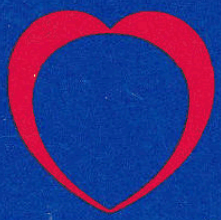
Research Annual Report 1985/86

60 Years of Research 1926-1986



Baker Institute
Alfred Hospital

Baker Medical Research Institute
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Prahran Victoria, 3181
Australia
Telephone 522 4333
Telex ALFHOSP AA31371

Annual Report 1985/86

Baker Medical Research Institute

Affiliated with Alfred Hospital and Monash University

Sixth Annual Report of the Baker Medical Research Institute

Thirty-seventh Annual Report of the Clinical Research Unit, Alfred Hospital

Twenty-ninth Annual Report of Ewen Downie Metabolic Unit, Alfred Hospital

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.

AIM 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.

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R. MILLER, LL.B.,LL.M.

"BEFORE 2000 HEART APPEAL"

Committee

Chairman
Sir LAURENCE MUIR

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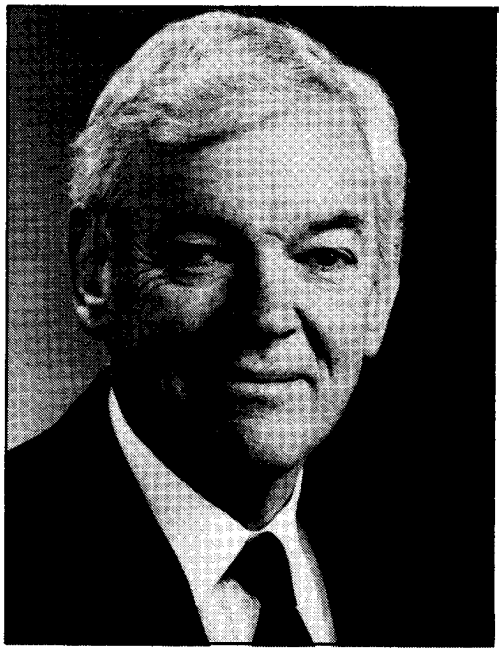
B. PASCOE
M.G. DOWNES
F. VERLINDEN
(Appeal Co-ordinator)

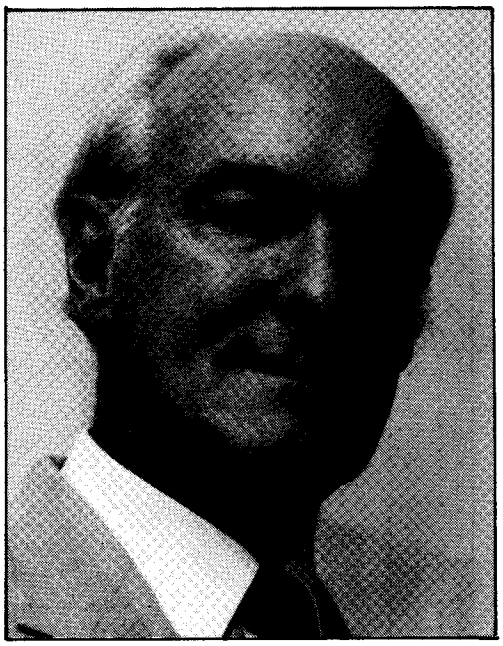
BOARD OF MANAGEMENT

Sir Laurence Muir President, was senior partner of Potter Partners until 1980. He is Chairman of the Canberra Development Board, a Member of the Federal Parliament House Construction Authority and a member of the Board of Australian and International Companies



Mr. J.D. Moir Vice-President, is a Senior Partner in the legal firm, Gillotts

Mr. W.J. Bailey Honorary Treasurer, is Managing Director of the ANZ Banking Group

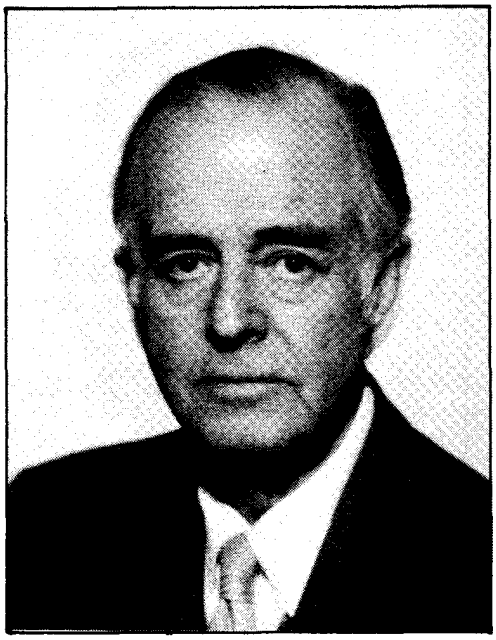


Dr. R. Cumming is Secretary of the National Health and Medical Research Council

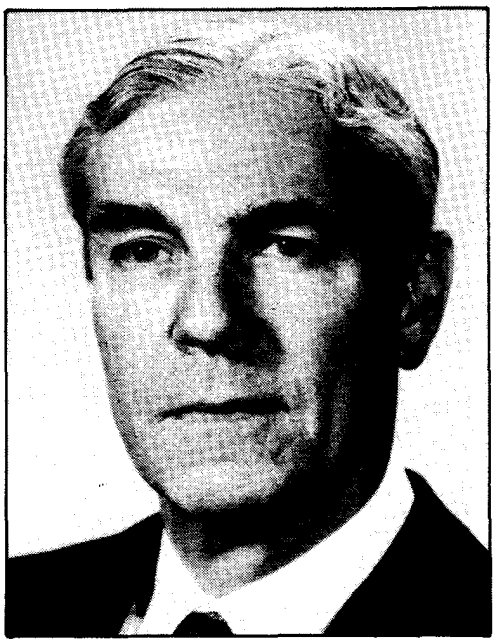
Professor P.I. Korner is Director of the Baker Medical Research Institute

Dr. T.J. Wood Secretary to the Board, is Chief Executive Officer of the Alfred Hospital

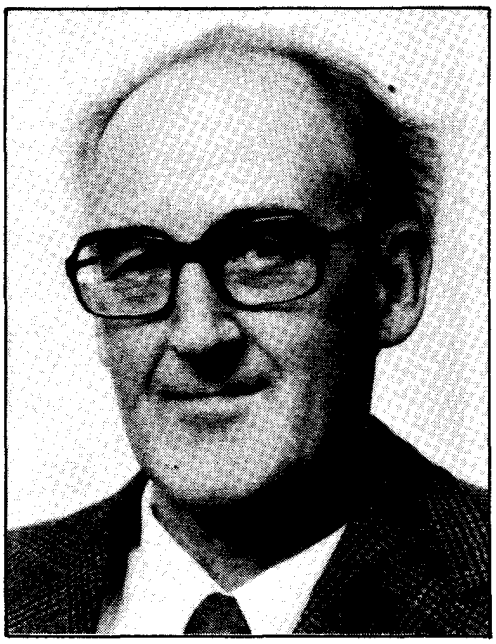
Mr. D.F. Hogarth is Chairman of Directors of Kodak (Australasia) Pty. Ltd.



Emeritus Professor R.R. Andrew was Foundation Dean of Monash Medical School and is President of the Baker Institute Alumni Association and a Past Vice-President of the Institute

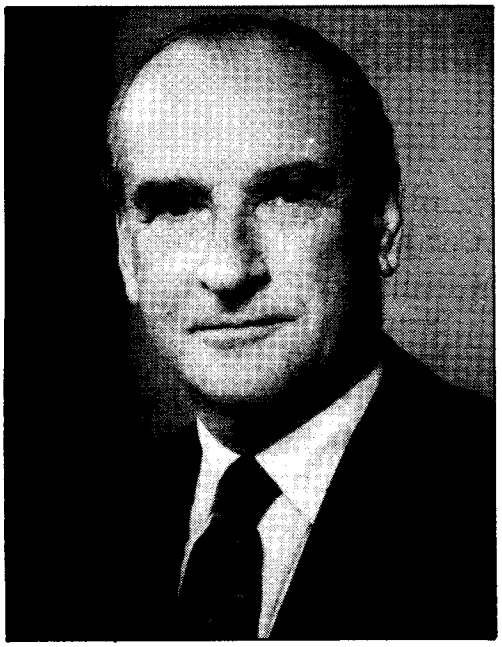


Mr. J.C. Habersberger is a Past President of the Institute and of the Alfred Hospital. Until his retirement in 1976, he was managing Director of Kodak (Australasia) Pty. Ltd.



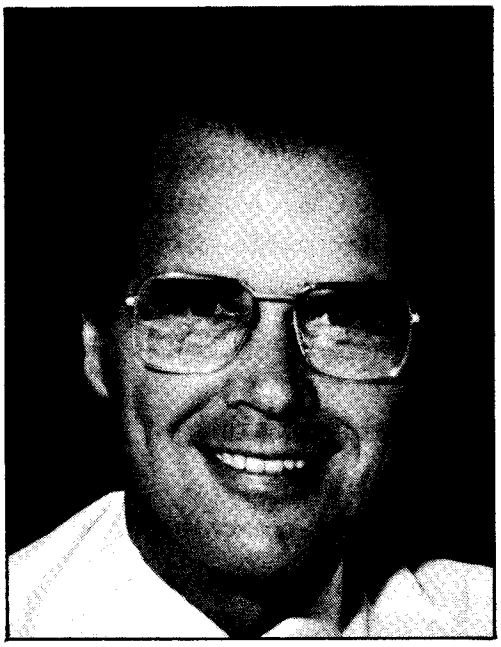
Professor G.C. Schofield is Dean of the Faculty of Medicine of Monash University

Mr. W.D. McPherson is Chairman of the Chamber of Manufacturers Insurance Ltd and a Director of BHP Ltd.



Dr. H.B. Kay is a member of the Alfred Hospital Board of Management.

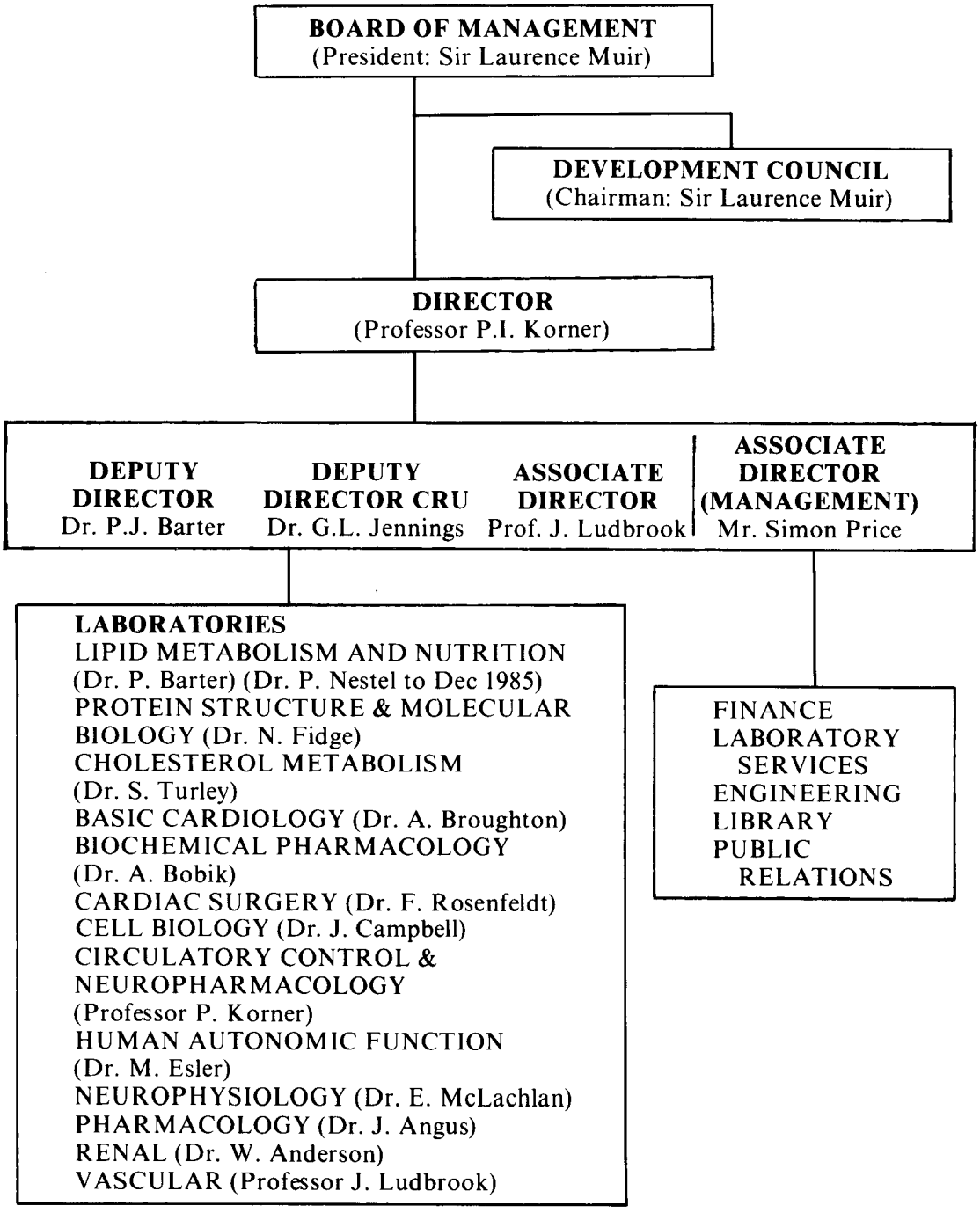
Mr. R.J. Barcham is a Company Director



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

Contents

Organisation Chart



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July 1986)
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F.R.A.C.S.,F.R.C.S.
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Head
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M.B.,B.S.(Syd.),M.D.(Newcastle),
F.F.A.R.A.C.S.,Research Fellow,(till
December 1985)
Dr. Zhu Jia Long, Physiology(Beijing)
Visiting Scientist, (from May 1986)
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Visiting Scientist (from April 1986)
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1986)
Ms. C. Duch (till January 1986)
Miss S. Penrose (from April 1986)
Research Student
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(Melb.),Ph.D.,(till April 1986)

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B.Sc.,M.Sc.(Thailand)
Technical Staff
Ms. K. Walls

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Ms. O. Christie, B.Sc.,M.Sc.(Mon.)

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Dr. E. Laufer, Senior Research Associate
Dr. G. Hasking, M.B.,B.S.,F.R.A.C.P.
Dr. K. Sudhir, M.B.,B.S., Visiting Scientist (from April 1986)
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Ms. E. Dewar, B.Sc.
Ms. M. Kalantzis, B.Sc.(Hons.)
Ms. L. Nelson, B.Sc.
Mr. G. Lambert, B.Sc.

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Mrs. A. MacPherson (till December 1985)
Mr. S. Wood (till January 1986)
Mr. M. Hume-Cook, B.Sc.,(from January 1986)
Mr. A. Gilchrist, B.Sc.(Hons.)(Vic.NZ)
Ms. J. Lineham, B.Sc.(Hons.) (Melb.) (from January 1986)
Mr. L. Colson, B.V.Sc.(from April 1985 to January 1986)

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Ms. S. Smith (till January 1986)
Mr. P. Coles, B.Sc.(Zool.) (Latr.) (from January 1986)
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Technical Staff
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Ms. A-L. Leb (from November 1985)
Ms. K. Pempel (till October 1985)

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Dr. Chen Xiao Zhong, M.B.,B.S.(Shanghai),M.S., Visiting Scientist
Dr. He Guo Wei, M.B.,B.S.(An Hui), M.S., Visiting Scientist
Dr. M. Newman, M.B.,B.S.,F.R.A.C.S.

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Ms. J.D. Rogers
Ms. N. Puglisi

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Mrs. M.J. Black, B.Sc.(Hons.)
Ms. R. Rennick, B.Sc.(Hons.)
Ms. S. Koudounas, B.Sc.(Hons.)
Ms. E. Ng, B.Sc.(Hons.)

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Head
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Dr. J. Pavia, B.V.Sc.(Hons.)

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Sr. J. Jennings, S.R.N. (from March 1986).

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B.Sc.,Ph.D.(Mon.)(till December 1985)

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Ms. A. Hulse (from March 1986)

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Ms. M. Delafield

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Mr. P.J. Griffin (from April 1986)
Ms. J. Pritchard

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Mr. A. Bons, Officer in Charge

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Ms. K. Isherwood (from July 1985)

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ALFRED HOSPITAL - BAKER INSTITUTE

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Dr. A. Broughton, M.B., B.S., Ph.D., F.R.A.C.P., Assistant Physician
Dr. M.D. Esler, M.B., B.S., F.R.A.C.P., Assistant Physician
Dr. A. Lim, M.B., B.S., F.R.A.C.P., Assistant Physician
Dr. P. Weissberg, M.B., B.S., M.R.C.P.(U.K.), Honorary Clinical Assistant

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Dr. G. Jackman, Ph.D.(Syd.), M.Sc., A.R.A.C.I., A.R.C.S.
Dr. P. Little, Ph.D.(Syd.), M.Sc., B.Pharm.

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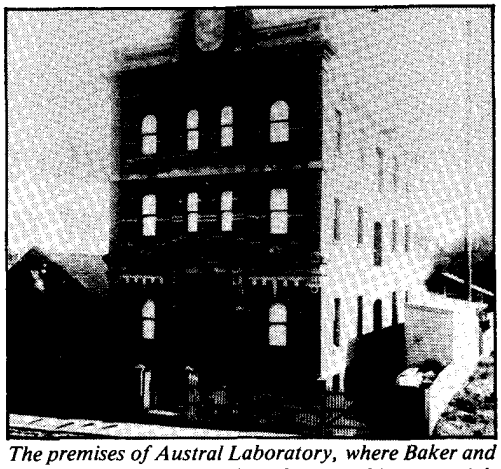
M. Kalantis, B.Sc.(Hons.)
G. Mill, B.Sc.(Hons.)
L. Nelson, B.Sc.
C. Oddie, M.Sc.
H. Skews, B.Sc(Hons.)
S. Timmins, B.Sc.
Bee Ho Teow
S. Scealy, S.R.N.
M. Henderson, S.R.N.
A. Bruce, S.R.N.

Secretary
C. Harwood

History

The Baker Medical Research Institute was founded in 1926 from funds provided by Thomas Baker, his wife Alice and his sister-in-law Eleanor Shaw. Baker was born in England in 1854, and his family emigrated to South Australia in 1865. He became a pharmaceutical chemist and practised in Maryborough, in Queensland, before finally settling in Melbourne in about 1880. There he went into partnership with John J. Rouse in a business for manufacturing photographic materials. The business flourished and later amalgamated with the London Kodak Company, the precursor of today's Kodak (Australasia) Pty Ltd. The Institute has maintained a link with the firm ever since.

One of Baker's friends in Melbourne was John Mackeddie, senior physician and chairman of the Alfred Hospital's medical staff. By the early 1920s Mackeddie had become convinced of the urgent need to establish clinical pathological services in all Australian teaching hospitals. He was keen that the Alfred Hospital should be in the vanguard of this development and persuaded Baker to provide the necessary funds. Mackeddie particularly wished to develop a biochemical laboratory at the hospital (which was important in relation to the newly introduced insulin treatment of diabetics) and to provide other clinical pathological services. However, Baker hoped that more could be accomplished. The Deed of Agreement with the Hospital which founded the "Thomas Baker, Alice Baker and Eleanor Shaw Institute" stipulated that this should also serve as a medical research department.



The premises of Austral Laboratory, where Baker and Rouse established their photographic materials manufacturing.

Thomas Baker died in 1928 soon after the establishment of the Institute. In his will, Thomas Baker established the Baker Benefactions, the trustees of which are independent of the Institute. The Benefactions support several charities through annual grants and have provided unfailing support to the Institute to the maximum of their capacity. In 1986, this support will amount to $715,000, or about 18% of our current expenditure.

Until the end of World War II, the Institute functioned in accordance with Mackeddie's original concept and the service needs of the Alfred Hospital took precedence over research work. However, in 1946, the clinical pathology departments moved into the hospital as autonomous departments and after this the Institute devoted its entire effort to research. Until 1974 this encompassed many fields of medicine but, from 1975 onwards, the Institute has concentrated on two of the major cardiovascular disorders - hypertension and atheroclerosis.

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 has a staff of 130 people. The research is carried out in 13 laboratories and in the Alfred Hospital's Clinical Research Unit.

President's Report

Sir Laurence Muir

In preparing this report on the Institute, I am reminded how much has been achieved since last year. As 1985 opened, the Board had committed itself to upgrade the technology available to our research scientists by launching a capital appeal to raise $2.0 million. Almost all our laboratories needed some replacement of old and obsolete equipment and we urgently needed to complete the establishment of our Protein Chemistry Laboratory. Although our Appeal still has some way to go, with the very generous help of our many supporters, particularly Sir Thomas Ramsay, Mr. Ted Lustig and Mr. Max Moar and their families, the Smorgon Family, Dame Elisabeth Murdoch, The Brockhoff Foundation, Mr & Mrs Bertalli, Esso Australia, Kodak Australasia, ACI Australia, the ANZ Bank and many others, the Institute faces the future with renewed confidence that it will make a significant contribution to the fight against heart attacks and strokes "Before 2000". In our armoury we now have a new JEOL 1200 EX Electron Microscope in the Sir Thomas Ramsay Electron Microscope Suite, a new Immunochemistry facility in the Lustig & Moar Laboratory and our Protein Chemistry laboratory is fully equipped at a cost of $500,000. We have also established the Esso Scholarship scheme which enables us to collaborate with scientists from other parts of Australia and from overseas for brief intensive studies over a few weeks. We need a further $750,000 over the next four years before we can say that we've done what we set out to do - but we are well on the way.

1986 is a milestone in the history of the Institute in as much as on 1st April we celebrated our 60th Anniversary. I think Thomas Baker, who made the original endowment sixty years ago, would be well pleased with what the Institute has achieved.

Without question, the Baker Institute has "arrived" internationally. Our thanks are due to our international advisers Dr. A.C. Barger (Harvard), Dr. R.L. Havel (Cardiovascular Research Institute, San Francisco), Professor Y. Yamori (Shimane University, Japan) and Professor A. Zanchetti (Milan) who have all guided our development. The level of interest in the Institute

from all round the world is almost overwhelming. At the present time we are enjoying the company and collaboration of colleagues from USA, Canada, India, Thailand, China, Japan and the U.K.

This year we are preparing our case for a further Block Institute Grant from the National Health and Medical Research Council. Last year I was critical that our annual funding from NH&MRC had not grown at the rate we anticipated. I particularly wish to acknowledge therefore the extra support for equipment we received in our 1986 funding. The review of our Block Institute grant will take place in 1987 and will include review by an international panel. I am confident that the substantial achievements of the Institute's scientists, which are impressive, will stand us in good stead.

I should like to conclude by thanking my Board, Development Council and Appeal Committee colleagues for their unfailing support and wisdom in the management and development of the Institute. Their energy and guidance is a cornerstone of the Institute and very much appreciated. I should also like to congratulate our Director and his staff who have once again excelled at all they have attempted. Science is about individual excellence and team performance. The Baker Institute team goes from strength to strength.

Directors Report and Overview

Professor P.I. Korner

This year marks the 60th birthday of the Baker Institute and our second decade as an exclusively cardiovascular research centre. At the end of 1985 we bade farewell to Paul Nestel, who was Deputy Director of the Institute for the past 9 years. His work on basic physiology of cholesterol metabolism and on the role of diet in the prevention of coronary artery disease has been outstanding and represents a lasting contribution to knowledge. He left to take up appointment as Chief of the Division of Human Nutrition of the C.S.I.R.O. and a personal Chair in Medicine in the Flinders University in Adelaide. It would be hard to imagine anyone better qualified for the appointment and I wish him every success. Our loss is the nation's gain.

