound significantly more macrophage-conditioned lipoprotein (862.4 ± 88.2 ng/mg cell protein). This indicates that macrophages modify the lipoprotein through oxidation, since probucol is a known antioxidant.

These studies extend the role of the macrophage in lipoprotein metabolism of the artery wall, demonstrating that this cell not only scavenges lipid but modifies the β-VLDL particle such that it is more readily bound and taken up by smooth muscle cells.

Collagen synthesis by cultured rabbit smooth muscle cells – alteration with phenotype

A.H. Ang, J.F. Bateman, G.R. Campbell, J.H. Campbell

Enzymatically-isolated smooth muscle cells express a contractile phenotype in the first few days of primary culture, then spontaneously modulate to the synthetic phenotype and proliferate. These cells are capable of reverting to the contractile phenotype upon reaching confluency if they have not undergone more than five population doublings (reversible synthetic (RS) phenotype). However, if the cells have undergone more than five population doublings before achieving confluence, they do not revert to the contractile state but remain permanently synthetic (irreversible synthetic (IRS) phenotype).

In the present study, the rate of collagen synthesis and collagen type distribution by smooth muscle cells were investigated in relation to the alteration in phenotypic expression. Reversible synthetic smooth muscle cells in primary and secondary cultures synthesised collagen at a rate which was 25-30 times that of contractile smooth muscle cells in early primary culture. At the same time, non-collagen protein synthesis increased only 5 to 6 fold, indicating a specific stimulation of collagen synthesis. The association between phenotype and collagen synthesis was further demonstrated by a 50% decline in collagen synthesis when RS cells in primary culture returned to the contractile phenotype upon reaching confluency. In contrast, when RS cells did not revert to the contractile phenotype but remained permanently synthetic (IRS phenotype), collagen synthesis was maintained at a high level.

SDS-PAGE analysis indicated a slight alteration in the collagen type distribution of smooth muscle cells consequent to phenotypic modulation. With modulation to the RS phenotype, the relative proportion of type I collagen synthesised increased from 78% to 90% with a concomitant decrease in type III collagen from 18% to 6%. This enrichment of type I collagen was removed when RS cells returned to the contractile phenotype and synthesised 83% type I and 13% type III collagen. IRS cells synthesised similar proportions of types I and III collagens as RS cells. Relative proportion of type V

collagen synthesised always accounted for 4-5% irrespective of the phenotypic state of the cells.

Although phenotypic modulation to the synthetic state was shown to be associated with enrichment of type I collagen, the enrichment was relatively small in magnitude. Additionally, type I collagen was always the predominant collagen species synthesised. Thus modulation to the synthetic phenotype was characterised by enhanced synthesis of total collagen rather than shifts in the distribution of collagen types.

These findings are in accord with observations made in vivo of normal and atherosclerotic tissues. Hence, enzymatically-isolated aortic smooth muscle cells in primary culture with its associated phenotypic modulation is a useful in vitro model system for understanding the pathogenesis of atherosclerosis in relation to extracellular matrix synthesis, and thus the fibrous nature of the plaque.

The effects of intravenously injected - very low density lipoprotein on small and large arterial injuries

J.A. Manderson, R. Klein, J.H. Campbell, G.R. Campbell

One of the earliest morphological changes in animals fed a high-cholesterol diet is the adhesion of monocytes to the arterial endothelium followed by their infiltration into the intima. However, animals fed a cholesterol-supplemented diet show a massive (30- to 50-fold) increase in plasma cholesterol levels, which cannot be equated to the human situation. By administering β-VLDL daily, thereby producing pulse-like changes in plasma cholesterol levels, we have tried to mimic sporadic insults that may occur fron consumption of high fat meals.

A series of daily injections of β-VLDL was administered over 4-5 days to rabbits whose arteries contained either experimental circumferential lesions or areas of intimal thickening. The circumferential lesions were similar to those that occur spontaneously and were produced by the application of longitudinal tension. The intimal thickening was produced by denuding the endothelium with a balloon catheter. Over the period of injection of β-VLDL the plasma cholesterol levels rose in a pulse-like manner from 60 to 100 mg/dl. Following cessation of injections the cholesterol levels initially rose further and then decreased to normal levels within 4 weeks. Injections of β-VLDL, commencing 1-2 days after production of the circumferential lesions, resulted in an increase in the number of mononuclear leukocytes (primarily macrophages) and in a moderate accumulation of lipid by these cells and the medial smooth muscle cells. If the injections were started 14 days postinjury there was some accumulation of lipid in the large lesions but none in small lesions. There was no lipid accumulation in any lesions if the β-VLDL was administered 3 months later. A very slight accumulation of lipid occurred in the intimal thickening, or neo-intima, following a series of β-VLDL injections given to rabbits 2 or 6 weeks after balloon catheter injury. The series of injections produced a significant increase in the number of mononuclear leukocyte profiles per area of the neo-intima, suggesting an increased infiltratiion of these cells into the injured artery. These results suggest that a small transient increase in the plasma concentration of cholesterol-carrying lipoproteins may lead to increased infiltration of mononuclear leukocytes into areas of intimal thickening or areas of "spontaneously occurring" injury.

Extracellular matrix components influence the phenotype of vascular smooth muscle cells

E.S. Stadler, J.H. Campbell, G.R. Campbell

The functional unit of the media of the normal mammalian artery may be considered to be the vascular smooth muscle cell plus its extracellular matrix. Enzymatically dispersed medial smooth muscle cells are deprived of a basal lamina and an extracellular matrix. These cells consequently undergo a change of phenotype from a more contractile to a more synthetic state within the first few days in primary culture and proliferate logarithmically.

Ultrastructural stereological techniques were used to quantitate the phenotype of vascular smooth muscle cells grown in primary culture on a plastic substrate (control), on and within matrices of collagen type I and on collagen type III, collagen type IV or basement membrane Matrigel. The volume fraction of myofilaments (Vvmyo) of freshly isolated smooth muscle cells at day 0 was 54.2 ± 2.0%. By day 5 in culture, the Vvmyo of cells grown on collagen type I and collagen type III had decreased significantly to 14.2 ± 2.1%, 16.5 ± 2.4% and 16.3 ± 2.9% respectively. In contrast, the Vvmyo of cells grown within matrices of collagen type I, on collagen type IV and basement membrane Matrigel remained significantly higher at 30.7 ± 1.5%, 30.8 ± 4.2% and 32.1 ± 3.1% respectively.

These findings demonstrate that modulation of smooth muscle cell phenotype early in culture is arrested by the presence of extracellular matrix components and suggests that the matrix synthesised and secreted by smooth muscle cells in the normal vessel wall contributes to the maintenance of their contractile functional state.

Cellular Biochemistry Laboratory

Head: Dr. E.A. Woodcock

Projects

Phosphatidylinositol turnover in the heart: mechanisms and functional significance.

Signal-transduction pathways in the adrenal zona glomerulosa and their relationship to the synthesis of aldosterone.

Importance of the phosphatidylinositol turnover pathway in the control of the renal function.

Summary

The maintenance of life in complex organisms requires communication between the different, specialised organs. This is achieved either by the release of hormones into the blood stream or by activation of specific neuronal pathways which release neurotransmitters onto the target cells. Cells therefore require the ability to recognise these extracellular signals and to undergo appropriate responses to each. The first of these requirements, recognition, is achieved by the hormone or neurotransmitter binding specific receptors on the cell surface. This binding initiates a cascade of events within the plasma membrane and the cytoplasm. The large range of hormones, neurotransmitters and growth factors together with the equally large number of possible responses might suggest a complex array of receptor signal transduction pathways. However, evidence available to date overwhelmingly supports the concept of a seemingly small number of signal transduction mechanisms which mediate a wide range of responses. How these complex "signal transduction pathways" are controlled in order to initiate only the appropriate response is still poorly understood and is the major interest of the Cellular Biochemistry Laboratory. This laboratory was established in the Institute during 1989.

Our recent interests have centred on responses initiated via the phosphatidylinositol (PI) turnover pathway in tissues of importance in cardiovascular regulation. In this pathway, binding of a hormone or neurotransmitter to its receptor triggers the hydrolysis of a plasma membrane lipid phosphatidylinositol (4,5) bisphosphate (phosphatidylinositol with two extra phosphate groups). This hydrolysis generates two signalling molecules, diacylglycerol (DAG) and inositol (1,4,5) trisphosphate. DAG is very lipophilic and remains in the plasma membrane where it stimulates a specific protein kinase called protein kinase C. Inositol 1,4,5 trisphosphate (1,4,5 IP_3) being highly charged moves into the cytoplasm and binds its own receptors on the endoplasmic reticulum initiating Ca^{2+} release. Subsequently, 1,4,5 IP_3, possibly in concert with some of its metabolites, activates entry of extracellular Ca^{2+}, but the mechanism of this extremely important control mechanism is not yet understood. In most tissues the metabolism of 1,4,5 IP_3 is complex involving both phosphorylation and dephosphorylation pathways. But the significance of this complexity and the wide range of highly phosphorylated products formed remains a mystery.

The complexity of the PI turnover pathway suggests the possibility of flexibility in its operation in different tissues or even within the one cell type when subjected to different stimulatory factors. Such flexibility may be of great importance in ensuring appropriate responses to stimulation. For instance, in vascular smooth muscle cells PI turnover is involved in mediating both the mitogenic response and the contractile response. Both of these responses are perturbed in disease states such as hypertension. It is of obvious importance that the appropriate response occurs following stimulation by hormone, neurotransmitter or growth factor. To gain insight into the relationship between PI turnover and cellular responses, the Cellular Biochemistry Laboratory has made a detailed study of the pathway in a number of different tissues and within the one cell type under stimulation by different factors.

Phosphatidylinositol turnover in the heart: mechanisms and functional significance

I.J. Kuraja, E.A. Woodcock

While extensive studies of PI turnover have been carried out in a number of different cell types, few detailed studies have been performed in heart tissue. Cardiac PI turnover is known to be stimulated via α_1-adrenoceptors and possibly initiates the positive inotropic response to adrenergic agonists. In studies using both the Langendorff perfused heart preparation and isolated left and right atria we have made a detailed study of the PI turnover pathway in adult rat heart stimulated by adrenergic agonists. These studies have shown that the pathway in heart differs from that described in most other tissues. In heart tissue, addition of noradrenaline causes a rapid and transient release of 1,4,5 IP_3 followed by a slower sustained rise. This profile of release is similar to that described in other tissues. However, metabolism of 1,4,5 IP_3 in the heart was solely via dephosphorylation to $4IP_1$ and no products of the phosphorylation pathway were observed at any time after addition of noradrenaline. Qualitatively similar profiles were observed in ventricle and in left and right atria. However, in quantitative terms, right atria responded more strongly to noradrenaline than left atria or right or left ventricle. This higher responsiveness of right atria was not due to a higher density of $\alpha 1$ adrenoceptors

relative to the other chambers of the heart.

To understand the significance of this abbreviated PI turnover pathway in the adult heart, experiments have been performed using myocytes prepared from two day old rats whose myocytes are not yet fully differentiated and still have mitotic activity. Addition of noradrenaline to neonatal myocytes caused a stimulation of release of 1,4,5 IP_3 but the observed metabolism of this compound was by both phosphorylation and dephosphorylation pathways. Thus the pathway in the neonatal myocyte appears to be similar to that described in many other cell types but the pathway in the highly specialised adult myocyte present in intact tissue is different. These results indicate that this complex pathway of 1,4,5 IP_3 metabolism is suppressed in the adult heart. Whether this constitutes a suppression of responses mediated via this pathway is an interesting possibility which requires further investigation.

In other studies we investigated the cardiac actions of the newly described vasoconstrictor peptide endothelin. Endothelin is present in vascular endothelial cells and is the most potent vasoconstrictor substance yet described. Endothelin has been shown to have positive inotropic activity in rat atria and to stimulate release of atrial natriuretic peptide. Endothelin produced a dose-dependent stimulation of inositol phosphate accumulation in right and left atria. As with the noradrenaline stimulation, right atria responded more profoundly than left atria. The profile of release of 1,4,5 IP_3 following endothelin stimulation was similar to that observed with nor-adrenaline - a rapid and transient release followed by a slower sustained rise. However, the initial response to endothelin was slower than the response to noradrenaline. The inotropic response to endothelin also was slower to develop than the noradrenaline response. Thus the rate of release of 1,4,5 IP_3 may be the limiting factor determining the rate of development of increased tension.

Signal transduction pathways in the adrenal zona glomerulosa and their relationship to the synthesis of aldosterone

E.A. Woodcock, L.M. Caroccia, J.K. Tanner

Studies have been undertaken to address the question of how cells recognise different extracellular stimuli when these activate the same second messenger pathway.

Phosphatidylinositol turnover in the adrenal glomerulosa is stimulated by three vasoconstrictor peptides, angiotensin II, vasopressin and endothelin. In these cells vasopressin is mitogenic, whereas angiotensin II primarily has steroidogenic activity. Endothelin produces a small steroidogenic response and other possible responses have not yet been investigated. The overall inositol phosphate response to vasopressin was small compared with responses to angiotensin II and endothelin which were similar.

An examination of the details of the PI turnover pathway provided an explanation for these observed differences. All three agonists caused the rapid and transient release of 1,4,5 IP_3 followed by a slow gradual rise. However, the metabolism of the released 1,4,5 IP_3 was different under stimulation by the three different peptides. Under vasopressin stimulation 1,4,5 IP_3 was metabolised largely by dephosphorylation to $4IP_1$. With angiotensin II, metabolism was initially by dephosphorylation but after 1 min with hormone considerable metabolism by phosphorylation was observed. In the case of endothelin, phosphorylation was observed immediately on adding the peptide. The initial, transient increase in 1,4,5 IP_3 was slower in response to endothelin than to vasopressin or angiotensin II. Angiotensin II produced a higher rise in 1,4,5 IP_3 than either of the other two peptides and this correlated well with a higher response in cytosolic free Ca^{2+} to angiotensin II stimulation.

Experiments were undertaken to investigate the mechanism of the

Dr. Elizabeth Woodcock (right) and Jenny Tanner, examine HPLC results.

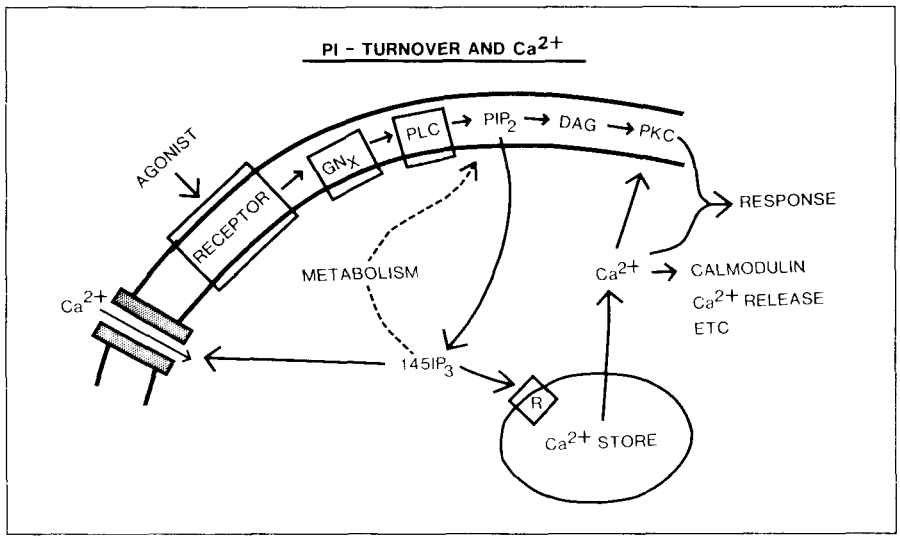


Fig. 1 The relationship between phosphatidylinositol (PI) turnover and changes in cytosolic calcium concentrations. Stimulation of the cell causes the release of inositol 1,4,5 trisphosphate (1,4,5 IP_3) and sn 1,2 diacylglycerol (DAG). 1,4,5 IP_3, possibly together with its metabolites, controls the cytosolic free calcium concentration. Changes in free calcium, often in concert with activation of protein kinase C (PKC) initiate many different cellular responses including contractility changes and mitogenesis.

control of 1,4,5 IP_3 metabolism in adrenal glomerulosa cells. Effects of Ca^{2+} were investigated by incubating cells in Ca^{2+} free medium to reduce the cytosolic free Ca^{2+} changes to stimulation. Such reduction in Ca^{2+} resulted in reduced 1,4,5 IP_3 phosphorylation activity in the presence of any of the three peptides. Thus Ca^{2+} can have a role in regulating the metabolism of 1,4,5 IP_3 but is unlikely to be the full explanation since Ca^{2+} responses to vasopressin and endothelin were similar while their 1,4,5 IP_3 metabolic pathways were different.

The implications of these results are that cells have the ability to discriminate between different signals even when these activate the same signal transduction pathway. This mechanism provides flexibility in cellular responses but also means that erroneous responses are possible if the system is perturbed under pathological conditions.

Importance of the phosphatidylinositol turnover pathway in the control of renal function

I.J. Kuraja, E.A. Woodcock

The kidney has been poorly studied in terms of PI turnover, even though changes in Ca^{2+} are likely to be important in controlling tubular function and mesangial cell contractility. Study of PI turnover in renal tissue poses problems associated with high background activity in the absence of added stimulatory hormone or neurotransmitter. Furthermore, the complex array of cell types present in the kidney can make interpretation of results from tissue slice experiments difficult. We have developed methods for the preparation of proximal tubules and papillary collecting ducts and we have made a preliminary study of PI turnover in these regions of the kidney.

Lysyl-bradykinin (kallidin) is produced by kallikrein in the distal tubule, but the significance of this localised production has not been fully understood. In our studies, bradykinin was found to be an effective stimulator of PI turnover in papillary collecting tubules. This provides evidence for a direct tubular action of bradykinin and provides an explanation for reported interactions between bradykinin and vasopressin in controlling solute reabsorption in renal collecting ducts.

In other studies we showed that endothelin stimulated PI turnover in papillary collecting ducts. Receptors for endothelin are present at high density in this region. Further studies are required to investigate tubular actions of endothelin.

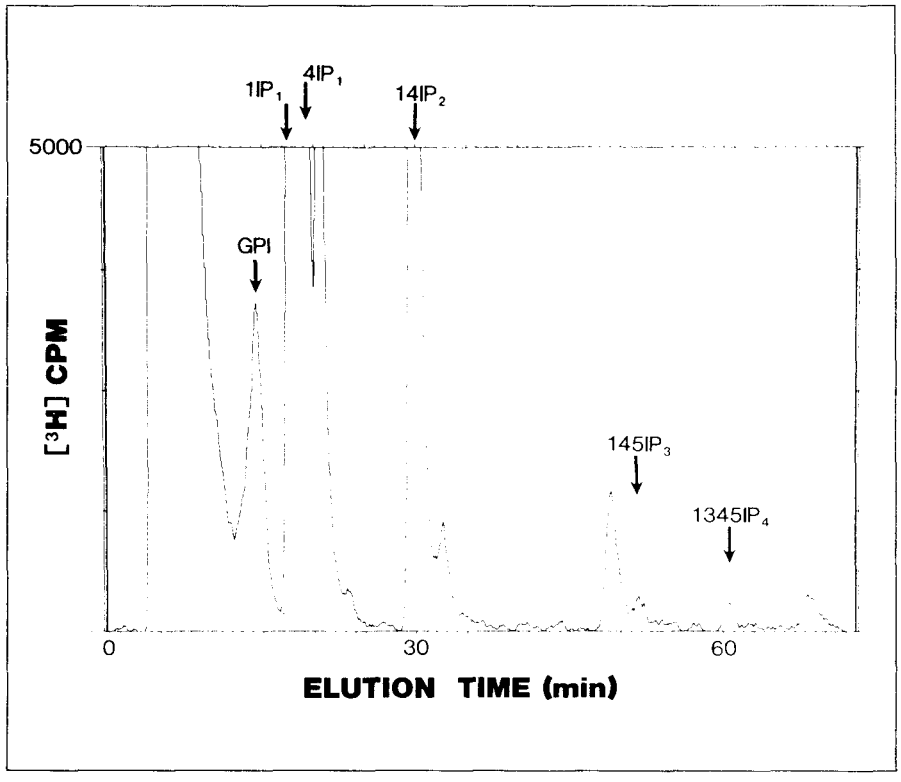


Fig. 2 Separation of inositol 1,4,5 trisphosphate (1, 4, 5 IP_3) and its metabolites is achieved by anion-exchange high performance liquid chromatography. These are quantitated automatically by an on-line computerised radioactivity detector.

Circulatory Control/ Neuropharmacology Laboratory

Head: Professor P.I. Korner

Projects

Central Nervous System

Contribution of forebrain noradrenergic pathways on the action of α-methyldopa.

Mechanisms involved in the central pressor action of angiotensin II.

Spinal noradrenergic pathways in the central action of α-methyldopa and 6-hydroxydopamine.

Reflexes

Effects of acute hypertension on renal sympathetic baroreflex.

Assessment of gain of tachycardia and bradycardia responses of cardiac baroreflex.

Afferent regulation of pressor hormones and vasoconstrictor response to haemorrhage.

Baroreceptor heart rate reflex in spontaneously hypertensive rat.

Renal sympathetic baroreflex in two strains of Israeli rabbits.

Power spectral analysis of blood pressure variability.

Summary

Our aim is to determine how the brain regulates blood pressure and blood flow in conscious animals and how some of the mechanisms are altered in experimental hypertension. The work has ranged from examining the structure and function of different pathways, to examining the efferent (motor) and afferent (sensory) mechanisms that contribute to the physiological responses to a range of physiological disturbances.

Visiting scientist, Dr. Carol-Ann Courneya studies neural regulation of the circulation.

Over the last few years, our analysis of central nervous mechanisms has focused on neurons (nerve cells) that, when stimulated, release specific amines from their endings and thereby activate adjoining neurons in pathways involved in cardiovascular control. The central noradrenergic neurons are cells that release from their endings the same transmitter as the peripheral sympathetic nerves. Our interest arises from the fact that the central action of drugs such as α-methyldopa (α-MD, Aldomet) and clonidine are critically dependent on the integrity of these pathways. Our most recent work has shown that the slowing of heart rate produced by α-MD is mediated through a special noradrenergic pathway which enhances vagal responses to arterial pressure changes. This pathway travels from the nucleus tractus solitarius region (which receives the sensory endings from the baroreceptors) to the adjoining motor nucleus in the hindbrain (the vagal nucleus) via a long and circuitous route through higher brain centres. It illustrates the complexity of integration of sensory information by the brain when a pathway travels such a long way to influence the activity of an adjoining structure. Fine-tuning of information from a number of sensory receptors obviously involves a large number of brain centres in different regions of the brain.

The peptide angiotensin (Ang) II is a transmitter released from certain brain endings in the medulla that produce sympathetic excitation, probably in conjunction with other transmitters. Our work this year has helped in the functional localization of the brain renin-angiotensin system. We have shown that receptors very sensitive to Ang II are localized in a very small region of the hindbrain near a group of cells called the subretrofacial nucleus. Activation of this area by Ang II raises blood pressure dramatically and its central effects greatly enhance sympathetic nerve responses to blood pressure changes. However, preliminary evidence in intact rabbits suggests that the system is not tonically active, at least in normal rabbits.

We have recently shown that during acute hypertension there is marked depression of the gain and range of the renal sympathetic baroreflex, partly as a result of activation of some of the cardiac baroreceptors. The heart acts, so to speak, as a brake on the load against which it has to pump. There is similar depression of the vagal component of the baroreceptor heart rate reflex in chronic hypertension, which has advantages for the heart itself. We have studied this in the spontaneously hypertensive rat in the course of post-natal development: depression of the vagal component of the reflex occurs in parallel with the development of left ventricular enlargement, suggesting that it too arises from sensory influences from the heart.

We have completed an analysis on the role of cardiac and arterial baroreceptors on the autonomic and hormonal responses during haemorrhage in the rabbit. Both sets of receptors are involved in the secretion of the pressor hormone, arginine vasopressin (AVP), but neither plays a role in renin release, which occurs directly as a result of the pressure and flow changes on the kidney. By examining the circulatory responses following 20% removal of the blood volume in intact rabbits, we obtain a 'model' of circulatory control, where the constrictor responses are entirely mediated

through neural mechanisms of the autonomic nervous system. In this model, blood pressure is well-maintained through a highly integrated pattern of autonomic activity involving both cardiac stimulation and constriction of the peripheral blood vessels. The 'drive' for these responses comes from the arterial and cardiac baroreceptors. In autonomically blocked rabbits, AVP and renin release are greatly enhanced. Without a functioning autonomic nervous system, baroreceptor influences are hard to demonstrate and blood pressure is poorly maintained. AVP and Ang II produce more than double the normal constriction of peripheral blood vessels but, for maintaining blood pressure, this is of little benefit without stimulation of the heart. The autonomic nervous system does this most effectively, but AVP and Ang II do not.

Finally, we have developed a new method for assessing the intrinsic sensitivity of the baroreceptor-heart rate reflex separately during rises in blood pressure on the one hand and falls in blood pressure on the other. The first is largely mediated through the vagus and the second through the cardiac sympathetic. We have shown that increasing the cardiac load enhances the intrinsic sensitivity of the vagus, both when induced acutely and in chronic hypertension.

Role of forebrain noradrenaline pathways in acute central effects of α-methyldopa and 6-hydroxydopamine

M. van den Buuse, G.A. Head, P.I. Korner

We examined the role of the ascending noradrenergic projections to the forebrain – the dorsal and ventral bundles (DBVB) – on the responses of mean arterial pressure (MAP) and heart rate produced by two drugs which enhance the activity of the central noradrenergic pathways through increased transmitter release. Lesions or sham-operations were performed by local stereotaxic injections of, respectively, small doses of 6-hydroxydopamine (6-OHDA) or ascorbic acid vehicle into the DBVB region. We compared the acute responses to intracisternal (i.c.) 6-OHDA and α-methyldopa (α-MD) in lesioned or sham-operated rabbits. We studied the effects of the drugs in conscious rabbits with intact arterial baroreceptor afferents, and in another group of sino-aortically denervated (SAD) rabbits.

Lesions in the ascending bundles completely abolished the bradycardia evoked by i.c. α-MD in rabbits with intact baroreceptors, but not that of SAD rabbits. The effects on MAP were slight. The findings after DBVB lesions were identical to our earlier finding after electrolytic lesions of the A1 and A2 noradrenergic cell groups of the dorsal medulla.

The acute pressor responses to i.c. 6-OHDA were unaffected by the lesions in intact rabbits, as was the biphasic depressor response in SAD rabbits. However, DBVB lesions entirely abolished the bradycardia response in rabbits with intact arterial baroreceptors, which is largely vagal in nature. In SAD rabbits, 6-OHDA induces a biphasic fall, followed by a rise in heart rate, and the latter was significantly enhanced after the lesion. The effect in intact animals was not entirely simulated by lesions of A1 and A2 noradrenergic cell groups, but the SAD response was.