Atherosclerosis and high blood pressure remain the major health problems in Australia and their complications account for 50% of all deaths. Deaths from atherosclerosis account for about 25% of all deaths and there has been a substantial decline in mortality rate from this disorder since it reached its peak in 1968. This has come about through avoidance of various environmental 'risk' factors:- smoking, high dietary cholesterol intake, high blood pressure and obesity. That so much has been achieved is in large measure due to the education of the medical profession and public, by the Australian National Heart Foundation to whom the community is greatly indebted. Yet the mortality (and morbidity) from both the major cardiovascular disorders are still unacceptably high. At the clinical level there are complex interactions between hypertension and atherosclerosis, where a deeper understanding is required. For example, one major problem in relation to heart attack due to atherosclerosis of the large coronary arteries is that 1) hypertension greatly increases the risk of developing heart attacks, but 2) treatment of hypertension with standard blood pressure lowering drugs does not appear to diminish the risk of heart attacks, at least not within the framework of clinical trials that have been conducted so far.

I mention this to indicate that we considered it highly desirable to continue an integrated research programme

in both atherosclerosis and hypertension. We have been most fortunate in attracting Dr. Philip Barter to head the atherosclerosis programme in succession to Paul Nestel. Philip Barter comes from the Department of Clinical Biochemistry of the Flinders Medical Centre. He is a graduate in medicine from the University of Adelaide and has a Ph. D. from the Australian National University. He has just taken up his appointment as Deputy Director of the Baker Institute and his arrival, with a number of colleagues from South Australia, will make our atherosclerosis programme very strong indeed.

We have been equally fortunate that the key senior scientists in charge of the basic aspects of the atherosclerosis research programme have remained at the Institute. They include Dr. Noel Fidge, who is a world leader in lipoprotein biochemistry and in the molecular biology of atherosclerosis and Dr. Julie Campbell, who is similarly distinguished in the cell biology of atherosclerosis. The team also includes Dr. Stephen Turley an expert on the metabolism of cholesterol. The new Protein Chemistry Laboratory with Dr. Boris Grego forms part of the group, though its facilities will be equally important to other groups such as those engaged on examining the role of peptide hormones and transmitters in the regulation of blood pressure.

Dr. Garry Jennings has become Deputy Director of the Clinical Research Unit (CRU),succeeding Paul Nestel in that position. Garry Jennings has led the clinical research in hypertension, which have produced results of great therapeutic significance. In my report about this year's work of the Clinical Research Unit, which is part of Alfred Hospital, I have drawn attention to the problems facing the Victorian public hospital system at the present time. It is greatly to the credit of Garry Jennings and Murray Esler that the hypertension research programme has continuted at full steam. Alex Bobik, who heads Biochemical Pharmacology has become Associate Director (CRU Laboratories) and provides a tremendous resource to virtually every laboratory in the Institute, quite apart from the excellence of his own research programme.

We have abolished the three main research units within the Institute (Hypertension, Atherosclerosis and Cardiovascular Surgery) and believe that this will allow us to tackle the major scientific problems in a more closely integrated manner. Our research aims remain unchanged. Over the last 1-2 years there has been so much collaborative work initiated by individual scientists in the different disciplines to help them to solve problems of common interest. This is one of the real advantages of a multidisciplinary research institute. It seemed best to avoid 'overdepartmentalisation' by making the existing major research units larger or creating new units. The *different laboratories* are now the major scientific units. This provides a more integrated approach to problem solving and better interaction between the hypertension and atherosclerosis research programmes. Parenthetically, the 'chiefs' of all the 12 main laboratories of the Institute have all become international figures in their own right and are enthusiastic about the new more integrated organisational structure.

This year 15 scientists from Britain, Canada, China, France, Germany, India, Japan, the United States of America and Thailand have worked at the Baker Institute for periods ranging from 3 months to 2 years. This is a greater number of visiting scientists than we have ever had before. I am happy to report that we also have visiting

scientists from other Australian States outside Victoria. With the help of Esso (Australia) we have started a national research programme, which brings interstate scientists to the Institute for periods of 2- 3 months. In this way the Baker Institute will come to be regarded as a national resource for collaborative cardiovascular research.

Research Overview

We have made good progress with our new strategy for investigating and treating hypertension, with the work on the high density lipoprotein (HDL) receptor in atherosclerosis and with our extensive effort related to the sympathetic nervous system. I will refer briefy to the work of our two pharmacology groups on the role of the endothelium on arterial reactivity and our effort to develop a new cardiac stimulant. (For the other interesting projects the reader should refer to the scientific reports of the different laboratories.)

Hypertension

From studies of chronic experimental hypertension due to narrowing of the renal artery in dogs, Warwick Anderson and I found that the basic cause accounted for only about 25% of the rise in blood pressure. The remaining 75% of the hypertension was due to amplification of all stimuli resulting from the enlargement of the muscle coat of the arteries and heart. The enlargement occurs as a result of the extra work of contraction against the high blood pressure. Such muscle enlargement and amplification of stimuli is present also in human primary hypertension, where the basic cause of the hypertension is still unknown. Indeed, the magnitude of the amplifier properties complicates our ability to detect the basic cause. With this in mind, Garry Jennings and colleagues examined whether in human hypertension the enlargement of the muscle coat could be reversed, by lowering blood pressure by standard drug treatment. It took about 2 years of treatment to produce adequate reversal of muscle enlargement and the greater the drug-induced reversal the slower the subsequent redevelopment of hypertension when medication was stopped. We believe that the slow redevelopment of hypertension at this time simulates the development of hypertension earlier in life.

By good fortune Garry Jennings, Murray Esler and Paul Nestel were engaged in an investigation to see whether there was a physiological basis for the epidemiological finding that habitual activity improved the outlook for not developing heart disease. We found, unexpectedly, that in normal subjects exercise produced *longterm* lowering of the blood pressure. We have now shown that such a longterm fall in blood pressure also occurs in patients with hypertension. In addition, in about 40% of patients with initially severe hypertension normal blood pressure has been maintained for over 1 year without further drugs. By a programme of 3 times per week exercise, about 75% of patients with previously untreated mild-moderate hypertension were maintained at normal pressures for over a year, without requiring initial drug treatment. Murray Esler had discovered that exercise acts by switching off the activity of sympathetic nervous system to the heart and blood vessels.

We have also begun a new programme to try to detect the causes of

human primary hypertension, by looking for various 'faults' in the blood pressure regulating machinery before and after reversal of muscle enlargement. In parallel with the work in human patients, Alex Bobik, Mike Adams and Jim Angus are studying the spontaneously hypertensive rat (SHR) where some of our hypotheses can be examined more rigorously. We are greatly indebted to Professor Yukio Yamori, of the Shimane Medical School in Japan, for generously supplying us with these animals with genetic hypertension, that were first bred in Japan. We are now breeding a colony of our own at the Baker Institute.

Atherosclerosis

Cholesterol is a normal constituent of all body cells, but when excessively high in the blood plasma it becomes an important *risk factor* for developing atherosclerosis. The mechanisms that are important in the transport of cholesterol in and out of cells have become a matter of great interest from the viewpoint of causation of coronary artery disease. Cholesterol is carried in the plasma attached to special proteins called lipoproteins, which maintain it in soluble form. One type of lipoprotein carrying cholesterol is low density lipoprotein (LDL). When the LDL cholesterol in plasma is high the chances of developing atherosclerosis increases greatly. The reason for high LDL plasma cholesterol is a genetic deficiency (and occasionally complete absence) of a special protein in the cell membrane called the LDL receptor. This was discovered by Brown and Goldstein in the United States, who were awarded the 1985 Nobel Prize in Medicine for this work. These important findings have recently led to development of new drugs for the treatment of atherosclerosis, that can suppress cholesterol synthesis, resulting in a 'compensatory' increase in LDL receptor numbers and a reduction in plasma cholesterol.

Noel Fidge (Protein Structure and Molecular Biology Laboratory) has been working with another type of 'cholesterol' receptor called the high density lipoprotein (HDL) receptor. This helps to take cholesterol *out* of cells, a process termed reverse cholesterol transport. If this process could be accelerated in patients with atherosclerosis it would help to diminish the size of cholesterol-containing plaques in the arteries. Noel Fidge's laboratory currently leads the international race to purify the receptor protein and define its chemical structure. Moreover, Philip Barter has discovered a new protein that is important in the transfer of cholesterol from the HDL receptor to plasma and in the regulation of receptor numbers. The capacity to manipulate receptor regulation may complement current therapeutic approaches related to the LDL receptor and provide an additional form of useful medical treatment.

Other work by Stephen Turley addresses the relationship between overweight and alterations in metabolism of cholesterol and triglycerides in genetically obese rats. However, cholesterol is by no means the only factor in the atherosclerotic plaque. Recent work by Julie Campbell (Cell Biology) indicates that an inflammatory scavenger cell normally present in blood and tissues, called the macrophage, plays an important role in plaque formation. It secretes a chemical substance which acts locally on the smooth muscle cell of the artery wall and causes it to migrate next to the endothelium which lines the artery. The macrophage changes the way in which the muscle cell utilizes fat. These repre-

sent other potential targets for therapeutic intervention.

Sympathetic Nervous System

The sympathetic nerves innervate the heart and blood vessels and play a key role in the regulation of blood pressure. About half of our laboratories have projects related to sympathetic function. Thanks to the recent efforts of Colin Anderson, Elspeth McLachlan, Alex Bobik and John Cassel a large range of immuno-histochemical techniques are now available for mapping the pathways of the different types of nerve cells in the central nervous system (CNS). A new technique for direct recording of sympathetic nerve activity in the conscious animal has been introduced by Pat Dorward and has provided us with new insights about the role of pressure-sensitive receptors from the heart in circulatory regulation. Geoff Head, Emilio Badoer and I have developed new methods for studying the role of the central catecholamine-releasing nerve cells. Together with Alex Bobik and colleagues we have investigated the sites of action in the CNS involved in the blood pressure and heart rate-lowering actions of the anti-hypertensive drug alpha-methyldopa.

John Ludbrook has investigated the short-term mechanisms of reflex circulatory regulation in exercise, particularly those leading to the initial suppression of the arterial baroreceptor reflex. He and Peter Rutter have shown that in haemorrhage, when blood loss exceeds about 20-25% of the blood volume, there is release of morphine-like compounds near the sympathetic nerve endings which leads to failure of transmission in these nerves, a phenomenon which July Oliver and I had previously demonstrated. The sympathetic failure is antagonised by the morphine antagonist naloxone, which may become useful in the treatment of shock.

Studies in humans by Murray Esler and colleagues have shown that sympathetic nerve activity is increased in only about 20% of patients. A pointer that this may be so comes from the demonstration that there is impairment of the noradrenaline re-uptake pump at the nerve endings. This type of membrane 'fault' cannot be readily compensated through the normal CNS regulating system and could be cause of the hypertension in this subset of patients.

Murray Esler has developed a new method for assessing sympathetic nerve function in man using a pharmacokinetic approach. Together with Greg Hasking he has investigated patients with heart failure and shown that in the absence of treatment sympathetic activity to the heart is almost maximal, which is responsible for the limitation of their performance during exercise. In collaboration with Ian Willett he has measured sympathetic activity in patients with severe cirrhosis of the liver. The latter have very high *venous* pressures in their gastro-intestinal veins, and gastro-intestinal bleeding from ruptured veins is one of the common causes of death in this disorder. Pressure can be reduced by lowering sympathetic activity with the drug clonidine, which will improve the outlook from this complication and may prolong life.

Pharmacology

James Angus, Tom Cocks and Julie Campbell have extended their work on the role of the endothelium (a single cell layer lining the inside of all blood vessels) in affecting the reactivity of large arteries. The endothelium releases

a chemical substance called endothelium-dependent relaxing factor -EDRF. When the endothelium is removed experimentally from a localised section of the artery and can no longer release EDRF, then many substances that previously caused the vessel to dilate, now cause it to constrict through this mechanism. Serotonin (which is released from blood platelets) can produce spasm of the coronary arteries when EDRF is absent due to local endothelial damage. By growing the endothelial cells in tissue culture EDRF can be released under highly controlled conditions. Jim Angus and Tom Cocks have developed a new type of bioassay and have shown that EDRF has a half-life of about 45 seconds. Watch this column in 1987 for further details about its composition!

The only good longterm cardiac stimulant drug available to-day for the treatment of heart failure is digitalis, which was discovered by William Withering about 200 years ago. It has the disadvantage that it is a relatively non-specific stimulant and also causes arterial constriction, which dissipates some of its beneficial effects on the heart. Recently numerous drugs were developed by the pharmaceutical industry that would stimulate the beta adrenoceptors of the heart. Garry Jennings showed in a series of clinical studies that these drugs were excellent short-term cardiac stimulants, but rapidly became ineffective in longterm use. The reason for this became apparent from tissue culture experiments performed by Alex Bobik, who found that this type of stimulant produced a decrease in beta receptor numbers. Subsequently, Alex Bobik and Graham Jackman in collaboration with colleagues from the Melbourne University Chemistry Department, are developing a new type of stimulant that by-passes the beta receptor in the heart muscle surface membrane, but stimulates the muscle through its action on an enzyme called adenylate cyclase. The stimulant substance is an analogue of forskolin (a natural compound in plants) which will specifically stimulate the enzyme present in heart muscle, but not in arteries.

Before 2000 Heart Appeal

The Before 2000 Heart Appeal was launched in January 1985 with the intention of raising $2.0 million to replace some major items of scientific equipment, to establish a protein chemistry laboratory which required a small number of very expensive equipment items, to upgrade the level of technology available in most laboratories to "state of the art" and to establish a Research Fellowship Programme.

The Appeal is a five year project. In the first year over $430,000 was actually received and promises of twice that amount were obtained. The injection of such a large amount of money, over and above other fundraising income has had a profound effect on the Institute. By mid 1986 our antiquated electron microscope was replaced. The protein chemistry laboratory is now fully equipped and we have been fortunate in recruiting Dr. Boris Grego to run it. Extensive redesigning of the Electron Microscope Suite has been carried out and modifications have been carried out to other laboratories. The Esso company has funded a scheme to allow us to undertake exchange fellowships with cardiovascular scientists from other parts of Australia and from overseas. The initial objectives of the Appeal have already been achieved. The major further objective is to turn the many promises of support into cash. If this can be achieved, there is every reason to believe that the Appeal will reach its target well ahead of time. The Appeal Committee, (Sir Laurence Muir, Mr. R. Pratt, Mr. R. Ansett, Mr. M. Downes, Mr. B. Pascoe) was enthusiastically supported by Mrs F. Verlinden. The Institute is much indebted to this group for their dedication in establishing the appeal headquarters and their energy in raising the funds so vitally needed for the Institute's development.

New Electron Microscope

Sir Thomas Ramsay generously donated $200,000 to the Baker Institute to purchase a new electron microscope. The existing microscope (Hitachi HU11E) is now almost 15 years old. The normal life span of an electron microscope is 10 years. The Hitachi has limited our ability to study the structure of lipoprotein particles as it has not been possible to use it at very high magnifications (x100,000). Given this opportunity to upgrade the electron microscope facility, we decided to buy a Joel 1200 EX. This instrument is one of the most up to date of the currently made machines having available all "state of the art" attachments. The Joel 1200 EX has several new features. One of these will be a 'ZOOM' magnification lens system as opposed to a step- wise change in magnification. Another feature will be that the image will not rotate as the magnification is changed. In older microscopes the image would suddenly rotate through up to 180 degrees at a certain change in magnification. In addition, there will be a computerised system for checking the critical alignment of the microscope and a memory for up to ten specimen locations. This means that the operator will be able to mark the X-Y co-ordinates of a particular organelle so that it is possible to move freely over the rest of the sections of the grid and be able to return directly to the marked object. For photography, there will be both a large format film camera and a 35mm camera system. With the 1200 EX it will also be possible to examine thicker sections than conventionally used because the accelerating voltage can be increased up to 1200KV. By rotating and tilting the specimen (an additional feature), three dimensional images can be obtained by viewing pairs of photographs taken at different angles with a stereo viewer.

It is envisaged that the installation of our new Electron Microscope will be completed by mid 1986. At present there are eight laboratories whose research will involve the use of the new electron microscope, these are -

The Cell Biology Laboratory for analysing fine cellular constituents of the arterial wall involved in the formation of atheromatous plaques

The Biochemical Laboratories of the Cardiovascular Metabolism and Nutrition Research Unit for identifying the shape of various subcellular lipoprotein - containing particles and their passage and localization within the cell.

Neurophysiology Laboratory for identifying the structural basis of the transmission of impulses from sympathetic nerves to the smooth muscle of blood vessels

Circulatory Control and Neuropharmacology for studying the structure of nerve cells within the brain involved in cardiovascular control

Renal Laboratory for studying effects of the hormone angiotensin II on subcellular components of the vessels and filtering units of the kidney

Basic Cardiology for studying the relationship of damage in enlarged muscle cells in chronic experimental hypertension to the architecture of the capillaries

Experimental Cardiac Surgery to help develop methods of cooling and preserving the heart during open heart surgery, that will do least damage to the heart muscle

Pharmacology for studying the role of endothelium in vascular reactivity in relation to the development of coronary artery spasm

Esso Fellowships

Scientists from South Australia, West Australia and Germany were the first recipients of Esso Medical Research Scholarships to study heart disease at the Baker Medical Research Institute, Melbourne.

The three, all established medical research scientists in their own right, were funded by Esso to undertake brief intensive collaborative research projects at the Institute.

Dr. Peter Howe, normally based at the CSIRO, Division of Human Nutrition in Adelaide collaborated with Professor Paul Korner, Director of the Baker Institute and Dr. Geoff Head in a project to investigate whether adrenaline cells located in the base of the brain are the driving force of the sympathetic nervous system.

Dr. Rod Dilley from the Department of Anatomy and Embryology at the University of Western Australia taught his experimental procedure for vein grafting into the aorta to Baker Institute staff and undertook a collaborative project with Drs Julie and Gordon Campbell on the changes in biology of smooth muscle cells in hypertension.

Professor Wilfred Jänig from the Physiology Institute, University of Kiel in Germany worked with Dr. Elspeth McLachlan on a project to find out how nerve cells that regulate constriction of the blood vessels (and thus blood pressure) differ in their behaviour from the nerve cells which control, for example, the bladder and the gut.

Protein Chemistry Laboratory

The establishment of a protein chemistry laboratory is nearing completion with Dr. Boris Grego joining the Baker Staff (April 1986) and the installation of a protein sequencer (May 1986) and a high performance liquid chromatograph, or HPLC (June 1986). The protein sequencer can determine the linear sequence of amino acids in purified peptides and proteins of levels down to 100 picomoles. This corresponds to 5 micrograms of a protein of 50,000 molecular weight. Obtaining reliable amino-acid sequence information is dependent on being able to purify and handle such small quantities of protein by techniques involving HPLC.

With the installation of a peptide synthesizer (August 1986) it will be possible to synthesize peptides for: (a) biology testing; (b) structure-function studies based on data generated by the protein sequencer; (c) raising specific antisera. Synthetic peptides will be purified by HPLC prior to use and analysed for correct composition either by the amino-acid analyser or protein sequencer.

The facilities above will enable, for example, the determination of the structure of the HDL binding protein and delineating those portions of the molecule important for its function in cholesterol metabolism. We also hope to commence sequencing the HDL "conversion protein" recently purified in Dr. Barter's laboratory and possibly the lipid transfer protein in collaboration with Dr. Dennis Calvert, Flinders University of South Australia.

Heart Risk Assessment Clinic

The Heart Risk Assessment Clinic now operates five days a week (previously three) and is free of charge.

Members of the public are invited to attend the clinic for assessment of their cardiovascular health. Weight, smoking, eating, drinking and exercise habits as well as hereditary factors are all taken into account in assessing the level of risk of heart attack or stroke in individual patients. Clinical tests include blood pressure and blood fats readings. Patients who have a need to be concerned about their cardiovascular health are offered dietary or other appropriate medical advice or treatment according to circumstances.

For appointment telephone 522 4333. An appointment is essential.

Visiting Scientists

The Seventh International Conference on Atherosclerosis held in Melbourne, October 6-10, 1985 gave us a welcome opportunity to host a number of visitors to the Baker Institute.

Professor Robert W. Wissler, the Donald N. Pritzler Distinguished Service Professor of Pathology at the University of Chicago, spent 10 weeks with Dr. Julie Campbell working on specialized antibody staining techniques for distinguishing smooth muscle cells of different phenotype from non-muscle cells as part of a world-wide programme on "Pathobiological Determinants of Atherosclerosis in Youth".

Professor Nome Baker from California took the opportunity of being in Melbourne again to collaborate with Dr. Nestel during November on the kinetic modelling of lipoprotein metabolism.

Professor Julian Marsh from the Medical College of Pennsylvania, Philadelphia spent six weeks working with the Cardiovascular Metabolism and Nutrition Research Unit. He studied changes in various enzyme reactions associated with the triglyceride-lowering action of fish oil.

Dr. Murray Huff from the University of Western Ontario, London, spent four months in the Unit collaborating in studies on the metabolism of triglyceride-rich lipoproteins by macrophages. Murray previously spent some time in the Unit with Dr. Nestel on a Canadian Postdoctoral Research Fellowship.

Other visitors:

Dr. Michael Adams from the Department of Physiology, University of Western Ontario, has recently joined Dr. Bobik's Biochemical Pharmacology Laboratory on a two-year Postdoctoral Fellowship from the Canadian MRC to study the role of the autonomic nervous system in the development of hypertension.

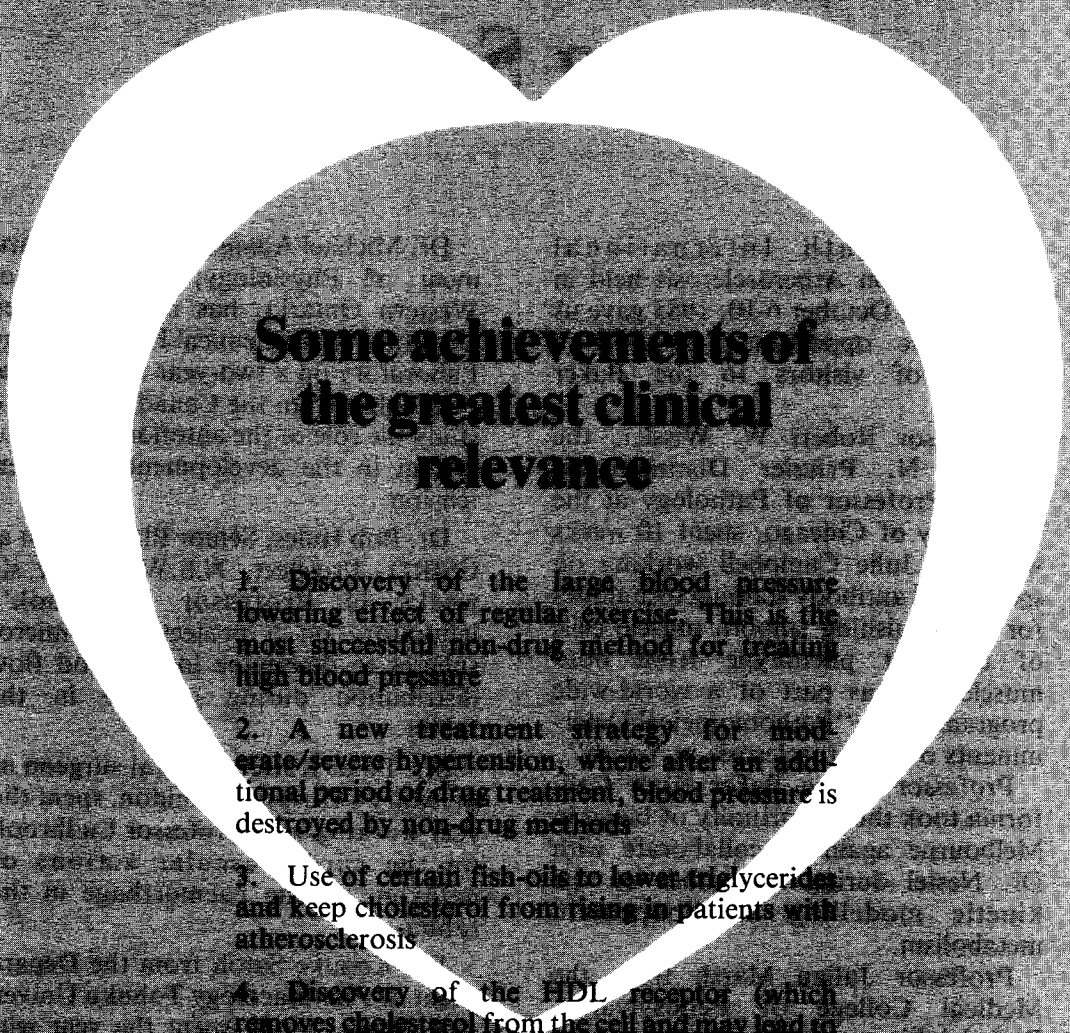
Dr. Bob Hales, Senior Physiologist at CSIRO, Prospect, N.S.W., spent six weeks in Professor Ludbrook's laboratory on a project with microspheres to determine local blood flow distribution during exercise in the rabbit.

Mr. Peter Rutter, general surgeon at St. Mary's Hospital, London, spent the year working with Professor Ludbrook on the cardiovascular actions of naloxone during haemorrhage in the rabbit.

Dr. Keisuke Satoh from the Department of Pharmacology Tohuku University, Sendi, Japan spent the year with Dr. Jim Angus and Dr. Tom Cocks in the Pharmacology Laboratory working on the classification of receptors on endothelial cells.

Dr. Peter Weissberg, a lecturer in Cardiovascular Medicine at the University of Birmingham, joined Dr. Broughton's and Dr. Bobik's laboratories as an MRC travelling fellow. He is studying the control of intracellular pH.

Dr. Chen Xiou Zhong from Shanghai Chest Hospital, China joined the Cardiac Surgery Laboratory in February 1985, to work towards an M.D. degree on atrial preservation and postoperative arrhythmias.



Some achievements of the greatest clinical relevance

1. Discovery of the large blood pressure lowering effect of regular exercise. This is the most successful non-drug method for treating high blood pressure

2. A new treatment strategy for moderate/severe hypertension, where after an additional period of drug treatment, blood pressure is destroyed by non-drug methods

3. Use of certain fish-oils to lower triglycerides and keep cholesterol from rising in patients with atherosclerosis

4. Discovery of the HDL receptor (which removes cholesterol from the cell and may lead to better treatment of established atherosclerosis)

5. A better understanding of the causation of arterial spasm underlying certain types of heart attack

6. Development of a new device for optimal cooling of the heart in open-heart surgery

7. Discovery of a new method for measuring sympathetic nerve function in humans. Apart from its importance in hypertension, a by-product of this work has been improved treatment in patients with liver disease

8. Establishment of a cardiovascular RISK DETECTION AND REDUCTION CLINIC to

Laboratory Reports

Atherosclerosis Unit

Lipid Metabolism and Nutrition Laboratory

Head: Dr. P. Nestel
(till December 1985)

Protein Structure and Molecular Biology Laboratory

Head: Dr. N. Fidge

Cholesterol Metabolism Laboratory

Head: Dr. S. Turley

Over the past 9 years Dr. Paul Nestel provided strong leadership for the unit, named Cardiovascular Metabolism and Nutrition Research Unit. The work focused on community prevention of atherosclerosis by lowering blood cholesterol and triglycerides through dietary measures. Last year Dr. Nestel resigned his position to take up appointment of Chief of the Division of Human Nutrition of the Commonwealth Scientific and Industrial Research Organisation in Adelaide. We will miss him at the Baker Institute, but it is good that someone of his scientific eminence will shape national awareness of the role of nutrition in maintaining good health.

His place as Deputy Director of the Institute has been taken by Dr. Philip Barter, who has a distinguished record in lipoprotein and atherosclerosis research. The change in leadership has resulted in some re-organisation. The cohesiveness of the molecular biology and physiology and cell biology groups will, of course, be maintained. In addition, we hope that the molecular biology facility and the new protein chemistry laboratory will join in collaborative projects outside the atherosclerosis field.

The most significant development that has occurred has been the greater use of molecular biology techniques in understanding the structure and regulation of lipoproteins. Dr. Noel Fidge, who has led the effort from the Baker Institute has engaged in a fruitful collaborative programme with several molecular biologists from the University of Melbourne's Biochemistry Department. In collaboration with this group many successful cDNA clones and a number of monoclonal antibodies have been produced to help determine the characteristics and role of some of the cellular receptors important in fat transport. Dr. Fidge and Dr. Mariko Nagashima, a young molecular biologist recently appointed to the Institute, have begun a programme to determine some of the factors important in apolipoprotein synthesis using recombinant DNA and hybridisation techniques. We have now assembled some of the basic equipment of the new Protein Chemistry Laboratory (amino acid analyzer and sequencer and peptide synthesizer) and have appointed Dr. Boris Grego to help run the new facility.

Summary

The use of molecular biological tools has already been referred to for determining lipoprotein structure and function and for studyng interactions between lipoproteins and cells of the

arterial wall. Studies have begun to determine factors which regulate apolipoprotein synthesis by recombinant DNA and hybridisation technology.

We have used cell culture methods to provide information about the metabolic mechanisms by which macrophages accumulate large amounts of fat in the arterial wall. The receptor mechanisms responsible for lipid accumulation by these cells have been investigated by Dr. Murray Huff, a visiting scientist from Canada, during a 3 months stay at the Institute. Another visitor, Dr. Julian Marsh, (Philadelphia, USA) spent several months in Paul Nestel's laboratory investigating some of the biochemical pathways of fatty acid esterification, to help explain the unusual effect produced by highly unsaturated fatty acids in fish oil in lowering triglyceride levels in plasma.

Dr. Fidge and colleagues have continued work on the identification and partial purification of one of the very important lipoprotein receptors - the HDL receptor. This receptor plays an important role in the transport of cholesterol out of cells, including those of the arterial wall. Facilitating this movement may provide a new approach to the treatment of established atherosclerosis. The purification of the HDL binding protein and a knowledge of its structure is the first step to understanding the normal regulatory mechanisms and whether it is possible to put it to use in treatment.

Coronary heart disease, gallstones and insulin-independent diabetes are frequently associated with obesity (fatness) in humans. Overweight subjects are more prone to develop heart disease partly because of their high plasma cholesterol and triglyceride levels. There have been significant advances in understanding the biochemistry of hypertriglyceridemia in fat subjects, but we still do not understand why they have an elevated plasma cholesterol and secrete cholesterol into bile at a fast rate. Since the liver is a major site of cholesterol metabolism, changes in cholesterol synthesis or conversion to bile acids could alter the balance of cholesterol in the body. In recent years certain strains of genetically obese animals have been developed for investigating cholesterol metabolism under conditions when the liver is enlarged and its fat content increased. One of these strains is the SHR/N-corpulent rat, which develops obesity, insulin-independent diabetes and elevated plasma cholesterol and triglyceride levels. We are investigating the mechanisms underlying the disorders of cholesterol metabolism in these animals.

LIPID METABOLISM AND NUTRITION LABORATORY

Head: Dr. P. J. Nestel

Projects

Fish oil fatty acids - clinical nutrition.

Fish oil fatty acids - metabolism in liver.

Impaired lipolysis as a cause of chylomicronaemia.

The macrophage and foam cell formation.

Modelling very low density lipoprotein metabolism in man.

Fish oil fatty acids - clinical nutrition

P. Nestel

We have continued to evaluate the clinical usefulness of these unusual, highly polyunsaturated fatty acids. Our initial findings were that plasma triglycerides were lowered to a remarkable degree but that the effect on the plasma cholesterol concentration was minor. This aspect was re-examined in another way: the potential of fish oil to modify the cholesterol - raising capacity of dietary cholesterol was studied in normal individuals. Egg yolk cholesterol was eaten by the test subjects with or without fish oil for periods of several weeks. During a third period, fish oil was consumed within a low-cholesterol diet. Egg yolk cholesterol failed to raise low density lipoprotein cholesterol levels when fish oil provided the bulk of the dietary fat. Marine fatty acids can, therefore, limit elevation of plasma cholesterol, apart from lowering plasma triglyceride concentration.

Fish oil fatty acids - metabolism in liver

P. Nestel, S. Wong, J. Marsh, L. Faehse

Since we had found that the striking triglyceride lowering effect of marine fatty acids was related to events in the liver, we have pursued these studies with isolated liver cell lines, both rodent and human.

The human cell line Hep G2 was used to test the effects of fatty acids on the production and secretion of triglyceride and of apoprotein B, the structural protein of very low density lipoprotein (VLDL). We found that, as the fatty acids became longer and more unsaturated, the formation of triglyceride and apoB was reduced. Thus, oleic acid, which has one double bond and 18 carbon atoms, showed the highest stimulation, whereas docosahexaenoic acid (found in fish oil), which has six double bonds and 232 carbon atoms, led to virtually no triglyceride and apoB production. Other fatty acids of 18-20 carbons and 2-4 double bonds showed intermediate effects.

Our previous studies had indicated that fatty acids such as docosahexaenoic acid inhibit fatty acid synthesis and stimulate fatty acid oxidation in rat liver. However, additional effects were likely, including inhibition of enzymes of fatty acid esterification (glyceride formation). Professor Marsh from Philadelphia has undertaken the assays of the activities of key enzymes of fatty acid esterification from livers exposed to the relevant fatty acids.

Impaired lipolysis as a cause of chylomicronaemia

P. Nestel, T. Billington

We had previously found in subjects with severe hyperlipidaemia causing accumulation of chylomicrons in plasma, that the large chylomicron remnants were being removed more slowly than the VLDL remnants. This was established by comparing the removal rates of the two apoproteins, apo B48 and apo B100, which are specific for chylomicrons and for VLDL respectively. One possibility was that the chylomicrons were not as susceptible to lipolysis by lipases as were the VLDL. This has been tested by measuring the rates of removal of the two lipoproteins (labelled and reinjected), before and after lipolysis was induced with heparin. The hypothesis was confirmed in that only the VLDL removal was accelerated.

Interestingly, chylomicron removal in these patients could not be

significantly stimulated. The lipase normally associates with these particles and may then participate in the specific receptor-mediated uptake of the remnant in the liver. We do not know whether the abnormality resides in the lipoprotein structure or in the receptor.

The macrophage and foam cell formation

P. Nestel, M. Huff, T. Billington, B. Meyer, P. Nugent

The circulating monocyte has the capacity to take up lipoprotein lipid, become attracted to arterial endothelium, migrate into the artery wall and there, by taking up further lipid, become the foam cell of atheroma. We have previously shown that large triglyceride-rich lipoproteins interact with specific receptors on the macrophage surface and that the ligand is apoprotein E.

This class of lipoproteins includes a wide range of particles, including chylomicron remnants derived from dietary fat, and VLDL remnants secreted from the liver. Dr. Huff, on a repeat visit from Canada, has played an important role in this study. We have aimed to define which particle of the triglyceride-rich lipoproteins consistently loads the macrophage with excess cholesterol. Surprisingly only chylomicron remnants showed this potential, whether obtained from patients with pathological chylomicronaemia or from normal subjects after a fatty meal. It is possible that macrophages possess a receptor analogous to the chylomicron receptor, believed to occur only in the liver. This hepatic receptor is also not down-regulated through the uptake of cholesterol in the chylomicron remnant. Macrophages are therefore susceptible to cholesterol overload and transformation to foam cells.

Modelling VLDL metabolism in man

H. Barrett, N. Baker, P. Nestel

The transport of VLDL triglyceride and apoprotein B from the liver, through the various catabolic pathways in plasma, can be defined through multicompartmental modelling of labelled VLDL. We have completed a series of studies in which triglyceride metabolism was studied after injecting ^{3}H-labelled glycerol and apo B after reinjecting ^{125}I-VLDL.

Our model of VLDL catabolism differs from simpler models previously reported, one reason being our experimental design. VLDL comprise a heterogeneous group of particles which include a cascade of precursor and product lipoproteins. By analyzing the kinetics of these several lipoproteins individually, but simultaneously, we have recognised the highly complex nature of overall VLDL metabolism. The new model has been developed in collaboration with Professor Baker from California, who worked here in 1984 and who has now been on his second visit.

PROTEIN STRUCTURE AND MOLECULAR BIOLOGY LABORATORY

Head: Dr. Noel Fidge

Projects

High density lipoprotein receptor.

The receptor-binding domain of human HDL apolipoproteins.

Monoclonal antibodies to apolipoproteins.

Molecular biology of apolipoproteins.

a) The amino acid sequence of rat apolipoprotein AII deduced from the nucleotide sequence of cloned cDNA.

b) The influence of hormones and nutrients on levels of mRNA for liver apolipoprotein.

c) Exploitation of E-coli produced apolipoprotein for producing monospecific antisera.

High density lipoprotein receptor

N. Fidge, H. Edelsbacher and P. Vadiveloo

Unlike LDL, which primarily donates cholesterol to cells, HDL is probably involved in reverse cholesterol transport (removal of cellular cholesterol) from some tissues, delivery of sterol to others (eg. steroidogenic tissues) and both removal from, and delivery of cholesterol to the liver. Thus, the processing of HDL may involve different mechanisms for different cells. Some insight into these mechanisms may be gained by studies at the molecular level of the ligand-membrane interaction. Our work is designed to provide information on the nature and tissue distribution of the binding protein in plasma membranes obtained from rat liver, kidney and adrenals.

A significant breakthrough was achieved when we identified an HDL binding protein firstly in adrenal cortical membranes and later in the kidney and liver. Following separation of solubilised membrane proteins on polyacrylamide gel slabs and 'Western' blotting, one major band was identified which bound HDL_3, or apo AI or apo AII. The protein, which was present in both liver and kidney membranes, was partially purified by repetitive preparative SDS-polyacrylamide gel electrophoresis and although accompanied by considerable loss of binding activity, could still be detected by the ligand-blotting procedure used initially to detect its presence in cell membranes. The product contains one major protein (of M.W. approx 75,000) in addition to two minor bands of higher M.W. Because treatment with mercaptoethanol reduced the concentration of the higher M.W. bands it is possible that they are disulfide linked aggregates of the major protein.

The protein has been transferred to nitrocellulose sheets and antisera to it has been successfully produced in rabbits. This provides another tool to help in the rapid purification and characterisation of this putative HDL receptor.

The receptor-binding domain of human HDL apolipoproteins

N. Fidge, J. Morrison and E. Gruner

To investigate the specific regions of the apolipoproteins of HDL which interact with the HDL receptor, preliminary studies have been undertaken using fragments of AI produced by chemical cleavage (cyanogen bromide) or following enzymatic cleavage (trypsin). At least four major fragments representing N-terminal, middle region and C-terminal sequences were produced by chemical cleavage, which were then crudely separated by gel filtration techniques. Each fragment was labelled with ^{125}I and assembled with phospholipid, into artificial "lipoproteins". The initial experiments showed that only the N-terminal fragment interacted with adrenocortical cells with characteristics of specific binding, which suggests this region may be recognised by the receptor. Difficulty in producing 'clean' fragments will

be overcome by HPLC separation techniques which should focus on the narrower sequence involved in binding. Because apo AII also binds to cells, similar studies will be performed using AII fragments to allow a comparison between the two apolipoproteins and receptor interaction.

Monoclonal antibodies to apolipoproteins

N. Fidge, M. Gordon, E. Gruner and H. Edelsbacher

To investigate the potential of monoclonal antibodies as probes for determining the receptor binding domain of apolipoproteins, a number of antibodies to apo AI have been produced by hybridoma technology. Several dozen antibodies have also been produced to human apo B. The AI antibodies will be tested to determine if any of them inhibit binding of HDL or AI apolipoprotein to HDL binding sites. In conjunction with studies involving cleaved fragments of apo AI or apo AII (see above) it may be possible to select antibodies which uniquely interact with only one fragment, which will both confirm or assist in interpretating the sequences involved in receptor binding.

The apo B monoclonal antibodies will be used to focus attention on the region of apo B recognised by the B/E receptor. We are using apoB monoclonals to probe the interaction of triglyceride rich lipoproteins with cells; three antibodies have been found which inhibit the binding of VLDL, but not LDL to cells while another two inhibit LDL but not VLDL binding. This suggests that the receptor domain of apoB is "masked" in some lipoproteins and becomes exposed during catabolism, or else the apolipoprotein undergoes conformational change during the process of transformation. These studies highlight the potential use of McAbs to study the modelling and remodelling of lipoproteins in relation to physiological function.

Molecular biology of apolipoproteins

Our group has closely collaborated with Drs. Geoff Howlett and Gerhard Schreiber from the Biochemistry Department, Melbourne University in studies designed to throw light on the major factors which regulate lipoprotein metabolism at the cellular level. This has resulted in the production of cloned cDNA for several major human and rat apolipoproteins. We are using these clones for probing the regulation of apolipoprotein synthesis and for studying the structure of the peptides.

a) The amino acid sequence of rat apolipoprotein AII deduced from the nucleotide sequence of cloned cDNA

M. Nagashima, G. Morris, G. Howlett, N. Fidge, and G. Schreiber.

From a human liver cDNA library constructed in the expression vector λgt11Amp3, a human apo AII cDNA clone was isolated by immunochemical screening using antiserum to human apo AII. Nick translated human apo AII cDNA was then used as a probe to identify a rat apo AII cDNA clone from a rat liver cDNA library by *in situ* hybridization of bacteriophage plaques under high stringency conditions. The cDNA insert from this clone was characterized by DNA sequencing providing the amino acid composition which matched well with that of an earlier report. While the rat apo AII cDNA insert did not code for the entire presegment, it had the same COOH-terminal residues of the presegment as well as the same prosegment (Ala-Leu-Val-Arg-Arg) as in human preproapo

AII. These results suggested that rat apo AII contains 79 amino acids. Residue 6 of mature rat apo AII is Asp, while it is Cys in human apo AII, and this would account for the absence of dimeric forms of rat apo AII in plasma. While the overall sequence homology between rat and human apo AII is about 50%, the NH_2-terminal domain has higher homology. Furthermore, hydrophobic residues in corresponding positions are remarkably well conserved. The size of rat apo AII mRNA was estimated to be about 600 nucleotides. Analysis of mRNA levels in different tissues demonstrated that the liver was the chief site of synthesis of rat apo AII.

b) The influence of hormones and nutrients on levels of mRNA for liver apolipoproteins

M. Nagashima, N. Fidge and G. Howlett

Changes in the level of mRNA for various apolipoproteins in response to hormones and exogenous fatty acids were investigated in Hep G2 cell using recombinant DNA techniques. Following 24 h incubation, relative levels of specific mRNAs were estimated by dot blot hybridization of cytoplasmic RNA preparations with ^{32}p-labelled specific cDNAs. Results of preliminary experiments indicated that addition of insulin (0.5 μg/ml) (or 90nM) to the minimum essential medium led to a decrease in the levels of hepatic apo AI and AII mRNA while no significant effect was observed with dexamethasone (30 nM). Incubations of Hep G2 cells with 2 mM oleic acid bound to albumin apparently resulted in slight increases in mRNA levels for both apo AI and AII when compared with incubations of cells with albumin. This system provided a suitable model for studying the regulation of synthesis of apolipoproteins.

c) Exploitation of E-coli produced apolipoprotein for producing monospecific antisera

M. Nagashima, N. Fidge, G. Howlett

Antisera to apolipoproteins, if pure, provide valuable tools for quantitating and probing the structure of lipoproteins. However, there is considerable difficulty producing immunologically pure apolipoproteins by conventional protein chemical isolation and consequently also in producing monospecific antisera.

We are attempting to exploit the cDNA clones to apo AI and E by growing sufficient quantities of the corresponding E Coli to produce enough hybrid protein (β galactosidase linked to apo E or AI) for immunising rabbits. Individual human apolipoprotein was synthesized as a fused protein by E coli that had been infected with recombinant bacteriophage derived from λgt11-Amp 3. The fused protein was partially purified from the lysate by gel filtration on a Sephacryl S-300 column followed by hydroxylapatite column chromatography. Using these proteins as antigens, weak titres have so far been obtained for apo AI antisera and more recently stronger antisera to apo E have been produced. With further boosting, these animals may provide antibodies of sufficient strength against genetically engineered apolipoproteins thus ensuring the production of antibodies with indisputable purity.

CHOLESTEROL METABOLISM LABORATORY

Head: Dr. S. Turley

Projects

Metabolism of high density lipoprotein in the obese rat.

Effect of fish oil on cholesterol metabolism in the rat.

Regulation of hepatic cholesterol production in the obese rat.

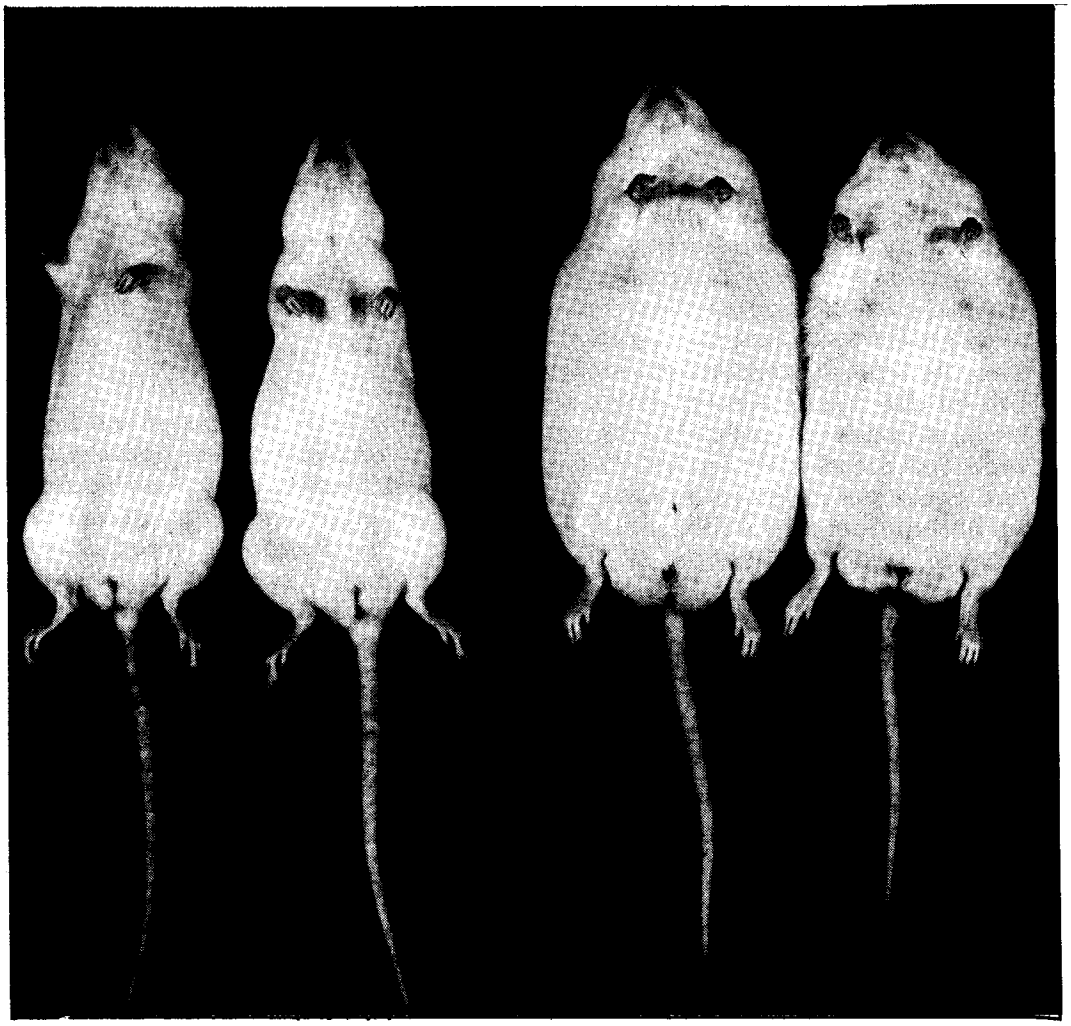
Bile acid metabolism in the obese rat.