We conclude that ascending noradrenergic projections to the forebrain are part of a pathway involved in the facilitation of baroreflex-mediated cardiac vagal activity that occurs during administration of both α-MD and 6-OHDA. DBVB lesions abolish this facilitatory response. It confirms earlier findings regarding the importance of forebrain structures in the modulation of baroreflexes. The effects on heart rate in SAD rabbits, due to transmitter release, were sympathetically mediated through pathways other than the arterial baroreceptors and this pathway too is influenced by the long ascending loop of the DBVB noradrenergic pathway.

Evidence for a central renin-angiotensin system that affects sympathetic tone to blood vessels

G.A. Head, N.S. Williams, S.J. Godwin

We examined the question whether there is a central endogenous renin-angiotensin system. Comparison of dose-response curves to Ang I, II and III given into the fourth ventricle of conscious rabbits showed that all three drugs evoked similar maximum pressor effects, with the order of potency consistent with conversion of Ang I to Ang II to Ang III. Pretreatment with the Ang-converting enzyme inhibitor

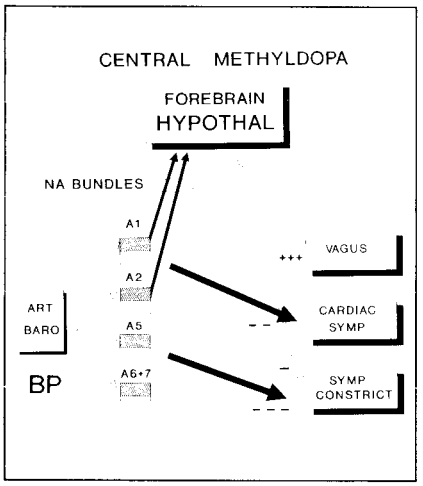


Fig.1 Schema shows the pathways by which α-methyldopa reduces blood pressure and heart rate, which arise from the noradrenergic nerve cell groups (A1, A2, A5) and project as shown by solid arrows to sympathetic mononeurons to the heart and blood vessels. We have found that elevation of cardiac vagal tone is mediated through a supraspontine pathway via the noradrenergic bundles.

enalapril, given centrally, produced a dose-dependent inhibition of the pressor response to Ang I, suggesting that normally, the effects of the latter were entirely mediated through conversion to Ang II. All three peptides given centrally elicited constriction of the renal and mesenteric vascular beds and vasodilatation of the hindlimb, as demonstrated in rabbits instrumented with Doppler flow probes.

We studied the pressor responses to microinjections of Ang II into various hindbrain regions, in urethane-anaesthetized rabbits. Pressor responses were observed only in the region corresponding to the subretrofacial nucleus (SRN) of the ventrolateral medulla. Dose-response curves of Ang II injected at this site produced maximum increases in blood pressure that were lower than those produced by injections into the fourth ventricle; however, the ED50 was about 1000 times lower compared with i.c.. Local injection of an Ang II antagonist into the SRN selectively blocked the pressor responses to Ang II, both when given locally and into the fourth ventricle. Thus, our data supports the presence of a brain renin-angiotensin system, capable of modulating sympathetic vasomotor tone, with an important component of the system placed in the subretrofacial region.

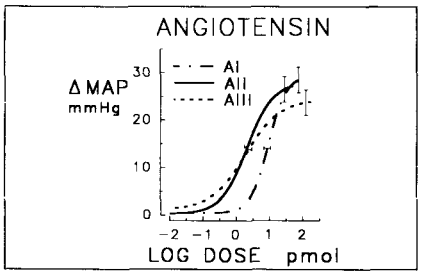


Fig. 2 Dose-response curves to fourth ventricular administration of angiotensin I, II and III in conscious rabbits. Angiotensin I is about 5 times less potent than the others, presumably because it must first be converted to Angiotensin II to produce its central increase in blood pressure.

Role of central angiotensin II on renal sympathetic and cardiac baroreflexes

P.K. Dorward, C.D. Rudd

This study reports the effects of angiotensin II (Ang II) injection into the fourth ventricle on the properties of the renal sympathetic and cardiac baroreflexes in conscious rabbits. A renal sympathetic nerve electrode had previously been implanted and perivascular balloons placed around the intrathoracic descending aorta and inferior vena cava for eliciting graded rises and falls in mean arterial pressure (MAP). Sigmoidal curves were derived in these animals relating MAP to (i) renal sympathetic nerve activity (RSNA) and (ii) to heart rate.

Central Ang II (2.5 - 25 ng/min) caused a transient small increase in renal sympathetic nerve activity and a small rise in MAP. The reflex properties were markedly altered: the upper RSNA plateau level increased two-fold, with corresponding rises in RSNA range and gain. For the cardiac baroreflex, there was a similar, but smaller augmentation for the upper (tachycardia, i.e. sympathetic) plateau and for the range and gain. Central enalapril had no effect on the properties of either baroreflex, suggesting that under normal circumstances, the central Ang II receptor sites are not tonically active.

Effects of acute hypertension on renal sympathetic baroreflex

P.K. Dorward, L.B. Bell, C.D. Rudd

We used conscious rabbits, in which reflex properties were evaluated before and at the end of 40 minutes increase in BP by +30 mmHg and +45 mmHg above resting. Three groups were studied: (i) with all afferents intact; (ii) cardiac 'denervated' rabbits, following instilling of intrapericardial procaine; (iii) rabbits given i.v. naloxone (4 mg/kg bolus, 0.12 mmHg/kg/min). In normal rabbits, the rises in BP produced reduction in renal sympathetic nerve activity (RSNA) range by 34% and 67%, respectively; and corresponding reduction in gain by 64% and 87%.

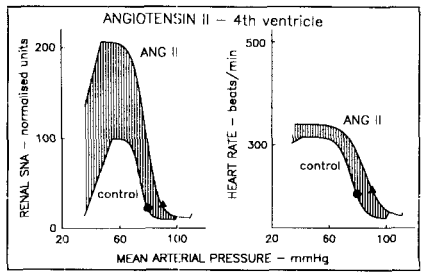


Fig. 3 Renal sympathetic nerve (left) heart rate (right) baroreflex curves in conscious rabbits. Shading shows effect of a constant fourth ventricular infusion of angiotensin II.

One-third of this substantial attenuation of reflex properties was prevented by pericardial procaine, but none of it was blocked by naloxone. The absence of naloxone-sensitive mechanism was in stark contrast to the effects of haemorrhage (fall in BP), where similar attenuation of the reflex was entirely abolished by naloxone. Thus, the sources of reflex depression in acute hypertension do not include a central opioid mechanism, which helps to distinguish them from the pathway activated during haemorrhage. It suggests that different afferent mechanisms are activated by the two disturbances.

Relationship between baroreceptor heart rate reflex changes in hypertension and development of cardiovascular hypertrophy

G.A. Head, M.A. Adams, A. Bobik

We have previously found that there is reduction in heart rate (HR) range in adult spontaneously hypertensive rats (SHR), owing to a diminished vagal capacity to slow the heart. We examined the baroreceptor-heart rate reflex properties in closely-matched SHR and Wistar Kyoto (WKY) rats, aged from 6 - 20 weeks, in relation to the development of cardiac and vascular hypertrophy in the SHR. Baroreflex properties were determined by the steady-state method, using vasoactive drugs, while vascular hypertrophy was assessed subsequently by determining the vascular resistance of the perfused hindquarter preparation during maximum constriction and maximum dilatation; cardiac hypertrophy was assessed from left ventricle (LV)/body weight (BW) ratios. The vagal component of the HR range was similar in SHR to WKY at 6 weeks' of age, but was significantly reduced in SHR at 9 weeks and maximally reduced by 14 weeks (−57%). These changes closely paralleled the development of cardiac

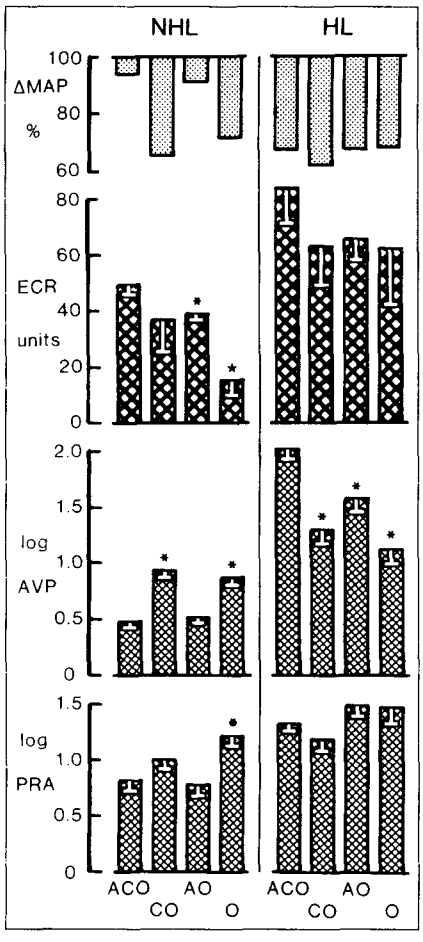


Fig 4. Responses to 20% loss of blood volume in rabbits with neural, humoral and local effectors intact (NHL, left) and autonomically blocked rabbits with only the last two intact (HL). Afferent status denoted by presence of arterial (A) and cardiac (C) baroreceptors and all other (O) afferents. MAP = mean arterial pressure (% control); ECR = estimated constrictor responses; AVP = plasma arginine vasopressin (pg/ml); PRA = plasma renin activity (ng AI/ml/h).

hypertrophy, as assessed by LV/BW. By contrast, most of the vascular hypertrophy had occurred earlier when there was no difference in baroreflex properties between SHR and WKY. Thus, the changes to the baroreceptor-heart rate reflex in SHR appear to be related to the development of cardiac hypertrophy.

Assessment of gain of tachycardia and bradycardia responses of the cardiac baroreflex

P.I. Korner, B.A. Kingwell, G.A. McPherson

Mean arterial pressure (MAP)- heart rate (HR) reflex curves were obtained in humans by the vasoactive drug method and in conscious rabbits by the drug and perivascular cuff methods, which provide somewhat different sources of afferent drive. The MAP-HR relationship was depicted by a sigmoidal curve, consisting of the two halves of separate logistic functions, each centred on the resting value, one for the tachycardia response, the other for the bradycardia response. Estimates of the upper and lower plateaus, of the HR Range and of the average gain of the entire curve differed only slightly from those obtained by a single symmetrical function. The new method allowed separate assessments of the gains of the tachycardia and bradycardia responses, Gt and Gb, and of the corresponding normalized (range-independent) gains, Ct and Cb. In both humans and rabbits, Gt was often greater than Gb, in agreement with earlier findings by others. The relative magnitude of Gt to Gb depended (i) on the location of the resting HR on the HR range of the compound curve and (ii) on the intrinsic sensitivity of the reflex, which was assessed by Ct and by Cb. The influence of resting heart rate often masked the effects of intrinsic sensitivity on the tachycardia and bradycardia responses.

We examined the HR responses by both cuff and drug methods and found quantitative differences in Ct and Cb between them, consistent with an earlier hypothesis of method-related differences in the afferent drive. There were also differences in Ct/Cb of the vagal component of the reflex between humans and rabbits, using the drug method. We conclude that normalized gain is a valuable parameter for assessing intrinsic sensitivity, independently of the HR Range. It is an essential parameter for assessing the biological significance of differences in gain of the tachycardia and bradycardia responses during different physiological disturbances.

Afferent mechanisms in haemorrhage

C.A. Courneya, P.I. Korner, J.R. Oliver, R.L. Woods

We completed analysis of the role of the arterial and cardiac baroreceptors in the regulation of (i) the secretion of the pressor hormones arginine vasopressin (AVP) and plasma renin activity (PRA), the latter serving as a marker for production of angiotensin II (Ang II); (ii) the hindquarter's vascular resistance response during haemorrhage. The rabbits were bled at a constant rate of about 3% of the blood volume per minute until mean arterial pressure (MAP) had fallen to about 45 mmHg, when the blood was immediately reinfused.

Hormone secretion.

Plasma AVP and PRA responses were studied after selective denervation of the arterial baroreceptors, of the cardiac receptors and of the both sets of receptors. We obtained blood volume hormone concentration curves, but the concentrations just before cessation of haemorrhage provided the greatest discrimination. We studied rabbits with intact autonomic nervous system, in which hormone concentrations rose only after more than 25% of the blood volume had been removed and rabbits with autonomic blockade, in which hormone secretion was greatly enhanced.

For AVP secretion in rabbits with intact autonomic nervous system, the role of the arterial baroreceptors during haemorrhage was clear cut, but that of the cardiac receptors was equivocal. However, in autonomically blocked rabbits, both sets of afferent input made a definite contribution to the response; input from the arterial baroreceptors provided about two-thirds of the afferent drive, whilst that from cardiac receptors provided about one-third, with little increase in secretion after combined denervation. In intact rabbits, the fall in blood pressure and blood volume during haemorrhage was related to

Dr. Geoff Head (left) and Dr. N. Minami

the animal's afferent status, whilst in autonomically blocked rabbits, the falls in MAP and blood volume were all the same, whatever the animal's afferent status.

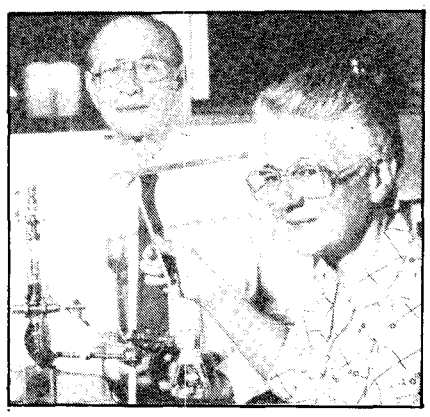
With PRA, neither arterial nor cardiac baroreceptors were involved in the increased levels during haemorrhage. We assume that renin is released largely in response to direct haemodynamic signals on the renal 'barostat'.

Vasoconstrictor response.

We have previously found that in normal rabbits with functional autonomic neural, hormonal, and local effector mechanisms, removing 20% of the blood volume evokes a response solely through neural (constrictor) and local (dilator) mechanisms, with no contribution from the pressor hormones. The latter come into play only with more extensive haemorrhage. By contrast, 20% BV loss during autonomic blockade produces enhanced release of AVP and PRA, so that circulatory regulation is almost entirely through the hormonal constrictor effects. Thus, autonomically intact rabbits and autonomically blocked rabbits provide, respectively, neural and hormonal 'models' of circulatory regulation.

In the autonomically intact rabbits, the initial phase of good maintenance of MAP was due to both cardiac support and peripheral vasoconstriction, with the latter somewhat above the local dilator response component. The arterial and cardiac baroreceptors together accounted for about 70% of the drive for the reflex vasoconstriction during haemorrhage, with about 30% of the response presumably mediated through other afferent mechanisms. The drive by the cardiac receptors was responsible for about 20% of the constrictor effect, whilst that arising from the arterial baroreceptors accounted for at least 50% of the total constriction.

In autonomically blocked rabbits, MAP was poorly maintained due to the absence of autonomic support to the heart. However, the degree of peripheral vasoconstriction was about double that of autonomically intact rabbits, due to enhanced AVP and PRA concentration. After combined sino-aortic and cardiac receptor denervation, the vasoconstrictor response was virtually unaffected, because renin (which is not under baroreceptor control) rose to levels close to those producing maximum constriction. These models serve to illustrate the fine-tuning capacity of the baroreceptor-mediated vasoconstrictor response through neural effector mechanisms. This is lost in the hormonal model, partly because only AVP is under baroreceptor control. However, even if both hormones were under baroreceptor control, their inability to stimulate the heart makes this much less effective as a means of maintaining blood pressure than neural autonomic control.



Professor Korner and his long-time research assistant Judy Oliver

Renal sympathetic baroreflex properties in two rabbit strains

P.K. Dorward, C.D. Rudd and M. Weinstock*

We used the perivascular cuff and drug methods to examine the renal baroreflex properties in two genetically related strains of rabbits, bred in Israel (Strains I and II). We determined the involvement of (i) cardiac afferents, using 5% pericardial procaine, and (ii) of an endogenous opiate mechanism, by examining the effects of intravenous naloxone. In both strains, the gain of the baroreflex, as determined by the drug method, was half that obtained by the cuff method. In contrast to earlier findings with the baroreceptor heart rate reflex, there were no differences in gain between strains with either method, and the difference in gain was unaffected by naloxone.

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The mechanism of action of aldomet: role of the spinal noradrenergic fibers

Carol-Ann Courneya, G.A. Head.

Alpha-methlydopa (α-MD) is an anti-hypertensive agent, which has been successfully used in patients to reduce blood pressure through a combined effect of decreasing cardiac output and decreasing peripheral resistance (dilating peripheral vascular beds). α-MD is converted to an active metabolite (α-methyl noradrenaline), which acts in the noradrenaline-containing fibers in the spinal cord to inhibit the post-ganglionic sympathetic motor fibers. We are currently testing this hypothesis in rabbits which have been instrumented with flow probes around the lower aorta to measure hindquarter blood flow. The blood pressure and blood flow response to α-MD are being compared between rabbits with intact noradrenaline-containing fibres, and those animals which have undergone local destruction of those fibers. This will establish whether these fibers are involved in the mechanism of action of α-MD.

Sir Thomas Ramsey Electron Microscopy Laboratory and Morphology Laboratory

Head: S.E. Luff and R.J. Dilley

Projects

Electron microscopy

Neuroeffector relationships in the kidney.

Quantitative electron microscopy of neuroeffector junctions in muscular arteries of normal and spontaneous hypertensive rats.

Development of the innervation in muscular arteries in normal and spontaneous hypertensive rats.

Morphology

Effects of IGF-1 transgene expression on cardiovascular remodelling.

Cell size, number and reactivity in SHR renal hypertension.

Summary

Research conducted in these laboratories has two major emphases; the structural arrangement and biochemical content of axon terminals innervating blood vessels; and structural mechanisms of remodelling in the cardiovascular system.

We have now firmly established that specialised neuromuscular junctions occur in almost all muscular arteries, and almost all axon varicosities form specialised junctions. This strongly suggests that in small resistance arterioles, neuromuscular transmission occurs via these specialised junctions and not via distant release of neurotransmitter. These important findings could change the previously accepted views on the mechanism of neuromuscular transmission in blood vessels. They have formed the basis of our more recent studies on the innervation of muscular arteries in the kidney and spontaneous hypertensive rats.

There is considerable evidence to suggest that the sympathetic innervation of blood vessels is involved in the development of hypertension, possibly by promoting increased wall mass of resistance arteries in this condition. For example, it is known that there are more axon terminals in the spontaneously hypertensive rats (SHR) arteries than in those from normotensive (WKY) animals. However, it is not yet established if there is a difference in the density of neuromuscular junctions, which is a more relevant factor. In conjunction with the Circulatory Control and Neuropharmacology Laboratory, we are comparing arteries from two strains of rat to ascertain if there is a difference in junction density.

Another major aspect of the research this year has been studies of the innervation of the kidney cortex in conjunction with the Renal Laboratory. There is considerable controversy concerning how the sympathetic innervation of the kidney controls renal function. Last year we reported that we had found two different types of sympathetic axons, a finding which has not been reported previously and which could be central to our understanding of sympathetic neural control of kidney function. This year we have gathered more detailed information on the shape, size, distribution and contacts of these two types of axons with different cell types in the rat and rabbit.

We have also been analysing the innervation of the macula densa and extraglomerular mesangial region of the kidney. In animals that have been treated with enalapril to block the conversion of angiotensin I to angiotensin II, the smooth muscle cells of the glomerular arterioles change from being predominantly contractile cells to ones capable of synthesising and secreting renin. We have commenced experiments to study the sympathetic innervation of these cells in enalapril treated rabbits to compare them with normal animals. This work will provide information on whether innervation of smooth muscle cells change if their function is changed.

In conjunction with the Biochemical Pharmacology Laboratory, we have been investigating the development of the sympathetic innervation of muscular arteries in young normal and spontaneous hypertensive rats. Little is known about the development of the peripheral sympathetic nerve terminals. This project involves immuno-histochemical studies on the biochemical content of developing terminals and ultrastructural studies on innervation development in muscular arteries of 2-12 day old normal and spontaneously hypertensive rats.

The projects on cardiovascular structural remodelling aim to examine mechanisms of cardiovascular growth. In collaboration with Dr Stephen Schwartz in Seattle, USA, we have used a transgenic mouse model to study the cardiovascular effects of increased growth hormone gene levels. These mice have increased body weight, as well as greater heart and vessel wall mass and so are particularly useful as models of growth. These "giant" mice may also help to explain why human patients with acromegaly or gigantism, who also have increased levels of growth hormone, have a higher frequency of hypertension and cardiac hypertrophy. Our studies to date have shown that hypertrophy in the transgenic mice is selective for those vessels which supply larger organs, and thus appears to occur in response to greater demands for blood flow rather than as a direct effect of growth hormone. Growth occurs primarily by increasing cell numbers in the heart and existing vessels. We have extended these studies this year to include transgenic mice which have increased gene levels of insulin-like growth factor I, a molecule

which is also strongly implicated in acromegaly and gigantism. Transgenic animals with this gene also exhibit increased body weight and we have completed quantitative analyses to describe cardiovascular hypertrophy in this model.

Dr. Rod Dilley of the Morphology Laboratory.

Spontaneously hypertensive rats (SHR) also exhibit cardiovascular hypertrophy, in that they have thicker vessel walls than normal animals (WKY) and a greater number of smooth muscle cells per length of vessel. By comparison, when renal hypertension is produced in normal animals by restricting blood flow to the kidney, there is evidence to suggest that the size of the smooth muscle cells increases, but not number. We are interested in how these different remodelling mechanisms arise. One possibility is that vessels of SHR strain rats respond most readily to hypertension by increasing cell number. In fact, smooth muscle cells from SHR aorta have a greater ability for cell proliferation in cell culture. Another possibility is that SHR hypertension and renal hypertension are induced by different mechanisms, and thus invoke different mechanisms of vessel wall growth. We are currently investigating whether cell number or cell size increases after we combine these two models. We produce additional increases in SHR blood pressure by applying renal hypertension on top of the existing hypertension. In this collaboration with the Biochemical Pharmacology Laboratory we are also studying the functional effects of increased wall structure. To date, we have successfully produced elevated pressure in these animals and found that the heart and vessels are significantly larger than those of the normal SHR. We are currently extending these studies to include quantitative analyses of cell number and size.

Neuroeffector relationships in the kidney

S.E. Luff, S.G. Hengstberger, W.P. Anderson, K.M. Denton, E.M. McLachlan*

We have been conducting a detailed ultrastructural study on the innervation of the kidney cortex in the rabbit and rat. We have adopted a new approach in this investigation, by determining the structure and distribution of specialised neuronal junctions. This approach is based on our own recent findings that sympathetic axons innervating almost all muscular arteries form specialised junctions with smooth muscle cells and in the small resistance arterioles. This project has involved serial section analysis and three dimensional reconstruction of individual axons in the region of the glomerular arteriole and juxtaglomerular apparatus. We have previously reported the finding that there are two structurally distinctive types of axons innervating the glomerular arterioles of the rabbit kidney. We have now completed a detailed analysis of these axons and compared them with our existing data on the innervation of the submucous arterioles and other muscular arteries. We have found that these two types of axon (I & II) are also structurally different from these innervating most of the muscular arteries we have studied in adult rats, rabbits and guinea pigs. The major characteristics of these vessels are detailed below.

Type I axons

These axons are not typically varicose. They exhibit only small changes in axon diameter along their length having neurotubules and filaments running through the whole length of the axon segments we have analysed (i.e. 40μm). At various points along their length synaptic vesicles are found clustered towards the region of axonal membrane that is forming a junction with a target cell, usually a smooth muscle cell. The diameter of the intervaricose regions of these axon are significantly greater than those of the Type II axons.

Type II axons

These are typically varicose. The varicosities are usually a simple fusiform shape and frequently very small. The intervaricose regions are similarly very small, their diameters being about one tenth of the Type I intervaricosities.

We have now established that all the axons that innervate this region of the kidney in the rabbit are catecholaminergic. We have also established that these two types of axons are not restricted to the rabbit afferent and efferent arterioles as they are also found in the rabbit interlobular arteries and the rat glomerular arterioles.

Differences between the innervation of the renal vessels compared with that of other vessels.

The arrangement of the innervation is generally longitudinal to the vessel axis with few instances of branching axons. This compares with the complex branching network found around most other vessels, including arterioles. There is a high incidence of single axons. Single axons segments are frequently observed without any association with a Schwann cell. Such axons may or may not be surrounded by basal lamina and may have a loose association with other cell types such as fibroblasts. Such axons are able to form junctions with smooth muscle cells. These structural features could be indicative of a more dynamic and plastic neuronal system in the kidney. The mean varicosity volume of Type I axons is similar to that of other vessels (i.e. 1.05 $\pm$ 0.46 μm^3, and 1.2 $\pm$ 0.25 μm^3). A similar proportion (82%) of the Type I axon varicosities form junctions with target cells (i.e. predominantly smooth muscle cells) to those in other vessels of similar size. The Type II axons are similar in that they are varicose but

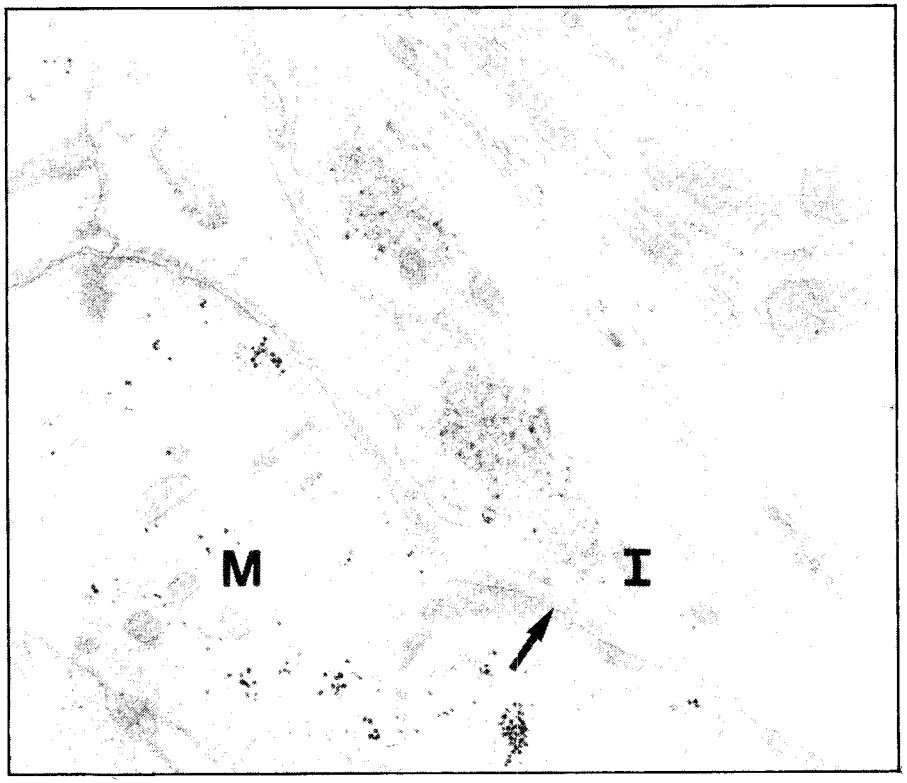

Fig. 1 Type I axon forming junction with an afferent arteriole. M = smooth muscle cell of afferent arteriole, arrow = junction, I = Type I axon terminal.

differ in that the size of the varicosities are frequently much smaller than those innervating other vessels. A smaller proportion (71%) of Type II axons form junctions, but the varicosities that do not form junctions are predominantly the small (< 1μm diameter) varicosities. There are a similar proportion of junctions that have presynaptic membrane specialisations in the kidney to that found in submucous arterioles and, as we also found in the latter vessels, they are restricted to the larger (> 1μm diameter) varicosities.