Biliary lipid secretion in the obese rat.

Metabolism of high density lipoprotein in the obese rat

J. Cohn, P. Nestel, S. Turley

Obese rats have significantly higher plasma levels of high density lipoprotein (HDL) than their lean littermates. However, the chemical composition of the HDL is similar in both types of animal except for an enrichment of C apoproteins in the HDL from the obese rats. From the measurement of HDL protein turnover and pool size it was determined that the production rate of HDL in the obese animals was significantly higher than in the lean rats. In separate studies the catabolism of HDL was determined by measuring the uptake in vivo of HDL containing cholesteryl ester that had been labelled



SHR/N—corpulent rats and their lean littermates.

with a non-degradable marker. In the obese rats the total amount of HDL cholesteryl ester taken up by the tissues significantly exceeded that in the lean animals. In both cases the liver was the principal site of catabolism. It was concluded that in the obese rat, the higher levels of HDL are due predominantly to overproduction and not diminished catabolism, and that the liver remains the most important site for HDL cholesteryl ester catabolism in these animals.

Effect of fish oil on cholesterol metabolism in the rat

S. Wong, P. Nestel, S. Turley

Recent studies have shown that fish oil dramatically lowers plasma triglyceride levels in man and in obese rats with hypertriglyceridemia. This occurs because particular fatty acids present in the fish oil suppress the secretion of very low density lipoprotein from the liver. At least in some animal models fish oil also lowers plasma cholesterol levels, although the mechanism through which this occurs is not known. The effect could be mediated through the amount of lipoprotein cholesterol that is taken up by the liver or on other pathways involved in the synthesis and esterification of cholesterol within the liver. Studies are now being carried out to examine these possibilities.

Regulation of hepatic cholesterol production in the obese rat

S. Turley

Although many of the metabolic disorders present in the obese rat mimic those found in overweight humans, this animal model does not manifest the marked overproduction of cholesterol that is characteristic of obesity in man. This difference occurs because in the rat the rate of cholesterol production in the liver is greatly suppressed whereas in overweight humans it remains unchanged. This suppression is not caused by the delivery of excess dietary cholesterol from the intestine to the liver because feeding a drug that blocks cholesterol absorption does not eliminate the suppression. It is more likely that the suppression represents a compensatory response to the production of excess cholesterol in the small intestine and adipose tissue, the mass of which is much greater in the obese animals. The mechanism through which excess cholesterol produced in these extrahepatic tissues reaches the liver is not known, but it may involve a lipoprotein transport system that is not present in obese humans.

Bile acid metabolism in the obese rat

S. Turley

In the liver, cholesterol can be utilised in several different pathways. One of these involves an irreversible conversion to bile acids (which are known as the degradation products of cholesterol). The bile acids are secreted in the bile and on reaching the intestine become part of a large pool of bile acids that plays an active role in facilitating the absorption of cholesterol from the intestine into the blood. In overweight humans and in the obese rat, the size of the bile acid pool is greatly expanded. The cause of this is not known. It may be due to an increase in the quantity of bile acids produced in the liver or it may reflect a more efficient reabsorption of bile acids from the small intestine. The obese rat is being used as a model to examine these possibilities and to determine what effects an increased bile acid

pool has on cholesterol metabolism in the liver.

Biliary lipid secretion in the obese rat

S. Turley

The liver secretes into the bile micelles composed of bile acid, phospholipid and cholesterol. Under normal conditions the cholesterol remains in micellar form and crystals do not develop. However, in obese humans the amount of cholesterol secreted into the bile often increases to the extent that it is not completely solubilised. This results in the formation of cholesterol crystals in the gallbladder and over many years these become stones which may eventually obstruct the flow of bile from the liver to the intestine. The source of this excess cholesterol in the bile is not known but it is related to the overproduction of cholesterol within the body as a whole. In the obese rat such overproduction does not occur and biliary cholesterol secretion is not increased. Thus in the obese rat there is a mechanism of control of cholesterol production that is not present in overweight humans.

If this mechanism could be elucidated it may provide an explanation for the way in which the production of excess cholesterol in the tissues of the obese individual ultimately causes an increase in the quantity of cholesterol secreted by the liver into the bile.

Basic Cardiology Laboratory

Head: Dr. Archer Broughton

Projects

Effects of arterial blood pressure on coronary blood flow distribution in the normal heart.

Coronary circulation and ventricular performance of the hypertensive heart.

pH control within heart muscle cells - role of bicarbonate ions.

Summary

We have examined the intrinsic factors that determine the amount and distribution of coronary blood flow in the heart in chronic experimental hypertension. In hypertension, enlargement of the heart muscle is an important compensatory mechanism, which allows maintenance of pumping performance against the higher arterial pressure load. Yet enlargement of the muscle cells makes these more vulnerable to reduction in the oxygen supply of the heart. We have examined in a highly controlled preparation previously developed at the Baker Institute the amount and distribution of the coronary blood flow across the entire thickness of the left ventricle. These studies indicate that the blood supply of the layer of enlarged heart muscle right next to the ventricular cavity becomes compromised sufficiently to impair the heart's contractility, even with relatively minor reduction in arterial pressure.

The other major project concerns the factors that safeguard the acidity (pH) of the cells of the heart. Intracellular acidosis is commonly associated with cells surrounding dead heart cells after a heart attack and leads to electrical instability, disturbances of the rhythm of the heart and sometimes death. This problem has been investigated with electrophysiological techniques, using a new type of pH-sensitive microelectrode. The work has pointed to the critical importance of the bicarbonate ion in preventing intracellular acidosis.

Projects

Effects of arterial blood pressure on coronary blood flow distribution in the normal heart.

J. Smolich, P. Weissberg, A. Broughton, P. I. Korner

The left ventricle (LV) of the normal heart has a thick muscular wall which can be subdivided into inner (endocardial) and outer (epicardial) layers. The endocardial muscle lies adjacent to the high pressure cavity and must develop a much higher level of wall force in order to shorten during contraction than the epicardial muscle. Endocardial heart muscle thus expends more energy than epicardial muscle and normally receives more coronary blood flow per unit of muscle weight.

The normal pattern of regional coronary perfusion which favours the endocardium is disturbed by many cardiac diseases especially coronary disease and cardiac hypertrophy. The resulting perfusion deficits involve a redistribution of flow towards the epicardial muscle layers. The severity of the flow abnor-

mality has been gauged from the change in the ratio of endocardial to epicardial flows. However, the significance of such an alteration in the pattern of coronary perfusion is not completely clear since the factors controlling regional perfusion have not been adequately studied even in normal hearts.

We have studied the relationship between arterial blood pressure and myocardial blood flow distribution in the hearts of normal dogs under general anaesthesia. We made every effort to control other factors known to alter blood flow through the heart such as the intensity of the cardiac contraction, heart rate and the extent of cardiac filling. Autonomic reflex alterations in cardiac performance were eliminated by ganglion blockade. We kept heart rate constant by pacing and controlled LV filling pressure using a left atrial 'reservoir'. Arterial pressure was reduced in stages by altering the extent of arteriovenous shunting through a wide-bore fistula connecting the aorta to the jugular vein. LV performance was monitored throughout from the rate of rise of cavity pressure 40 mmHg above end-diastolic pressure, (dPdt)DP40. Total and regional LV blood flow was measured with radioactive microspheres. In 7 dogs, arterial pressure was reduced on average from the resting value of 116 mmHg to 90, 75 and then 59 mmHg. With each pressure decrease there was a progressive flow redistribution away from the endocardium, the endocardial to epicardial flow ratio falling from 1.16 to 0.76 by the lowest pressure. However, there was no detectable change in LV contractility as judged from the isovolumic indices.

We conclude that myocardial flow distribution is sensitive to moderate arterial pressure reduction in the normal heart. The simplest interpretation of the data is that even marked reductions in the endocardial to epicardial flow ratio do not necessarily indicate the development of perfusion abnormalities since function was not impaired.

The coronary circulation and ventricular performance of the heart with left ventricular hypertrophy due to chronic hypertension

J. Smolich, P. Weissberg, A. Broughton, P. I. Korner

Heart muscle cells within the LV enlarge by a process of adaptive growth known as hypertrophy in response pressure-overload such as hypertension. There is some concern that the cells may 'outgrow' their blood supply. The hypertrophied heart is reported to be predisposed to coronary flow abnormalities including subendocardial profusion 'deficits' which may account for the development of cardiac failure in untreated hypertensive patients. However, previous studies have not obtained the simultaneous measurements of coronary flow and ventricular performance required to prove a deleterious impact of the demonstrated flow alterations.

We have examined in normal dogs and in those with left ventricular hypertrophy, the relationship between coronary blood flow distribution and ventricular performance during acute reductions in arterial pressure. The experiments were performed under highly controlled conditions (see above) with heart rate and cardiac filling pressure held constant and autonomic reflexes eliminated.

Arterial pressure was reduced by increasing the flow through an arteriovenous shunt. Total LV blood flow was similar in both groups at rest. Pressure reduction to 90, 70 and then 60

mmHg reduced total coronary flow and caused a flow redistribution away from the endocardium in both groups. But the changes were much more marked in hypertensive compared to normal hearts. The ratio of endocardial to epicardial flow fell from 1.27 to 0.35 in hypertensive animals. Concomitantly the index (dPdt)DP40 also declined, indicating a deterioration in LV contractility. Although total coronary flow was closely similar in both groups at the lowest pressure studied (89 mls/min/100 gm in normal dogs; 81 mls/min/100 gm in hypertensive dogs) there was significantly less redistribution away from the endocardium within normal hearts and no accompanying decline in LV contractile performance.

We concluded that coronary perfusion becomes inadequate in the hypertensive heart at moderately low arterial pressures where a reduction in global coronary flow combined with a marked redistribution of flow away from the endocardium produces contractile impairment. Comparable flow reduction occurs in normal hearts but the redistribution of flow is less marked and contractile impairment does not develop.

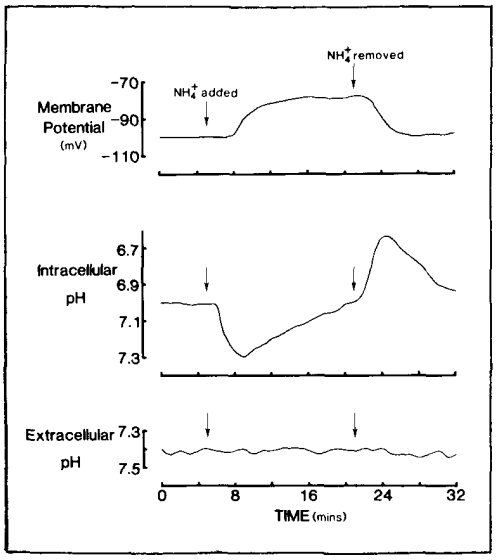
pH control in cardiac cells - the role of bicarbonate ions

A. Broughton, M. Ross-Smith

The distribution of permeable ions across the cardiac cell membrane is strongly influenced by membrane voltage. Normally the electrical potential measured inside a resting ventricular muscle cell is about 95 millivolts more negative than the extracellular potential. This electrical gradient favours the entry of positively charged ions like H^+ (the hydrogen ion). The intracellular H^+ concentration (usually expressed as pH) must be precisely controlled for normal cellular homeostasis. The most profound disturbances occur during acute myodcardial infarction when sudden reduction in coronary blood flow leads to an accumulation of hydrogen and other ions within the cardiac cell. These ionic disturbances destabilize the cardiac electrical properties and are responsible for lethal arrhythmias in infarct patients. We would like to understand how the controlling mechanisms regulating intracellular H^+ concentration become impaired in cardiac disease. However, we first need to clarify how the mechanisms operate to regulate intracellular pH in the normal heart.

Under normal conditions intracellular pH of the ventricular muscle cell is about 7.1. This value is at least 0.5 pH units more alkaline than the value appropriate for the voltage difference across the cell membrane. Several mechanisms have been proposed which would allow the normal cell to actively extrude hydrogen ions. These include firstly, sodium-hydrogen exchange, and secondly, chloride-bicarbonate exchange. We have begun to investigate the role of such mechanisms in quiescent papillary muscle strips from the guinea pig. Conventional microelectrodes were used to record the intracellular membrane potential. Intracellular pH was measured by ion-selective microelectrodes containing the H^+ sensitive resin dodecylamine. These electrodes are sensitive to even small changes in intracellular pH. Fig. 2 shows the recordings obtained with the pH-sensitive electrodes when ammonia (15mM) is added to alkalinise the cell, then withdrawn to provoke acidification.

Our initial experiments suggest that the bicarbonate ion may play a more important role than sodium-hydrogen exchange under the conditions we



Changes in membrane potential and intracellular pH, observed in guinea-pig myocardium, in response to the addition and removal of ammonium (NH_4+) ions.

employed. Amiloride ($3x10^{-4}M$) which depresses sodium- hydrogen exchange did not alter intracellular pH. When extracellular bicarbonate concentration was reduced from 21 to 7.5 mM to reduce extracellular pH from 7.4 to 6.95, intracellular pH fell by 0.15 units. This response was not affected by amiloride. Extracellular bicarbonate was then completely replaced by Hepes buffer and the preparation gassed with pure oxygen instead of 5% carbon dioxide to maintain the extracellular pH normal. Intracellular pH initially alkalinised (reflecting the washout of carbon dioxide) but then progressively acidified over the next 40 - 60 minutes. These studies suggest that the bicarbonate is critical for combating intracellular acidosis to a much greater extent than has been recognised.

Biochemical Pharmacology Laboratory

Head: Dr. A. Bobik

Projects

Forskolin binding sites and adenylate cyclase activation.

Partial purification of adenylate cyclase.

Structure-activity studies of 'forskolin-like' compounds.

Mechanisms regulating sodium transport in vascular smooth muscle.

Effects of 6-hydroxydopamine on central noradrenergic neurons.

Purification of rabbit adrenal phenylethanolamine-N-methyl-transferase.

See also Clinical Research Unit Report

Summary

This year we have continued to examine how biochemical processes in cardiac and vascular smooth muscle affect their ability to respond to changes in the level of autonomic activity. The enzyme adenylate cyclase facilitates the function of many intracellular "messengers" which, in conjunction with other membrane transport processes, determine the overall responsiveness of cardiac and vascular tissue.

Our previous studies have suggested that it may be possible to improve the function of failing hearts by stimulating adenylate cyclase with drugs which interact at the catalytic unit. The potential advantage of this type of approach to treating heart failure over that where the drugs stimulate beta receptors is that tolerance should not develop to such drugs. This has been the major reason why drugs which activate adenylate cyclase indirectly through membrane receptor systems have failed. We have shown that the naturally occurring diterpene forskolin is a more potent activator of cardiac than either liver or lung adenylate cyclase. The reason for this appears to be related to the properties of specific sites which bind forskolin on the three tissues. The binding sites on cardiac membranes have a higher affinity for forskolin than those of the other two tissues. Partial purification ofthe adenylate cyclase indicates that these binding sites are closely associated with the catalytic unit of adenylate cyclase. We are proceeding with our purification studies to gain a better insight into the structure of the catalytic unit which will explain why forskolin is more selective for the heart than the other two tissues. This will lead to the development of more selective drugs for stimulating the catalytic unit. We are collaborating with Dr. G. Feutrill and Professor D. Cameron from the Department of Organic Chemistry, Melbourne University to determine which parts of the forskolin molecule are essential for selective stimulation of the adenylate cyclase in the heart.

Another project has examined how salt may be involved in the development of hypertension. One suggested mechanism is that substances circulating in blood which reduce the rate at which sodium is eliminated from cells by inhibiting the level of activity of the sodium-potassium pump. Such substances resemble the actions of the

cardiac stimulant drug digoxin and can be detected by using specific antibodies to digoxin; but their concentration in blood is only marginally increased in experimental renal hypertension. Their concentration in blood is not affected by either increasing or decreasing the level of sodium intake in hypertensive or normotensive animals. It therefore appears unlikely that they contribute to any rise in blood pressure during the development of hypertension. In more basic studies on the regulation of sodium transport in smooth muscle cells of blood vessels we have demonstrated that the cell membrane is quite impermeable to sodium. We discovered that sodium entry into cells is critically regulated by specific proteins in the membranes which exchange extracellular sodium ions for intracellular hydrogen ions. We have fully characterised the system and are now examining how different hormones and receptor systems might regulate its activity.

Our neurochemical studies have tried to identify which catecholamine-containing neurons in the brain might be involved in blood pressure regulation. We have focussed on identifying which noradrenergic cell bodies may be affected by the neurotoxic agent 6-hydroxydopamine. Administration of this agent into the central nervous system abolishes the ability of alpha-methyldopa to lower blood pressure. It also reduces noradrenaline content in several discrete areas of the central nervous system. We have also developed a sensitive method for measuring adrenaline content in the brain. The high sensitivity is obtained by combining our previously developed microdissection technique of the brain with a radioenzymatic assay for adrenaline. This results in minimal diluation of the radioactive label (^{3}H) S adenosyl-L-methionine used in the assay. This technique helps to assess the role of adrenaline containing neurons in regulating blood pressure.

Projects

Relation between (^{3}H) forskolin binding sites and adenylate cyclase activation

G. Jackman, A. Bobik

We have previously reported that the ability of forskolin to activate adenylate cyclase in the heart was 5-10 times greater than in liver. Rat lung adenylate cyclase was only poorly activated by forskolin. We have now investigated the reasons for its selectivity. The greater ability of forskolin to stimulate cardiac adenylate cyclase than either that of liver or lung could not be accounted for by a larger number of adenylate cyclase catalytic units or by any differences in the properties of the stimulatory nucleotide binding proteins. Sodium fluoride (10mM) stimulated adenylate cyclase in all three tissues to a similar extent. Differences in the ability of forskolin to stimulate the three tissues were related to the number of specific forskolin binding sites. Cell membranes purified from rat hearts and livers possess a single population of binding sites for (^{3}H) forskolin. The affinity (dissociation constant) of (^{3}H) forskolin for these binding sites averaged 0.25 μM and 1.43 μM for heart and liver membranes respectively. No such binding sites could be detected in membranes prepared from rat lungs. In these membranes only very low affinity (dissociation constant 100 μM) could be detected. Our results suggest that stimulatory nucleotide binding proteins do not influence the level of adenylate cyclase stimulation by forskolin, rather (^{3}H) forskolin binding sites appear to determine the effect of forskolin in the different tissues.

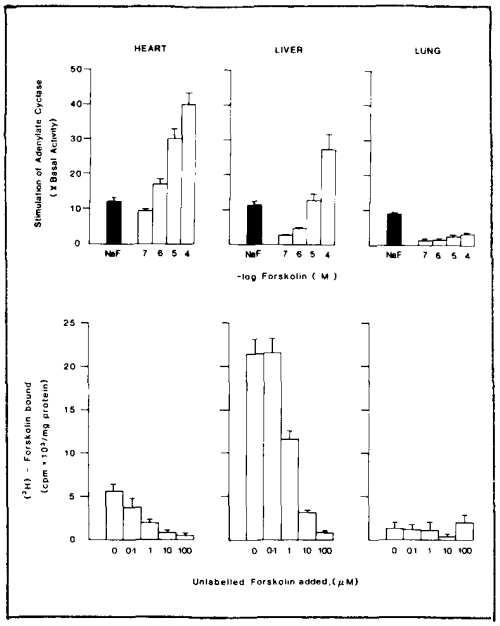


Fig 3
Upper Panel: Comparisons of the ability of sodium fluoride (NaF) and forskolin to activate adenylate cyclase from the heart, liver and lungs of rats.
Lower Panel: Specific binding sites detected by (^{3}H) forskolin on membranes isolated from the heart, liver but not lungs of rats.

Partial purification of adenylate cyclase

G. Jackman, M. Frigo, A. Bobik

We have commenced to purify the catalytic units from rabbit cardiac and liver cell membranes. Adenylate cyclase was solubilised from these membranes with 0.1% Lubrol 12A9. The solubilised fraction was chromatographed on a DEAE Sepharose anion exchange column and enzyme activity eluted with a 200 mM sodium chloride-Tris buffer. Proteins in fractions containing adenylate cyclase activity were precipitated by adding ammonium sulphate to 50 per cent saturation. After 6 h of dialysis the adenylate cyclase preparation was further chromatographed on a strong anion exchange Mono Q column using fast protein liquid chromatography. Adenylate cyclase which could be activated by 0.1mM forskolin but not by 10mM sodium fluoride or beta adrenoceptor agonists was eluted from the ion exchange column with a 300 mM sodium chloride-tris buffer. SDS-polyacrylamide gel electrophoresis of the fractions containing adenylate cyclase activity indicate mainly three proteins of high molecular weight which range between 105 and 138 KD.

Synthesis of substituted dihydroxydecalin analogues

A. Teo, G. Feutrill*, D. Cameron, G. Jackman*

The bicyclic trans decalin ring structure of forskolin with its 1,9-hydroxyl substituents appears to be the critical component in the forskolin structure which influences its ability to activate adenylate cyclase. To test this hypothesis we have synthesised a variety of substituted 1,9-dihydroxydecalin derivatives which will be initially examined for their ability to activate adenylate cyclase in different tissues of the rat. In brief the bicyclic ring systems have been synthesised via the Diels-Alder reaction from 1-trimethylsiloxybutadiene and 2,6-dimethylbenzoquinone. The Diels-Alder product is catalytically reduced to the dihydroxydecalin with cis-ring fusion. Treatment with acid or alkali inverts the cis dihydroxydecalin into the trans isomer. 1-(2H)-Napthalenone, octahydroxy-1, 8-dihydroxy-2, 8a-dimethyl (1 α, 4a α, 8 $\alpha\beta$) (structure A) which resembles the first two rings of forskolin (structure B) is a typical compound synthesised by the abovementioned procedure. Overall yield for the dihydroxydecalins is about 25 percent.