We are currently analysing the axons that are situated in the juxtaglomerular region close to the macula densa. We have found varicosities closely apposed to the basal lamina of the macula densa. To ascertain if these axons are forming specialised junctions will require three dimen-sional reconstruction of these axons which is presently in progress. We have so far not observed any axons close to the extra-mesangial cells. We are also currently investigating the density of junctions on the renin-secreting cells in normal and enalapril treated animals.

* Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Queensland.

Frequency of neuro-muscular junctions in the adult spontaneously hypertensive and Wistar Kyoto rat

S.E. Luff, P.I. Korner, E.M. McLachlan

There is considerable evidence in the literature that the innervation of blood vessels has a humoral influence in the development of vascular smooth muscle cells and on the development of structural hypertension. When spontaneously hypertensive rats (SHR) are compared to normotensive WKY rats, increased density of axon varicosities is evident quite early in development. The aim of this study was to determine if there is any difference in the frequency of junctions in these two strains of animals. We have completed a study on four different sized mesenteric arteries in 16 week old animals and have found no significant difference in the junction frequency in the vessels of these two strains of rats. We are now investigating the possibility that the effect of the innervation may occur earlier in development and are studying 4 week old rats.

Effects of IGF-1 transgene expression on cardio-vascular remodelling

R.J. Dilley, S.M. Schwartz*

We have shown that the heart and aorta of IGF-1 transgenic mice are larger than those of normal animals. These increases are in proportion to body weight increases and appear to be stimulated as part of normal adaptive growth to supply a larger body mass with blood. The growth is accomplished without an increase in cell number in the heart

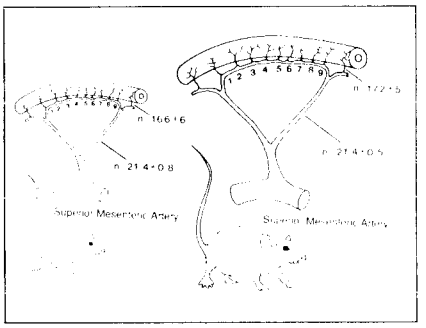

Fig.2 Growth hormone transgenic mice (right) are larger than normal mice and have a heavier and longer small intestine. The number of mesenteric artery branches supplying the small intestine is not altered but each vessel is longer.

or aorta, suggesting that cells become larger to increase the overall tissue mass. Furthermore, in comparison to growth hormone transgenic mice, where the brain and the vessels supplying it are the same size as normal animals, the IGF-1 transgenic animals have a 30% larger brain mass. This increase is greater than that due to body weight change. Surprisingly, we did not find an increase in size of the common carotid artery of the transgenic animals, even though it supplies a significantly larger brain mass. These results suggest that there may be some elements of vessel specific responses to growth factors in these models.

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Human Autonomic Function Laboratory

Head: Dr. M.D. Esler

Projects

Release of amine neurotransmitters and their metabolites from the brain.

Effect of physical conditioning on the sympathetic nervous system: regional pattern of sympathetic inhibition.

Investigation of sympathetic nervous function by concurrent measurement of nerve firing and transmitter release.

The neural deficit in autonomic insufficiency syndromes.

Release of noradrenaline from the heart during simulated stressful life events.

Investigation of the contribution of cardiac sympathetic nervous overactivity to cardiac arrhythmias.

Neuronal uptake of noradrenaline in healthy and hypertensive humans and in laboratory animals.

Studies on neuropeptide Y release in man.

Noradrenaline release and reuptake in cardiac failure.

Whole-body and neuronal adrenaline kinetics in man and laboratory animals.

Summary

The sympathetic nervous system is a stimulant division of the autonomic (automatic) nervous system which is very important in regulating a range of body functions, particularly those involving the heart and blood vessels. The Human Autonomic Function laboratory has developed unique methods for studying the human sympathetic nervous system, from measurements of the rate of release of its neurotransmitter ("chemical messenger") noradrenaline from individual organs of the body. This has led to new knowledge on both the normal functioning of this branch of the ner-

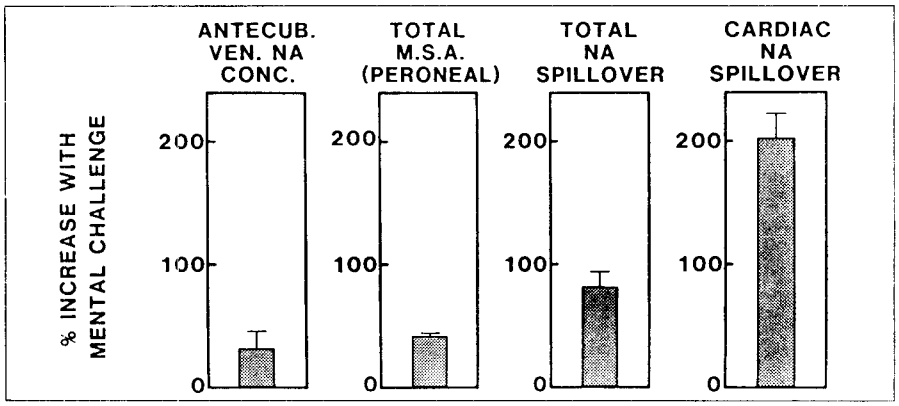

Fig.1 Responses to the sympathetic nervous system to laboratory mental stress (forced mental arithmetic) were measured. The sympathetic nerves to the heart were activated more than those passing to other sites, including muscle (small response only in muscle sympathetic activity, "M.S.A.").

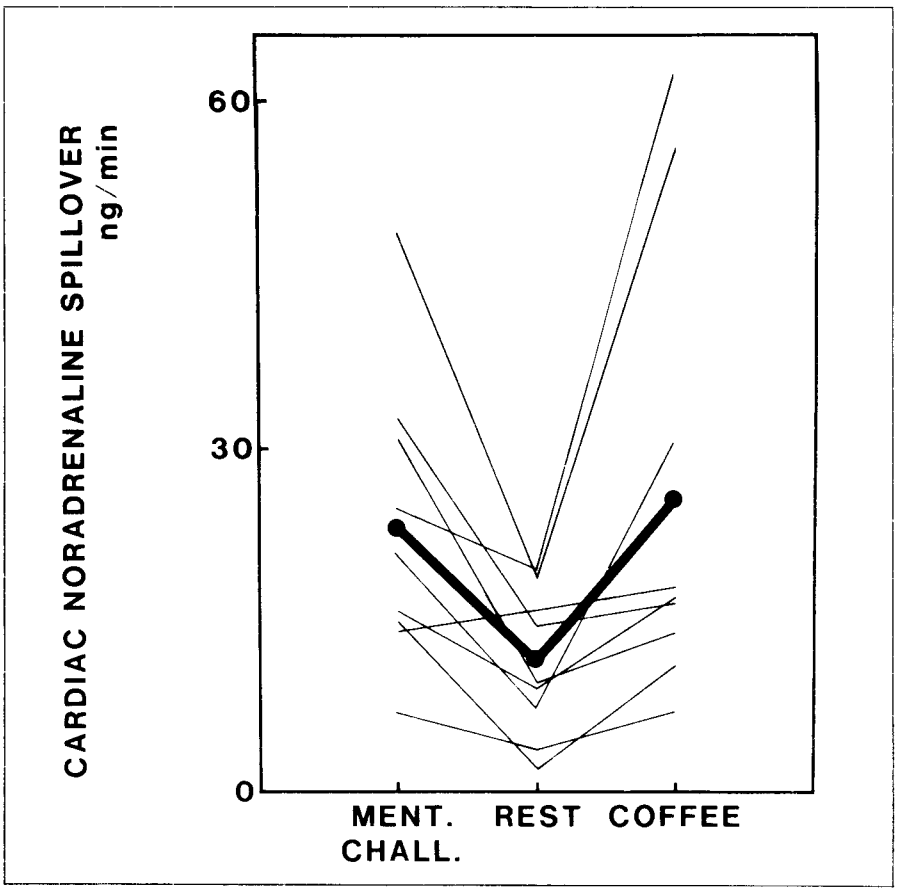

Fig.2 Coffee drinking stimulates the nerves to the heart to a degree similar to that seen with laboratory mental stress.

vous system and its derrangements in disorders such as high blood pressure and heart failure. A recent focus of our research concerns the responses of the sympathetic nervous system to stress. We are now also able to measure electrical firing in sympathetic nerves, in addition to noradrenaline release (Figure 1), and in a study performed in collaboration with a distinguished visiting scientist, Professor Gunnar Wallin demonstrated that mental stress preferentially stimulates the nerves to the heart. A range of common life events, such as coffee drinking and exercise (Figure 2) also activate the cardiac sympathetic nerves. We are continuing this line of research, to test whether stress really can cause heart disease.

Another goal of the laboratory is to develop methods (and apply them in patients) for studying some of the chemical bases of brain function. A particular interest is the transmitters of the central nervous system (particularly noradrenaline) which regulate the activity of sympathetic nerves and blood pressure. We find, using sampling catheters passed from an arm vein to the internal jugular veins, which carry blood from the brain, that noradrenaline overflow from the brain to the blood stream occurs. We now have strong evidence that noradrenaline in the brain is important in controlling the activity of the sympathetic nerves (Figure 3), and preliminary but fascinating results suggesting that increased activity of noradrenaline in the brain contributes to the development of high blood pressure.

Investigation of sympathetic nervous function by concurrent measurement of nerve firing and transmitter release

M. Esler, G. Wallin*, G. Eisenhofer, G. Lambert, H. Cox, P. Dorward, I. Meredith, G. Jennings

The two most powerful methods for studying human sympathetic nervous system function are electrical (measuring nervous firing) and biochemical (measuring neurotransmitter release). We have recently simultaneously applied both methods to learn more about the responses of the nervous system to stress. While healthy research volunteers were subjected to mild laboratory mental stress (solving arithmetic problems while being distracted) and to an isometric exercise challenge (sustaining a handgrip for ten minutes), nerve recordings were made from very small electrodes previously painlessly placed in sympathetic nerves to skeletal muscle. The release of noradrenaline from the heart was measured concurrently. The mental stress (arithmetic) preferentially activated the nerves to the heart, leaving the muscle sympathetic nerves relatively silent. Isometric exercise stimulated nerves to heart and muscle proportionately (Figure 1). The results confirm earlier clinical suspicions that it is the heart which is particularly influenced by nervous reactions to stress.

*visiting scientist

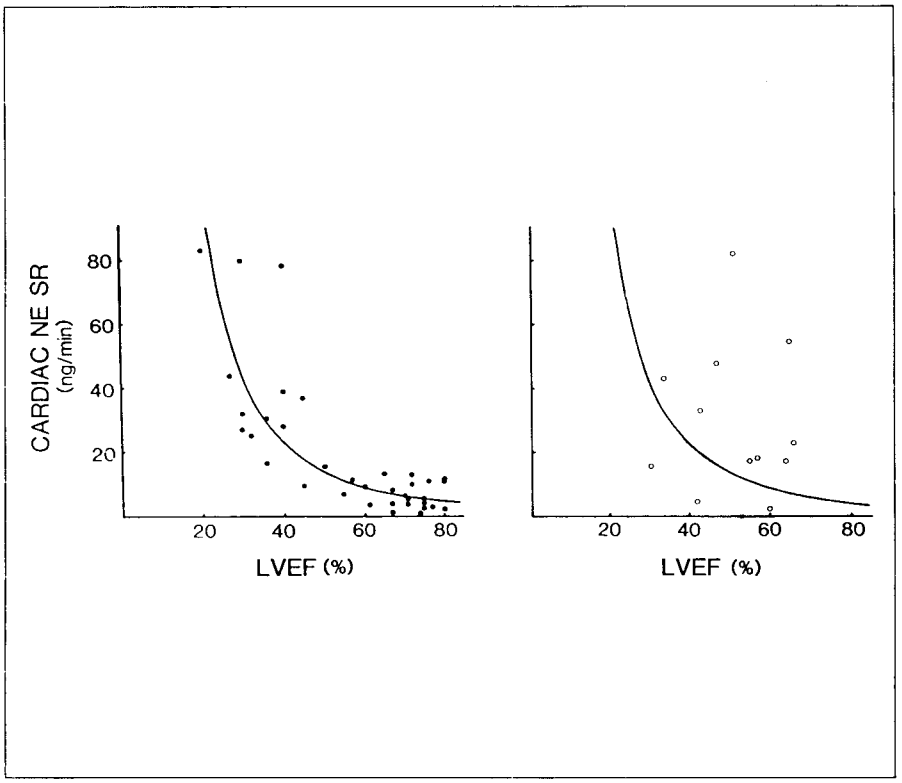

Fig.3 A curvilinear relationship exists between the pumping efficiency of the heart (left ventricular ejection fraction, "LVEF" and neurotransmitter release from the sympathetic nerves of the heart ("cardiac NE SR"). In patients with heart failure, with low LVEF, the cardiac sympathetic nervous outflow is stimulated.

Release of noradrenaline from the heart during simulated stressful life events

I. Meredith, M. Esler, G. Lambert, G. Eisenhofer, H. Cox, G. Jennings

Following up the observations noted above, we have performed a systemic investigation of activation of the sympathetic nerves of the heart by mimicing real-life circumstances in the laboratory. The responses to mental stress, coffee drinking, cigarette smoking and isometric exercise have been studied. These have been chosen because they have been incriminated (either on strong scientific grounds or in medical folklore) in the development of heart disease. Cigarette smoking caused adrenaline release from the adrenal gland but had little effect on the

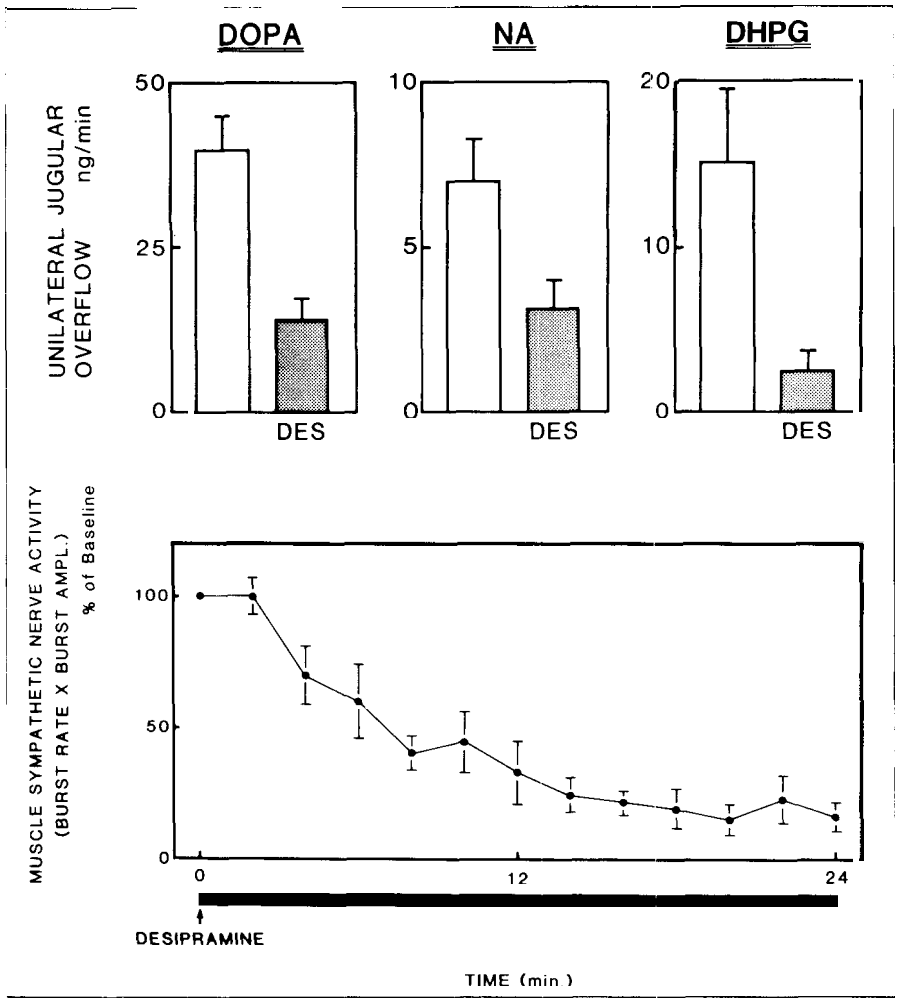

Fig.4 With desipramine, overflow of noradrenaline, its precursor DOPA, and its breakdown product DHPG from the brain is reduced. There is an accompanying 85% fall in muscle sympathetic nerve firing rate.

sympathetic nerves of the heart. Coffee drinking and isometric exercise activated the sympathetic nervous outflow to the heart, but in proportion to the stimulation of sympathetic nerves passing to other organs (Figure 2). It was mental stress alone which preferentially activated the nerves to the heart. Our research evaluating whether this stress response is involved in causing heart attacks (by dangerously altering heart rhythm or by causing coronary artery spasm) continues.

Noradrenaline release and reuptake in cardiac failure

I. Meredith, M. Esler, G. Lambert, G. Jennings

Our previous research has demonstrated that in patients with failing hearts the sympathetic nervous system is intensely stimulated. This may to some extent facilitate the pumping of the diseased heart muscle, but is thought to have adverse consequences, such as causing dangerous disturbances of heart rhythm and consequent sudden death. Our continuing research shows that, of all sympathetic nervous outflows, it is that to the heart which is most stimulated (a tenfold increase in noradrenaline release is detected). A "law of the heart" exists (Figure 3) relating, in a curvilinear fashion, the extent of cardiac sympathetic nerve activation to the degree of failure of the heart. An important point is that even in early, mild cardiac failure substantial stimulation of the cardiac nerves is present. It is known that such patients, with only mild heart failure, are nevertheless very prone to sudden death.

Release of amine neurotransmitters and their metabolites from the brain

C. Ferrier, M. Esler, M. Horne*, G. Eisenhofer, H. Cox, G. Lambert, G. Jennings

The activity of the sympathetic nervous system is regulated by the brain. We have developed techniques for studying neurotransmitter ("chemical messenger") release in the brain, by measuring overflow of transmitter into the internal jugular veins which drain from the brain. Sampling catheters can be passed painlessly from an arm vein, with X-ray viewing control, to lie in both jugular veins. We are particularly interested in noradrenaline release in the brain because of its importance in the central nervous system control of sympathetic nerve fibre activity and blood pressure. We have detected release of noradrenaline from the brain into both internal jugular veins, along with its precursor (DOPA) and one of its breakdown products or metabolites (DHPG) (Figure 4). We have found that a drug, desipramine, which strongly reduces sympathetic nerve firing also markedly lowers noradrenaline turnover in the brain (Figure 4). This CNS effect of desipramine appears to be the mechanism by which nerve firing is reduced. We are currently studying CNS noradrenaline turnover in patients with high blood pressure, to learn if their increased sympathetic nerve firing is attributable to a CNS cause.

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Lipoprotein Metabolism Laboratory

Head: Dr. P.J. Barter

Projects

Evidence in vitro that hepatic lipase reduces the concentration of apolipoprotein A-I in rabbit high density lipoproteins.

Interaction of apolipoprotein A-II with reconstituted HDL containing egg phosphatidylcholine, unesterified cholesterol and apolipoprotein A-I.

Synergistic effects of lipid transfers and hepatic lipase in the formation of very small high density lipoproteins during incubation of human plasma.

Lipoprotein lipase prevents the hepatic lipase-induced reduction in particle size of high density lipoproteins during incubation of human plasma.

The interaction of cholesteryl ester transfer protein and unesterified fatty acids promotes a reduction in the particle size of high density lipoproteins.

Effects of acipimox on plasma lipoproteins of hyperlipidaemic human subjects.

Summary

Our primary interest in the Lipoprotein Metabolism Laboratory has continued to be the regulation of high density lipoproteins (HDL) with particular reference to factors which regulate (i) the particle size distribution of HDL and (ii) the partitioning of cholesterol between HDL and low density lipoproteins (LDL). In the latter studies we have made a discovery of major importance.

The importance of understanding factors which influence the partitioning of cholesterol between different plasma lipoproteins is highlighted by the observation that the risk of developing coronary heart disease correlates positively with the concentration of cholesterol in LDL and negatively with that in HDL. Most of the cholesterol in plasma exists as cholesteryl esters which reside in the hydrophobic core of plasma lipoproteins. In human plasma, the cholesteryl esters readily exchange between different lipoprotein fractions in a process of equilibration catalysed by the cholesteryl ester transfer protein (CETP). In the case of HDL and LDL, we have shown previously that the rate of the CETP-mediated exchange is rapid relative to the rate of lipoprotein catabolism; as a consequence, the pools of cholesteryl esters in these two lipoprotein fractions are close to equilibrium in vivo. In any given subject, therefore, the distribution of cholesteryl esters between the LDL and HDL fractions is a function of: (i) the relative molarities of the two lipoprotein fractions and (ii) the relative capacities of lipoprotein particles in each fraction to accommodate cholesteryl esters. Much intersubject variation in the distribution of cholesteryl esters between LDL and HDL can be explained in terms of differences in the lipoprotein molarities which, in turn, reflect variations in either the synthesis or the receptor-mediated cellular uptake of lipoproteins. We have now discovered that the capacity of lipoprotein particles to accommodate cholesteryl esters is also subject to variation. We have found that the capacity of HDL particles to accommodate cholesteryl esters is markedly reduced by an interaction between CETP and unesterified fatty acids. Thus, unesterified fatty acids disrupt the CETP-mediated equilibrium between LDL and HDL and there is a net mass transfer of cholesteryl esters from HDL to LDL until, at a new steady state, a greater proportion of the cholesteryl esters is partitioned in the LDL fraction. This novel effect of unesterified fatty acids is concentration dependent and is subject to inhibition by fatty acid-poor but not by fatty acid-rich human serum albumin. We conclude

Dr. P. J. Barter

that unesterified fatty acids may be important physiological regulators of the partitioning of cholesteryl esters between the non-atherogenic HDL and the atherogenic LDL fraction. Furthermore, the increased concentrations of unesterified fatty acids in association with obesity, smoking, diabetes and chronic stress may well underlie the low concentrations of HDL cholesterol in these conditions.

Evidence in vitro that hepatic lipase reduces the concentration of apolipoprotein A-I in rabbit high density lipoproteins

M.A. Clay, K-A. Rye, P.J. Barter

Interest in factors which regulate the concentration of HDL has been stimulated by the observation of an inverse relationship between the concentration of HDL cholesterol and the risk of developing coronary heart disease. In general terms, the concentration of HDL in plasma may be regulated at three levels: (i) the synthesis of individual HDL constituents; (ii) the removal of HDL from the plasma and (iii) the remodelling of mature HDL within the plasma compartment. The present report focusses on the issue of the remodelling of HDL in plasma and asks the question: do primary changes to HDL lipids result in secondary changes to HDL apolipoproteins? Specifically, we have tested the hypothesis that when the HDL particle size is decreased following a primary reduction in the concentration of HDL core lipids, a proportion of the apolipoproteins on the HDL surface will become redundant and, as a consequence, be shed from the particle. To this end, we have exploited the fact that rabbit HDL is naturally enriched with triacylglycerol and that when rabbit plasma is incubated in vitro with hepatic lipase, HDL triacylglycerol is hydrolysed and the particle size of HDL decreases. We now report that these changes are accompanied by a dissociation of a proportion of the apoA-I from the HDL.

Whereas the apo A-I is confined to the HDL fraction in non-incubated rabbit plasma, following incubation of rabbit plasma with canine hepatic lipase there is an appearance of up to 30% of the apoA-I in the lipoprotein-free fraction of plasma. This dissociation of apoA-I from HDL has been demonstrated independently by ultracentrifugation of plasma at a density of 1.21 g/ml, by size exclusion chromatography of plasma on columns of Superose 6 and Superose 12 connected in series and by immunoblot analysis of plasma separated by non-denaturing gradient gel electrophoresis on 4-30% polyacrylamide gels. The dissociation of apoA-I from HDL has been shown to be unrelated to hydrolysis of HDL phospholipids. It does, however, correlate with the reduction in HDL particle size in experiments designed to show both the time course of the phenomenon and its dependence on the concentration of hepatic lipase in the incubation.

These in vitro studies are consistent with a proposition that the concentration of apoA-I in HDL is determined in part by intraplasma factors which regulate the metabolism of HDL core lipid constituents.

Interaction of apolipoprotein A-II with reconstituted HDL containing egg phosphatidylcholine, unesterified cholesterol and apolipoprotein A-I

K-A. Rye

The high density lipoproteins (HDL) in human plasma are heterogeneous, consisting of particles of varying size and composition. Studies addressing the regulation of HDL particle size usually involve the modification of native HDL with plasma factors under conditions which alter both the core and surface components of HDL. The resulting modified particles either acquire or shed surface constituents in order to conserve their structural integrity. Although such systems have afforded a great deal of information about the relationship between the size of HDL and the composition of the core and surface, there is, at present, little known about the influence of surface components alone on the size and stability of the particles. The current study addresses this issue in a model system of reconstituted HDL (r-HDL). The preparation of r-HDL containing various phospholipids, apolipoproteins and a range of concentrations of unesterified cholesterol has been reported by several investigators. The present study describes the preparation of r-HDL containing both apolipoprotein (apo) A-I and apo A-II. R-HDL with 100:1 (mol:mol) egg PC:apo A-I and 0 (Series I), 5 (Series II) or 10 (Series III) mol% unesterified cholesterol were prepared by the cholate dialysis method. The resulting complexes had a Stokes' radius of 4.7 nm and contained two molecules of apo A-I per particle. When the r-HDL (2.0 mg apo A-I) were supplemented with 1.0 mg of apo A-II, one of the apo A-I molecules was replaced by two molecules of apo A-II. This modification was not accompanied by a loss of phospholipid nor by major change in particle size. The addition of 2.5 or 4.0 mg of apo A-II resulted in the displacement of both apo A-I molecules from a proportion of the r-HDL and the formation of smaller particles (Stokes' radius 3.9 nm), which contained half the original number of egg PC molecules and three molecules of apo A-II. The amount of apo A-I displaced was dependent on the concentration of unesterified cholesterol in the r-HDL: when 2.5 mg of apo A-II was added to the

Series I, II and III r-HDL, 44, 60 and 70% respectively of the apo A-I was displaced. Addition of 4.0 mg of apo A-II did not promote further displacement of apo A-I from any of the r-HDL. By contrast, the association of apo A-II with r-HDL was independent of the concentration of unesterified cholesterol and was a linear function of the amount of apo A-II which had been added. It is concluded that (1) the structural integrity of egg PC:unesterified cholesterol:apo A-I r-HDL which contain two molecules of apo A-I is not affected when one of the apo A-I molecules is replaced by two molecules of apo A-II, (2) when both apo A-I molecules are replaced by apo A-II, small particles which contain three molecules of apo A-II are formed and (3) the displacement of apo A-I from r-HDL is facilitated by the presence of unesterified cholesterol in the particles.

Synergistic effects of lipid transfers and hepatic lipase in the formation of very small high density lipoproteins during incubation of human plasma

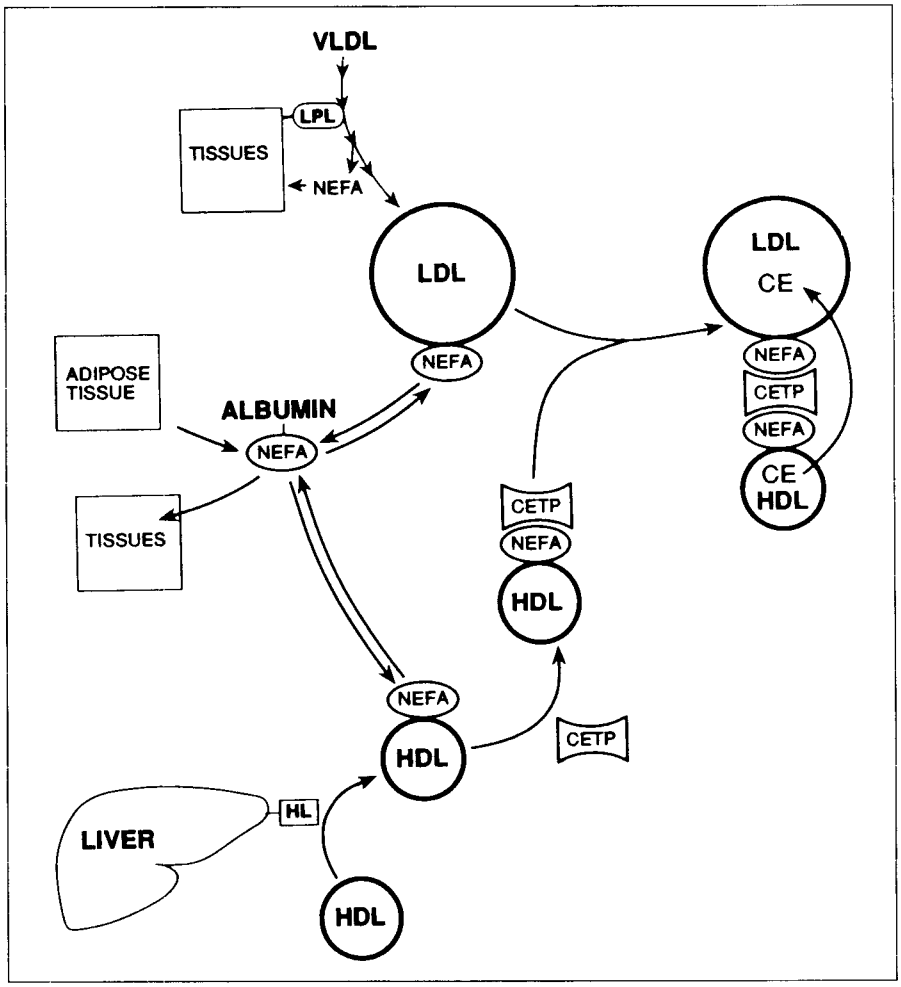
H.H. Newnham, P.J. Barter

Studies have been performed to determine the involvement of very low density lipoproteins (VLDL), cholesteryl ester transfer protein (CETP) and hepatic lipase (HL) in the formation of very small HDL particles. Human whole plasma has been incubated for six h at 37C in the absence and in the presence of various additions. There was minimal formation of very small HDL in incubations of non-supplemented plasma or in plasma supplemented with either VLDL, CETP or HL alone; nor were small HDL prominent after incubating plasma supplemented with mixtures of VLDL plus CETP, VLDL plus HL or CETP plus HL. By contrast, when plasma was supplemented with a mixture containing all three of VLDL, CETP and HL, incubation resulted in an almost total conversion of the HDL fraction into very small particles of radius 3.7 nm. The appearance of these very small HDL was independent of activity of lecithin:cholesterol acyltransferase. It was, however, dependent on both duration of incubation and on the concentrations of the added VLDL, CETP and HL. The effect of these incubations was also assessed in terms of changes to the concentration and distribution of lipid constituents across the lipoprotein spectrum. It was found that not only did lipid transfers and HL exhibit a marked synergism in promoting a reduction in HDL particle size but also that HL, although deficient in intrinsic transfer activity, enhanced the CETP-mediated transfers of cholesteryl esters from HDL to other lipoprotein fractions.

Lipoprotein lipase prevents the hepatic lipase-induced reduction in particle size of high density lipoproteins during incubation of human plasma

H.H. Newnham, G.J. Hopkins, S. Devlin, P.J. Barter

Human plasma lipoproteins or human whole plasma have been incubated in vitro with canine hepatic lipase (HL) and bovine milk lipoprotein lipase (LPL) to determine the effects of these lipases on the particle size distribution of HDL, as determined by native gradient gel electrophoresis. Incubation of the d<1.21 fraction of plasma with varying concentrations of HL and LPL and subsequent analysis of the distribution of



Postulated mechanism by which nonesterified fatty acids (NEFA) promote the redistribution of cholesterol from the non-atherogenic HDL to the atherogenic LDL. CETP, cholesteryl ester transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; CE, cholesteryl esters; VLDL, LDL and HDL, very low, low and high density lipoproteins respectively.

constituents across the lipoprotein spectrum after size exclusion chromatography showed that the lipid and lipoprotein specificities of the lipases used were similar to those reported previously. HL preferentially hydrolysed HDL triglyceride while LPL hydrolysed predominantly VLDL triglyceride, but neither lipase altered HDL particle size. However, when both VLDL and CETP were added to incubations of either the d<1.21 fraction of plasma or whole plasma, HL promoted marked reduction in HDL particle size from the initial populations of 5.3 and 4.3 nm Stokes' radius to a uniform population of particles of 3.7 nm radius. These effects of HL were dependent on the concentration of VLDL. Under the same conditions LPL was virtually without effect. When both LPL and HL were included in the same incubation however, LPL inhibited the effects of HL on HDL particle size. These results suggest that HL has a direct effect on HDL particle size in a process which is dependent on concurrent lipid transfers between HDL and VLDL and that LPL inhibits the effect of HL by reducing the concentration of VLDL.

The interaction of cholesteryl ester transfer protein and unesterified fatty acids promotes a reduction in the particle size of high density lipoproteins

P.J. Barter, L.B.F. Chang, H.H. Newnham, K-A. Rye, O.V. Rajaram

Purified human cholesterylester transfer protein (CETP) has been found under certain conditions to promote changes to the particle size distribution of high density lipoproteins (HDL) which are comparable to those attributed to a putative HDL conversion factor. When preparations of either the conversion factor or CETP are incubated with HDL3 in the presence of very low density lipoproteins (VLDL) or low density lipoproteins (LDL), the HDL3 are converted to very small particles. The possibility that the conversion factor may be identical to CETP was supported by two observations: (i) CETP was found to be the main protein constituent of preparations of the conversion factor and (ii) an antibody to CETP not only abolished the cholesteryl ester transfer activity of the conversion factor preparations but also inhibited changes to HDL particle size. In additional studies the changes to HDL particle size promoted by purified CETP were inhibited by the presence of fatty acid-free bovine serum albumin; by contrast, albumin had no effect on the cholesteryl ester transfer activity of the CETP. The possibility that albumin may inhibit changes to HDL particle size by removing unesterified fatty acids from either the lipoproteins or CETP was tested by adding exogenous unesterified fatty acids to the incubations. In incubations of HDL with either VLDL or LDL, sodium oleate had no effect on HDL particle size. However, when CETP was also present in the incubation mixtures the capacity of CETP to reduce the particle size of HDL was greatly enhanced by the addition of sodium oleate. It is concluded that the changes in HDL particle size which were previously attributed to an HDL conversion factor can be explained in terms of the interacting effects of CETP and unesterified fatty acids.

Effects of acipimox on plasma lipoproteins of hyperlipidaemic human subjects

P.J. Barter, L. Nelson, S. Devlin, P. Jenkins

Ten subjects with combined hypertriglyceridaemia (mean TG 4.92 mM) and hypercholesterolaemia (mean cholesterol 8.04 mM) were treated for six months with Acipimox (750 mg/day) in order to define the effects of the drug on the lipids, apolipoproteins and particle size distribution of individual lipoprotein fractions. Effects on the concentrations of constituents in whole plasma were consistent with previous reports: plasma TG was reduced by 45% ($p<0.05$), plasma cholesterol was reduced by 19% ($p<0.05$) and plasma apoB was reduced by 17% ($p<0.05$); the plasma concentration of apoA-I increased by 6% (n.s.) while apoA-II was unchanged. The reduction in plasma cholesterol was confined to the VLDL fraction which was reduced by 61% ($p<0.01$). LDL cholesterol was increased by 5% and HDL cholesterol by 10%, but neither change was significant. The ratio of HDL cholesterol:LDL cholesterol increased by 9% (mean for the ten subjects) although again this was not significant. The mass ratio of apoA-I:apoB in whole plasma, however, changed from 0.98 ± 0.22 (mean ± SD) in pretreatment samples to 1.28 ± 0.34 after Acipimox, an increase of 31% which was significant ($p<0.05$). A change in TG was significant in all lipoprotein fractions, with reductions of 46%, 37% and 33% in VLDL, LDL and HDL respectively. In the case of apoB, the VLDL concentration was reduced by 52% ($p<0.01$) while that of LDL was unchanged. The particle sizes of LDL and HDL were assessed by both size exclusion chromatography and non-denaturing gradient gel electrophoresis. Before therapy, the particle size of both LDL and HDL in these hyperlipidaemic subjects was smaller than in normolipidaemic individuals. After six months of therapy with Acipimox, all subjects displayed an increase in the particle size of LDL; in seven of the ten subjects there was also a small increase in the particle size of HDL. It is concluded that the major effect of Acipimox is a reduction in the concentration of both lipid and apolipoprotein constituents of VLDL, although its role in normalizing the particle size of LDL may also be important.

Lipoprotein Structure & Function Laboratory

Head: Dr. N.H. Fidge

Projects

Structural studies on the HDL receptor complex.

Immunochemistry of the HDL receptor.

Determination of the structural domain of apo AI recognised by high density lipoprotein receptors.

Investigation of the receptor binding specificity of HDL apoproteins: studies on apo AII.

Regulation of hepatic high density lipoprotein binding proteins following administration of simvastatin and cholestyramine to rats.

The effect of lovastatin on the interaction between high density lipoprotein and cultured rat adrenocortical cells.

Novel separation of apoproteins by HPLC techniques.

Synthetic peptides of apoproteins and receptors.

Summary

The laboratory has continued to focus attention on the mechanisms involved in the interaction between HDL and cells. Our aim has been to assemble information about the major components of the system which include (a) purification and cloning of the HDL receptor to determine its structure, (b) identification of the ligand recognised by the receptor, and (c) identification of factors which regulate activity of the HDL receptor. These studies have combined the skills of protein chemistry, molecular biology and immmunochemistry established in our section at the Baker Medical Research Institute. The goal is to understand the biochemistry involved in the binding between HDL and cell surfaces so that ultimately we can manipulate the function of these receptors and influence HDL metabolism.

A protocol has been established enabling successful purification of the HDL receptor complex which, according to ligand blot information appears to comprise two proteins of 120 and 100 kDa which we have designated HB1 and HB2 respectively. Both proteins are very hydrophobic, as revealed by their behaviour on reverse phase HPLC, and are present in low concentrations, thus resulting in very low yields. These factors have made purification difficult but sufficient quantities have been produced to obtain limited amino acid sequence of regions of HB2.

Shona Devlin

Some highlights of the work over the past year have been progress in further characterisation of HB1 and HB2. Both have been identified as glycoproteins with low pI values, and we have produced an antibody to HB2 which inhibits binding of HDL to plasma membranes of rat liver. Other monoclonal antibodies have been produced against regions of the ligand which also inhibit binding and significant progress has been made on identification of the receptor binding domain on one of the principal HDL apolipoproteins.

Structural studies on the HDL receptor complex

H. Hiroya, N. Fidge, T. Tetaz, J. Andreou

The identification of two binding proteins in rat liver plasma membranes (HB1 and HB2) which bind HDL3 is not inconsistent with data obtained in whole cell or membrane binding studies, which suggest that more than one binding site may be involved. Possibly, some initial recognition of the ligand stimulates activation of a high affinity process involving at least two (and perhaps more) HDL binding proteins. Whether or not these exist as a complex or as separate entities which become interactive following activation remains to be determined. Another explanation is that HB1 and HB2 simply act as mere "anchor" proteins, docking HDL to enable transfer of components between the lipoprotein and cell.

Clues to understanding the role of HB1 and HB2 in HDL metabolism require more structural information, and this necessitates the isolation of larger quantities of the receptor proteins than we achieve by present methods. Recent experiments have identified HB1 and HB2 as glycoproteins and a significant improvement in purification has resulted from this discovery. Using wheat germ lectin affinity columns, which bind N-acetyl- glucosaminyl residues, most of the HDL binding proteins are recovered in active form in the bound fraction. Many other glycoprotein contaminants remain in the preparation, so final purification still requires preparative SDS gels.

Due to problems with recovery of these proteins from gel slices, we have undertaken a new method of enzyme cleavage called in situ enzyme hydrolysis. After removing SDS from stained gels, the gel is crushed and dried and incubated with trypsin. Peptides are then recovered by extraction with 0.1% trifluoroacetic acid and separated by reverse phase HPLC and purified peptides can then be sequenced if available in sufficient quantities.

Further characterisation of the HDL binding proteins has shown that both HB1 and HB2 are acidic proteins with a pI of approximately 4.2 and may comprise at least two

isoforms in each case. Two dimensional gel electrophoresis reveals that at least one binding protein (HB2) retains activity following this procedure which may later be exploited for sequencing analyses following transfer to PVDF membranes.

Immunochemistry of the HDL receptor

J. Kanellos, N. Fidge, C. Allan

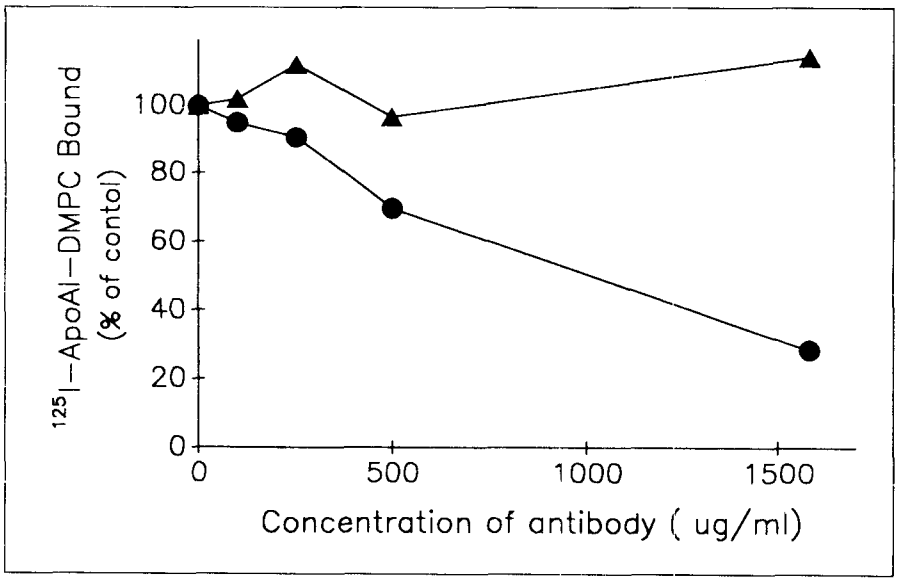
We have recently obtained confirmatory evidence, from immunochemical studies that purified HB2 functions biologically as an HDL binding protein. A peptide was synthesised, corresponding to a small region of HB2 recently sequenced in the Protein Chemistry laboratory and conjugated to ovalbumin. Following injection into rabbits, antisera was produced which, after absorption of ovalbumin and other contaminating antibodies, yielded a highly monospecific anti HB2 antibody (HB2). Several experiments have been performed to probe HB2 activity. First, when added in increasing concentrations to incubations containing ^{125}I-labelled HDL and plasma membranes, HB2 inhibited binding of HDL to its receptor. Secondly, when sections of rat liver were incubated with HB2 followed by fluorescein labelled second antibody, the resultant images showed that HB2 bound only to plasma membrane regions of intact parenchymal cells. In a third series of experiments involving ligand blots of HB2, the antibody was shown to inhibit HDL binding which was restored when the synthetic peptide was added to the incubation mixture, suggesting that the peptide competed with HB2 for the antibody binding site.

As a result of these trials, the antibody will be used for screening rat liver cDNA libraries as described under projects of the Molecular Biology laboratory.

Determination of the structural domain of apo AI recognised by high density lipoprotein receptors

J. Morrison, N.H. Fidge

There is good evidence that HDL interacts with high affinity sites present on hepatocytes. The precise nature of the ligand recognised by putative HDL receptors remains controversial although there is a consensus that apo AI is involved. This suggestion would be strengthened if a biologically active site demonstrating a high affinity for the receptor could be isolated. Cyanogen bromide fragments (CF) of apo AI (CF1- CF4) were complexed with phospholipid and their ability to associate with the receptor compared in various binding studies. Careful analysis of the concentration-dependent association of ^{125}I-labelled dimyristoyl phosphatidylcholine (DMPC) recombinants to rat liver plasma membranes revealed high and low affinity binding components. As all DMCP-recombinants displayed the low affinity binding component, it was postulated that this interaction was independent of the protein present in the particle and may well represent a lipid-lipid or lipid-protein association with the membranes. Only ^{125}I-labelled CF4-DMPC displayed a high affinity binding component with similar Kd and Bmax (8×10^{-9} M, 1.6×10^{-12} Mol/mg plasma membrane protein) to that of ^{125}I-labelled AI-DMPC (7×10^{-9} M, 1.4×10^{-12} Mol/mg plasma membrane protein). Further, ligand blotting studies showed that only ^{125}I-labelled CF4-DMPC associated specifically with HB1 and HB2, two HDL binding proteins recently identified in rat liver plasma membranes. We conclude that a region within the carboxy-terminus of apo AI is responsible for the interaction with putative HDL-receptors present in rat liver plasma membranes.



Inhibition of HDL receptor on liver plasma membranes by antibodies against a synthetic peptide engineered from a region of the receptor.

Investigation of the receptor binding specificity of HDL apoproteins: studies with LpAII particles

P. Vadiveloo, N. Fidge

Most studies investigating the identity of potential apoprotein ligands have employed artificial recombinants of single apoproteins and phospholipid but these complexes do not resemble the native lipoprotein recognised by the cell, since their size, shape and lipid composition are usually quite different to that of HDL3. These differences may be important in terms of function, including ligand-receptor interaction. Previous studies in our laboratory, utilizing native HDL3 and specific antibodies, have suggested that both apoproteins AI

and AII are involved in the interaction between HDL3 and cells.

With an aim to further define the role of apoproteins of HDL3 in binding, we reasoned that the native lipoprotein, changed only in apoprotein content, may provide a more suitable physiological model for probing ligand-receptor function. Thus, we prepared HDL3-like particles which were enriched in apo AII and depleted in apo AI (LpAII). The advantage of this approach is that the only difference between the particles is the apoprotein content, with no alterations in size, shape or lipid composition.

LpAII particles were shown to bind to cells with similar affinity and capacity as HDL3. Further experiments indicated that HDL3 and LpAII competed with each other for binding and displayed similar affinities for a common binding site(s). The results suggest that apo AII, as well as apo AI plays an important role in the process of HDL recognition by putative HDL receptors.

Regulation of hepatic high density lipoprotein binding proteins following administration of simvastatin and cholestyramine to rats

D. Mathai, N. Fidge, M. Tozuka, A. Mitchell

Whether or not the HDL binding proteins (HB1 and HB2) play a significant physiological role in HDL metabolism remains to be determined; if they do provide a mechanism for regulating HDL transport into or out of cells it could be expected that their binding activity may respond to a variety of biochemical processes affected by HDL transport and metabolism. Such postulated changes in activity may result from altered rates of their synthesis or from modifications to their binding activity at the membrane surface. In order to determine whether the binding activities of HB1 and HB2 are regulated, we have treated rats with drugs or diets known to affect lipid metabolism and lipoprotein concentrations, and compared the activities of liver HDL binding proteins in these treated animals with those of control rats. To measure the activities, we have extended the process of ligand blotting used initially for detecting the presence of HB1 and HB2 during their isolation and purification, to a system for quantitation which depends on the radioassay of ^{125}I-labelled HDL bound to HB1 and HB2 following ligand blotting of purified plasma membrane proteins.

Of the various treatments used, only simvastatin (S) or simvastatin plus cholestyramine (ST) produced significant changes, with reductions of up to 40 and 60% respectively for HB1 and HB2. The effect on the binding proteins was not associated with changes to serum or liver cholesterol concentrations which did not change significantly following S or ST administration. Other treatments which markedly altered plasma or liver cholesterol levels did not influence HB1 or HB2 expression, supporting this suggestion. We thus provide evidence of a biochemical role for HB1 and HB2 in HDL metabolism and suggest that their regulation is influenced by some unknown cellular mediator rather than through changes in cholesterol concentrations. The demonstration of HB1 and HB2 regulation provides another important criterion for their acceptance as a functional HDL receptor. An understanding of its exact role awaits further investigation.

The effect of lovastatin on the interaction between high density lipoprotein and cultured rat adrenocortical cells

D. Sviridov, N. Fidge

This project was initiated as a result of the collaboration between medical scientists in the USSR and

Mirella Rizzo and Barbara Meyer use the FPLC in their studies of lipoproteins

Australia. Recently, several compactin-related drugs have been developed which inhibit cholesterol synthesis by suppressing the activity of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG CoA) reductase and have found useful application in treating hypercholesterolaemia. However, the effect of these drugs on cellular metabolism may be more complex than originally envisaged. For example, in addition to its suppression of cholesterol synthesis and upregulation of low density lipoprotein (LDL) receptors, administration of these drugs is accompanied by increased levels of circulating HDL and induction of HMG CoA reductase and HMG CoA synthase synthesis accompanies the initial inhibition of its activity by lovastatin.

We found that preincubation of rat adrenocortical cells with lovastatin inhibited high density lipoprotein (HDL3) interaction with rat adrenocortical cells in a dose and time dependent fashion. Lovastatin also caused a decrease in the number of binding sites, a moderate increase in the affinity of HDL binding to cells and produced a dose and time dependent inhibition of cholesterol synthesis in these cells. Dose dependence, but not time-dependence, of the inhibition of cholesterol synthesis correlated with that of HDL binding ($r = 0.91$, $p < 0.004$). Incubation of cells with lovastatin for up to 24 h did not change their free or total cholesterol content. The inhibitory effect was apparently not due to a modification of HDL by lovastatin. These results suggested that lovastatin causes a down-regulation of HDL receptors on cultured rat adrenocortical cells.

Novel separation of apoproteins by HPLC techniques

T. Tetaz, N. Fidge

Purification of the various plasma apolipoproteins has enabled investigation of their structure and function and consequently the assignment of physiological roles to many of them. Of special interest to this laboratory has been the separation of the medium molecular weight apolipoproteins, apo AIV, apo AI and apo E. These proteins have traditionally been purified by a series of steps including gel filtration, anion exchange or chromatofocussing, usually in the presence of denaturants such as urea or guanidinium hydrochloride. However, none of these techniques produce satisfactory purification of apoproteins and most lipoprotein laboratories use preparative gel electrophoresis as a final step to obtain homogeneous preparations of apoproteins, particularly when these proteins are needed for antibody production or functional investigation.

We have developed a method for the rapid separation of the medium molecular weight apolipoproteins AIV, AI and E by high performance liquid chromatography. Separations were achieved using a commercially available column of very low hydrophobicity (TSK Phenyl-5PW) in the reversed-phase mode rather than the conventional mode of hydrophobic interaction. Delipidated apolipoproteins were dissolved in 20 mM orthophosphoric acid (pH 2.3), applied to the column which was pre-equilibrated with the same buffer, and eluted with an increasing gradient of acetonitrile. Purified apolipoproteins were identified by a combination of SDS-polyacrylamide gel electrophoresis, amino acid analysis and N-terminal sequence analysis. In one step the method can be used to separate the major human chylomicron apolipoproteins AIV, AI and E, following preliminary removal of the C apolipoproteins by size-exclusion chromatography.

Synthetic peptides of apoproteins and receptors

J. Andreou, T. Tetaz, N. Fidge

The availability of amino acid sequences of important lipid transport proteins such as

Moira Clay

lipoproteins, plasma transfer proteins including cholesteryl ester transfer protein and receptors, provides an opportunity to probe specific regions for biological activity or antigenicity. Computer modelling has enabled prediction (from the sequence and from plots of hydrophilicity and hydrophobicity) of potential antigenic regions which may be used for antibody production while suspected folding regions, such as helices, can be used to probe for lipid protein interactions.

We have recently synthesised several peptides which have provided useful antibodies as well as probes for structural investigations. Having recognised that the COOH terminus of apo AI contains the receptor binding site, we have made two peptides and produced polyclonal antibodies to each. Similarly, peptides from regions of human CETP have been synthesised and antibodies produced to enable identification of CETP if expressed in the transgenic mice containing human CETP genetic material.

More recently, peptides from selected regions of the HDL receptor HB2, have been made and antibodies produced. These peptides are also coupled to affinity columns which enables purification of antibodies in large quantities.

Molecular Biology Laboratory

Head: Dr. A. Mitchell

Projects

Cloning of two HDL binding proteins.

The regulation of hepatic lipase activity in the rabbit.

The effect of cholesterol-lowering drugs on the expression of apolipoproteins in the rat.

Studies in the rat and rabbit on cholesteryl ester transfer protein and its possible role in the development of atherosclerosis.

Summary

The Molecular Biology laboratory has now been functioning for two years. Originally set up at the Baker Institute to assist with the cloning of proteins being studied in the laboratories of Lipoprotein Metabolism and Protein Structure and Function, this laboratory is now involved in a number of different projects dealing with the general issue of the regulation of plasma cholesterol. Much of our effort is concentrated on the role of high density lipoproteins (HDL) in cholesterol metabolism.