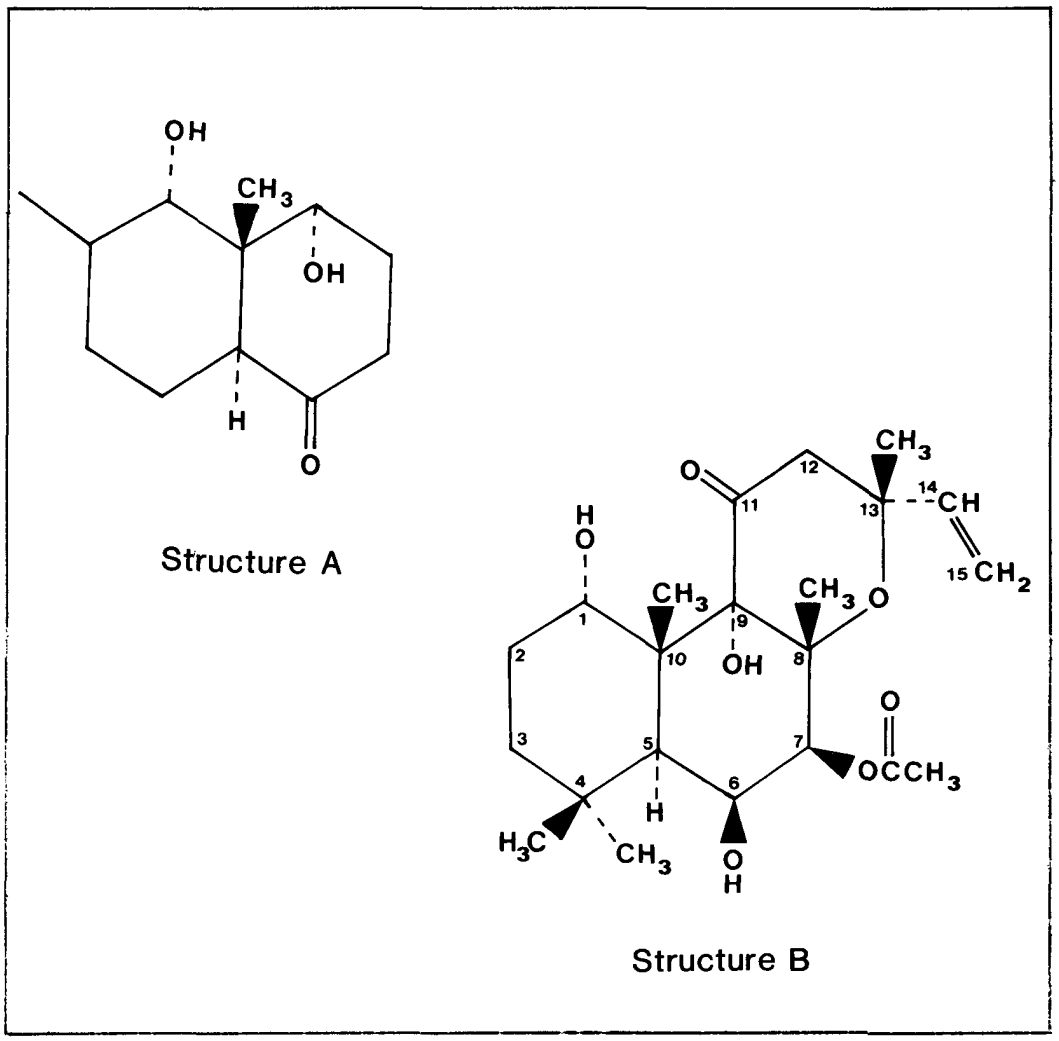


Fig 6
Structural formulae depicting the close similarity between a synthetic analogue of forskolin (structure A) and naturally occurring forskolin (structure B).

To date twenty analogues of the dihydroxydecalins have been synthesised.

*Organic Chemistry Dept., University of Melbourne.

Effects of salt intake levels on vascular Na-K pump activity in normotensive and renal hypertensive rabbits

A. Bobik, P.J. Little, P. Scott

We examined the relationship between salt intake levels, the concentration in plasma of putative endogenous digoxin-like regulators of Na-K pumps, which interact with "digoxin specific" antibodies and vascular Na-K pump activity. Experiments were carried out in adult rabbits 4 - 6 weeks after either sham operation or bilateral cellophane renal wrapping to induce hypertension. Both groups received diets in which sodium content was either low, normal

or high. Two weeks after commencing the diets, 24 h mean urinary sodium excretion in both groups of rabbits was similar averaging 5, 13 and 34 mmoles for the low, normal and high salt diets respectively. Digoxin-like immunoactivity was 19 percent higher in plasma of hypertensive than in normotensive rabbits. However the concentration of digoxin-like immunoactivity measured in plasma was not dependent on the level of sodium intake in either group of animals. Na-K pump activity in aortic strips from the hypertensive animals was reduced by approximately 22 percent. The different salt diets had no effect on vascular Na-K pump activity. These results indicate that both the concentration of digoxin- like immunoactivity in plasma as well as vascular Na-K pump activity are not dependent upon the level of sodium intake in either normotensive or renal hypertensive animals.

Na-H exchange is a major pathway for sodium influx in vascular smooth muscle

P.J. Little, P. Weissberg, A. Bobik

We have previously demonstrated that the rate at which sodium enters cultured rat aortic vascular smooth muscle could be greatly reduced by the diuretic drug amiloride or by lowering extracellular pH. This suggested that sodium was entering the cells via a Na-H exchange process. We have now obtained conclusive evidence that this exchange process is the major pathway for sodium entry into these smooth muscle cells. Firstly ethylisopropylamiloride, specific inhibitor of Na-H exchange was approximately 100 times more potent than amiloride at inhibiting sodium influx into smooth muscle. In a 135mM sodium containing medium,

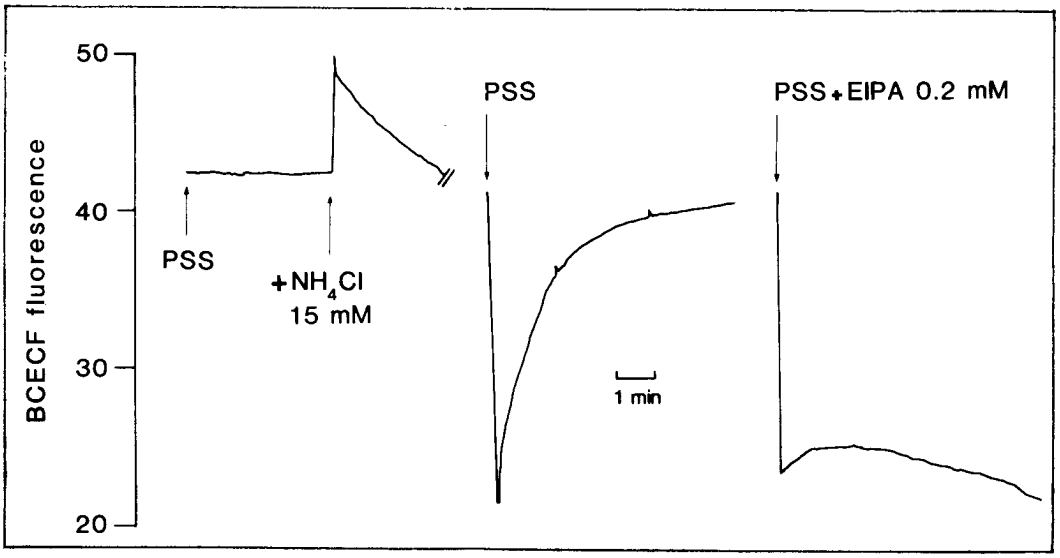


Fig 7
Measurements of intracellular pH in rat aortic smooth muscle cells, grown in culture on glass cover slips, using the fluorescent pH probe Bis(carboxyethyl) carboxyfluorescence (BCECF). Addition of ammonium chloride (NH_4Cl) to the cells alkalinizes the cell cytosol (an increase in BCECF fluorescence). Rapid removal of the ammonium chloride by washing the cells with a physiological salt solution (PSS) induces an intracellular acidification. Intracellular pH is gradually restored to normal. Addition of ethylisopropylamiloride (EIPA) impairs the ability of smooth muscle to normalise pH by inhibiting sodium hydrogen exchange.

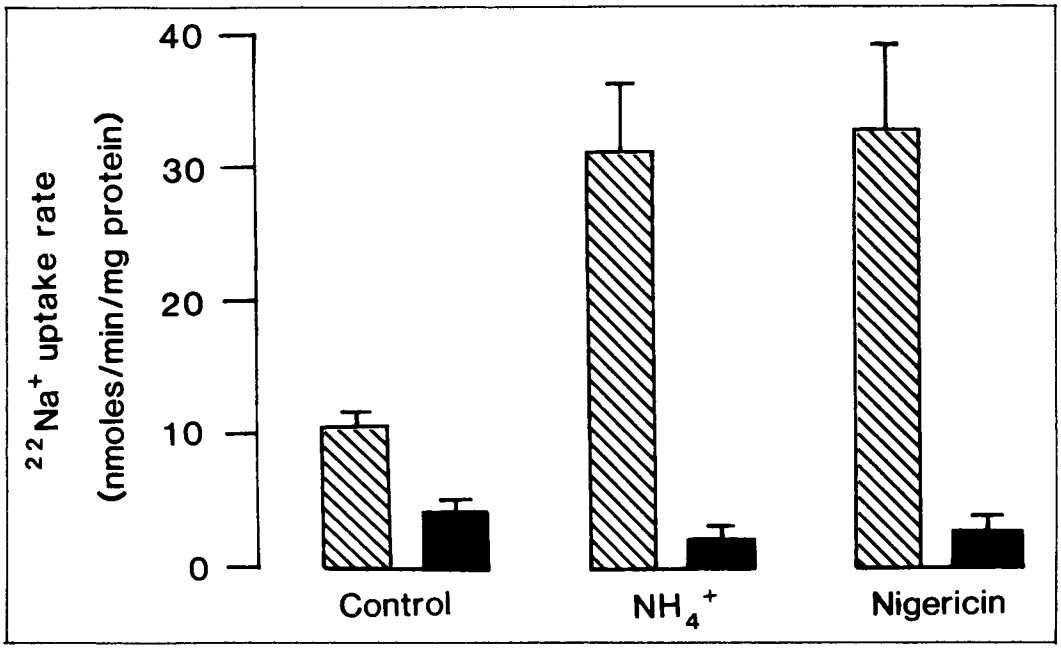


Fig 8
Sodium (^{22}Na) uptake into cultured rat aortic smooth muscle cells in the absence (▨) and presence of 100 μM ethylisopropylamiloride (■). The difference between these two measurements represents sodium uptake via the Na-H exchange mechanism. Pre-exposure of the cells to ammonium chloride (NH_4Cl) as well as exposure to nigericin increases the rate of sodium uptake by this mechanism.

both drugs reduced sodium influx rate by about 80 percent. By inhibiting Na-H exchange ethylisopropylamiloride also induced an intracellular acidification in a concentration dependent manner similar to its effects on sodium influx rate. Secondly addition of 0.7 μM nigericin to the cells in a sodium free-choline chloride medium reduces intracellular pH from 6.6 to 5.9. This reduction in intracellular pH increases the rate of $^{22}Na^{+}$ influx approximately 4-fold. Ethylisopropylamiloride prevented the increase in sodium influx rate. Similarly ethylisopropylamiloride prevented the increase in $^{22}Na^{+}$ influx rate brought about by acidifying cell cytosol by pre-exposing cells to 15 mM ammonium chloride. In this instance the recovery in cytosolic pH was also prevented by the Na-H exchange inhibitor. Thus the Na-H 'pump' is important for controlling the rate of sodium influx in vascular smooth muscle and it also appears to regulate intracellular pH.

Relationships between the effects of 6-hydroxydopamine on cardiovascular responses and its effects on central noradrenergic neurons

A. Bobik, C. Oddie, P. Scott, G. Mill, P. Korner

Release of catecholamines (CA) from neurons of the brain by the selective neurotoxic agent 6-hydroxydopamine (6-OHDA) initially activates and then destroys both the CA pressor and depressor pathways. We have examined the relationship between the abilities of various doses of 6-hydroxydopamine to affect these pathways in discrete areas of the brain. Intracisternally administered 6-OHDA displayed no dose-

related selectivity for either pathway. In rabbits whose carotid sinus and aortic baroreceptors were denervated, 6-OHDA initially caused a fall in blood pressure which was maximal at about one hour, after which blood pressure rose. Both the low dose of 6-hydroxydopamine (100 μg/kg) and the high dose (600 μg/kg) initiated responses of similar magnitude. However, the relationship between dose of 6-OHDA and spinal cord noradrenaline concentration was dose dependent. In addition the ability of alpha-methyldopa to activate this pathway and induce falls in blood pressure was inhibited by 30%, 55% and 100% two weeks after administration of either 100, 300 or 600 μg/kg i.c. doses of 6-hydroxydopamine. The effects of 6-OHDA on blood pressure correlated closely with the reductions in noradrenaline content in the intermediolateral column of the spinal cord. Reductions in noradrenaline content were also observed in several hypothalamic nuclei whilst in the midbrain reductions were mainly localised to the A1, A2 and A5 cell groups. Thus, CA cell bodies with axons terminating in the spinal cord may be closely associated with the depressor pathways activated by alpha-methyldopa.

Cardiac Surgery Laboratory

Head: Dr. F. L. Rosenfeldt

Projects

Effect of venous cannulation and cooling techniques on atrial temperature.

Electrophysiological and biochemical study of the effects of venous cannulation and cooling techniques on myocardial preservation.

The response of the hypertrophic heart to hypothermic cardioplegia..

Long term results of cardiac surgery.

Summary

To date most emphasis in preserving the function of the heart during cardiac surgery has centred on the left ventricle which pumps blood to the different organs. Little work has been done on protecting the atria which function as a reservoir and pacemaker. One reason is that the atria have been considered to be resistant to intraoperative damage.

However, it is almost universal experience that 20- 30% of the patients recovering from coronary bypass surgery develop disturbances of atrial rhythm, especially atrial fibrillation. A high proportion of patients also develop delays in the conduction of the excitatory impulse between the atria and the ventricles, which may last for minutes, hours or even days after surgery. Usually the disturbances of postoperative cardiac electrical activity cause only minor discomfort or inconvenience, but they may delay recovery and sometimes threaten the patient's survival. This year we have begun to study whether better protection of the atria makes the heart less vulnerable to the development of rhythm disturbances.

Our previous work had shown that good ventricular protection during surgery is practically synonymous with effective cooling. However, it seems possible that certain simplified techniques of drawing blood away from the heart during surgery, which are now widely used, may cause damage to the atria. Thus, we are studying whether various techniques of venous drainage affect the temperature in the atria and other regions of the heart. We have also used the experience gained at Alfred Hospital during corrective surgery for cardiac arrhythmias, to study the effect of various venous drainage techniques, on the postoperative cardiac rhythm.

We have continued work on cardiac hypertrophy (muscle thickening) and the response of the hypertrophic heart to induced cardiac arrest and cooling (hypothermic cardioplegia). The work suggests that hypertrophic heart muscle has a greater rate of resting metabolism than normal, which could account for its greater susceptibility to damage during cardiac surgery.

We have examined some of the long term results of cardiac surgery and have concentrated on the results in 800 patients operated on during the first 10 years of coronary bypass surgery at Alfred Hospital, 1969-1979. All operation records have now been reviewed and coded and nearly all surviving patients have been interviewed in the last two years. The analysis will concentrate on the late results, i.e. survival,

freedom from angina or need for reoperation. We will also seek to identify factors such as age, sex and type of operation, especially the use of coronary endarterectomy, which may be associated with good or bad long term results.

Projects

Comparison of the effect on myocardial temperature of various techniques of venous cannulation and cooling

Dr. X. Z. Chen, Dr. F. L. Rosenfeldt

We used dogs, to investigate the effects on myocardial temperature during 30 minutes of hypothermic cardioplegia, of several methods of atrial cannulation for venous drainage during bypass. Methods studied included: two stage atriocaval (a-c) cannula; two snared cannulae; two snared cannulae plus right atrial perfusion with cold Hartmann's solution or blood. The results for atrioventricular node temperature are illustrated in Fig. 9. During cardioplegia, atrioventricular node temperature averaged 24°C with the a-c cannula, 21°C with dual snared cannulae and 17°C with right atrial perfusion. A similar pattern was shown in recordings of right atrial wall temperature.

Our previous studes have shown that hypothermic protection of the myocardium is greatly reduced at temperatures above 20°C. Hence, we conclude that hypothermic protection of the right atrium against ischaemia is inadequate when a single a-c cannula was used. Protection is improved by the use of two snared caval cannulae especially when combined with right atrial perfusion.

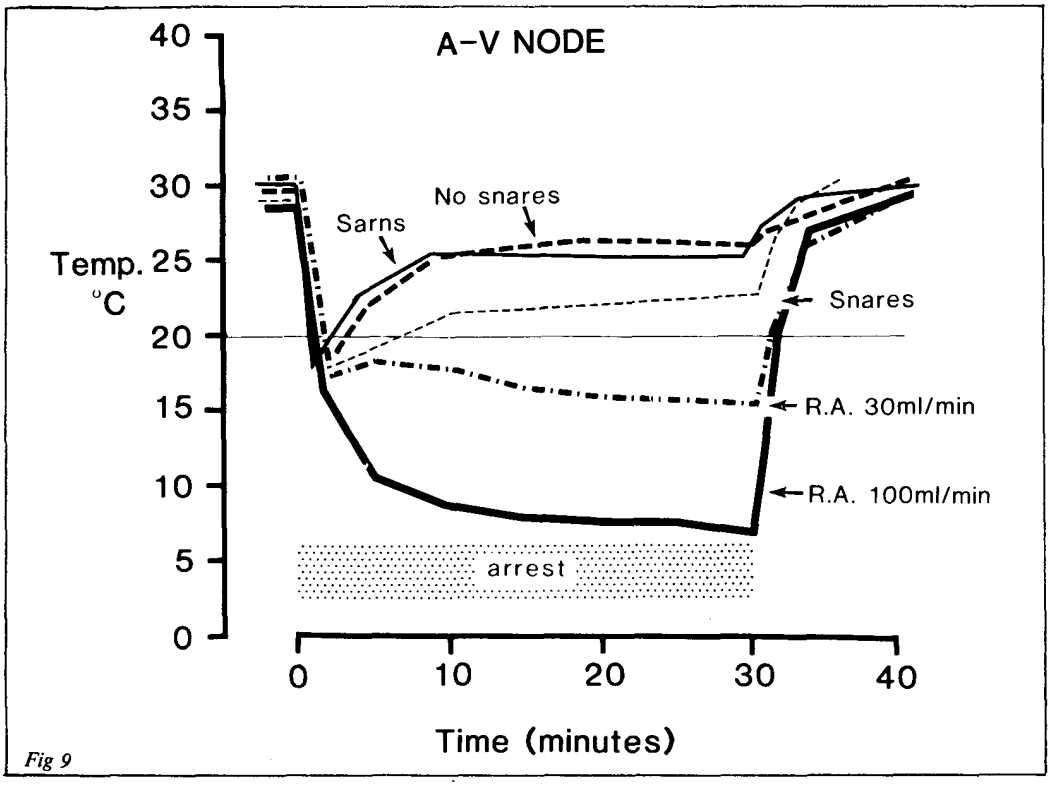


Fig 9

Electrophysiological and biochemical study of the effects of venous cannulation and cooling techniques on myocardial preservation.

Dr. X. Z. Chen, Dr. F. L. Rosenfeldt, Dr. Newman.

We investigated the effect of two methods of venous cannulation and cooling on the electrophysiological function of the atria and conducting system and on myocardial biochemistry. One group of dogs placed on cardiopulmonary bypass underwent 2 hours of arrest using cold blood cardioplegia, topical cooling and a single atrio- caval (a-c) cannula. A second group was treated similarly except that the a-c cannula was replaced by two snared caval cannulae and the interior of the heart was perfused continuously via the right atrium with cold blood from the heart lung machine. Measurements were made before and after arrest of trans-atrial and atrioventricular conduction times, atrial refractory period and sinus node recovery time. Measurements were also made of high energy phosphate and lactate levels in biopsies taken at intervals from several regions of the myocardium.

Persistent atrial electrical activity during the cardioplegic period was more common in the a-c cannula group than in the right atrial perfusion group. This phenomenon of incomplete electro-mechanical arrest during cardioplegia has been shown by others to be associated with myocardial damage. Our preliminary results also suggest that dual cannulation and right atrial perfusion improves the rate of recovery of atrioventricular conduction and reduces lactate accummulation in the atrium.

Clinical applications and devices

Our investigations into the physiological effects of hypothermia on the ischaemic myocardium have demonstrated that cooling from 37°C to 25°C greatly increases protection of the heart; but there was further benefit with cooling to lower temperatures down to 4°C. Myocardial temperatures below 20°C can be readily achieved in the isolated heart in the laboratory, but this is more difficult in the human heart at the time of surgery. In 1979 we developed a disposable recirculating circuit for clinical use, which could induce profound cooling by irrigating the heart with cold saline circulated through a coil in an ice bath. This device was produced commercially by a Melbourne manufacturer, Kal Life Systems. It has now been used in over 3000 cases at the Alfred Hospital and has been adopted by four other Australian hospitals and also in a modified form in Yugoslavia!

Another device which has commercial potential is our Microprocessor Drug Infusion Controller. This device was developed in 1980-81 in collaboration with the Royal Melbourne Institute of Technology. It can accurately reduce and maintain the blood pressure in a hypertensive patient at a desired level by adjusting the infusion rate of a hypotensive drug by measuring the patient's own blood pressure response and altering the infusion rate through a feedback circuit. Following trials in animals and postoperative patients, an improved version of the of the controller was completed in 1984. A $10,000 grant was awarded by the Victorian Government to promote the commercial development of the device. The Newtronics Company Ltd. of South Melbourne was engaged to produce two commercial prototypes. These are due for delivery

and further evaluation early in 1986.

In 1979 the Cardiac Surgery Unit at the Alfred Hospital began corrective surgery for cardiac arrhythmias. This followed pioneering work at Duke University in the U.S.A. and work by Dr. J. B. Uther and colleagues in Sydney. In this type of surgery, the key to success is accurate localisation of abnormal conducting fibres by electrical mapping of the heart at operation. The initial design for the requisite equipment came from Dr. Uther's group at Westmead Hospital in Sydney. Modification of this design, construction and testing of the mapping equipment was a joint venture between the Baker Institute and the Electronics Department of the Alfred Hospital. By the end of 1985, over 50 surgical procedures had been carried out at the Alfred Hosptial for the treatment of the Wolff-Parkinson-White (arrhythmia) syndrome, with a 95% success rate. Interest has been expressed by several electrophysiology groups in Europe, the United States and in Asia. Demand for such specialised equipment was judged insufficient to make commercial production feasible. However, we have supplied circuit diagrams, layouts and detailed descriptions to those interested at a moderate cost.

Cell Biology Laboratory

Head: Dr. Julie Campbell

Projects

Morphometric analysis of smooth muscle phenotypic change in culture - correlation with proliferative ability.

Collagen synthesis by smooth muscle cells of different phenotype.

Ploidy of smooth muscle cells in resistance vessels.

Modification of smooth muscle β-VLDL metabolism by endothelium.

Macrophage - smooth muscle interactions.

Summary

Atherosclerosis is characterized by a fibrous plaque of modified smooth muscle cells overlying a grumous core of fat. In the Cell Biology Laboratory, we are studying interactions between the different cells of the artery wall in an attempt to elucidate the cellular mechanisms of how and why atherosclerotic plaques develop.