HDL is a plasma component which participates in the removal of excess cholesterol from tissues and its transport to the liver for either excretion or recycling, in a process known as "reverse cholesterol transport". Either directly related to this function or as a consequence of some other role, HDL seems to have a protective effect against the premature onset of atherosclerosis.

Cholesterol associated with HDL particles comes into contact with many other plasma proteins, including enzymes, which influence its fate. Important among these are hepatic lipase (HL) and cholesteryl ester transfer protein (CETP). When the circulating HDL particles return to the liver, their removal from the blood is a critical step in influencing plasma cholesterol metabolism.

Regulation of the activity of HL and CETP and the basis for the species differences in the levels of these two proteins are being investigated in our laboratory. Dr. Rod Warren is studying HL expression in the rat and rabbit and factors which influence expression. The rabbit is of interest because it has very low levels of HL compared to the rat, and is highly susceptible to atherosclerosis. We have found that the gene is present in the rabbit and that hepatic mRNA levels for HL are comparable to those in rat.

The presence of CETP in the rat is a subject of debate. If present, it is at a very low level of activity which might occur if a small amount of protein were formed, or if a specific inhibitor were present in rat plasma. Since rats have little tendency to develop atherosclerosis, it is possible that a lack of CETP contributes to this resistant state. Dr. David Ebert, a scientist from the U.S.A. who is currently working in our laboratory, has obtained evidence that the rat does possess a gene with homology to the human CETP gene, although expression of the gene has not been demonstrated either at the mRNA or protein level. Sequencing of putative coding regions of this rat gene is in progress. The other major project in Molecular Biology is the search for clones encoding HDL binding proteins, HB1 and HB2. We have been working on various aspects of this project, including purifying the protein, raising antibodies, screening cDNA libraries and analysing putative clones.

Cloning of two HDL-binding proteins

A. Mitchell, N. Fidge, J. Kanellos, T. Tetaz

Two proteins which specifically bind high density lipoproteins (HDL) have been identified in preparations of liver plasma membranes. The larger of these proteins (HB1) has a molecular weight of 120 kDa while the smaller (HB2) is approximately 100 kDa. Both have been purified to homogeneity and HB2 has been obtained in sufficient quantity to allow partial internal sequencing of the molecule.

Work is continuing in the Protein Structure and Function Laboratory to obtain primary sequence information for HB1. Meanwhile, we have made oligonucleotide probes to three regions of HB2. For each peptide, two oligonucleotides were synthesized. One of these was a unique sequence or "guessmer", where the nucleotide at the third position of the codon was selected following consultation of codon usage tables for mammalian genes. The second was a mixed probe of limited degeneracy in which deoxyinosine was included at positions with three or four nucleotides at the third codon position. The probes, ranging in size from 32 to 38 nucleotides in length, will be used to screen a rat liver cDNA library.

A second approach to obtaining clones for HB1 and HB2 is to screen expression libraries with antibodies raised to either the purified protein, or a synthetic peptide corresponding to a region for which amino acid sequence is available. The latter is available for HB2 and is currently being characterized.

Finally, oligonucleotides have been synthesized for use as primers in PCR amplification of cDNA sequences between two regions of peptide sequence.

The availability of clones for the putative HDL receptor molecules will facilitate many studies at the molecular level of these important proteins.

The regulation of hepatic lipase activity in the rabbit

R. Warren, A. Mitchell

The rabbit has low levels of the enzyme hepatic lipase (HL) present in plasma after the injection of heparin, compared with either humans or rats. We were interested

Peter Griffith shows Dr. Alana Mitchell the results of DNA sequencing of CETP

in gaining information on the reasons for this species difference, particularly since the rabbit is very susceptible and the rat resistant to developing atherosclerosis on cholesterol feeding.

To establish whether the rabbit possessed a gene homologous to rat HL, DNA was prepared from rabbit blood digested with restriction endonuclease EcoRI and subjected to Southern blotting following fractionation on agarose gels. The membrane-bound DNA was probed with a full length rat HL cDNA clone (from Dr. M. Schotz of the U.S.A.) to reveal fragments with homology to the rat gene under conditions of moderate stringency.

Next, expression of this gene was monitored by probing rabbit liver polyA RNA, after size fractionation on agarose gels and transfer to nitrocellulose, with the rat cDNA clone. Using this system it appeared that rabbit HL was expressed at only about 5% of the level of rat HL. However, to establish the possible role played by species differences, a human HL clone was isolated from a liver cDNA library and the hybridizations repeated to reveal a greater homology between rabbit and human than rabbit and rat HL sequences.

We have used this knowledge to identify clones coding for HL from a rabbit liver cDNA library and are currently sequencing this gene.

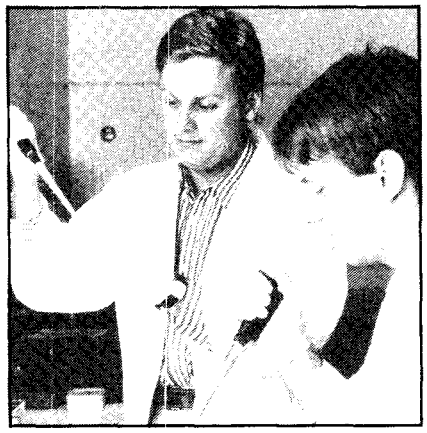
Another aspect of the regulation of HL in rabbits which we have examined is the effect of cholesterol feeding on HL activity and hepatic mRNA levels. Within two days of placing rabbits on a cholesterol-enriched diet, the enzyme activity of HL increases by approximately 3-fold, after which it drops slowly to level out above the pre-cholesterol control value. This observed change can be only partially accounted for by elevations in mRNA levels, suggesting that other post-transcriptional events are also involved.

The effect of cholesterol lowering drugs on the expression of apolipoproteins in the rat

A. Mitchell, N. Fidge, P. Griffiths

Several drugs are now widely used to lower cholesterol levels in patients at risk of developing premature coronary artery disease. One type of drug is represented by cholestyramine, a bile acid sequestrant which lowers serum LDL-cholesterol levels by stimulating bile acid production and upregulating LDL receptors. A second type of drug, for example simvastatin, lowers cholesterol levels by inhibiting HMG CoA reductase, a key enzyme in the biosynthesis of sterols. Apart from their major function in lowering cholesterol, these drugs cause additional changes to cellular biochemistry which have not been well-defined.

We have investigated changes in plasma concentrations and hepatic mRNA levels of the apolipoproteins AI, AIV, E and B following the inclusion of simvastatin and cholestyramine in rat diets. Simvastatin caused significant changes to mRNA levels and plasma concentrations for both apo AIV (with appreciable decreases to both components measured) and apo AI (for which mRNA levels rose somewhat, while plasma levels decreased). Simvastatin also caused a marked lowering of apo B mRNA levels which was not reflected in a change to plasma concentrations of apo B. Cholestyramine had little effect but in combination with simvastatin it potentiated the effect on apo AI mRNA and also caused apo B mRNA and plasma content to decrease significantly. Thus, expression of the various apolipoprotein genes of rat is influenced differentially by the cholesterol lowering drugs cholestyramine and simvastatin.



Dr. Rod Warren (left) and visiting scientist from the USA, Dr. David Ebert.

Studies in the rat and rabbit on cholesteryl ester transfer protein and its possible role in the development of atherosclerosis

D. Ebert, A. Mitchell

Rats, unlike humans, are not susceptible to the development of atherosclerosis, and differ in certain aspects of their lipoprotein metabolism. For example, rat plasma is deficient in a cholesteryl ester transfer protein (or CETP) which is important in transferring cholesterol from HDL to the atherogenic lipoprotein particles. This deficiency may arise by various mechanisms. For example, the protein may exist in plasma, but be prevented from acting by the presence of a specific inhibitor, or the gene for CETP may be absent, or present but not be expressed.

We have begun to examine the molecular basis for the apparent lack of CETP by probing rat hepatic mRNA with human CETP cDNA (obtained from Genentech in California) and by seeking evidence for CETP sequences in rat genomic DNA fragments.

Using a variety of conditions

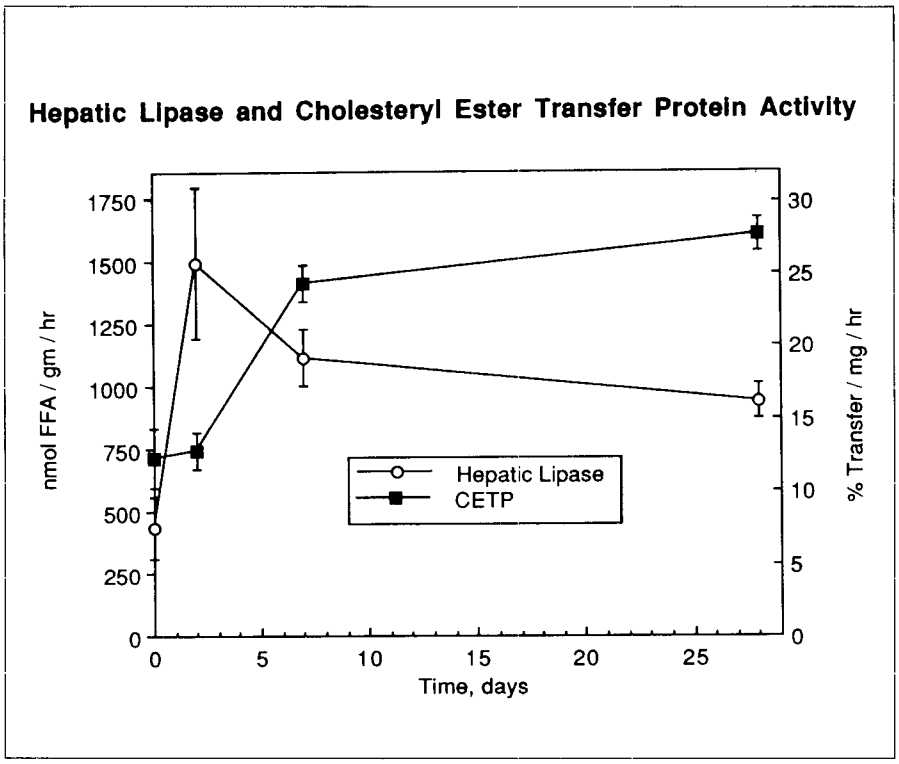


Fig.1 The changes in hepatic lipase and cholesteryl ester transfer protein in rabbits fed a cholesterol-supplemented diet. Cholesterol feeding began at zero time and was continued for 28 days.

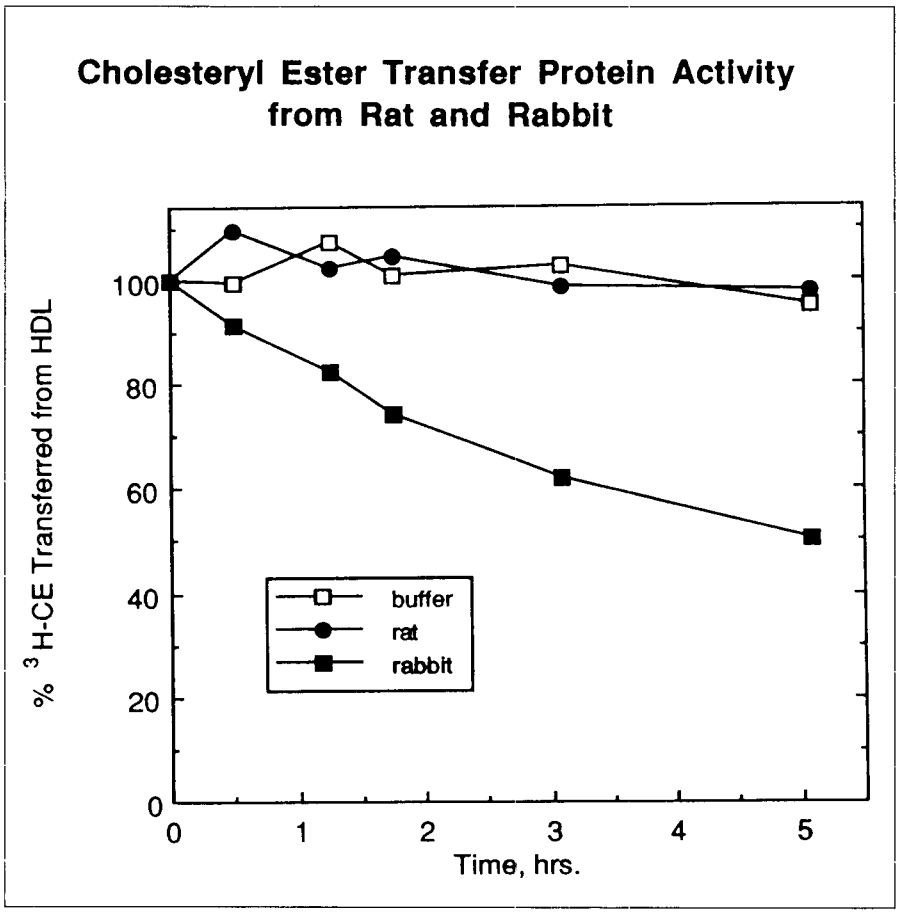


Fig.2 A comparison of the activity of cholesteryl ester transfer protein from rat and rabbit as measured by transfer of radioactive cholesteryl ester (CE) from HDL to LDL.

for hybridization and washing of filters, we have found no evidence for mRNA coding for CETP in the rat. However, rat DNA does appear to possess some homology to the human CETP sequence when washed under conditions of 0.3 M NaCl and 62°C. Interestingly, it appears that the 3′ portion of the cDNA for human cDNA has no counterpart in the rat genome whereas both the 5′ and middle sections were found to hybridize to rat DNA restriction fragments. Since the activity of CETP resides in the carboxyl-terminal 26 amino acids, this observation may explain the apparent lack of transfer activity in rat plasma.

To pursue this point further, we have screened a rat genomic library and located clones for CETP. We are now in the process of subcloning putative structural regions into M13 for sequencing. Initially this will allow comparison of homology between human, rabbit and rat CETP genes. Depending on the outcome of this preliminary investigation, we shall examine regulatory regions of the gene, or use the clone to screen a cDNA library and to probe mRNA.

Despite its possible role in the development of atherosclerosis, little information is available on regulation of CETP activity. We have begun a study to examine whether plasma cholesterol transfer activity changes in response to cholesterol feeding in rabbits. In the same animals we are also measuring hepatic lipase activity, since these two proteins may interact in the transport of plasma cholesterol. In vitro transfer activity from HDL to LDL increases by approximately 2-fold after 7 days of cholesterol-feeding, with a distinct lag period of 2 days. This response is quite different from that of HL which reaches a peak at 2 days, suggesting that the two proteins are subject to different regulatory mechanisms. Measurement of hepatic mRNA levels is being performed to understand more of the regulatory processes.

Pharmacology Laboratory

Head: Dr. J.A. Angus

Projects

Coronary collateral vessel reactivity.

Human skin small artery reactivity in congestive heart failure.

Human small coronary artery reactivity.

Human vein reactivity in primary hypertension.

Endothelium-derived vasoactive factors, EDRF and endothelin.

Characterization of N-type voltage operated calcium channels in the cardiovascular system.

Potassium channels in vascular smooth muscle.

Serotonin-receptors in human coronary arteries.

Mathematical modelling of vascular resistance in hypertension.

Summary

Two broad areas of research are conducted in the Pharmacology Laboratory. The first is the identification of mechanisms of action of novel drugs, endogenous factors or toxins, particularly in relation to blood vessels and the heart. Here we seek information that may unravel how normal physiological processes occur or help identify new targets for potential drug development. The second area is the study of the reactivity of blood vessels in various abnormal conditions with particular reference to clinical problems such as hypertension, congestive heart failure, atherosclerosis, coronary vasospasm or collateral vessel development after conduit artery occlusion.

This year Dr. Tom Cocks has continued to investigate the release of endothelium- derived relaxing factor (EDRF) and the powerful vasoconstrictor peptide endothelin from cultured endothelial cells. These cells that line all blood vessels release endothelin when grown in culture without serum. He and Dr. Robyn Woods found that oxyhaemoglobin doubled the production of endothelin as measured by bioassay or by radioimmunoassay. This could explain the delayed cerebral vasoconstriction observed after sub-arachnoid haemorrhage.

EDRF is now considered to be the simple molecule nitric oxide, formed from the enzymic conversion of the amino acid L-arginine. However, this reaction can occur in many different cell types including macrophages, leucocytes, liver cells and in the brain. We are working to identify (i) any special features of endothelial cell NO production, (ii) whether NO can mimic all the actions of EDRF and (iii) the physiological importance of basal release of EDRF/NO in vascular reactivity.

Dr. Grant McPherson has continued his characterization of the K+-channel on vascular smooth muscle cells opened by the vasodilator drug cromakalim. This novel site of action does not seem to be unique to vascular smooth muscle since 3 antagonists of cromakalim in blood vessels were equally effective against cromakalim-induced relaxation in trachea and gut (ileum) smooth muscle. The search is on to determine whether there is an endogenous K+ channel opener which regulates smooth muscle tone which could be involved in the development of hypertension.

Dr. Didier Pruneau, a visiting pharmacologist from Fournier Laboratories, Dijon, France, has spent 12 months in the Pharmacology Laboratory studying the cardiovascular actions of a novel toxin, ω-conotoxin GVIA, derived from a fish-eating snail. Electrophysiology studies showed that this toxin blocks a specific calcium channel in excitable tissue - the N-type channel. Dr. Pruneau has used this tool to characterise where N-channels are located and inhibit transmitter release from nerves. This important work suggests that N-type channels control the release of noradrenaline from sympathetic nerves and to a lesser extent acetylcholine from parasympathetic nerves, with little evidence of activity on the postsynaptic membrane in heart or blood vessels.

This is the second year of major support of the Pharmacology Laboratory by Glaxo Australia. This helpful support is in 3 areas of research including (i) the understanding of receptors involved in human coronary artery reactivity, (ii) characterization of factors released by the endothelium and (iii) blood vessel reactivity during collateral development and ischaemia. This latter area is part of our second major theme of our work, to determine vessel reactivity during abnormal conditions. In collaboration with the Alfred-Baker Medical Unit, we have found a marked loss of contractile response of small arteries from buttock skin biopsies of patients with congestive heart failure. In addition, drug therapy that blocks local production of angiotensin II from the precursor peptide can be determined in the skin vessels of these patients. Peripheral vascular disease related to loss of blood vessels - rarefaction or loss of large arteries leading to collateral vessel development - is poorly understood. Mrs. Jane Ward (PhD student) is currently investigating the reactivity of collateral arteries in dog hearts and rabbit hindquarters after occlusion of the major conducting arteries. An understanding of the regulation of these collateral channels is important because of their crucial role following

Dr Michael Lew has returned from postdoctoral studies in the USA

coronary occlusion and the lack of effective drug therapy for peripheral ischaemia.

Little is known about the response of human small coronary arteries to vasodilator or constrictor stimuli. With the co-operation of the cardiac surgeons at the Alfred Hospital and Dr. Arch Broughton, a cardiologist and Honorary Research Fellow, we have completed an initial study of the interaction between the endothelium and the artery wall in microvessels dissected from the right atrium. We found a most unusual oscillatory depolarisation and contraction of these arteries to acetylcholine and other constrictor agents. The pathological implications of these findings are not yet clear. Aging, endothelial dysfunction and perhaps long term drug therapy are questions to be answered. The excellent collaboration with the Alfred Hospital and the close proximity of the cardiac theatres to the laboratory make these important studies a reality.

Endothelium-derived vasoactive factors

T.M. Cocks, S. King, J.A. Angus

Endothelial cells lining all blood vessels are now thought to produce a powerful vasodilator substance EDRF (nitric oxide, NO), and a long acting vasoconstrictor peptide endothelin.

EDRF/nitric oxide

Nitric oxide is produced by the enzymatic conversion of L-arginine to NO and citrulline within endothelial cells. The physiological role of NO is however still unknown. Some have used the competitve substrate analog N-monomethyl-L-arginine (L-NMMA) (which is not converted to NO and as such inhibits the enzyme) to claim a role for EDRF in vitro and in vivo in keeping blood vessels tonically relaxed. Thus, application of L-NMMA has been shown to contract only endothelium-intact isolated vessels. It also increases blood pressure in vivo. Both these responses have been taken as evidence for removal of the tonic vasodilator effects of EDRF.

We have examined the effects of L-NMMA on the isolated dog coronary artery, one of the most sensitive preparations to EDRF. Firstly, we observed only poor inhibition by L-NMMA of relaxation responses to a range of EDRF agonists. Even when the experiments were repeated in the absence of extracellular Ca^{++} in order to reduce the amount of EDRF released per unit stimulus, L-NMMA remained a weak inhibitor of all the EDRF agonists tested.

We also examined the effect of L-NMMA on relaxation responses in the coronary artery to EDRF released from small columns of cultured endothelial cells. In this bioassay, the source of EDRF (cultured cells) is separated from the detector (endothelium-denuded ring of artery), thus allowing perturbations to be performed between the cells and the artery. To our surprise, we obtained complete inhibition of the relaxations to EDRF released from the cultured cells, but little or no block of the same reponses to EDRF released from cells in situ. We are currently investigating whether this discrepancy indicates more than one EDRF.

We can also demonstrate endothelium-dependent contractions to L-NMMA in both endothelium-intact rings of coronary artery and in the cell culture-bioassay system. However, we have other evidence which suggests that these reponses are not due to removal of the effects of basal EDRF, but possibly due to the release of another endothelium-dependent vasoactive factor. If these findings are confirmed, they question the use of L-NMMA to determine the physiological role(s) of EDRF.

Endothelin

(T.M. Cocks, S.J. King, R.L. Woods, J.A. Angus)

Endothelin (ET) is a novel 21 amino acid vasoconstrictor peptide released from endothelial cells. It is now known that there is a family of endothelins, three mammalian peptides (ET-1,2,3) as well as a number of ET-like peptides (sarafatoxins) found in the venom of the Israeli snake.

Our contribution to the understanding of the physiology of these intriguing peptides has been to demonstrate that (i) large veins are more sensitive to ET than large arteries in both man and dog, (ii) ET is vascular bed selective in its effects in the conscious ganglion-blocked rabbit, and (iii) oxyhaemoglobin stimulates endothelial cells to increase either their synthesis and/or release of ET. The latter finding may have important implications clinically in the understanding of cerebral vasospasm following haemorrhagic stroke.

K+ channels and the regulation of vascular smooth muscle tone

G.A. McPherson, S. Keily, J.A. Angus

The level of tone displayed by resistance blood vessels is dependent ultimately on the level of intracellular calcium. This in turn depends to some extent on the resting membrane potential (Em). We have become interested in the role of a K+ channel which regulates smooth muscle tone by altering Em. Drugs such as cromakalim and pinacidil open a K+ channel in the plasma membrane which allows K+ to flux out of the cell. This results in membrane hyperpolarisation and, in blood vessels with active tone, in relaxation. Due to this novel mechanism of action, drugs which activate this channel are the subject of considerable research due to their potential use in hypertension and other conditions resulting from smooth muscle hyperactivity such as asthma.

We discovered in 1988 some selective antagonists of the cromakalim mediated vasorelaxant responses. These antagonists, phentolamine, alinidine and glibenclamide, have been used as tools in a number of studies designed to increase our understanding of the mechanisms involved in the regulation of vascular smooth muscle tone. We asked whether differences in the nature of the K+ channel opened by cromakalim in a number of smooth muscle types could be unveiled using these three antagonists. In vitro studies were performed in vascular (thoracic aorta) and non-vascular (trachea and ileum) smooth muscle

from the guinea pig. Our results showed that the K+ channel opened by cromakalim was present in all tissues studied since cromakalim was a powerful vasorelaxant in all cases. The lack of potency difference between tissues would indicate that the K+ channel is homogeneous. This idea was supported by the finding that the antagonists studied displayed equal potency in the three preparations studied.

Recent studies have concentrated on whether there is an endogenous cromakalim which affects vascular smooth muscle tone in vitro and in vivo. Studies with phentolamine, alinidine and glibenclamide in rat small mesenteric arteries in vitro have shown that these compounds depolarise the vascular smooth muscle cell directly. One explanation is that there is a basal release of an endogenous cromakalim-like compound which is inhibited by the antagonists. The notion of an endogenous K+ channel opener which regulates vascular tone and whether defects in this system could be involved in the etiology of hypertension are yet to be explored. Using the tools developed at the Baker Institute, however, we are well placed to test these intriguing hypotheses.

Characterization of N-type voltage operated calcium channels in the cardiovascular system

D. Pruneau, J.A. Angus, P. Arnold, P. Coles

Electrophysiological studies have characterized three separate Ca^{++} ion channels on excitable cell membranes as N-, L- and T- based on the channel opening time. The roles of these distinct channels may now be evaluated since the discovery of organic Ca^{++} entry blocking drugs for the L-type channel and a newly discovered 27-amino acid peptide, ω-conotoxin GVIA (ω-CTX) from the fish-eating marine snail, Conus geographus.

We have used ω-CTX as a tool to investigate the physiological role of N-type VOCCs in the sympathetic neurotransmission to the vasculature and to heart in vitro and in vivo. In isolated rat small mesenteric arteries (150-350 μm in internal diameter) set up in the Mulvany myograph, we found that ω-CTX (0.1 to 3nM) inhibited in a concentration-dependent manner contractions resulting from electrical nerve stimulation. The excitatory junctional potentials, small bursts of depolarisation due to postsynaptic excitation by the released neurotransmitter, were also abolished by ω-CTX (3 nM) (Fig. A). Since the contractions induced by exogenously applied noradrenaline or potassium chloride were unaffected by ω-CTX, we concluded that ω-CTX acted on nerve terminals to block the release of noradrenaline and ATP.

In anaesthetized rats in which the spinal cord was destroyed physically, we demonstrated for the first time that ω-CTX (1.6 and 3.2 μg/kg i.v.) was a powerful inhibitor of pressor responses and tachycardia due to electrical stimulation of the sympathetic outflow to the vasculature and the heart.

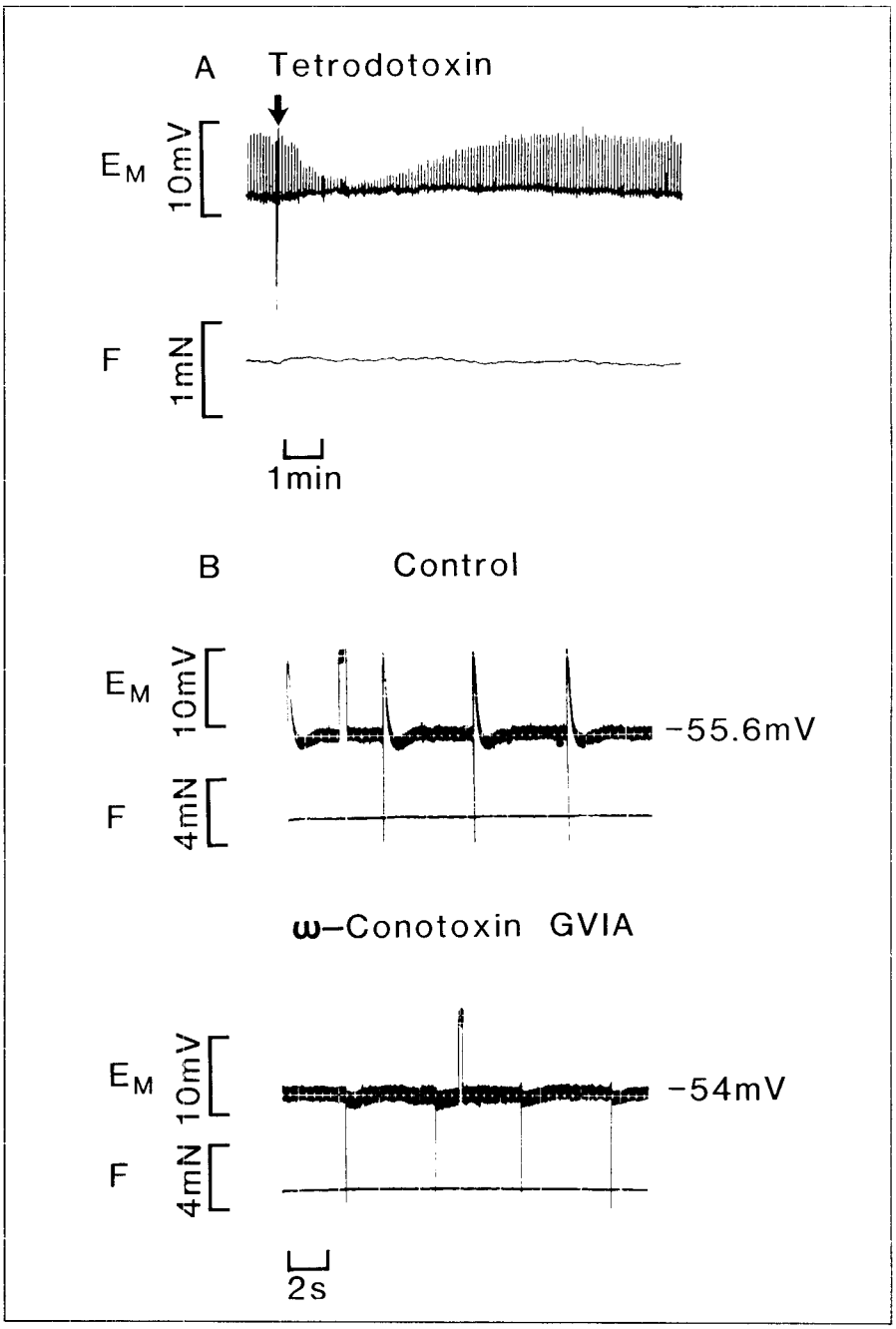


Fig. A Effect of tetrodotoxin (A) and ω-conotoxin GVIA (B) on the excitatory junction potential (ejp) in a mesenteric small artery of the rat during transmural nerve stimulation (0.2 Hz). Note that tetrodotoxin inhibits the ejp, in a reversible manner.

ω-CTX had a slow onset and long duration of action. In accordance with in vitro studies, responses to i.v. noradrenaline were unaffected by ω-CTX.

In chronically instrumented rabbits, ω-CTX 3 μg/kg slowly reduced blood pressure, and increased heart rate and hindquarter and renal conductances. Reflex tachycardia in response to sodium nitroprusside was markedly attenuated by ω-CTX 3 g/kg but the bradycardia to phenylephrine i.v. or "Bezold-Jarisch like" reflex to serotonin injection and the naso-pharyngeal reflex were little affected by ω-CTX. These experiments indicate that ω-CTX acts prejunctionally to inhibit sympathetic transmission in vitro and in vivo presumably by blocking N-type Ca^{++} channels, but has little effect on the vagus. These N-type channels may offer a new target for selectively reducing sympathetic activity and blood pressure.

Pharmacology of collateral vessels

J.A. Angus, J.J. Smolich, J. Ward, G.A. McPherson

Gradual occlusion of a major coronary artery from atheroma in man results in the transformation of small diameter native collateral vessels into larger diameter collateral vessels to prevent ischaemia. Little is known of the reactivity of these collateral arteries to vasoconstrictor stimuli. We have now examined the quantitative reactivity in vitro and morphology of segments of collateral arteries from greyhound hearts. Six months prior to these experiments, casein constrictors had been implanted on the origin of the left circumflex coronary artery to cause gradual occlusion of this major conduit vessel. Mature collateral vessels are easily identified on the epicardial surface of the canine heart (Fig. B). These vessels were removed, together with similar sized branches arising from the non-occluded left anterior descending coronary artery, and prepared for studies on the Mulvany myograph. Small arteries (150-1000 μm internal diameter) were mounted in a myograph to measure isometric force responses. After normalisation, the diameters of the normal arteries were 680.1 ± 50.9 μm internal diameter, and for the collateral arteries, 562.6 ± 68.9 μm (n = 8). Concentration response curves to both constrictor and dilator agents were performed to examine the pharmacological reactivity of these arteries. The vessels were then prepared for light microscopy and morphometric analysis using a calibrated digitising tablet. The maximum active pressure response (P, where P = Tension/radius) to K+ depolarization, endothelin-1 (Et) and the thromboxane A2 mimetic, U46619 were reduced in the collateral arteries. In contrast, an augmented response was observed for vasporessin (AVP).

In vessels precontracted with K (30-40 mM), the range and sensitivity (EC50) of the vasodilator reactivity to acetylcholine, sodium nitroprusside and cromakalim were unaltered in the collateral vessels compared with the normal arteries. Quantitation of the structural differences between normal and collateral arteries of the same size demonstrated a thickening of the adventitia, a relatively thin media and the development of a substantial neo-intima in the collaterals.

These findings indicate that mature collateral arteries cannot be considered structurally normal. The agonist non-specific loss of constrictor capacity in these vessels is probably related to the paucity of contractile

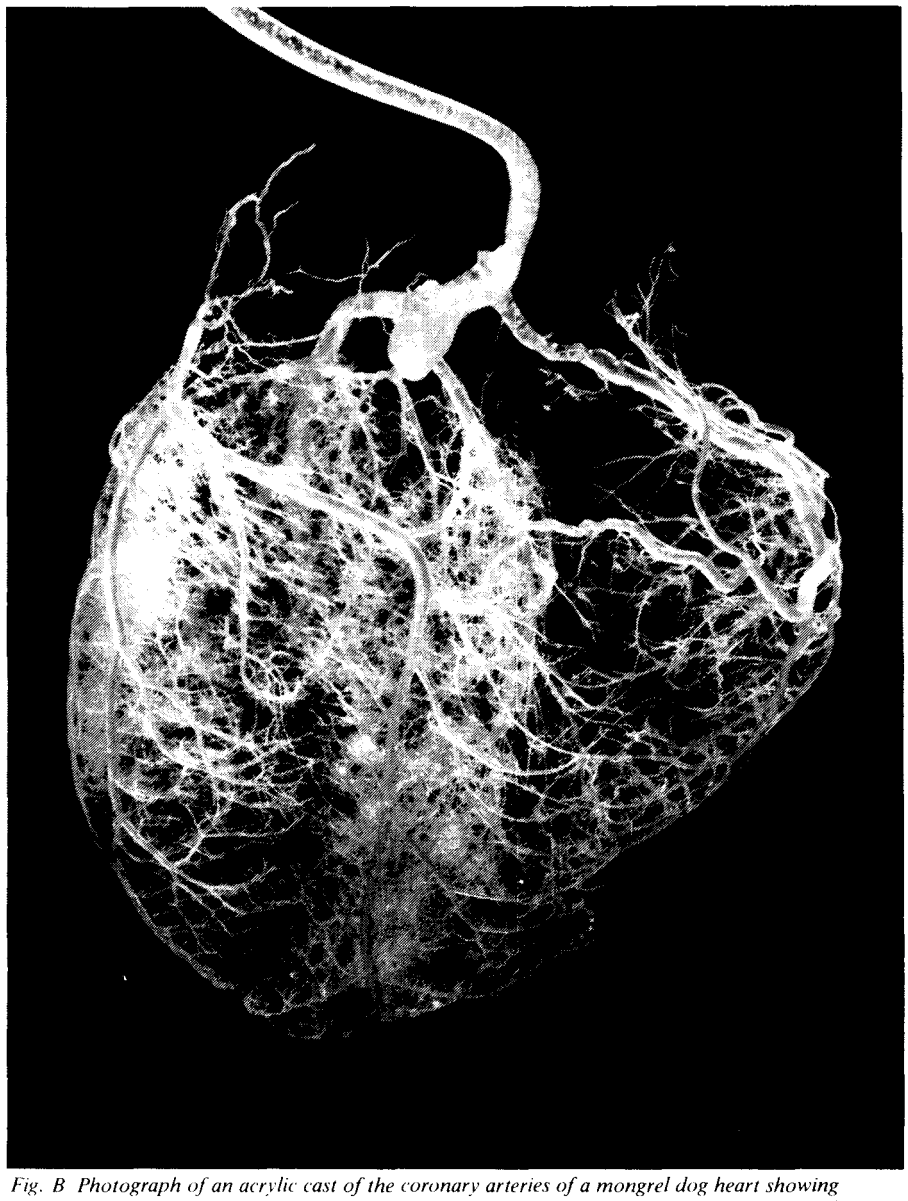


Fig. B Photograph of an acrylic cast of the coronary arteries of a mongrel dog heart showing the development of tortuous collateral arteries on the epicardium 6 months after occlusion of the circumflex coronary artery.

medial smooth muscle. In addition, the lumen encroachment by the neo-intimal thickening would play an important role in compromising blood flow. While the collateral arteries are vital in supplying the myocardium in the previously ischaemic zone, they are not endowed with enhanced vasoconstrictor activity, maintain their relaxant activity, but have a compromised lumen that is probably flow-limiting.

Current research is directed towards understanding the pharmacology of peripheral vascular disease and collateral vessel reactivity in the ischaemic hindlimb of the rabbit.

Pharmacology of human small coronary arteries

J.A. Angus, A. Broughton, G.A. McPherson, M. Ross-Smith

Acetylcholine generally causes relaxation of large and small arteries by acting on endothelial cells to release a powerful local factor, endothelium-derived relaxing factor (EDRF) now thought to be nitric oxide. Contraction of human large coronary arteries in response to acetylcholine has been reported but has generally been taken as evidence of endothelial dysfunction at sites of atheroma. We found that in human coronary resistance arteries (average internal diameter = 155 μm) freshly dissected from the tip of the right atrial appendage of patients undergoing open-heart surgery, and mounted in a Mulvany myograph, acetylcholine causes a most unusual depolarisation (30-40 mV) and oscillation (0.5 Hz) of the smooth muscle cell membrane potential and contraction in the absence of any atheroma. This response pattern is dependent on Ca^{++} ext but was unaltered by the removal of extracellular Na or by high concentrations of the dihydropyridine L-type Ca^{++} channel inhibitor felodipine (0.1 μM). The endothelium was mostly intact as judged by morphology. In addition, the EDRF releasing agent substance P repolarised and relaxed the artery in the presence of acetylcholine (Fig. C). By contrast, in resistance arteries dissected from human buttock skin biopsies, acetylcholine caused only hyperpolarisation and relaxation. These studies suggest that the human coronary resistance arteries from cardiac patients are unusually excited by muscarinic receptor stimulation and emphasise the capacity of EDRF to antagonise membrane depolarisation and contraction.

Human vein reactivity in primary hypertension

K. Sudhir, J.A. Angus, M. Esler, G. Jennings, G. Lambert, P.I. Korner

Enhanced vascular reactivity in hypertension may reflect a generalised abnormality of smooth muscle involving both arteries and veins. We studied the in vitro reactivity of human vein, biopsied from the forearm of 15 patients with untreated primary hypertension and 14 normotensive subjects. Veins of hypertensive patients were stiffer since the slope of their wall tension/circumference relationships was steeper than in normotensive subjects. There was no difference between groups in their sensitivity (location of the EC50) or in normalised maximum contractile force (Fmax/unit radius) in response to potassium, or to serotonin. However, veins from hypertensive subjects were less sensitive to noradrenaline and the selective α2-adrenoceptor agonist UK14304, and showed a fall in normalised Fmax with all α-adrenoceptor agonists. In contrast, in veins from hypertensive subjects, normalised Fmax was 3.7 times higher in response to angiotensin II than in veins from normotensive subjects. Despite the reduced responses to exogenous α-adrenoceptor agonists, contractile responses to transmural field stimulation were enhanced in hypertension. The effect of selective α2-adrenoceptor blockade suggested a decrease in pre-junctional auto-inhibition, while the effect of desipramine on field stimulation and tritiated noradrenaline efflux suggested decreased neuronal amine uptake in veins of hypertensive patients. There was no evidence of medial hypertrophy in the veins of hypertensive subjects. We conclude that veins in hypertension are stiffer, have reduced α-adrenoceptor responsiveness, and generate a greater maximal force in response to angiotensin II. Importantly, these veins show enhanced responses to nerve stimulation possibly due to decreased α2-adrenoceptor mediated auto-inhibitory feedback, and reduced neuronal amine uptake, when compared to veins from normotensive subjects.

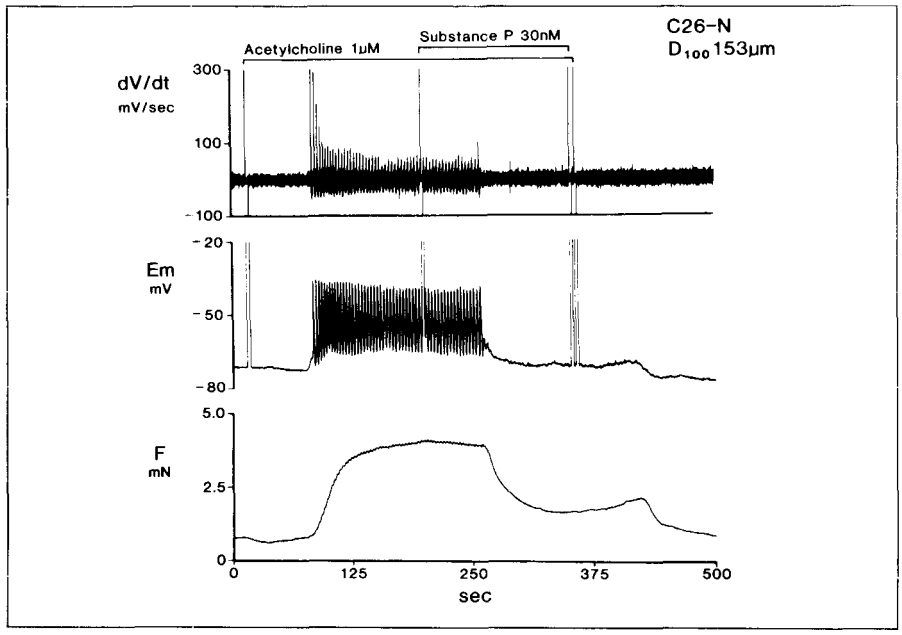


Fig. C Membrane potential (Em), its derivative dV/dt, and contractile force (F) responses to acetylcholine applied to a human isolated small coronary artery (153 μm i.d.) in a myograph. The unusual oscillatory response to acetylcholine was abolished by substance P infusion.

Renal Laboratory

Head: Dr. W.P. Anderson

Projects

Site of action of endothelin in the kidney.

Mechanism of action of endothelin in the kidney.

Regional vascular effects of ANP.

Regulation of glomerular function - micropuncture analysis.

ANP role of renal nerves.

Renal vascular actions of angiotensin II morphometric analysis.

Stimulating the release of a renal medullary depressor substance.

Summary

Work this year has concentrated on three putative endocrine factors of great potential importance to the kidney - atrial natriuretic hormone, endothelin and medullipin. We have used our well-developed conscious dog and rabbit micropuncture preparations to gain information which is not possible by other means.

Atrial natriuretic peptide was discovered in the early 1980s, but its physiological significance is yet to be fully understood. This is partly because there have been relatively few studies of its cardiovascular actions in whole, conscious animals. Our most interesting finding was that ANP causes an increase in peripheral vascular resistance (especially noticeable when autonomic reflexes are blocked), rather than a decrease as previously assumed. A decrease was assumed because arterial blood pressure usually falls after ANP infusion, but the hypotension is actually due to a very marked reduction in cardiac output. This year we have worked out exactly where ANP causes vasoconstriction, and this seems mainly to be the heart and gastrointestinal tract. We now plan to test whether the vasoconstriction is a primary action of ANP or is due to the release of another vasoconstrictor substance. We will also study the functional significance of these mesenteric and myocardial vasoconstrictions. Other studies on the physiology of ANP have included its interactions with renal nerves and its effects on cardiovascular reflexes.

Endothelin is a recently discovered polypeptide, made by endothelial cells. It may be of special importance in the kidney, because of the role of the endothelial cells in renal function. Micropuncture studies in our laboratory have shown that endothelin is a potent renal vasoconstrictor, constricting both pre-glomerular and post-glomerular vessels. This powerful vasoconstriction was confirmed in conscious dog experiments. The vasoconstriction seems to be progressive at high doses, and may eventually cause a complete cessation of renal blood flow. We have also shown that the effects of endothelin in the kidney appear to be ameliorated by volume expansion and accentuated by cyclo-oxygenase inhibition. These findings hint of a role for endothelin in the development of renal failure.

We have recently begun to study whether the kidney makes an "anti-hypertensive" factor, and what role this factor may play in the regulation of blood pressure. For the last ten years, there have been reports that the kidney may release from its medulla a lipid (medullipin) which, under certain circumstances, lowers arterial pressure. To date, experiments on this substance have been limited to rats, and mostly to narrow experimental circumstances (e.g. following release of renal artery stenoses). We have begun to study whether this lipid has a more general role in blood pressure regulation, studying two additional species (dogs and rabbits). Current experiments are determining the range of blood pressures over which the kidney releases a depressor substance, and its mode of action.

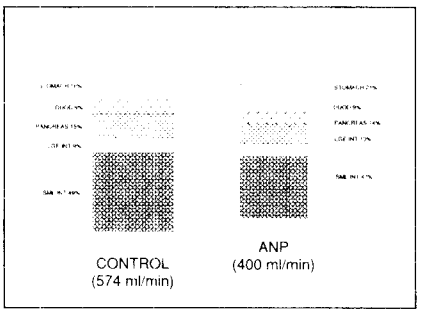


Fig. 1 ANP reduces blood flow to all gastrointestinal vascular beds.

Renal vascular effects of endothelin in the rabbit

K.M. Denton, W.P. Anderson

We have examined the effects of endothelin on the renal vessels to determine its site of action within the kidney, and also examined its effects on the filtering ability of the kidney. To measure the resistance changes within the kidney in response to endothelin infusion, it was necessary to utilize the micropuncture facilities of the Renal Laboratory. These facilities have enabled the direct measurement of pressures in the glomerulus and in renal veins, enabling calculation of pre- and post-glomerular resistance.

Our findings show that endothelin is a very potent vasoconstrictor substance in the kidney. It causes a progressive increase in renal resistance and adversely affects renal blood flow and glomerular filtration rate. The increased renal vascular resistance was due to an increase in both pre- and post-glomerular resistances. Endothelin is of importance since it may well be involved in the local control of blood vessel tone and could play a role in pathological conditions that are associated with a reduction in flow to the kidney, i.e. renal failure.

Regional vascular effects of ANP

R.L. Woods, J. Smolich

In a recent study we demonstrated an unexpected but striking cardiovascular response to atrial natriuretic peptide (ANP) in conscious dogs. With the autonomic

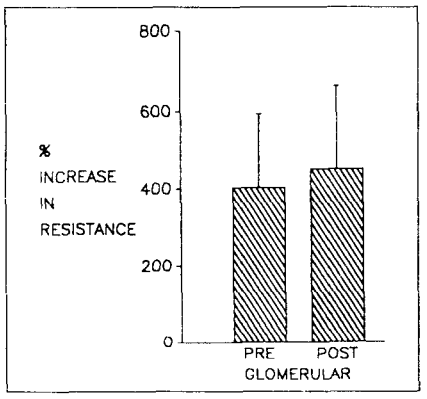


Fig. 2 Endothelin increase both pre- and post-glomerular vascular resistance.

reflex responses blocked, ANP caused marked vasoconstriction and negative inotropy, combining to reduce cardiac output and lower blood pressure. In order to determine in which organs the vasoconstriction was occurring and whether there were blood flow changes in the heart which may be responsible for the reduction in contractility, we injected radioactive microspheres into conscious dogs to measure total and regional blood flow changes after infusion of ANP to steady-state. We found that the greatest effects of ANP occurred when the autonomic nervous system was blocked by the ganglion blocking agent, pentolinium, thereby preventing the buffering activity of this system. ANP reduced blood flow to the gastrointestinal tract by 200 ml/min and by 180 ml/min in myocardial muscle. The effects in the gut were evident even when the autonomic reflexes were intact, and were consistent with our previous findings of falls in mesenteric flow as measured by indwelling Doppler flowmeters. The changes in cardiac blood flow may be directly related to the fall in cardiac output since, in the intact animals, there were neither significant falls in cardiac output nor in myocardial blood flow.

Intrarenal infusion of endothelin in conscious dogs

W.P. Anderson, R. Heguilen, R.L. Woods, T. Vanderstap

The physiological effects of endothelin may be of particular interest in the kidney because of the important role the endothelium plays in renal function. To investigate the effects of endothelin on the renal circulation and function, endothelin has been infused locally into the renal artery of trained conscious greyhounds (0-20 pM/kg/min). Preliminary results show that endothelin causes a dose-related renal vasoconstriction, which is progressive at higher doses. It also reduces GFR and filtration fraction, particularly at higher doses, indicating a substantial effect on pre-glomerular vessels. Preliminary results also indicate that the effects of endothelin on renal blood flow and GFR may be attenuated by fluid loading the animals, and accentuated when prostanoid production is inhibited (aspirin or ibuprofen). Thus, endothelin appears to have potent renal effects, which may be modulated by the volume status of the animal and by prostanoids.

A renal medullary depressor substance

W.P. Anderson, R.L. Woods, I. Christy, C.A. Courneya, C. Thomas, T. Vanderstap, J.R. Oliver

Over the last decade, there have been several reports that the interstitial cells of the renal medulla can release at least two substances which lower blood pressure. One of these substances is platelet activating factor, the other is an as yet unidentified neutral lipid, designated medullipin. Previously, these substances have been studied only in the rat. We have begun experiments in dogs and rabbits to test whether these depressor substances are important in other species, to determine the factors which cause their release from the kidney, and to determine their hemodynamic effects. Unlike in previous experiments, we have developed methods of perfusing the kidneys of anaesthetized dogs and rabbits in situ. Preliminary results indicate that the kidneys of both species do release a depressor substance in response to increased perfusion pressure.

The rate at which blood pressure falls is more rapid in the rabbit than in the dog.

Trish Vanderstap, and friend

Glomerular ultrafiltration in the rabbit

K.M. Denton, I. Christy, W.P. Anderson

Using the unique rabbits with superficial glomeruli in our colony, we have now established a stable preparation to allow direct study of the properties of the glomeruli. Previously most information on the factors controlling ultrafiltration in the glomerulus has come from rat studies. This is the first report of measurements in rabbit glomeruli. The rabbit has advantages over the rat in that blood samples can be taken without circulatory effects and more complex studies can be performed because the surgery involved (e.g. renal nerve studies, intrarenal infusions) are easier in these larger animals.

Blood pressure in rabbits is about 70 mmHg and in rats about 115 mmHg, and the major differences in filtration between these species comes from this fact. Rabbits have a filtration rate lower than rats, whereas the glomerular permeability filtration coefficients are very similar. One of the major consequences of this is that filtration is occurring along the entire length of the glomerular capillaries in the rabbit, whereas in the rat, filtration equilibrium occurs before the end of the capillaries. It is believed that this situation in the rabbit – filtration disequilibrium – also occurs in human kidneys. Thus, rabbits may be more appropriate for studying the factors controlling glomerular filtration.

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Staff Activities

Dr Murray Esler was awarded the prestigous Wellcome Australia Medal and Award for 1989. This annual award is the highest accolade given to an Australian scientist who is considered to have made the most outstanding discovery in the field of human health. This award brings well-deserved recognition in Australia for Murray's careful, thoughtful and novel approach to his field of research which has attracted international interest over many years. Murray's work began by developing a technique to study the function of the sympathetic nervous system. He used a tracer technique to measure the rate of release of transmitter - the chemical messenger between the nerve and the target organ. More recently, he has refined the method to learn more about specific regional release from the brain, kidney, intestine and heart. Not only has he defined what happens in normal volunteers but importantly what occurs in hypertension, mental illness and liver cirrhosis. These clinical studies have taken great courage, conviction and patience, a point sometimes overlooked by the those who easily work with less organised systems such as isolated cells or tissue. Murray's laboratory has recently expanded to cover the refinement of metabolite assays under the guidance of Dr Graeme Eisenhofer, Helen Cox and Gavin Lambert.

The announcement and presentation of the Wellcome award was made at a formal dinner at the Great Hall, National Gallery of Victoria on 12 October. The presentation to Murray was made by Professor Antony Basten, Director of the Centenary Institute of Cancer Medicine and Cell Biology, in the presence of 300 guests. In his address, Professor Basten acknowledged the support by the Wellcome Trust world wide of 50 million and by Wellcome Australia in supporting medical research through this medal, Wellcome Senior Research Fellowships and research agreements with universities and the CSIRO. The Managing Director, Mr Ken Roberts, spoke of the desire of Henry Wellcome to support "the pursuit of excellence" in medical research where the funds arising from pharmaceutical sales would be returned to support research. Previous winners of the Wellcome Australia Medal are Prof. Basten,

Dr. Murray Esler (left) and Dr. Garry Jennings celebrate Murray's award of the Wellcome Medal.

Prof. John Chalmers, Prof. Ian McCloskey, Prof. Ian Gust, Prof. Len Harrison, Dr G. Mitchell and Prof. John Funder.

Murray's acceptance speech was a magnificent example of this humble scientist paying tribute to his parents as they educated him in Geelong; to his PhD training with Prof. Nestel at the Australian National University; to his overseas experience with Dr. Stevo Julius in Michigan; to Paul Korner who encouraged Murray to accept no lesser rigor and excellence in clinical research than he would in basic science; to Garry Jennings, his close collaborator over the last ten years, and finally to his wife, Rosemary, who "is the wind beneath my wings".