An early event in atherogensis is the migration of monocytes through an intact endothelium into the sub-endothelium where they become macrophages. This is particularly prominent in hyperlipidaemia. We have been studying the influence that monocyte-derived macrophages have on smooth muscle cells, particularly any effect on phenotype and proliferation. Since fat preferentially accumulates in regions of a previously denuded vessel where the endothelium has recently regrown, we have investigated the effect of both quiescent and proliferating endothelial cells on lipid accumulation in smooth muscle cells of different phenotype. Also, since the smooth muscle cells of atherosclerotic plaques synthesize large amounts of collagen and other extracellular matrix molecules, the type and amount of collagen synthesized by cells of different phenotype has been examined, as has the direct relationship between the phenotype expressed by smooth muscle and its capacity to proliferate.

Projects

Morphometric analysis of smooth muscle phenotypic change in culture

Elizabeth Ng, Gordon R. Campbell and Julie H. Campbell

Morphometric analysis of the young

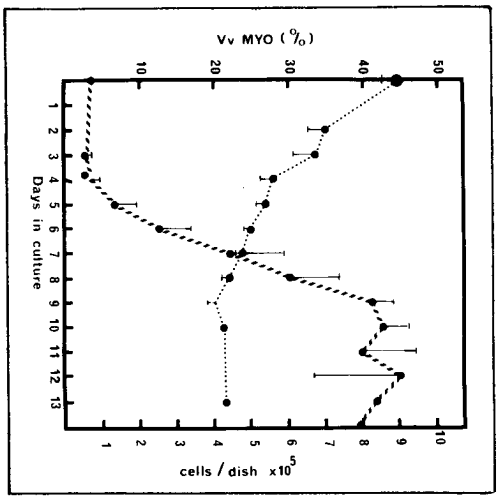


Fig 10

rabbit aortic wall showed that the constituent smooth muscle cells have a myofilament volume fraction of 45%, while the myofilament volume fraction of enzymatically isolated smooth muscle cells two days in culture is 35% decreasing to 27% by day 4. After day 4, the decrease in myofilament volume fraction is much more gradual and tends to plateau to about 22%. In parallel with the ultrastructural change, proliferation begins after day 4 with cells dividing in a logarithmic fashion for the next few days (Figure 10). These findings suggest that phenotypic modulation is reflected ultrastructurally in a sudden, sharp decrease in the volume fraction of myofilaments with subsequent smooth muscle cell proliferation.

Monocytes in atherosclerosis

Robyn Rennick, Julie H. Campbell and Gordon R. Campbell

Monocyte-derived macrophages are present as lipid- laden foam cells in the atherosclerotic plaque from an early stage, suggesting that they play a role in the etiology of the disease.

To study the interaction between monocyte/macrophages and arterial smooth muscle cells, the two cell types were grown in co-culture in modified Rose chambers. The presence of monocyte/macrophages stimulates the phenotypic modulation of smooth muscle from the contractile to synthetic state and stimulates the proliferation of the synthetic state cells. Lipid- loading the monocytes with prior incubation in β-VLDL enhances the proliferative effect.

The macrophage-derived mitogen is a non-dialysable protein of molecular weight 13,000-18,000. The identity of the factor stimulating the change in phenotype is unknown, however heparan sulphate degrading endoglycosidases, which are known to be produced by macrophages, are under investigation.

Collagen synthesis by smooth muscle of different phenotype

Ang Aik Hooi, John Bateman, Janet D. Rogers, Gordon R. Campbell, and Julie H. Campbell

Atherosclerotic plaques are characterized by enhanced synthesis of connective tissue particularly collagen. As such it is of interest to determine if smooth muscle cells of different phenotype in culture exhibit differences in collagen synthesis.

Cells have been pulse labelled with tritiated proline for 4 hours and total proteins recovered from both the cell layer and the labelling medium. The amount of newly-synthesized collagen with the incorporated tritiated proline has been determined by the collagenase assay. It was found that both the reversible- and irreversible-synthetic cells synthesize two times more collagen than contractile cells. The distribution of types I, III and V collagens was also examined. The cells were pulsed under the same labelling conditions as mentioned above. Collagen and high molecular weight proteins were precipitated with 75% ammonium sulphate, and subsequently subjected to limited pepsin digestion. An aliquot was then taken for SDS-PAGE electrophoresis and the gels fluorographed for visualization of the collagen bands. These bands were cut out, hydrolysed and counted for radioactivity. Contractile cells synthesized 80% type I and 13% type III whereas both the reversible- and irreversible- synthetic synthesized 90% type I and 5.6% type III. In all the 3 phenotypes, the proportion of type V collagen synthesized remained constant, ranging from 6-7%.

Polyploidy of vascular smooth muscle in hypertension

Jane Black, Lucy Popadynec, Nella Puglisi, Gordon R. Campbell, Julie H. Campbell

A change in ploidy (increase in the number of chromosomes from 2N to 4N) occurs in large elastic arteries such as the aorta during hypertension. This study is investigating the ploidy of smooth muscle cells from vessels with a gradation of diameters, from large elastic arteries through to small arterioles. It is the resistance vessels with only 2 to 3 smooth muscle cell layers in the media of the blood vessel wall that play a major haemodynamic role in hypertension. Does polyploidy occur in resistance vessels?

We have recently developed a technique to isolate the smooth muscle cells from very small vessels with little contamination of other cell types. The method involves enzymatic dispersion with collagenase and a further break down of extra-cellular matrix with trypsin versene.

The smooth muscle cells of the aorta (large elastic artery), the caudal artery (muscular artery), the superior mesenteric artery (small elastic artery) and the mesenteric arterioles (resistance vessels), of the spontaneously hypertensive rat (SHR) and Wistar Kyoto (WKy) controls are being studied. The ploidy of the smooth muscle cells is determined in two ways. Firstly a suspension of cells is stained with propidium iodide (PI), a fluorochrome with stoichiometrically binds to DNA, and a flow cytometric DNA analysis is performed using a flow activated cell sorter (FACS). This method provides statistical data on the frequency of cells in different stages of the cell cycle. An increase of 4N cells compared to controls is indicative of polyploidy.

Alternatively, freshly isolated smooth muscle cells are smeared on slides, fixed and stained using a Feulgen technique. Intensity of fluorescence of stained nuclei is measured using an MPV photometer.

The results to date confirm that a large increase in ploidy occurs in the smooth muscle cells of the aorta during hypertension. Results from the MPV photometer also suggest that a further increase in ploidy with greater than 4N chromosomes also occurs. However, in the smaller vessels (the caudal artery, superior mesenteric and mesenteric arterioles), there is fluctuation in the frequency of 4N cells suggesting that division of the cells is occurring.

As it is the resistance vessels and not the aorta which play a major haemodynamic role in hypertension, polyploidy would appear to hold little relevance to the etiology of hypertension and probably occurs as result of the disease.

Circulatory Control/ Neuropharmacology Laboratory

Head:- Professor P. I. Korner

Projects

Reflex Regulation

Properties of renal sympathetic baroreceptor reflex.

Uniformity of population of renal sympathetic efferent units.

Resetting of the arterial baroreceptors during directional changes in blood pressure.

Autonomic and local components of the circulatory response to haemorrhage.

Role of arterial and cardiac baroreceptors in mediating vasopressin release during haemorrhage.

Determinants of the circulatory response to atrial natriuretic peptide.

Central Transmitters and Receptors

Localization of the different noradrenergic (NA) cell groups in rabbit.

6-OHDA induced transmitter release to characterize the cardiovascular role of the central catecholaminergic (CA) neurons in the rabbit.

Role of different CA cell groups in the circulatory response to alpha-methyldopa (MD) and to clonidine in the rabbit.

Role of neurons in the A1 region in mediating vasopressin release during haemorrhage

Organisation of central CA pathways in the rat.

Role of C1 adrenaline neurons in regulating blood pressure in the rat.

Summary

Reflexes

We have been studying how the autonomic nervous system, which innervates the heart and blood vessels, functions during a variety of disturbances in the conscious animal. Such a disturbance alters the activity of various sensors, including those that signal changes in the blood pressure in the arteries and heart (baroreceptors). This information is processed in different 'centres' of the brain, which in turn alters the activity of the vagus and sympathetic nerves to the heart and of the sympathetic nerves to the arteries and

veins of the different organs of the body.

Dr. Pat Dorward, developed a technique for longterm recording of the activity from the renal sympathetic nerve in conscious rabbits, and from the nerves that carry information to the brain from the arterial baroreceptors. This allows a very precise type of input-output analysis of reflex circulatory behaviour. One drawback of sympathetic nerve recording is that the fragile nerves last for only about 8-10 days after implanting the electrodes. Although this represents something of a world record it is much shorter than the 'life' of the measuring device that can be implanted to record blood flow and vessel calibre which remain serviceable for about 2-3 months. The latter methods can under suitable conditions provide us also with information about the effects of the disturbance on *local* regulatory mechanisms in the different organs. Clearly, both neural and haemodynamic methods complement one another in circulatory analysis.

We have used the method of sympathetic nerve recording for a comprehensive analysis of the role of the baroreceptors of the heart and arteries on renal sympathetic nerve activity in the conscious animal, in response to graded changes in blood pressure. This was done by inflating small balloons previously implanted around the aorta and vena cava. This work has shown that, contrary to current opinion, baroreceptors from the heart influence sympathetic activity at all times, and not only in emergencies. With small changes in pressures in the arteries and heart, the information from both sets of receptors determines the level of renal sympathetic nerve activity in an additive manner. But, when there is a large fall and the heart has become relatively empty, the baroreceptors from the heart 'switch off' the renal sympathetic nerve activity, nullifying the messages that the brain receives from the arterial baroreceptors. This tends to raise sympathetic activity to help maintain the blood pressure. This switching off is analogous to what occurs in fainting. With poor pumping by the empty heart switching off the nerve activity helps the blood return to the heart. This maintains at least *some* pumping but at the cost of an even lower blood pressure.

The method of direct sympathetic nerve recording has provided us with new evidence of the role of many other sensory receptors on renal sympathetic nerve activity other than the various groups of baroreceptors. These sensory mechanisms can produce colossal increases in sympathetic activity. In the intact animal this action is almost totally suppressed, through the action of both groups of baroreceptors and only becomes obvious after baroreceptor denervation. We have extended the work in the conscious animal by examining in experiments under anesthesia whether the function of the fibres in the nerve is uniform. This appears to be so. The fibre population appears to be fairly uniform, at least to the sensory stimuli tested so far.

A new method, using blood flow recording with pulsed Doppler flow meters has allowed quantitative assessment of the circulatory response to haemorrhage in the conscious rabbit. One of the current controversies relates to the relative importance in circulatory regulation of the autonomic nervous system on the one hand, and the hormone vasopressin secreted by the pituitary gland on the other. Our work has shown that up to a loss of blood volume of about 25% the role of the autonomic nervous system is much more important than vasopressin in maintaining blood pressure.

Central Transmitters

When a nerve cell (neuron) in the brain is excited by an electrical nerve impulse, small packets of chemical transmitter are released. The transmitter diffuses across the gap (synapse) lying between the nerve ending and the adjoining nerve cell and alters the excitability of the latter. Different types of nerve cells release different chemical substances at their endings. We have been examining the functions on the regulation of the circulation of neurons that release catecholamines (CA) (noradrenaline and adrenaline) and of others that releases serotonin (5HT) at their endings. These two groups play a major role in the action of two important anti-hypertensive drugs, alpha-methyldopa (MD) and clonidine.

We discovered several years ago that release of the transmitters can be selectively induced by the administration into the spinal fluid bathing the brain of two selective neurotoxins 6-hydroxydopamine (6-OHDA) and 5, 6-dihydroxytryptamine. The work to date has shown that not all the CA cells have the same effect on the blood pressure. The same applies to 5HT neurons. For example, some CA neurons raise blood pressure through nerve fibres that ascend to higher brain 'centres', whilst other CA neurons that send fibres down to the spinal cord lower blood pressure. It is through the latter pathway that the anti-hypertensive drugs MD and clonidine act to lower blood pressure.

We have tried to pin-point exactly the circulatory functions subserved by the many individual CA cell groups. These are distributed in several discrete cell clumps which can be accurately localized in anaesthetized rabbits; they can then be selectively destroyed by passing small electric currents. One problem in this type of study is that each CA cell group is intermingled with many other types of neurons, that are also destroyed by the lesion. We have developed new tests which permit analysis of the specific role of the CA neurons after the lesion based on the CA release response and the circulatory effects of central administration of MD and of clonidine. The principle of the method is that 2-4 weeks after the lesion the nerve fibres from the CA neurons in the region have degenerated and lost their transmitter at the endings. In a normal rabbit the acute release of CA transmitter and the central circulatory effects of MD and of clonidine depend on the integrity of CA neurons. Hence, if a given cell group is part of the central pathways involved in the above responses there will be a detectable difference between lesioned and control rabbits in their responses to 6-0HDA induced transmitter release and to central administration of the anti- hypertensive drugs. From these tests we have found that the different noradrenergic neuron groups regulate different circulatory central pathways (see projects).

Following Dr. Geoffrey Head's return after a two year period in the Netherlands and USA on a Life Insurance Overseas Medical Research Fellowship we have been studying the function of the CA pathways in different strains of rats. Using the 6-OHDA induced acute transmitter release response it appears that there are differences in the presence or importance of the various CA pathways regulating blood pressure in genetically different strains of rat. For example, the suprapontine pressor response is absent in the spontaneously hypertensive rat (SHR) with genetic hypertension, but is present in the Wistar rat. Even in the latter, the pressor response is not as large as in the rabbit. The finding is of particular interest in relation to the search for genetically determined 'faults' in neuronal mechanisms subserving circulatory regulation.

Projects

Cardiac and respiratory rhythmicities and reflex responses to baroreceptor, chemoreceptor and nociceptor inputs in single renal postganglionic neurons in the rabbit.

P. K. Dorward, S. L. Burke, W. Janig and J. Cassell*

Postganglionic neurons supplying the kidney were studied for their reflex responses to stimulation of the arterial baroreceptors, central and peripheral chemoreceptors and cutaneous nociceptors and for the rhythmicity of their spontaneous activity in anaesthetised rabbits. A vasoconstrictor response pattern was seen in all units. The largest excitation occurred during hypoxia and injections of CO_2 saturated solutions into the carotid artery while hypercapnia only slightly increased firing. Perivascular balloon-induced falls in blood pressure doubled firing rates while pressure rises silenced 90% of units and reduced firing in the rest. Nociceptive responses were more variable. Reflex responses in units were similar to those recorded in the whole renal nerve. Unit spontaneous activity displayed both respiratory and cardiac rhythmicity. With one exception, no silent units were found which responded to the afferent inputs studied. Nor was there a small-spike fibre group which was excited by angiotensin, as has been previously suggested.

Baroreceptor and chemoreceptor reflex responses under alfathesin anaesthesia were double those found with chloralose-urethane. Both rhythmicities were increased by hypoxia with chloralose-urethane, whilst alfathesin prevented this reflex interaction. However, with both anaesthetics, parameters characterising rhythmicities and reflex responses were unimodally distributed with no apparent subgrouping of units on quantitative grounds. We conclude that renal sympathetic neurons are a functionally uniform population which behave like vasoconstrictors.

**Department of Physiology, Christian Albert University, Kiel, FGR. (Esso Overseas Visiting Scientist).*

Rapid resetting of rabbit baroreceptors and reflex heart rate responses by directional changes in blood pressure

S. L. Burke, P. K. Dorward and P. I. Korner

Function curves relating blood pressure to (1) activity both in single aortic baroreceptor units and in the whole aortic nerve and (2) heart rate were constructed during slow ramp changes in pressure away from and back to resting level, in anaesthetised and conscious rabbits. Pressure changes were produced by inflating perivascular balloons on the aorta (and vena cava) and deflating them again. The resultant curves formed typical hysteresis loops. Within 30 seconds the unit and whole nerve function curves were reset in the direction of the primary pressure change by the same amount as we had previously found over a 15 min to 2 hour period.

During falls in pressure in conscious rabbits, resetting of reflex heart rate curves was the same as baroreceptor resetting. But during pressure rises heart rate resetting was double baroreceptor resetting, suggesting that other pressure-sensitive receptors were involved, possibly those in the heart. We conclude that baroreceptor and baroreflex hysteresis loops are a very rapid form of resetting. This mechanism allows

relatively high gain reflex responses, during slow directional pressure changes and greatly extends the operating range of the reflex.

Quantitative analysis of cardiovascular response to haemorrhage

*P. I. Korner, J. R. Oliver, J. Gipps, F. Hanneman, J. R. S. Hales**

Instrumented conscious rabbits are bled at a constant rate of 4 ml/min till their blood pressure falls, but by not more than 20 mmHg. The animals are not disturbed by this and the blood is immediately re-infused. Instantaneous blood volume-circulatory response curves are derived by an on-line computer programme. These relationships include that of changes in blood volume to 1) blood pressure; 2) heart rate; 3) cardiac output; 4) blood flow in hindlimb and superior mesenteric artery. In collaboration with Dr. J. R. S. Hales we have also determined the distribution of the peripheral blood flow during moderate haemorrhage, using the radioactive microsphere technique.

We have studied the rabbits (1) under normal conditions; (2) after total autonomic blockade, with the blood pressure held at normal levels by a constant infusion of noradrenaline; (3) after blockade of the cardiac afferents and efferents using Professor Ludbrook's method of pericardial instillation of procaine; (4) role of arterial baroreceptors on blood pressure response and contribution of central catecholaminergic neurons.

**Division of Animal Research CSIRO, Sydney.*

Localization of the main noradrenergic neuron groups in the rabbit and the effect of cathodal lesions

E. Badoer, G. A. Head, J. A. Aberdeen, P. I. Korner

We have developed methods for stereotaxically localizing the major noradrenergic (NA) cell groups in the rabbit (A1, A2, A5, A6 + A7). For lesions of the A1 and A2 cells we used surface landmarks, including the obex. For localization of the pontine cell groups, which are situated more deeply from the brain surface, we mapped intracerebral structures including 1) the facial nerve for A5 and 2) the motor nucleus of the trigeminal nerve for A6 + A7. In the initial experiment we passed anodal currents through the electrode, using methods developed by other investigators. Making A1 lesions with this method gave rise to acute hypertension, pulmonary oedema and a high mortality, as described by these investigators. This syndrome was probably related to the toxic effects on the surrounding brain of ferric ions deposited at the electrode tip, because it disappeared entirely when we used cathodal currents to make the lesion. We have now made a total of 106 bilateral cathodal lesions in the above cell groups with an intraoperative mortality of 20%. Virtually all survivors remained indefinitely in excellent condition. In about 80% of survivors NA cell destruction greater than 75% was achieved.

Role of the different noradrenergic neuron groups on the circulatory response to 6-OHDA-induced central transmitter release

P. I. Korner, E. Badoer, G. A. Head

We studied the effects on the above response of bilateral lesions of the A1, A2, A1 + A2, A5 and A6 + A7 neuron

groups. As reference we used the acute circulatory changes in blood pressure (BP) and heart rate (HR) following intracisternal administration of 6- OHDA in sham-operated rabbits and determined whether these responses had become significantly altered by a given set of bilateral lesions. From studies in unlesioned sham-operated, pontine (decerebrate) and sino-aortically denervated rabbits, CA release affects the following pathways:- 1) a short latency bulbo-spinal depressor pathway (c, Fig. 11); 2) a long latency suprapontine pressor pathway (a, Fig 11); 3) a short latency pathway that slowed HR by increasing vagal tone (d, Fig. 11); 4) a short latency pathway that slowed HR by decreasing sympathetic tone (e); 5) a long latency pathway that reflexly slows HR through the vagus (f); 6) a long latency suprapontine pathway that accelerates HR through the sympathetic (b). Information about these pathways can be obtained from rabbits with intact arterial baroreceptors ('non-denervated' rabbits) (pathways a, d, f) and sino-aortically denervated (SAD rabbits) (pathways a, b, c, e). Accordingly, in this study we compared the responses between chronically lesioned 'non-denervated' and SAD rabbits with corresponding groups of sham-operated animals.

The average percentage destruction of noradrenergic cells was 85% and never less than 75%. We found that (i) the suprapontine pressor response (pathway a) was attenuated after A5 and A6 + A7 lesions; (ii) the late sympathetic tachycardia (pathway b) was abolished by A2 lesions; (iii) the short latency vagal bradycardia (pathway d) was abolished by A6 + A7 lesions; (iv) the short latency sympathetically medicated bradycardia (pathway e) was attenuated by A1 + A2 and A5 lesions. None of the lesions affected the bulbo-spinal depressor redsponse (pathway c), which may be response (pathway c), which may be mediated through adrenaline neurons (Fig. 11).

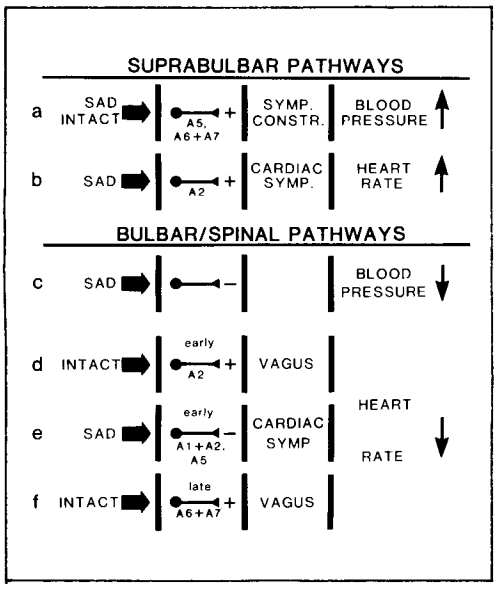


Fig 11

Role of central noradrenergic cell groups in the central actions of methyldopa and clonidine on blood pressure and heart rate

G. A. Head, E. Badoer, P. I. Korner

Central administration of MD and of clonidine lower blood pressure and heart rate. The effects of MD were completely abolished 2 weeks after intracisternal 6- OHDA, whilst the effects of clonidine were attenuated by about 50-60%. Only A2 lesions had a slight effect on the fall in blood pressure produced by MD. However, after chronic lesions of all cell groups studied (A1, A2, A1 + A2, A5, A6 + A7) the bradycardia response to MD was attentuated and after A1 + A2 lesions it was completely abolished. Clonidine-induced bradycardia was significantly attenuated after A2, A1 + A2 and A5 lesions but was unaffected by A1 and A6 + A7 lesions. All the above refers to rab-

bits with intact arterial baroreceptors. We also studied SAD sham-operated and lesioned (A1, A2, A1 + A2) rabbits; of these only after A1 lesions was there a significant change in the bradycardia response to MD and to clonidine. Since vagal tone is low in this preparation the findings suggest that A1 affects the cardiac sympathetic.

Differential blood pressure responses to intracisternal clonidine, alpha-methyldopa (MD) and 6-hydroxydopamine in spontaneously hypertensive and normotensive rats.

*G. A. Head and W. de Jong**

The cardiovascular effects of three drugs that activate central alpha-adrenoceptor mechanisms were investigated in conscious normotensive Wistar and Wistar-Kyoto (WKY) rats, and in spontaneously hypertensive rats (SHR). From dose-response curves, near-maximal intracisternal (i.c) depressor doses of clonidine, MD and 6-hydroxydopamine (6-OHDA) were determined. In all three strains of rats MD (1 mg i.c.) produced a 23-25% reduction in blood pressure. Clonidine 2.5 μg i.c.) and 6-OHDA (400 μg i.c.) produced similar falls in blood pressure in SHR to that observed with MD, but they were much less effective in the two strains of normotensive rats . Higher doses of clonidine *increased* blood pressure in Wistar rats, but reduced it further in SHR. The late pressor response observed 2-3 h after 6-OHDA administration in Wistar rats was not observed in SHR. These results support the view that clonidine and 6-OHDA, but not MD, have central pressor actions in the rat that oppose their antihypertensive action. The absence of this pressor effect in SHR indicates that there are significant differences in central noradrenergic pathways and/or α-adrenoceptor distribution among the three strains.