Dr Esler was an invited speaker at a symposium on "Psychosocial Influences On Cardiovascular Function" at the International Congress of Physiological Sciences in Helsinki, in July 1989, presenting a paper entitled, "The pathophysiology linking psychosocial stress to human cardiovascular disease". During this visit to Scandinavia, he also presented an invited paper, "Localised release of noradrenaline in humans as evidence of sympathetic differentiation" at the satellite meeting of the Congress in Gothenburg, "Sympathetic Control of Cardiovascular Function". Dr Esler attended by invitation the international meeting on "Mental Stress as a Trigger of Cardiovascular Events" in Italy in October 1989, delivering the paper "Biochemical Evidence of Sympathetic Hyperactivity in Human Hypertension". He was an invited guest at the World Congress of Cardiology in Manilla, in February 1990, presenting the paper "Neurohumoral Changes in Congestive Heart Failure". He was an invited participant at the American Heart Association workshop "Prevention Conference Hypertension" in Washington D.C. in April 1990. Dr Esler was one of a panel of speakers selected by the International Society of Hypertension to participate in an educational tour of medical centres of Malaysia and Thailand in May 1990, speaking on high blood pressure treatment and research. He will present the State of the Art Lecture, "Regional Sympathetic Activity in Hypertension", by invitation, at the meeting of the International Society of Hypertension in Montreal in June 1990. Dr Esler is also a founding editor of Clinical Autonomic Research and on the editorial board of Hypertension. He is a member of the Medical Research Ethics Committee of the NHMRC, and on the Scientific Advisory Board of the Mental Health Research Institute of Victoria.

Dr. James Angus visited Glaxo, Ware, England in May 1989 to present the first annual report of our activities to the Glaxo scientists under the Glaxo Australia-Baker Institute agreement. As a result of this visit, the budget was substantially increased to cover new initiatives. In December, Dr. Angus gave an invited lecture on "The role of presynaptic 2-adrenoceptors in the circulation" at a New York Academy of Science meeting in Philadelphia before visiting Glaxo Inc. at Research Triangle Park. In the future, the Baker-Glaxo agreement will be primarily linked with the cardiovascular group in Glaxo, USA. Dr. Angus serves on the editorial boards of the Journal of Hypertension and Journal of Cardiovascular Pharmacology. He is a member of the NHMRC Grants Committee and served as chairman of the Western Region RGIC in July. He was a member of the assigners panel of the NHMRC for Pharmacology.

Dr. Tom Cocks presented a paper on endothelin to the 1st International Symposium on endothelium-derived vasoactive factors in Philadelphia in May. He also visited Dr. Noel Cusack at Nelson Research Laboratories, Irvine, California.

Dr. Grant McPherson presented papers on K channel agonists and antagonists at the Vasodilator Mechanisms meeting in Strasbourg, and at the International Physiology Congress in Helsinki. He also gave lectures at ICI-Macclesfield and University of Manchester, Pharmacology Department. He gave an invited presentation in the Symposium on Young Pharmacologists on "Techniques in vascular pharmacology" at ASCEP, Sydney in December.

Dr Alex Bobik presented work at the European Hypertension meeting in Milan and the Physiology Congress in Helsinki. He also gave seminars on membrane ion transport processes in vascular smooth muscle and the mechanisms involved in the development of cardiovascular hypertrophy in genetic hypertension at the Cardiology Center, Moscow, the Physiology Department at Moscow State University and the A.A. Bogomeltz Institute of Physiology, Kiev, U.S.S.R. Dr Bobik also gave seminars at the Biochemistry Department, Cambridge and the MRC Blood Pressure Unit, Glasgow. He continues to serve on the editorial board of the Journal of Cardiovascular Pharmacology.

Dr Peter Little presented work on sodium transport processes at the Physiology Congress in Helsinki and at a symposium on intracellular pH regulation in Aarhus, Denmark.

Dr Fidge attended the American Heart Association meeting at New Orleans in November, 1989 and presented a paper with John Morrison on the binding domain of apoAI recognised by the HDL receptor. He was also a member of the National Heart Foundation Grant Review Committee (Sydney) and gave seminars on HDL metabolism to the CSL, Silenius Company, and Department of Biochemistry, Monash University.

Dr. Julie H. Campbell visited the Soviet Union in May/June 1989, as part of the Soviet-Australian scientific exchange programme. She also was an invited speaker at the Second Saratoga Conference on Progression and Regression of Atherosclerosis in Towada, Japan, October 1989. She continues to serve on the editorial board of the American Heart Association journal Arteriosclerosis. Together with Dr. Gordon Campbell (Department of Anatomy, University of Melbourne), she obtained a grant of $285,000

over two years for studies on newly developed calcium channel blockers from Servier Laboratories (Paris, France).

Ms. Robyn Rennick submitted her Ph.D. thesis entitled, "Smooth muscle cell- macrophage interactions in atherogenesis" in September 1989. She was awarded a British Heart Foundation Postdoctoral Fellowship (2 years) and an Australian Heart Foundation Travel Grant. She is currently in the Department of Anatomy and Developmental Biology, University College, London. Ms. M. Jane Black submitted her Ph.D. thesis entitled, "Polyploidy of vascular smooth muscle in hypertension" in December 1989. She is currently the recipient, together with Dr. Julie Campbell, of a National Heart Foundation Research Grant. Mr. Ang Aik Hooi also submitted his Ph.D. thesis in December 1989. The title of his project was "Collagen expression by smooth muscle cells alteration with phenotype". Ms. Sophie Horrigan won the Australian and New Zealand Society for Cell Biology Young Investigator Award for 1989, and also won the Australian and New Zealand Society for Experimental Pathology Young Investigator Award for 1989.

Dr. Warwick Anderson was an invited speaker at a symposium on "Intrarenal actions of angiotensin II", a satellite symposium of the meeting of the International Physiology Congress. The meeting was held in Budapest and Dr. Anderson's title was "Angiotensin II and the maintenance of GFR and renal blood flow during renal artery narrowing".

During 1989 Dr. Anderson was appointed to the Editorial Board of the journal, Hypertension, and re-appointed as an Executive Editor of the journal. He continued to serve as the Programme Secretary of the High Blood Pressure Research Council of Australia, and as a member of the Medical Research Committee, and Medical Research Ethics Committee, NHMRC.

Dr. Anderson continued many activities in the area of the welfare of experimental animals. He continues as the Chairman of the National Health and Medical Research Council's Animal Experimentation Ethics Committee, and a member of the Australian Council for the Care of Animals in Research and Teaching. During 1989, he was appointed to the inaugural National Consultative Council on Animal Welfare, advising the Federal Minister for Primary Industries and Energy. He was the invited speaker at the annual meeting of the Victorian Division of the Australian Veterinary Association, speaking on "Veterinarians on animal experimentation ethics committees: a scientist's expectations". He is scientific advisor to the Victorian Government's Animal Welfare Advisory Committee.

Dr. R.L. Woods attended the Physiology Congress in Helsinki, and a satellite meeting "Sympathetic control of cardiovascular systems", Gothenburg, Sweden. She also attended the Radiation Protection Course at Lucas Heights, NSW in February 1989.

Dr. Elizabeth Woodcock attended the 7th International Conference on Cyclic Nucleotides, Calcium and Protein Phosphorylation in Kobe (Japan) in October. She presented a paper entitled "Different inositol phosphate responses to angiotensin II and vasopressin in rat adrenal glomerulosa cells" which was selected for inclusion in the volume "Advances in Second Messenger Research". Dr. Woodcock and Ms. Ivana Kuraja also attended the meeting of the Australian High Blood Pressure Research Council held in Melbourne in December.

Dr Philip Barter was an invited speaker at an International Symposium on Lipoprotein Metabolism held in The Hague in The Netherlands in February 1989. In March, 1989, he presented an invited paper at the 5th International Colloquium on Atherosclerosis Research held in Brussels, Belgium. Dr Barter continued as a member of the National Board of the National Heart Foundation of Australia and as Chairman of its National Medical and Scientific Advisory Committee. He also remained a member of the Board of Governors of the Heart Research Institute in Sydney. He was appointed to the editorial board of the journal, Atherosclerosis.

Dr Frank Rosenfeldt was an invited speaker at the Pan-Pacific Symposium organised by the Australian Federation for Medical and Biological Engineering on the subject of lasers in clinical practice in July 1989. Dr Rosenfeldt was chairman of the organising committee for the Annual Meeting of the International Society for Heart Research, Australasian Section, held in Melbourne in April 1990. Dr Rosenfeldt teaches final year medical students at the Alfred Hospital and lectures to the post-graduate nursing course at Alfred Hospital. Dr Rosenfeldt was one of a team from the Alfred Hospital who attended a training course in the use of the Thoratec Heart Assist Device, held in Berkeley, California in September 1989. He also visited Cedars Sinai Hospital in Los Angeles to learn about laser treatment of coronary disease.

Ms Susan Luff visited the laboratories of Professor G. Burnstock, Department of Anatomy at University College London, United Kingdom. She also spent some time at the MRC, Anatomical Neuropharmacology Unit and the Department of Pharmacology at Oxford University where she presented a seminar. Ms Luff was also invited to present a lecture on "Innervation of Blood Vessels" in a symposium at the Australian Neuroscience Society Meeting in February, 1989.

Dr Geoff Head attended the Fourth European Meeting on Hypertension in Milan and a subsequent satellite meeting on "Cardiovascular structural changes in hypertension". He spent the period in July - August at the Necker Hospital in Paris on a return visit to Dr. Jean-Luc Elghozi and also attended the Physiology Congress in Helsinki.

Dr. Carol-Ann Courneya was

Popular and able member of the BRU Mr. Ted Turnbull retired this year.

awarded an Edward Wilson Memorial Fellowship by the Alfred Hospital to work at the Baker Institute during 1990 to study the central mechanisms of action of -methyldopa. She also attended the IUPS meeting in Helsinki.

Professor Korner gave an Invited Lecture at the Helsinki International Physiology Congress in July 1989; the topic was 'Baroreceptor and baroreflex resetting in circulatory control". He was an Invited Lecturer at a satellite conference in Rostock in East Germany, which he helped to organise on behalf of the Cardiovascular Commission of the International Union of Physiological Sciences, to promote East-West scientific dialogue. Interestingly, he reported that at that time, there was no evidence of the subsequent political changes that have since swept Eastern Europe. In February 1990, he attended a meeting in Dunedin on 'Future Trends in Hypertension Research". In December, he was re-elected as President of the Board of Management of the Alfred Group of Hospitals. He currently serves on a Victorian Government Committee to review research funding in the state.

Dr. Garry Jennings was an invited speaker at the Puijo Symposium, on Exercise and Hypertension (Finland), and the World Congress on Cardiac Rehabilitation, and was the "State-of-the-Art" lecturer at an International Symposium on Functional Capacity in Heart Failure.

Long serving member of the Biology Research Unit (Animal House) staff, Mr. Ted Turnbull, retired in 1989, after two decades of outstanding service to the Institute. A man of great compassion, he brought much skill and wisdom to his work with our animals, and wasa source of strength to many a younger staff member in times of difficulties in their private lives. For the last year of his time at the Institute, he was acting Supervisor of the Biology Research Unit.

Mrs. Nerida Aldred was appointed as Veterinary Nurse-in-Charge of the operating rooms during 1989, and has brought to the position much skill and experience.

The New Supervisor of the Biology Research Unit is Miss Debbie Ramsey. Debbie holds a Certificate of Animal Technology, and previously worked in the Renal Laboratory of the Institute. She is a member of the Victorian Executive of the Australian Animal Technician's Association. The position is very important in providing the highest standard of care to our animals, and there are many responsibilities under the recently adopted Victorian legislation and updated Code of Practice for the Care and Use of Animals for Scientific Purposes.

Miss Debbie Ramsey.

The Baker Oration* 7th November, 1988

ETHICS, LAW AND RESOURCES AT THE GROWING EDGE OF MEDICINE

Richard Lovell**

**This is a synopsis of the Oration which is published in full in the Australian and New Zealand Journal of Medicine.*

***Professor Emeritus, University of Melbourne. Chairman, Medical Research Ethics Committee of the National Health & Medical Research Council.*

Emeritus Professor Richard Lovell, AO, Chairman of the Medical Research Ethics Committee of the National Health and Medical Research Council (1982 – 1988)

There is a Greek saying that a doctor has opportunities for studying human nature which are given to no one else, wherefore a philosopher ought to begin his life as a doctor, and a doctor should end his life by becoming a philosopher. I am going to speak, to some extent philosophically, around the topic of constraint on medical resources. Having in mind the adage that those who do not remember the past are condemned to re-live it, let me start by telling the story.

THE STORY

In 1956, soon after I became Professor of Medicine and had been given charge of beds at both the Royal Melbourne and Alfred Hospitals, a young man was admitted to the Melbourne with acute nephritis. He soon developed severe renal failure, passing only a few drops of urine a day. This was a well known often fatal complication of nephritis. With careful management of fluid intake, the patient survived for many days and we kept hoping that the urine would start flowing again, but it did not. At that time, Hume's paper in the Journal of Clinical Investigation, which described recent attempts in Boston at treating patients like this with renal transplantation, had just reached Melbourne. Eventually believing the prognosis to be hopeless, I consulted my surgical colleague, Professor Maurice Ewing, and asked him to consider doing a transplant. After discussion with the

patient this course of action was decided upon.

Within days a kidney was obtained from a cadaver at the Alfred Hospital. It was driven across Melbourne and Professor Ewing transplanted it in the patient's thigh, which was a site tried in those early days. In spite of perfect surgical technique the kidney failed to function and the patient died. This was of course before the era of effective immunosuppressive drugs.

In the next few years, renal dialysis for patients like this one, with acute renal failure, was developed by Dr. Malcolm Whyte at the Kanamatsu Institute in Sydney and we flew some patients there for this life-saving treatment. Then dialysis was set up in Melbourne, largely through the initiative of Priscilla Kincaid-Smith from the University Department of Medicine and Vernon Marshall from the University Department of Surgery, both working initially at the Alfred.

A decade later, the first effective immunosuppressive drug, azothiaprine, became available and Priscilla Kincaid-Smith and Vernon Marshall started systematically to pioneer a cadaver transplant programme for patients with irreversible renal failure. An increasing number of patients who would otherwise have died were soon living full and active lives.

At a party in 1987 at the Royal Melbourne Hospital, 130 patients with transplants gathered to celebrate the 21st anniversary of the first survivors of renal transplantation. The Minister for Community Services and Health, Dr. Neal Blewett, greeted them and paid tribute to the success of the program not only in human terms but also in economic terms.

What comments can one make on ethics, the law, and resource constraint from this experience? What issues arose?

ISSUES

1. Decision-making with novel therapy

Maurice Ewing and I accepted responsibility for the decisions that had to be made about the first transplanted patient. There was of course discussion, in my team and in the surgical team, and in parts of the hospital's administration, and after the patient died I remember presenting the case at one of the regular meetings that used to take place to review deaths in the hospital. That was the way things were done in those days. In 1956 there were no such things as institutional ethics committees.

The novel aspect of the case bears interestingly on a question that is presently before the Medical Research Ethics Committee of NHMRC: what process is desirable before a novel therapy is tried or before there is a novel use of an existing therapy?

2. Law at the advancing front of medicine

At various times in the early part of this story mention may have been made of the law, but in the original case the only recollection I have of the law being mentioned was in a discussion on whether the police should be asked to assist the rapid transport of the donor's kidney from the Alfred Hospital to the Royal Melbourne Hospital. One of the arguments against was the danger of inflicting media publicity on the patient and relatives.

The law did of course come in in a very specific way later on, as the transplantation program developed, because of the desire for a legal definition of death that was relevant to obtaining kidneys from cadavers. With regard to transplantation of kidneys, the new statutes came after the medical research and the introduction of the new therapy, and thus of course after we had lived through the awkward period when it was not possible to say whether what was being done was research or treatment.

Law generally has to follow advances in medicine. It cannot lead because lawyers, like the rest of us, cannot read crystal balls. Until research is done it is hard to define precisely the matters on which you may want to legislate, or indeed whether it is wise to introduce new legislation.

If you legislate prematurely, you are liable to get things wrong and even find yourself confused about what you think you have said. In relation to IVF in Victoria we have had the singular spectacle of a law being passed by Parliament and the Attorney General then having to ask the Solicitor General what it meant. This hardly inspires confidence in the use of the law as a means of regulating the growing edge of biomedical research.

There are plenty of more flexible ways than new legislation with which to cope with many of the problems posed by medical research. This is not to say that there is no place for new laws. For example laws underpinning the framework on which regulation is based may well have a place. It is over the details of regulation that the legislative approach has justifiably attracted criticism.

3. Doctors, patients and costs of resources

In the 1960's, the pioneering of treatment by dialysis for patients with acute and possibly reversible renal failure led to discussions between doctors and administrators about cost. How could costs of flying our patients to Sydney be contained and condoned? How should dialysis apparatus be paid for? When the University Department of Medicine invested research funds in a badly needed and greatly improved dialysis machine, where could resources for technicians and nurses be found to use it?

That experience brought home to me the reality of the differences that exist between two roles that doctors, and I would say nurses also, must accept in society. One is to care for individual patients. The other is to help Governments to decide on the priority and distribution of medical resources. The two roles should not be confused.

4. Control over research

The development of Melbourne's early and remarkably successful renal transplantation programme resulted from the coincidence of skills and interests in renal disease among members of two University

departments. This could not have been foreseen. There is a lesson here: that the mechanism for controlling medical resources must not be too rigid. Tidy-minded planning is important, but there must be a capacity in medicine to seize the main chance when the right people are suddenly in the right place at the right time with the right idea. If there had been rigidity in planning in the 1960's we should not have had the capability that we soon developed for economic and effective treatment of irreversible renal failure.

I have mentioned coincidence as a noteworthy item. Let us consider a related factor that bears, in my story, on haemodialysis and the development of immunosuppressive drugs. In Australia major advances in high technology medicine are mostly made outside the country. For this reason alone, any attempt to control the directions of medical treatment by central control of research directions would be fruitless. In contrast with the law, medicine is not parochial. It is not state-based or even nationally based. It is based globally and responds to happenings across all national borders.

At a time when there are murmurings about increasing central control of research, we should also remember another lesson that history teaches us. Research initiated for one reason often has unforeseen consequences. We had cortisone for clinical use in 1948 because, during World War 2, funds were poured into adrenal steroid research for military reasons. The United States Government believed, wrongly, that Luftwaffe pilots were receiving steroids that enabled them to fly phenomenally high and see in the dark.

Furthermore, central controllers don't always get things right, and if they get them wrong the consequences can be very bad. The British Medical Research Council got penicillin wrong in 1939. They rewarded Florey's plea for help with a grant of twenty five pounds. The Rockefeller Foundation got it right. When he asked for sixteen hundred pounds, they gave it to him not once-off but for five years.

5. Who shall live, who shall die, and who shall decide?

When the transplantation programme for patients with irreversible renal failure got underway it was not long before the number of patients suitable for transplantation outnumbered those that could be coped with. The limiting factor was the number of dialysis machines available to provide the essential backup, and provision of the technical and nursing staff that is associated with dialysis. Stark decisions had to be made: which patients should be offered a chance to live and which ones should die? I can recall no formal discussions in the hospital about how these decisions should be made. The renal physicians and transplant surgeons accepted the decision-making as a responsibility that has traditionally been a part of a doctor's life. The sorting of multiple casualties in any disaster brings it home to one.

What happened with the kidney transplants at the Royal Melbourne Hospital was that the specialists concerned arranged a regular review of all possible patient candidates. In addition to the renal team, which included members of nursing staff, at every review the physician under whose charge each patient had been admitted, or his or her deputy, was invited to be present, and I, as one detached from the day-to-day transplantation problems but aware of what was going on, was asked to chair the meetings. The physicians putting patients up for consideration, who were mostly not renal specialists, usually asked their registrars to attend in their place. The relevant intern often came along as well and so each patient's case was presented by doctors totally familiar with them. Somehow, meeting after meeting, a consensus was reached and patients were classified as those who might be offered a transplant, those not to be offered one and those in whom a decision should be deferred.

At one time I became concerned that, with this approach, the members of the hospital's Board of Management, in whose hands the provision of facilities lay, were being insulated from the reality of the need for more resources. So I asked that a lay board member should attend these sessions. The response was to the effect that the job we were doing was a medical professional one, that the Board had confidence in the way in which we were doing it and that there was no case for adding a lay person to the group.

Reflecting on this experience twenty years later, was what we did right? The initiation of something as novel as a renal transplant programme was in the 1960's, would nowadays undoubtedly come before the ethics committee concerned with research in the hospital. If an ethics committee had existed at the time, could it have advocated a better procedure for dealing with the problem of patient selection?

I have been a strong proponent of the development of substantial participation of lay people in the regulatory system which governs ethical aspects of medical research. While I believe ethics committees are essential for preparing guidelines, for approving ethical aspects of medical research projects, and for monitoring projects, I have deep reservations about such committees intervening at the individual patient level in the hour-to-hour and day-to-day practice of medicine. I believe what was being recognized way back in the 1960's was that in medical practice a paramount value is the trust between doctor and patient, a trust which, if impaired, could undermine the basis on which doctors and patients relate to each other, the very basis on which medicine is practised. By all means let individuals professing expertise in ethics, or ethics committees, stand ready to join in professional consultations should patients or doctors wish them to. But let us remember that when committees, or worse still courts of law, intrude into the sick room, the trust between patient and doctor is eroded and the sick person is in danger of being treated not as a patient but as an interesting moral or legal case. The patient could end up with no doctor whom they could

trust to accept unqualified responsibility for trying to cure them, or relieve them, and always to comfort them.

MACRO ETHICS

Let me return to the role of the doctor in helping Governments to decide on the priority and distribution of medical resources. We don't seem always to be very good at it, and perhaps the time has come to build some new bridges, for if doctors and administrators and economists can together identify wasteful or ineffective components in the health services, constraints may turn out to be much less problematical. All that might be done might be able to be done.

It would, incidentally, be wrong to assume for a start that medical techniques which individually have high costs should by definition be targets for constraint. The real potential for savings is likely to be in lower cost and frequently used measures (Moloney and Roger, 1979), and, in the longer term, and perhaps most importantly of all, in today's preventive medicine and health promotion, whose effects will be seen some decades hence.

But, given that some constraints are after all necessary, what ethical principles should underlie decision-making? Continuing an approach based on lessons from history, one might take a recent case in which limited funds led to clear-cut decisions on resource allocation, and examine the notions that underlay the decisions that were made. The case occurred in the State of Oregon, USA, in 1987. (Welch & Larson 1988). The question identified was whether, for the next two years, to extend funding for basic health care to include 1500 persons not covered previously, or to continue to fund a programme of transplantation of bone marrow, heart, liver and pancreas, for a projected 34 patients. (Kidney transplants were not to be interfered with). The decision was made to extend the basic health care funding, and to stop the transplants.

The report on this matter did not, unfortunately for our purposes, reflect discussion of the principles that were taken into account in reaching the decision. It did however give four reasons why transplantation of the organs mentioned was the target. First, the transplantations represented easily identifiable large scale expenses. Second, they benefited relatively few people. Third, they represented a new type of therapy that was more easily foregone than other, longer established, ones. Finally, and this was a local administrative consideration, the transplantations were categorized as discretionary expenditures. Three of these reasons were essentially pragmatic ones. (Easy identifiability, novelty and the discretionary expenditure category). The reason that the transplantations benefited relatively few people embodied an ethical proposition, that the number of people who benefit should influence decision-making on resource constraint. I will come back to that.

Since this study did not take me very far in the search for principles that might assist in decision-making, I thought it might be helpful to explore another approach and ask: to what extent are ethical principles which underlie medical practice – that is, doctor/patient relationships – transferable to, or at any rate helpful in considering, medical resource constraints generally?

The first of these principles, beneficence, or trying to do good, when applied to a society rather than a patient, does not take one very far. It leads to comparing chalk and cheese, to weighing economic benefit for society against allowing people who might live to die, or who might live more comfortably to live less comfortably.

If beneficence does not take one very far, non-maleficence, never do harm, seems totally unhelpful. Any restriction of effective health promoting measures must do harm to some individuals.

The third ethical principle underlying doctor/patient relationships is autonomy, the notion of 'people being responsible to and for themselves, and therefore being able to determine what shall be done to them' (Morgan 1988). Where does this concept take us in guiding decisions about resource constraint? I think nowhere, but it leads to a secondary point that needs to be made in a country like ours with a federal-state system. When available medical resources are restricted, the concept of autonomy leads to an assertion that individuals should not be prevented from pursuing a resource that is not locally available to them – say a particular treatment – although in pursuing it they must not impose a burden on their community. In practice, the exercise of autonomy will naturally lead to a distinction within a community between have's and have not's, those able and those unable to buy their way out of the constraints imposed on them. Some may, for example, buy their way out through interstate or intercontinental travel, or by using privately provided medical services.

We should note that it has become apparent, in recent years, that the exercise of autonomy will skew even the most egalitarian form of health service. In the National Health Service in Britain, which was organized as the most egalitarian one of all, it soon became apparent that people who were better educated and better off benefited more from the service than did the less well educated and poorer people. The better educated knew how to make the best use of the service that was provided.

The fourth ethical principle in medical practice is justice or equity. I understand this to include the concept of being even-handed in relation to individuals, and in helping them without regard to their personal circumstances or backgrounds. I suspect that everyone would intuitively believe that justice should be a major consideration in any discussion of constraint in health care. To examine this further, let us again go back to real-life experience and consider what happened as in the early days of renal transplantation. What I suppose those involved were concerned with, when resources were very limited, was not simply

even-handedness in relation to individuals, but even-handedness in the way in which the whole operation was conducted. For with a novel resource – and these are always likely to be subject to constraints – the order in the queue cannot simply be determined by individual patients' needs. For example, knowledge has to be gained as quickly as possible about what sort of patients benefit and what sort do not. In the early days of renal transplantation there was an urgent need to know, for example, how well patients with diabetic nephropathy did as compared with those with glomerulonephritis.

We seem to have found two proposed ethical criteria that should bear on decisions about constraint of medical resources. One is that a constraint should be able to be operated equitably. This is, of course, quite different from saying that the constraint itself should be equitable, which would be a contradiction in terms. Secondly, there is the proposition, in the decision in Oregon, that account should be taken of the relative numbers of people who benefit. I personally find this notion very difficult because I'm not sure how we benefit from, say, a heart transplant, can be compared with primary health care. How many gifts of primary health care equate with one heart transplant?

DISCUSSION

The interaction between ethics, the law and constraint on resources in medical practice affects all our lives today. History shows that when constraints have occurred in special situations, as when novel forms of treatment have first been introduced, or when disaster has swamped existing resources, doctors have developed a framework for decision-making, which has guided their conduct.

The story of the introduction of renal transplantation between 20 and 30 years ago shows that the issues which arose included: making the decision to try a novel therapy in the first place, some aspects of law, decisions about costs and the use of resources, and controls over research. Given the limited resources initially available, decisions also had to be made on who should live, who should die, and who should decide.

In these sorts of circumstances, history tells us that decision-making has come within the framework of professional medical practice, and has been influenced by well known ethical principles. By contrast, ethical principles that might be useful in determining constraints which might be imposed on ordinary medical services, prove hard to define. The notion of the greatest good for the greatest number is too general, while the notion of equity, vital for the way in which constraints are exercised, seems not very helpful in deciding the aspects of health resources that should be constrained.

This rather negative, tentative, conclusion leads me to suggest that there is a real need for increased multi-disciplinary discussion to try to discover principles, other than purely economic ones, which should be taken into account when constraints on health resources are decided upon. Despite the conflict which doctors will find between the ethical principles which govern their day-to-day relationships with patients in practice and research, and the idea of constraining resources, I believe doctors must contribute to the debate. Maybe the realization that there is a conflict, and that there are separate roles to be played, may, for a start, make it easier for doctors to come to grips with some of the hard questions that have to be asked.

Moloney, T.W. & Rogers, D. 1979
Medical Technology: a different view of the contentious debate over costs.
New England Journal of Medicine **301**, 1413-19.

Welch, H.G. & Larson, E.B. 1988
Dealing with Limited resources.
New England Journal of Medicine **319**, 171-73

Morgan, J. 1988
Words we use.
Medical Research Ethics Committee Newsletter Vol. 1.
No. 3, page 8. National Health and Medical Research Council, Canberra.

Financial Report

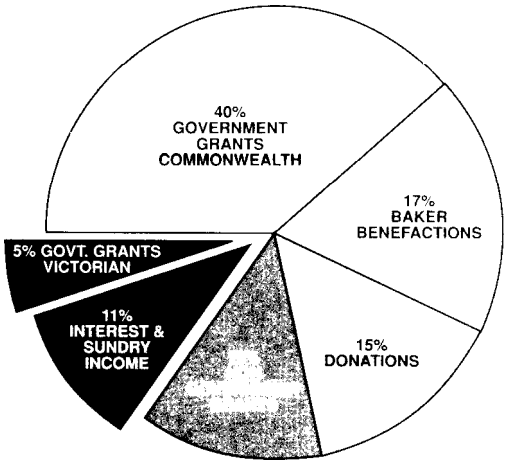
BAKER MEDICAL RESEARCH INSTITUTE

Year ended 31st December 1989

Income and Expenditure at a glance - Schedule 1

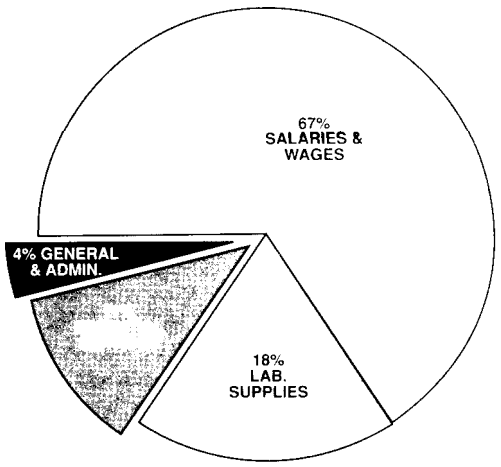
Income Derived from the Following Sources

1988 000s $	%		1989 000s $	%
900	17	Baker Benefactions	902	17
2,029	39	Government Grants - Commonwealth	2,191	40
250	5	Government Grants Victorian	300	5
549	10	Non Government Grants	632	12
963	19	Donations	828	15
541	10	Interest from Investments & Sundry Income	$593	11
5,232	100		5,446	100



Expenditure Distributed as Follows

3,545	66	Salaries and Wages	3,943	67
963	18	Scientific Equipment & Laboratory Supplies	1,024	18
720	13	Essential Services	669	11
184	3	General & Administration	229	4
5,412	100		5,865	100



Balance Sheets as at 31st December 1989 - Schedule 2

1988		$	1989
	Operating Fund		
	Accumulated Funds & Liabilities		
($707,919)	Accumulated (Deficit) (Schedule - 3)		($1,173,419)
	Bank Overdraft		$316,701
$748,553	Sundry Creditors & Accrued Expenses		$556,112
	Provision for Leave Entitlements:		
$211,505	- Annual Leave	$232,163	
$248,318	- Long Service Leave	$308,556	$540,719
$500,457			$240,113
	Represented by:		
	Assets		
$134,725	Cash at Bank		
$500	Cash on Hand		$1,200
$365,232	Sundry Debtors & Prepayments		$230,913
	Short Term Deposits		$8,000
$500,457			$240,113
	Endowment Fund		
	Accumulated Funds & Liabilities		
$1,790,584	Accumulated Fund - Schedule 4		$1,858,373
	Represented by:		
	Assets		
	Investments (at cost) held by the Institute		
$77,550	Govt & Semi Govt Stock	$202,600	
$412,627	Shares & Debentures in Companies	$385,738	
$65,032	Trust Units	$65,032	
41,393	Short Term Deposits	$320,861	
$33,200	Mortgage Loans	$29,109	1,003,340
$629,802			
	Held by A.N.Z. Executors & Trustees		
$43,794	Shares & Debentures in Companies	$46,046	
$357,972	Trust Units	$280,000	
$280,000	Short Term Deposits	$105,335	$431,381
$681,766			
$5,818	Cash at Bank		$47,557
$477,788	Sundry Debtors		$380,000
	Less: Liabilities		
($4,590)	Sundry Creditors		($3,905)
$1,790,584			$1,858,373

Research Scholarship and Other Funds
Accumulated Funds & Liabilities

1988		$	1989
	Accumulated Funds		
$556,501	Restricted Fund	Schedule 5	$575,424
	Edgar Rouse Memorial		
$98,309	Fellowship	Schedule 5	$91,809
$4,533	Research Fund	Schedule 5	$11,240
$38,098	Before 2000 Heart Appeal	Schedule 5	$1,574
$2,892	Laura Nyulasy Scholarship Fund	Schedule 5	$2,892
$40,300	William Buckland Research Fund	Schedule 5	$39,888
$97,984	Lang Research Scholarship Fund	Schedule 5	$103,249
$112,898	Bertalli Family Fund	Schedule 5	$114,933
$82,345	Ruby Wallace Travel Scholarship Fund	Schedule 5	$87,264
$132,611	Ethel Mary Baillieu Fund	Schedule 5	$131,643
$1,166,471			$1,159,916
	Represented by:		
	Assets		
	Investments (at cost) held by the Institute		
$1,183,977	Short Term Deposits		$1,054,122
	Held by A.N.Z. Executors & Trustees		
$8,665	Shares in Companies		$8,253
$34,577	Trust Units		$34,577
($50)	Short Term Deposits		($50)
$145,997	Cash at Bank		$123,414
$37,512	Sundry Debtors		$22,743
$1,410,678			$1,243,059
	Less: Liabilities		
$244,207	Sundry Creditors		$83,143
$1,166,471			$1,159,916

Statement of Movement of Accumulated Funds - Schedule 3

1988		$	1989
	Operating Fund		
$5,231,782	**Income**	$5,445,885	
$5,412,178	Less: Expenditure	$5,911,385	
($180,396)	Deficit for Year	($465,500)	
	Accumulated Deficit		
($527,523)	Opening Balance	($707,919)	
($707,919)		($1,173,419)	
	Operating Fund		
	Income		
	Donations from Baker Benefactions		
$11,569	Statutory Amount	$11,569	
$888,239	Yearly Block Grant	$890,112	
$899,808			$901,681
$962,847	**Other Donations (Net Transfers)**	$827,562	$827,562
	Grants in Aid of Research Projects		
$2,028,993	National Health & Medical Research Council	$2,191,223	
$260,225	National Heart Foundation of Australia	$324,600	
$134,030	Alfred Hospital	$125,956	
$49,545	North American Health Organisations		
	Sundry Grants	$57,190	
$2,472,793			$2,698,969
	Other Grants		
$2,106	G. F. Little Settlement	$1,392	
$19,314	J & E Borrowman Research Fund	$25,000	
$2,481	William Buckland Research Fund	$6,149	
$250,000	Victorian State Government	$300,000	
$370	L. Nyulasy Research Scholarship Fund	$339	
$20,000	Clive & Vera Ramaciotti Foundation	$0	
$4,467	Ruby Wallace Travel Scholarship	$8,818	
$38,252	E. E. Stewart Estate	$45,606	
$7,379	Bertalli Family Research Fund	$12,690	
$7,215	E. M. Baillieu Fund	$13,855	
$5,386	Lang Scholarship Research Fund	$10,529	
$356,970			$424,378
	Income From Investments		
	Held by The Trustees of the Baker		
$5,097	Institute Grant Trust	$5,097	
$243,730	Other Investment Income	$314,453	
$248,827			$319,550
	Other Income		
$198,046	Sundry Sales Recoveries & Refunds	$148,960	
$92,491	Clinical Services	$124,785	
$290,537			$273,745
$5,231,782	**Total Income**		$5,445,885

1988		$	1989
	Operating Fund		
	Expenditure		
$3,545,276	Salaries & Wages	$3,942,693	
$628,824	Laboratory Supplies & Isotopes	$736,939	
$334,730	Additional Equipment & Building	$287,545	
$51,877	Library Maintenance	$52,314	
$18,967	Postage	$14,677	
$38,670	Telephone	$43,059	
$75,666	Printing & Stationery	$51,585	
$110,121	Light & Power	$100,746	
$60,173	Insurance	$77,807	
$176,010	Repairs & Renewals	$126,974	
$83,671	Travelling Expenses	$85,740	
$38,983	Public Relations	$44,819	
$112,360	Fundraising Expenses	$109,951	
$8,941	Sundries	$11,606	
$21,167	Clinical Services	$8,753	
$7,271	Freight & Cartage	$14,400	
$21,570	Legal & Accounting	$32,594	
$2,980	Bank & Government Charges	$2,909	
$21,590	Staff Amenities & Advertisements	$48,826	
$12,403	Volunteers	$10,898	
$40,928	Alfred Hospital Services	$59,982	
$5,412,178	**Total Expenditure**		$5,864,817

Statement of Movement in Accumulated Funds - Schedule 4

1988		$	1989
	Endowment Fund		
$1,768,826	Opening Balance		$1,790,584
	Income		
$888,239	Donations - Baker Benefactions		
$1,309	Donations - Other		
$54,127	Interest - Investment	$40,780	
$136	Bank	$8,622	
$17,496	Profit on Sale of Shares & Redemption of Stock	$52,433	
$961,307			$101,835
	Expenditure		
$6	Federal Tax & Bank Charges	$266	
	Transfers to Operating		
$888,239	Baker Benefactions		
$51,304	Interest	$29,875	
	Accrued Transfers	$3,905	
$939,549			$34,046
$1,790,584	Closing Balance Schedule 2		$1,858,373

Statement of Movement in Accumulated Funds - Schedule 5
Research Scholarship and Other Funds

1988		$	1989
	Restricted Fund		
$160,335	Opening Balance		$556,501
	Income		
$11,569	Baker Benefactions - Statutory Amount		
$944,903	Grants & Donations	$810,277	
$22,742	Investment Income & Bank Interest	$8,499	
$17,411	Accrued Interest		
$996,625			$818,776
	Expenditure		
$11,569	Baker Benefactions Statutory Amount		
$531,576	Payments	$798,982	
$233	Bank Charges & Federal Tax	$871	
$57,081	Accrued Payments		
$600,459			$799,853
$556,501	Closing Balance - Schedule 2		$575,424
	Edgar Rouse Memorial Scholarship Fund		
$89,058	Opening Balance		$98,309
	Income		
$1,025	Donations	$3,669	
$7,629	Investment & Bank Interest	$11,237	
$4,200	Accrued Interest & Donations	$3,870	
$12,854			$18,776
	Expenditure		
$2	Federal Tax & Bank Charges	$45	
$1,665	Transfers to Operating	$7,677	
	Payments	$14,974	
$1,936	Accrued Transfers	$2,580	
$3,603			$25,276
$98,309	Closing Balance - Schedule 2		$91,809
	Research Fund		
$270	Opening Balance		$4,533
	Income		
$906,535	Donations	$577,079	
$157	Investment Income	$6,529	
$906,692			$583,608
	Expenditure		
$725,691	Transfers to Other Funds	$500,893	
	Payments - Other	$5,204	
$1,113	Federal Tax & Bank Charges	$2,804	
$175,625	Accrued Transfers	$68,000	
$902,429			$576,901
$4,533	Closing Balance - Schedule 2		$11,240

1988		$	1989
	Before 2000 Heart Appeal Fund		
$22,990	Opening Balance		$38,098
	Income		
$12,200	Donations	$6,620	
$1,751	Investment Income	$4,844	
$2,643	Accrued Interest & Donations		
$16,594			$11,464
	Expenditure		
$759	Transfer to Operating Fund	$1,723	
$5	Federal Tax & Bank Charges	$61	
	Payments	$46,204	
$722	Accrued Transfers		
$1,486			$47,988
$38,098	Closing Balance – Schedule 2		$1,574
	Laura Nyulasy Scholarship Fund		
$2,892	Opening Balance		$2,892
	Income		
$370	Investment Income		$339
$370			
	Expenditure		
$370	Grant to Institute		$339
	Payments – Other		
$2,892	Closing Balance – Schedule 2		$2,892
	William Buckland Research Fund		
$40,300	Opening Balance		$40,300
	Income		
$2,481	Investment Income		$6,149
	Expenditure		
$2,481	Grant to Institute		$6,149
	Payments – Other		$412
$40,300	Closing Balance – Schedule 2		$39,888
	Bertalli Family Research Fund		
$106,014	Opening Balance		$112,898
	Income		
$10,728	Investment Income	$13,151	
$3,535	Accrued Interest	$5,074	
$14,263			$18,225
	Expenditure		
$5,019	Transfers to Operating Fund	$9,307	
	Payments	$3,500	
$2,360	Accrued Transfers	$3,383	
			$16,190
$7,379			
$112,898	Closing Balance – Schedule 2		$114,933

1988		$	1989
	Ruby Wallace Travel Scholarship Fund		
$77,789	Opening Balance	$82,345	
	Income		
$6,537	Investment Income	$10,146	
$2,563	Accrued Interest	$3,713	$13,859
$9,100			
	Expenditure		
$2,758	Transfer to Operating Fund	$6,343	
$77	Federal Tax & Bank Charges	$122	
$1,709	Accrued Transfers	$2,475	
$4,544			$8,940
$82,345	Closing Balance – Schedule 2		$87,264
	Lang Research Scholarship Fund		
$92,564	Opening Balance		$97,984
	Income		
$7,746	Investment Income	$11,390	
$3,060	Accrued Interest	$4,404	$15,794
$10,806			
	Expenditure		
$3,346	Transfer to Operating Fund	$7,593	
$2,040	Accrued Transfers	$2,936	
$5,386			$10,529
$97,984	Closing Balance – Schedule 2		$103,249
	Ethel Mary Baillieu Fund		
$125,227	Opening Balance		$132,611
	Income		
$10,501	Investment Income	$15,357	
$4,100	Accrued Interest	$5,654	$21,011
$14,601			
	Expenditure		
$4,482	Transfer to Operating Fund	$10,085	
$2	Federal Tax & Bank Charges	$64	
	Payments	$8,061	
$2,733	Accrued Transfers	$3,769	
$7,217			$21,979
$132,611	Closing Balance – Schedule 2		$131,643

Statement of Investments (at cost) - Schedule 6

1988	Endowment Fund Held by the Trustees of the Institute	$	1989
	Goverment & Semi-Government Stock		
	M.M.B.W. Stock	$2,600	
	N.S.W. Treasury Bonds	$200,000	$202,600
	Other Stock		$100,000
	A.G.C. Debentures		
	Shares in Companies		
	Argo Investments Co. Ltd	$52,232	
	B.H.P. Ltd	$5,976	
	C.R.A. Ltd	$5,876	
	C.S.R. Ltd	$15,209	
	C.S.R. Conv. Notes	$14,703	
	Coles/Myer Ltd	$14,603	
	National Australia Bank	$4,310	
	North Broken Hill	$11,029	
	News Corp	$9,280	
	Westpac Banking Corp	$21,598	
	ANZ Banking Group	$16,631	
	Templeton Global Growth Fund	$50,000	
	Brambles	$11,304	
	General Property Trust	$17,265	
	Western Mining Corp	$11,032	
	Boral Industries	$13,602	
	W.M. Soul Pattinson	$11,088	$285,738
	Cash Management Trust Units		
	PP Cash Management Trust		$65,032
	Short Term Deposits		
	Toronto Dominion Australia Ltd	$20,889	
	Bank Bills	$300,000	$320,889
	Mortgage Loans		$29,109
	Sundry Debtors		
	Loans to Operating Fund		$380,000
			$1,383,368

Baker Medical Research Institute
Notes to and Forming Part of the Accounts for the Year Ended 31 December, 1989 - Schedule 7

1. Incorporation

On 1 August 1980, The Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute was incorporated as the 'Baker Medical Research Institute' under the Baker Medical Research Institute Act 1980. At this date the assets and liabilities of the original Institute were vested in the new Baker Medical Research Institute at book value.

2. Statement of Accounting Policies

The accounting policies of the Institute, which are consistent with those applied in previous years, are as follows:

(a) Historical Cost

The accounts of the Institute are prepared on the basis of historical cost and unless otherwise stated do not take into account the effect of changing money values or current valuations on non-current assets.

(b) Institute Funds, Income & Expenditure

The work of the Institute is financed from grants, endowments, donations and bequests of both general and specific natures. Income of a specific nature is taken to Restricted Funds where the terms of any relevant covenants apply to that income.

Income on investments is accounted for on an accrual basis.

Income from donations is accounted for on a cash basis.

Other income and expenditure is accounted for on an accrual basis.

Any deficiency arising therefrom is carried forward in the Operating Fund.

(c) Capital Expenditure and Depreciation.

Capital expenditure made by the Institute in respect of buildings, furniture and equipment in present and past periods has been charged against appropriate funds, grants or revenue accounts and expensed in the period in which it was incurred. Accordingly, no depreciation charge appears in the Institute's accounts.

The Insurance cover of such accumulated capital expenditure, including buildings, to 31 December 1989, was approximately $13,600,000.

3. Investments - Endowment Fund

The market value of shares in companies listed on the Australian Stock Exchange at 31 December 1989 was $816,202 (1988 $771,955).

Investments managed by the ANZ Executors & Trustee Company Limited are included in the balance sheet of the Institute in accordance with statements provided by the custodian company, giving details of the Institute's entitlements in securities held by the custodian company in its own name.

4. Contingent Liability

A contingent liability exists where the Institute has indemnified a former staff member in a libel brought against him in circumstances where he was representing the Institute. The action is presently pending and it is the opinion of the solicitors of the Institute and the Board of Management that the result of this action cannot be assessed at this time.

Auditors' Report to the Board of Management of Baker Medical Research Institute

We have audited the attached accounts as set out on Schedules 2 to 7 in accordance with Australian Accounting Standards.

As indicated in note 2(c), it is the Institute's policy to write off all capital expenditure as incurred.

In our opinion, with the exception of the effect of the omission of these assets, estimated to have a replacement cost of $13.6 million, the attached accounts are drawn up so as to give a true and fair view of the accumulated funds of the Institute and the net assets representing those funds at 31 December 1989, and the movements in those funds for the year to 31 December 1989, and have been made out in accordance with Australian Accounting Standards applicable to non-business entities.

Price Waterhouse
EA Alexander
A member of the firm
Chartered Accountants

Melbourne
30 April 1990

Baker Medical Research Institute
Statement by Board Members

In the opinion of the Board Members:

(a) the accounts are drawn up so as to give a true and fair view of the state of the Institute's affairs as at 31st December, 1989 and of its results for the year ended on that date;

(b) at the date of this statement there are reasonable grounds to believe that the Institute will be able to pay its debts as and when they fall due;

(c) the accounts have been compiled in accordance with Australian Accounting Standards, except in relation to the treatment of capital expenditure and depreciation of scientific assets as set out in the notes to the accounts and referred to in the Report of the Auditors.

Signed at Melbourne this 30th day of April, 1990 in accordance with a resolution of the Board.

John Moir

Paul Korner

Donations

Major Contributions
Received to 31st December, 1989

	$
Advanced Network Management	1,000
A N Z Banking Group	5,680
A N Z McCaughan	850
Arnhold Charitable Fund	2,000
Arnold, Mrs V	1,000
Ashton Mining Limited	1,000
3M Australia	1,000
Bailey Shaw & Partners Pty Ltd	1,000
Barbara Roche Estate	1,883
Bell Charitable Trust	6,500
Best Hooper	4,153
B P Australia	4,000
Brivis Australia Pty Ltd	1,000
B S Robbins Nurseries Pty Ltd	1,000
C E Heath Insurance	4,000
Chesters Fashions Pty Ltd	700
C I G Limited	1,000
Clive & Vera Ramaciotti Foundation	29,000
Comalco	2,000
Commercial Union Assurance	5,000
Commonwealth Banking Corp	1,000
C R A Limited	750
Dalgety Australia Holdings Ltd	1,000
Dillon, Miss M L	2,000
Distributed Data Processing Pty Ltd	1,000
Dorward, Dr Patricia K	5,025
Downes, Mr & Mrs M	850
Eisner, Mr K	2,750
E L & C Baillieu Limited	1,500
Elders I X L Limited	1,000
Emily E E Stewart Estate	39,546
Estate of the Late George Adams	1,000
Felton Bequest Committee	10,000
Fitzgerald, Mr L J	1,200
Fletcher Jones & Staff Pty Ltd	2,000
Gadsden, Mr & Mrs L A	1,000
Gann, Mrs R	1,000
George W Vowell Foundation	5,000
G & E Ramsay Charitable Trust	2,000
G & H Foulkes Charitable Trust	2,794
Habersberger, Mr & Mrs J C	1,510
Herald & Weekly Times Ltd	1,000
H M Schutt Trust	10,000
Hogarth, Mr D F	1,000
H P Blaich Estate	4,551
H P Williams Trust Fund	2,500
Imray, Rev F & Mrs F	2,000
James McNeill Trust	6,000
James N Kirby Foundation	2,000
J D & Lyla Harris Foundation	10,000
Jeffrey, Misses D & J	600
Joan Marsh Lillie Estate	20,000
Joe White Bequest	18,135
Kal Life Systems Pty Ltd	524
Kodak (Australasia) Pty Ltd	10,850
Kooronya Nominees Pty Ltd	850
Leopold Klein Charitable Foundation	2,000
Lustig & Moar Group	5,000
McCorkell Construction Company	1,000
McGrath, Lady	2,000
Macmillan Company of Australia Pty Ltd	1,000
McPhersons Limited	1,000
Marion & E H Flack Trust	7,700
M A S Robison	1,000
Minter Ellison	1,000
Muir, Sir Laurence	850
Murdoch, Dame Elisabeth	10,000
Myer, Mr S B	1,000
National Australia Bank	7,500
Nelson, Mr R E	5,000
Occidental Life Insurance Company	850
Peat Marwick Hungerfords	2,000
Percy Baxter Charitable Trust	12,500
Rotaract Club of Melbourne	600
Rotary Club of Balwyn	45,000
Scovell, Mr A M	600
Snowy Nominees Pty Ltd	3,000
T A & D Burgess Charity Trust	1,372
Telecom Australia	2,500
Terumo Corporation	1,000
The Age David Syme & Company	1,000
The George Hicks Foundation	2,000
The Hamilton Charitable Trust	3,500
The Ian Potter Foundation	10,000
The Jack Brockhoff Foundation	20,000
The Miller Foundation	1,000
The Shell Company of Australia	1,000
The William Angliss (Victoria) Trust	10,000
Victorian Ladies Bowling Association	80,000
Victorian Medical Insurance Agency Ltd	1,500
Wade, Mr E A	1,000
Webster, Mrs Ruth	1,200
Wellcome Australia	1,000
Westpac Banking Corporation	2,500
William Buckland Foundation	6,000
William Paxton Charitable Fund	2,500
Wooldridge, Mr & Mrs P	510
Wren, Mr J F	2,000

Brown Brothers (Milawa) have continued to generously support our seminars and VIP Tour functions.

A special thanks also must go to all our many other donors who, through their regular support, are equally important to the ultimate success of our research programme. Without their contributions, as well as those listed, we could not pursue our objectives as planned.
Cover photo donated by The Image Bank.

ANNUAL DINNER

Special acknowledgement and gratitude must be made to the Sponsors of the Baker Institute Annual Dinner held in December 1989.
They were: Australia and New Zealand Banking Group Ltd.
Commercial Union Assurance Company of Australia Ltd.
Kodak (A'asia) Pty. Ltd. – Medical & Health Divisions
Lustig & Moar Group.
Without their support and generosity this function could not have been held.

Thomas Baker Society 1989

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Mr D C Vollmerhause
Mr A C & Mrs J Weber
Mrs Ruth Webster
Mr O J Whelan
Mr R W Willmott
Mr S J Wintle

Century Club 1989

Membership of the Century Club now stands at 287, and space no longer permits us to list the names in this Annual Report, although they are recorded separately at the Institute.
Many new developments were made possible by the continuing, valued support of Century Club members. $27,475 was raised in 1989 which enabled specific research projects to be pursued in the following year.

Edgar Rouse Memorial Fund
in memory of

MR WAL HUGHES by 2/14 Aust Field Regiment
ALLAN W TATTERSON by 2/14 Aust Field Regiment
L T (LOU) PARR by 2/14 Aust Field Regiment
DAVID OLIPHANT by 2/14 Aust Field Regiment
H BAKER by 2/14 Aust Field Regiment
K MILLSTEAD by 2/14 Aust Field Regiment
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E BIRRELL by 2/14 Aust Field Regiment
J FULLER by 2/14 Aust Field Regiment
W SMITHERMAN by 2/14 Aust Field Regiment
W FREEMAN by 2/14 Aust Field Regiment
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MR K TAYLOR by 2/14 Aust Field Regiment
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IN HONOUR OF

MR B R SMITH	on their Anniversary by Mrs M J Smith
BETHOL R CAMPBELL	on Mother's Day by Mr A R Campbell
THE BAKER INSTITUTE	"For creating positive change in diet and life style" by Dr Geoffrey J Pullen on his 60th Birthday
MR H PETERS	by Stan & Eva Marks for Christmas
MR R C CARPENTER	by Mr D R Coustick

Baker Institute Club of 1000

In 1989, the Baker Institute established its 'Club of 1000', which is a club of leaders among commerce and industry. Each member company is entitled to nominate executives to be assessed and if necessary advised on risk reduction in the Institute's 'Heart Risk Evaluation Clinic'. They are also kept informed on the progress in our research work on heart disease and are included in VIP tours of the Institute.

Select companies are invited to join the Club of 1000 and the foundation members for 1989 are:

Ashton Mining Limited
Distributed Data Processing Pty
Terumo Corporation
Bailey Shaw and Partners Pty Ltd
McCorkell Construction Company
Brivis Australia Pty Ltd
Advanced Network Management
M A S Robison

We wish to thank these companies for their understanding of the importance of corporate support for our research efforts.