**Rudolf Magnus Institute for Pharmacology, University of Utrecht, The Netherlands.*

Contribution of adrenaline C1 neurons to the control of blood pressure

*G. A. Head, P. R. C. Howe**

There is now much circumstantial evidence to suggest that the above neurons are a major excitatory input to the sympathetic preganglionic cells of the spinal cord, so that they provide much of the vasomotor tone. While these cells project to the spinal cord, there is as yet no direct physiological proof that the adrenaline cells play the role suggested.

We have tried to inactivate the adrenaline neurons in rats. We used a drug that inhibits the biosynthetic enzyme that makes adrenaline; in another group we have given 6-OHDA to destroy the adrenaline cell. In anaesthetised SHR we compared the increases in blood pressure following stimulation of the adrenaline C1 region after previous treatment with LY134046 (enzyme inhibitor) and 6-OHDA. The latter attenuated the effects of stimulating the C1 area, suggesting that the adrenaline containing cells may be responsible for activity to the sympathetic system. In the dose used the enzyme inhibitor inhibited the enzyme and reduced adrenaline levels in the brain by about 30%; but it did not affect the cardiovascular response to stimulation. Thus either adrenaline is not the neurotransmitter for C1, or much greater reductions in level are necessary before function is abolished.

**Division of Human Nutrition, CSIRO, Adelaide.*

Human Autonomic Function Laboratory

Head: Dr. M. D. Esler

Projects

Influence of age on 'overall' sympathetic nervous system activity in essential hypertension.

The regional pattern of sympathetic nervous activation in essential hypertension.

Release of noradrenaline from the human heart during mental stress.

Clinical consequences of increased sympathetic nervous activity in cirrhosis of the liver.

Release of noradrenaline from human brain.

Flow-dependence of noradrenaline kinetics.

Neuronal uptake of noradrenaline in essential hypertension.

Sympathetic nervous function in cardiac failure.

See also Clinical Research Unit

Summary

We have validated use of measurements of the rate of release of the sympathetic nervous system transmitter noradrenaline, as a measure of the firing rate of the nerves. We have applied these methods to explore the way in which increased sympathetic nervous activity might cause diseases of the heart and circulation, in particular high blood pressure. Noradrenaline (NA) spillover to plasma is measured, during constant-rate infusion of noradrenaline labelled with a low dose of radioactivity, using the following relationships:

$$\text{Total NA Spillover} = \frac{^3\text{H NA Infusion Rate}}{\text{Plasma NA Specific Radioactivity}}$$

where ^{3}H NA = tritiated (radioactive) noradrenaline.

$$\text{Organ NA Spillover} = [(C_V - C_A) + C_A(NA^E)]\,PF,$$

where C_V = plasma NA conc. in organ vein; C_A = plasma NA conc. in artery; NA^E = fraction of tritiated NA extracted; PF = organ plasma flow.

We have studied the role of the sympathetic nervous system in essential hypertension, in cardiac failure and in cirrhosis of the liver. We have also carried out preliminary studies of noradrenaline release from the heart during mental stress, and of noradrenaline release from the brain.

Projects

Influence of age on 'overall' sympathetic nervous system activity in essential hypertension

M. Esler, G. Jennings, G. Lambert and P. Korner

The concentration of noradrenaline in plasma of hypertensive patients of different ages is about 25-35% higher than in subjects with normal blood pressure. This has been taken to mean that 'overall' sympathetic nervous system activity is increased in essential

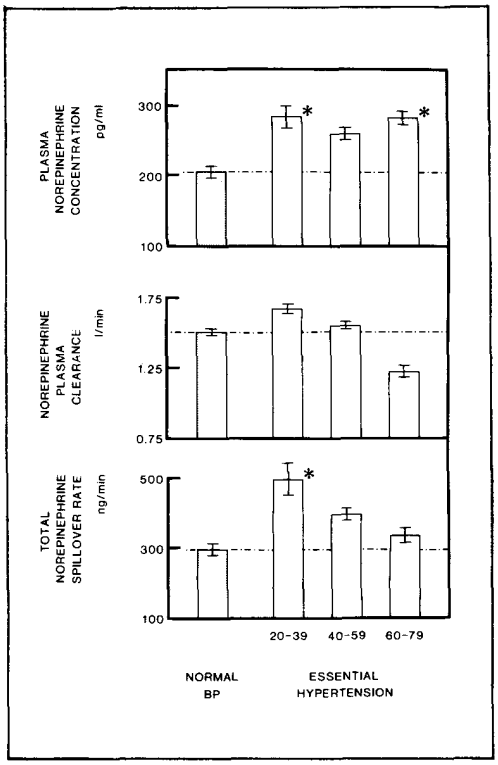


Fig 12
The overall rates of noradrenaline entry to and removal from plasma, and the plasma noradrenaline concentration, in patients with essential hyptertension and subjects with normal blood pressure. Noradrenaline release to plasma (and presumably sympathetic nervous 'activity') was increased significantly in younger hypertensive patients only.
**P<0.05.*

hypertension, however, when we measure the rates at which noradrenaline enters and leaves the circulation with the radioactive NA spillover rate method, we find that the rate at which noradrenaline is removed from the circulation falls with age in hypertensive patients (Fig. 12). This fall in plasma clearance in patients over 60 years is the cause of their elevated plasma noradrenaline concentration rather than increased noradrenaline release (Fig. 13). Noradrenaline release is increased only in patients under 40 years.

The regional pattern of sympathetic nervous activation in essential hypertension

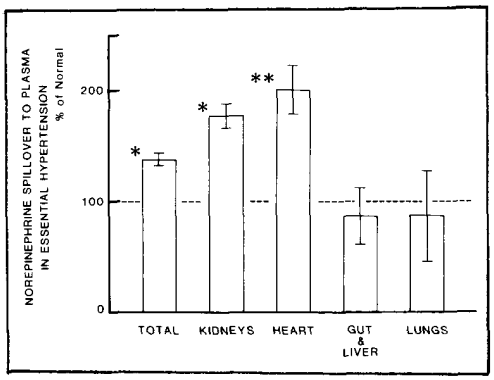


Fig 13
The pattern of activation of the sympathetic nervous system, based on measurement of noradrenaline (norepinephrine) release to plasma, present in patients with essential hypertension. Mean noradrenaline spillover in subjects with normal BP is indicated as 100%.
**P<0.05, ** P<0.01.*

M. Esler, G. Jennings, G. Lambert, J. Stockigt and P. Korner*

To see whether sympathetic neural activity was uniform in the different outflows, we measured the rates of noradrenaline spillover to plasma from individual organs including the heart, kidney, hepato-mesenteric bed, skin and muscle. This was done in 46 patients with essential hypertension and 22 subjects with normal blood pressure. On average, noradrenaline spillover from the heart was 100% higher, and from the kidney 79% higher than in subjects with normal blood pressure. Noradrenaline release from the lungs and splanchnic circulation was normal in hypertensive patients (Fig. 13). Sympathetic activity seemed normal in skin and skeletal muscle. The pattern of selective cardiac and renal sympathetic activation was particularly evident in younger patients (Fig. 14). Increased

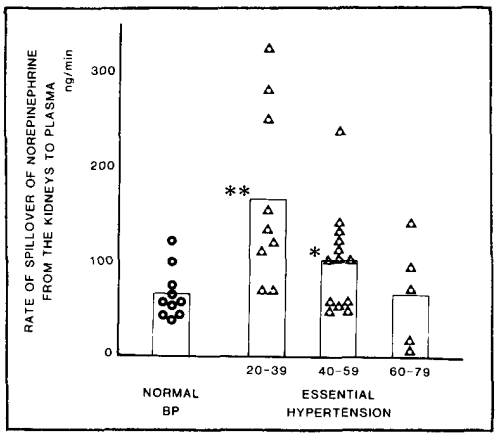


Fig 14
Renal noradrenaline release to plasma was increased particularly in younger hypertensive patients.
*$P<0.05$, **$P<0.01$.*

sympathetic nervous activity in the kidney could elevate blood pressure by causing body salt retention and by promoting secretion of the pressor hormone, renin. Increased cardiac sympathetic activity could raise blood pressure by enhancing the pumping action of the heart. The precise cause of these changes in sympathetic nervous function in hypertensive patients is not known. It probably is not mental stress, since splanchnic sympathetic activity increases in stress responses, but was normal in hypertension.

**Ewen Downie Metabolic Unit*

Release of noradrenaline from the human heart during experimental mental stress

M. Esler, G. Lambert, G. Jennings

Folklore has it that mental stress is an important cause of 'heart attacks', although the scientific evidence is not conclusive. One way in which mental stress could involve the heart is by increased cardiac sympathetic nerve activity, leading to abnormal heart rhythms and possibly death. We have measured the noradrenaline spillover rate from the heart in patients with essential hypertension during mild experimental stress (mental arithmetic). Although noradrenaline release for the whole body increased by only 53%, that from the heart was elevated by 360%, with a particularly large increase in some patients (up to ten-fold). With severe life stress the cardiac stress response could be very large indeed, and possibly sufficient to cause serious cardiac rhythm disturbances.

Clinical consequences of increased sympathetic nervous activity in cirrhosis of the liver

I. Willett, M. Esler, G. Jennings, G. Lambert,*
B. Crotty and F. Dudley**

We have previously demonstrated that noradrenaline spillover is increased in both the kidney and the splanchnic (gut) region of patients with cirrhosis. This may have adverse consequences. Increased renal sympathetic tone could aggravate the body salt and water overload that is typical of cirrhosis. Increased splanchnic sympathetic activity could enhance the high circulatory pressure found in the portal vein in portal hypertension, which would increase the probability of serious bleeding from the gastrointestinal tract, which is one of the major causes of death in cirrhosis.

In two experimental studies we have shown that the sympathetic suppressant, clonidine, which has been mainly used in hypertension, reduces the level of sympathetic activity in the gut of cirrhotic patients. Clonidine consistently lowered the portal pressure (on average

by 30%) and may prove useful in the long-term medical treatment of cirrhosis preventing some of the most disastrous complications.

*Gastroenterology Service

Release of noradrenaline from the brain

M. Esler, G. Lambert, G. Jennings

It is commonly believed that the noradrenaline which is released from the nerve cells within the brain does not escape into the circulation until it has undergone chemical breakdown. We now have evidence suggesting that this is not the case. In a pilot study in 7 patients with essential hypertension, blood samples were collected from the internal jugular vein via a catheter inserted into the circulation for clinical diagnostic testing. We used noradrenaline tagged with a low level of radioactivity to see if noradrenaline was released from the brain and found that NA spillover was 49 ngmin, equivalent to 12% of the overall release of noradrenaline to plasma for the body as a whole. We do not yet know whether this released noradrenaline is from the sympathetic nerves of the blood vessels of the brain, or from the nerve cells in the brain. If the noradrenaline we measure proves to be derived from brain cells, our technique will provide a measure of brain transmitter function.

Neurophysiology Laboratory

Head: Dr. Elspeth McLachlan

Projects

Functional innervation of arterioles

Innervation of the rat tail

Effect of temperature on neuromuscular transmission in cutaneous arteries.

Characterization of vascular and non-vascular sympathetic neurons.

Computer modelling of sympathetic neurons.

Synaptic potentials and currents in sympathetic neurons.

Descending projections to the thoracolumbar intermediate zone in rats and rabbits.

Summary

We are studying the cellular mechanisms of communication between nerve cells (neurons) of the sympathetic division of the autonomic nervous system, and between the endings of sympathetic nerve fibres and arterial vessels. Communication occurs through electrical signals (action potentials) that travel from the cell body along the nerve fibre and lead to activation of other neurons or of the smooth muscle cells of blood vessels which causes them to contract and narrow. This is the way in which the nervous system can increases to resistance to blood flow and so raises arterial blood pressure.

At the junctions (synapses) between two cells, a chemical transmitter substance is released from the nerve terminals, when the action potentials reach them. The transmitter interacts with 'receptors' on the membrane of the second cell and causes small ions to move either more or less easily through the neuronal membrane. Many hormones and other chemical substances can modify the ability of ions to move into or out of the cell. The result is sometimes excitation and sometimes inhibition of action potentials in the second cell, and eventually an increase or decrease in the calibre of the vessel.

We study these events by inserting fine glass micropipettes into nerve or muscle cells and then recording the transmembrane potential difference. In some experiments we use a special electronic circuit to control the potential at a predetermined level and to record current flow under these circumstances. This current reflects the movement of ions through pores (channels) in the membrane. There are several different kinds of channel in nerve and muscle cells. For example, one kind is opened by the action of transmitter at synapses. In sympathetic ganglia the transmitter is acetylcholine, while at the neuromuscular junctions on arterial vessels the transmitter substance is noradrenaline. In both cases, the movement of small cations, particularly sodium and potassium, is increased; this results first in membrane depolarization and then excitation. The time course over which the channels stay open and some of their responses to drugs are however quite different.

We have recently found that different sympathetic ganglion cells bear different types of channels that are not activated directly by the transmitter, but

are opened by depolarization of the cell as a result of ion movement. These channels are important because they determine the pattern of electrical discharge. We have identified a distinct difference between sympathetic neurones controlling blood vessels and the other functions e.g. with bladder contraction. Such differences may also exist between the smooth muscle of blood vessels in different organs, explaining differences in vascular reactivity, or, alternatively, the channels might become disordered in vascular disease. Understanding how other natural chemicals, hormones and drugs modify the behaviour of membrane channels could lead to new ways of treating disorders of sympathetic function.

We are using a range of anatomical techniques to obtain information about the structure and connection of neurons and synapses in the sympathetic nervous system. The sympathetic transmitter, noradrenaline can be demonstrated by fluorescence histochemistry and we have used the disappearance of fluorescent staining after nerve lesions to define particular nerve pathways to blood vessels. The recently developed immunohistochemical techniques combined with peroxidase 'tagging' to specific antibodies, are used to give a permanent record of the arrangement and size of varicose nerve terminals containing noradrenaline-synthesizing enzymes and neuropeptides. These methods are being applied to investigate the sympathetic endings on blood vessels and also the pathways within the brain and spinal cord involved with cardiovascular regulation.

At the ultrastructural level, we have studied sequential sections through individual sympathetic nerve endings on small arterioles. This has proved to be a more powerful analytical method than the classical method of studying a small number of sections obtained at random from the tissue. With serial sections we find that the vast majority of nerve-muscle contacts in blood vessels are structurally similar to other synapses, with the membranes of two cells coming into extremely close contact in specialized region. This observation is consistent with recent electrophysiological data on the mechanisms of nervous control of arterial smooth muscle (obtained over the last few years in David Hirst's laboratory). Traditional ideas on nerve-muscle excitation may require revision.

Projects

Functional innervation of arterioles

S.E. Luff, E. McLachlan, G.D.S. Hirst

We have been studying serial ultrathin sections in the electronmicroscope in order to discover how the sympathetic nerve endings around fine arterioles are arranged. Each ending has varicosities, which are swellings along the nerve terminal containing the vesicles that store noradrenaline (NAd). Previous studies by others have suggested that the varicosities are up to several μm from the smooth muscle cells of the blood vessel wall. It was therefore thought that NAd had to diffuse quite a distance before it reached the muscle cell.

Our observations on the fine arterioles (30-70 μm in diameter) of the submucosa of the guinea pig have shown that this description is incorrect. When *every* section is examined we find that more than 85% of the varicosities make direct contact with the arterial muscle cell. The gap between nerve and muscle cell membranes is less than 0.1 μm. The ultrastructural appearance of the neuromuscular junctions is virtually identical to that at nerve-muscle junc-

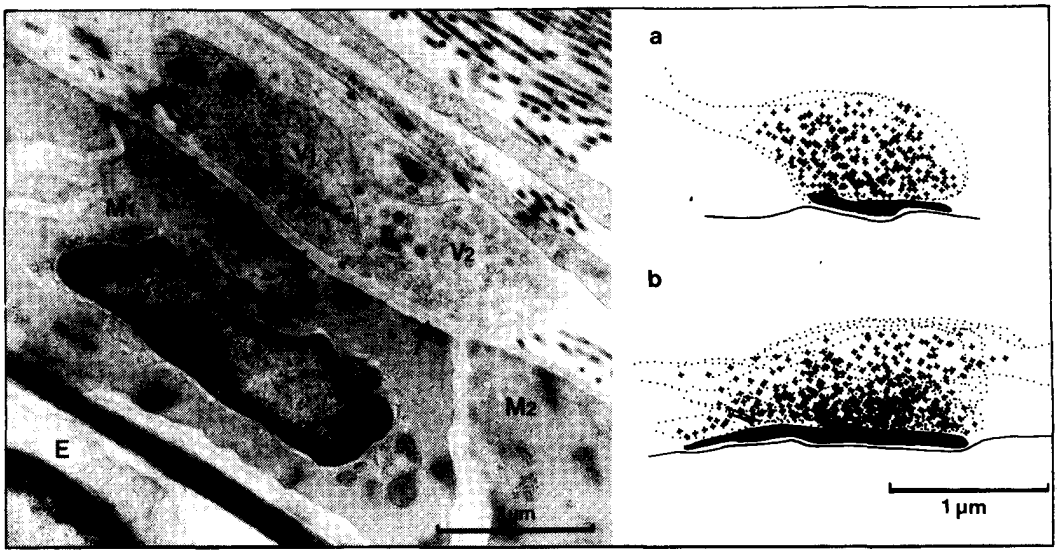


Fig 15
The structure of nerve muscle junctions on arterioles revealed by the electronmicroscope.
Left: Electronmicrograph of a nerve-muscle contact on an arteriole in the guinea-pig intestine. Varicosities (or swellings), V1, V2, of sympathetic nerve terminals contact smooth muscle cells, M1, M2, over the region shown with arrows. The endothelial cell, E, lines this small blood vessel, which is only one smooth muscle cell thick.
Right: Computer aided reconstructions of two contacts between sympathetic nerve terminals and the smooth muscle cells of arterioles. These were made by analysis of serial sections (0.1 μm = 0.0001 mm or 1/10000 mm thick) using the electronmicroscope.

tions in other muscles such as skeletal muscle, and at synapses between neurons. However, if the sections are sampled at random, we have found that the frequency with which contacts are encountered is low, as reported by others.

We have followed up these observations by applying NAd to the wall of a localized region of the living arteriole with an ionophoretic electrode. Responses similar to those obtained with nerve stimulation are found in clusters along the track of nerve bundles subsequently identified in the fluorescence microscope as corresponding to the sites of NAd-containing varicosities.

Innervation of the rat tail

T. Sittiracha

The tail of the rat is important for temperature regulation, and its blood vessels are densely innervated with sympathetic nerve fibres. It can be used as a model for the study of the control of the cutaneous innervation in man or other non-hairy mammals. There are a number of differences between the behaviour of the cutaneous vasculature and other beds such as skeletal muscle, particularly under conditions of emotional stress. We have been using two experimental approaches to determine the source of the innervation of the tail skin and blood vessels in the rat.

The first involves making lesions to the major nerve trunks running along the tail and determining whether the nerve endings on the arteries disappear. This is tested using fluorescence histochemistry for the demonstration of NAd, and also high pressure liquid chromatography to measure the amount of NAd present (kindly performed by Dr. C. Bell, Department of Physiology, University of Melbourne). The ventral collector nerve trunk is the main source of fibres to the caudal artery.

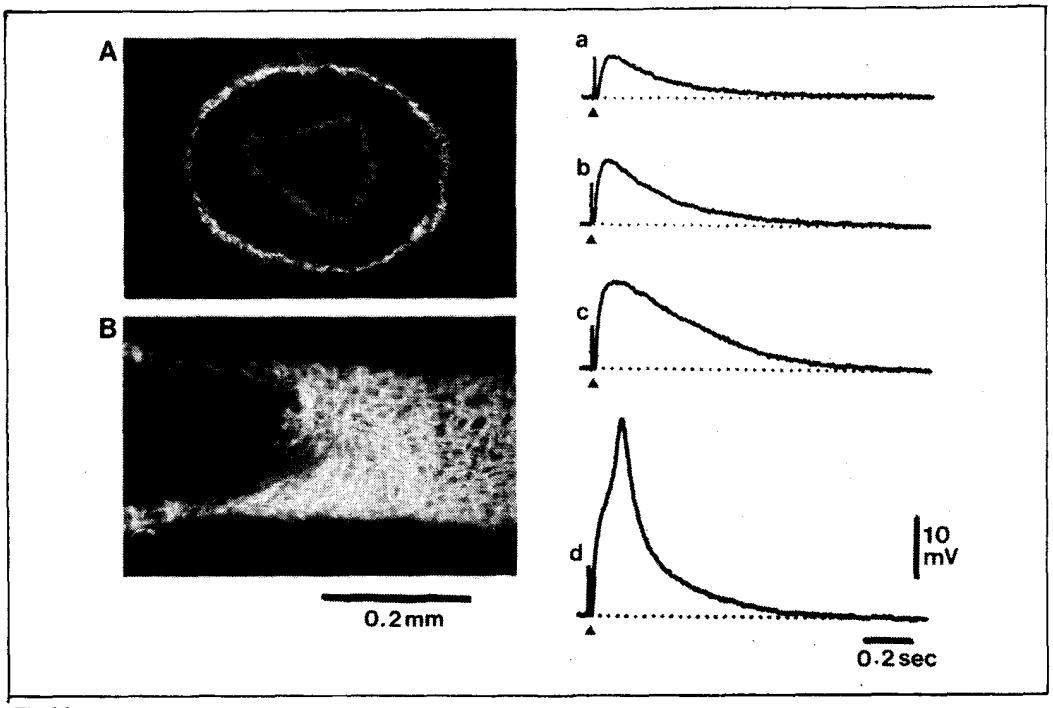


Fig 16
Innervation of arterial smooth muscle by noradrenergic sympathetic nerves.
Left: Photomicrographs of a rat tail artery as seen in the fluorescence microscope after formaldehyde fixation, showing the plexus of noradrenergic nerve terminals around the outside of the vessel, in cross-section (A) and longitudinal section (B).
Right: Electrical responses of the smooth muscle cells of the tail artery to stimulation of the nerve supply (at increasing strength from a-d). Excitatory junction potentials are recorded in the smooth muscle cells using an intracellular microelectrode. These potential changes lead to a muscle action potential in d, which is followed by muscle contraction and so constriction of the artery.

Secondly, we have applied a tracing substance, (horseradish peroxidase) to the axons of the major nerve trunks or to the nerve endings on the arterial wall and located the cell bodies giving rise to these axons in the ganglia of the sympathetic chain. Most of the cell bodies are found in the sacral chain beyond the hindlimb outflow, and are organised rostrocaudally in a manner similar to the sensory neurons in the same nerve.

Effect of temperature on neuromuscular transmission

T. Sittiracha, E. McLachlan, J. Cassell

Cutaneous blood vessels are particularly affected by sympathetic nerve impulses during emotional stress in man. In addition, the control of the calibre of the cutaneous blood vessels is important for the body temperature regulation in mammals. We have examined how the membrance channels of the smooth muscle of the arterial wall are affected by temperature changes. We found that the potential changes produced by nerve stimulation become smaller as the temperature falls because the amount of NAd released is reduced. However, this is largely compensated for by an increased sensitivity of the vessel to NAd via alpha-receptor activation. This latter produces a delayed depolarization that is very slow and seems to be enhanced by some of the metabolic effects of temperature. It involves a prolonged decrease in ionic

conductance, an effect opposite to that underlying the early excitatory junction potential.

Characterization of vascular and non-vascular sympathetic neurons

E. McLachlan, D. Lewis, J. Cassell

We have examined the lower thoracic and lumbar sympathetic outflow to the viscera and hindlimb to determine the differences between sympathetic neurons making contact with blood vessels and those innervating other organs. Different visceral sympathetic neurons are involved with inhibition of motility and control of intestinal secretion while others produce vasoconstriction. In contrast, much of the hindlimb sympathetic outflow goes to blood vessels.

Three types of voltage-dependent K+ channel are responsible for the different discharge patterns we have identified in these two neuron types. Vascular neurons are usually 'phasic' - i.e. they discharge readily in high frequency bursts - because of a slowly opening channel (called the M channel) that decreases cell excitability after the initial discharge. This channel is generally not present in non-vascular neurons projecting to the pelvic viscera, which discharge continuously to an ongoing stimulus. However, the rate of firing of these latter neurons is slowed because of the presence of a different channel (the A channel) which normally does little to modulate the activity of vascular neurons.

The third type of K+ channel is one opened by the inward movement of Ca++ ions during the action potential. This produces the ensuing after- hyperpolarization which decreases responsiveness, particularly to subsequent synaptic inputs. Lumbar vascular neurons usually have only small after-hyperpolarizations. In recent experiments on neurons of the coeliac ganglion, about 30% of cells have been found to have particularly pronounced and prolonged Ca-activated K+ conductances. The resultant after-hyperpolarization can silence discharge for several seconds. Although such cells discharge phasically, this behaviour seems more likely to be associated with motility of the gastrointestinal tract than with changes in splanchnic vascular resistance.

Computer modelling of sympathetic neurons

J. Cassell, E. McLachlan

We are using a computer model incorporating the electrical characteristics of sympathetic neurons to interpret the signals we record in experiments from the cell body under voltage clamp. The program for this model has been generously provided by Dr. J. Clements and Dr. S. J. Redman of the Experimental Neurology Unit at the John Curtin School of Medical Research, Australian National University.

The modelling helps determine the location of presynaptic varicosities on the dendrites of the cell. The shape of the synaptic currents recorded in voltage-clamp experiments after stimulation can be explained by simulating the effect associated with different anatomical distributions of synaptic sites in the model. We can then confirm our hypothesis by defining cell structure accurately with new intracellular dye injection techniques.

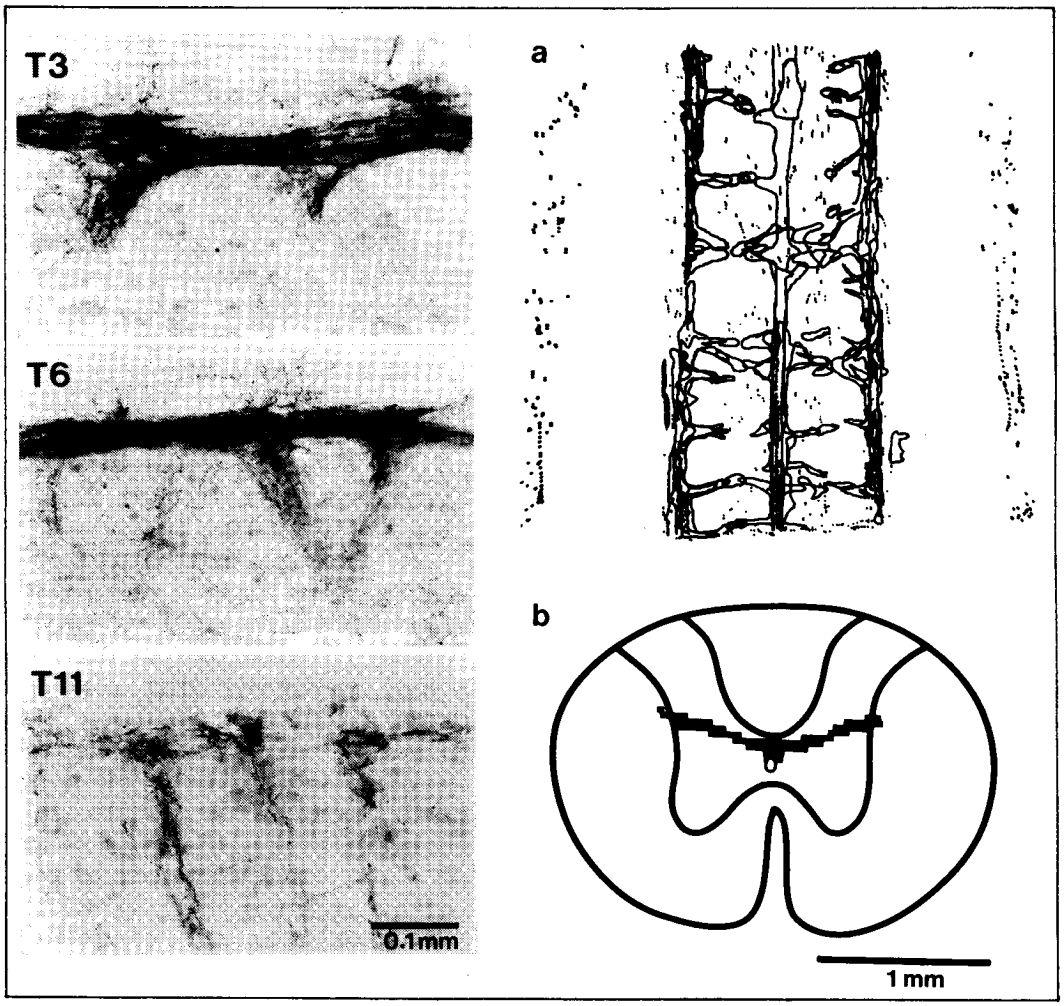
Voltage-dependent conductances modify the response of nerve cells to synaptic events and we have recently shown that the A current limits the size and duration of the synaptic potential in some neurons. As some of the peptides thought to be active in sympathetic ganglia may act by blocking A

channels, the stimulation observed with these compounds could occur by potentiation of ongoing synaptic events.

Synaptic potentials and synaptic currents in sympathetic neurons

G.D.S. Hirst, E. McLachlan, J. Cassell

There has been some confusion about the types of acetylcholine (ACh)-activated channels in autonomic neurons because the synaptic currents recorded in rat parasympathetic neurons were biphasic while those recorded in rabbit and guinea pig sympathetic neurons were monophasic. We have confirmed these findings in the rat and guinea pig. By averaging several hundred very small spontaneous currents (resulting from the release of



Descending adrenergic pathways terminating near sympathetic preganglionic neurons in the spinal cord of the rat. Left: Photomicrographs of horizontal section (30μm = 0.03 mm thick) showing varicose nerve terminals with immunoreactivity to PNMT (the enzyme present only in adrenaline-containing neurons). Clusters of endings have characteristic patterns at different levels of the thoracic spinal cord.

Right: Three-dimensional mapping of the arrangement of PNMT immunoreactive terminals in T6 segment using the computer. Horizontal sections are superimposed (a) and then rotated through 90° to show the distribution in a cross-section through the spinal cord (b).

single quanta of transmitter), we have shown that these differences also apply to quantal events. This means that the channels in rat neurons behave quite differently from those in cells from the other species, despite their indistinguishable pharmacological characteristics.

A second aspect of this study concerns the observed prolongation of synaptic potentials that occurs when a 'strong' suprathreshold preganglionic input is excited. This is particularly important for vascular pathways in which strong connections are always present in peripheral ganglia. We have identified a prolonged current activated by strong inputs to these neurones that is not explained by any conventional mechanism.

Descending projections to the thoracolumbar intermediate zone in rats and rabbits

C. Anderson, O. Srb-Christie

Several histochemical techniques now enable us to identify some of the chemical substances present in nerve terminals. The amine-containing pathways in the central and peripheral nervous systems can be demonstrated by formaldehyde-induced fluorescence, or by the newer immunohistochemical methods for peptides and for the enzymes associated with the synthesis of catecholamine.

To define the neuronal pathways that project from the brain stem to the sympathetic preganglionic neurons in the thoracolumbar spinal cord, we have devised computer-based techniques to make three-dimensional maps of the distribution of the terminals. These maps are being correlated with the position of sympathetic preganglionic neurons identified by retrograde labelling of different peripheral pathways using horseradish peroxidase. To date, we have shown the distributions of tyrosine hydroxylase (TH), phenylethanolamine-N-methyl transferase (PNMT), neuropeptide Y (NPY) and formaldehyde-fluorescence positive terminals in rat spinal cord.

Pharmacology Laboratory

Head: Dr. J. Angus

Projects

Isolation of endothelium derived relaxing factor (EDRF).

Endothelium dependent vasodilation after intimal damage.

Vascular responsiveness in renal hypertension.

Regional vascular responses to serotonin.

Comparison of large and small coronary arteries to antianginal drugs.

Alpha$_2$-adrenoceptors on endothelial cells.

Sympathetic vasconstriction and the role of alpha adrenoceptors.

Sympathetic transmission in the rat heart.

Summary

Our work concerns the different mechanisms that alter the calibre of large and small blood vessels, including those coming from (i) the endothelial cells lining all vessels; (ii) from the sympathetic nerves in the wall, and (iii) from hormones or drugs circulating in the blood. We wish to determine to what extent all of these contribute to the altered vascular responsiveness in disease states such as coronary artery spasm, coronary atherosclerosis, hypercholesterolemia and hypertension. Pharmacological methods are particularly appropriate to help unravel the role of these mechanisms that alter vascular function.

Endothelial cells form a fragile barrier between the blood stream and the wall of the blood vessel wall. Surprisingly, many circulating substances cause them to release a powerful substance that acts locally to relax the underlying smooth muscle. In turn, this increases the diameter of the lumen. The composition of this local 'hormone' is still unknown; it has been termed EDRF — endothelium derived relaxing factor. We are trying to determine its chemical composition which is the key to understanding its synthesis and metabolism. It may allow development of new analogues that mimic the action of EDRF, or of drugs that enhance its endogenous synthesis or inhibit its destruction. This type of drug could find therapeutic use in several types of vascular disease. So far we have led the international race to develop a bioassay for EDRF from cultured endothelial cells in collaboration with Dr. Julie Campbell (Cell Biology). With this bioassay and a new method of analysing concentration — response curves, we estimate that EDRF has a half-life of around 45 seconds; we know that it is negatively charged, is hydrophilic and binds to haemoglobin.

From the viewpoint of developing new drugs it is important to know whether loss of EDRF activity due to damage of the endothelium contributes to vascular disorders such as coronary vasospasm or even hypertension. In rabbits made hypertensive or fed a high

cholesterol diet, the hindlimb vasculature appeared to be unaltered in response to acetylcholine, which dilates vessels by releasing EDRF. By contrast, in large coronary arteries where there were concentric atheromatous lesions the EDRF response was absent. The lipid-containing foam cells in the neo-intima may provide a significant barrier to EDRF. However, the neo-intima is not by itself a barrier to the action of EDRF. In dog carotid arteries removal of endothelium by balloon inflation resulted 4 weeks later in a 2-20 cell thickened intima lined by endothelial cells. The response to vasoconstrictor agents was increased in sensitivity but responsiveness to acetylcholine was unaltered. It appears that provided neo-intimal cells are lipid free and covered by endothelium, EDRF release is normal.

Large and small arteries respond differently to many drugs and hormones. We have begun to determine the precise sites of action of drugs used in the treatment of angina. In conscious greyhounds we measured simultaneously the diameter of a large coronary artery (sonomicrometer crystals) and the resistance of the small blood vessels during intracoronary infusion of nitroglycerin or acetylcholine. Nitroglycerin was much more selective in dilating mainly the large vessels, whereas acetylcholine (which acts through EDRF release) dilated the bed more uniformly. Nitroglycerin and EDRF have been considered to act via the same intracellular messenger of smooth muscle cells to sequester calcium, but our results suggest that this may not hold for coronary vessels. In the same context a large group of chemicals loosely defined as calcium antagonists have a range of actions on the large and small coronary vessels, which may eventually lead to more precise therapy.

Projects

Half-life of EDRF

T. Cocks, J. Angus, J. Campbell, N. Puglisi.

Endothelial cells line the inside of blood vessels and are in close proximity to the smooth muscle cells in the wall. We devised a new bioassay system, where endothelial cells were grown in pure culture on plastic beads. Large numbers of cells (50 million) could be placed in a tube and perfused in a continuous system. The perfusate was passed along a catheter to drip over a contracted ring segment of coronary artery from which the endothelium had been removed, so the only source of EDRF was the culture system. Next bradykinin was injected through the cell column in different concentrations, causing the endothelial cells to release EDRF and relax the coronary artery. Thus, we obtained bradykinin concentration - relaxation response curves under varying circumstances.

Half-life was determined by altering the length of the catheter between the cell column and the artery, thus varying the time taken for EDRF released from the cells to reach the assay tissue. This technique allowed the construction of a family of bradykinin relaxation curves with delays of 0, 24, 48, 74 and 134 seconds. Theoretical analysis showed that an agonist drug acting via the release of an intermediate displaces the curves to the right and half-life can be determined from this shift. The half-life of EDRF was 45 seconds in this system at 37°C. To our knowledge, this is the first time that this method has been used to determine half-life of an endogenous substance.

Alpha$_2$-adrenoceptors are located on endothelium

K. Satoh, J. Angus, T. Cocks.

Adrenaline and noradrenaline stimulate endothelial cells to release EDRF in addition to their direct constrictor action on vascular smooth muscle. The EDRF- mediated action thus tends to limit the direct constrictor response. Our original observation in dog and pig large coronary arteries (Co) has been extended to small arteries as well as to the large carotid (Ca), femoral (F), renal (R) and mesenteric (M) arteries in the dog. All blood vessels were precontracted with the thromboxance analogue U46619, after establishing similar degrees of passive stretch from the Laplace relationship as devised by Mulvany for microvessels. In the presence of alpha$_1$ and beta-receptor blockade via prazosin and propranolol, noradrenaline relaxed the arteries in the order of magnitude Co>Ca>F>R>M. A similar order but with higher efficacy was observed for substance P, another EDRF-dependent vasodilator agent. In all cases removal of endothelium abolished the relaxation to noradrenaline and substance P. Evidence that the endothelial cell receptor was of the alpha$_2$ subtype was that the specific alpha$_2$, agonist UK14304 mimicked the relaxation curves to noradrenaline and that idazoxan (alpha$_2$ - antagonist) shifted the dose-response curves to the right. Thus endothelium-dependent relaxation to alpha$_2$ and substance P receptor stimulation is greatest in the coronary and weakest in the renal and mesenteric arteries.

Vascular responsiveness in renal wrap hypertension

C. Wright, J.A. Angus, P. I. Korner

The enlargement of the muscle coat that occurs in the small arterial blood vessels in all types of hypertension may alter the reactivity to constrictor and dilator stimuli. This property contributes considerably (about 75%) to the maintenance of the elevated blood pressure in established hypertension, indeed more than the underlying cause. We have previously found that the dose-response curve of the hindlimb resistance to three pressor substances had twice as steep a slope as curves obtained in control rabbits with normal blood pressure. For this work we used rabbits subjected to autonomic blockade, to allow evaluation of the *intrinsic* properties of the blood vessels. These findings have been challenged recently and we have re-examined our earlier findings. We used an improved preparation, in which we could determine full dose-hindquarter vascular resistance curves and assess vascular sensitivity from changes in ED_{50}. As above we used autonomic blockade, to allow assessment of *intrinsic* vascular properties. Cumulative dose-response curves to noradrenaline, methoxamine and angiotensin II were unchanged in sensitivity (ED_{50}) between sham and hypertensive rabbits. For each agonist the slope and range of the conductance curves was halved in hypertension, consistent with structural alterations in the resistance vessels. Similarly, the findings with vasodilator drugs (acetylcholine, serotonin adenosine) supported the hypothesis that the increase in vascular reactivity was non-specific and that there was no alteration in vascular sensitivity (ED_{50}).

These experiments extend our own previous work and also support Folkow's conclusions, which were based on analysis of full dose-response curves in the isolated hindquarter of rats perfused with an electrolyte solution. The present study is the first time full stimulus-response curves have been obtained in *vivo*. The structural changes

in hypertension make the resistance vessels an important non-specific amplifier of all types of stimuli.

Regional vascular responses to serotonin

C. Wright, J. Angus.

Serotonin is a powerful substance that can be released from platelets. Recently Cocks and Angus demonstrated that serotonin in dog coronary arteries (1) stimulated the release of EDRF and (2) directly constricted the smooth muscle. These two actions of serotonin are antagonistic. The receptors mediating the release of EDRF were classified previously as non-S_2 since ketanserin did not alter the relaxation effects of serotonin.

In the present work we studied regional response differences to serotonin in the vessels to the limb, gut and kidney in conscious instrumented rabbits. Pulsed Doppler flow transducers were used to measure blood flow and the autonomic nervous system was blocked. Intravenous infusion of serotonin (30 μg/kg/min) caused renal blood flow to fall abruptly to zero apparently as a result of renal artery spasm; at the same time hindlimb flow and conductance always increased, even at high infusion rate. Mesenteric conductance increased in 10 out of 16 rabbits and decreased slightly in 6. The serotonin antagonist methiothepin (1 mg/kg/iv) abolished the fall in renal conductance to serotonin and attenuated the dilator responses in the other beds. Ketanserin and methysergide (0.5 mg/kg) did not prevent renal artery spasm but shifted the threshold 4-10 fold. These antagonists had little effect on the dilator responses to serotonin in the other beds. We conclude that vascular actions of serotonin are not uniform and that there are at least two distinct classes of receptors, perhaps located on endothelial cells and smooth muscle cells. The recent availability of serotonin analogues which have selective vascular actions may help to clarify its complex vascular actions.

Comparison of large and small coronary artery reactivity to antianginal drugs

J. Angus, K. Satoh, T. Cocks.

Angina pectoris may be associated with two distinct mechanisms, one where large coronary artery segments may spasm and the other where insufficient coronary flow (high vascular resistance) limits the oxygen supply to the heart muscle during exercise. Clearly drugs which specifically dilate the large arteries will be useful for spasm while more general coronary dilator drugs are more suitable for exercise- induced angina.

Nitroglycerin is an old but most useful drug for the treatment of angina. It is considered to relax smooth muscle by generating the substance cGMP within the cell, resulting in the sequestration of calcium. This biochemical mechanism is identical to

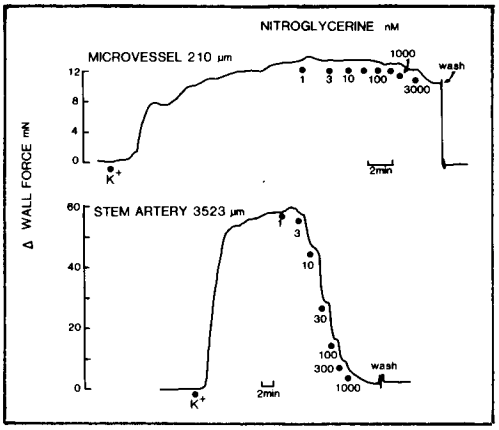


Fig 18
Nitroglycerin relaxes a canine large coronary artery segment (bottom trace) but is almost without effect in a very small artery (210 μm diameter) taken from the same heart.

that reported for EDRF-mediated relaxation of smooth muscle. We have compared the relaxation responses to acetylcholine and nitroglycerin in large (3-4.5 mm id) and small (150-350 μm id) coronary artery segments from greyhound hearts suspended in organ baths and on a Mulvany double myograph respectively. Cumulative additions of acetylcholine (0.01-10μM) relaxed the large and small vessels to near-maximum and with a similar potency. Nitroglycerin maximally relaxed the large arteries over a similar concentration range as for acetylcholine. In contrast, nitroglycerin was approximately 100 fold less potent in relaxing the small vessels. The calcium antagonists nifedipine, verapamil and nicardipine relaxed both vessels completely but the latter two drugs were more potent on the small vessels.

Thus, the loss of activity of nitroglycerin on small coronary arteries is not shared by EDRF - dependent vasodilators nor by the calcium channel antagonists. These data suggest that the failure of intracellular messenger cGMP generation may not be the explanation for the absence of activity of nitroglycerin in small vessels.

Sympathetic vasoconstriction and the role for alpha adrenoceptors

M. Lew, D. Hirst.

Sympathetic nerve stimulation in guinea pig mesenteric blood vessels causes excitatory junction potentials (ejp's), membrane depolarisation and contraction. These events are considered to be the result of noradrenaline released from nerve terminals in close proximity to alpha-adrenoceptors on smooth muscle cells.

Hirst and Neild found that high concentrations of many alpha-adrenoceptor antagonists were completely ineffective in blocking the post-synaptic electrical events associated with nerve stimulation. They concluded that noradrenaline may activate a new type of 'gamma' receptor that was distinct from alpha-receptors in these

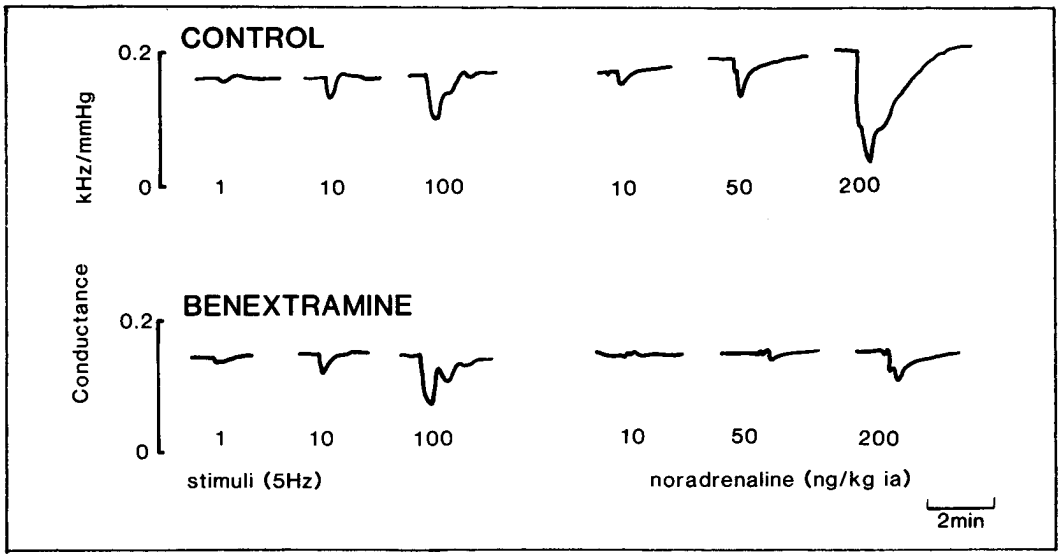


Fig 17
Traces of hindlimb vascular conductance in an anaesthetized areflexic rabbit. Left: traces are responses to lumbar sympathetic nerve chain stimulation that are resistant to the alpha receptor antagonist benextramine. Right: responses to intra-aortic injections of noradrenaline that are much reduced by benextramine.

vessels. This hypothesis, if true, offers a new target for antihypertensive drug therapy that may separate vascular actions of circulating adrenaline from nerve released noradrenaline.

In the present experiments contractile responses rather than electrical responses to nerve stimulation have been measured in small arteries from rabbits and guinea pigs. In a Mulvany double myograph, contraction responses to electrical field pulses were completely resistant to blockade by benextramine, an alpha-adrenoceptor antagonist, in agreement with the findings of Hirst and Neild. The relevance of these in vitro results were tested in an intact hindlimb vascular bed in the anaesthetised, ganglion-blocked rabbit. Falls in hindquarter blood flow elicited by lumbar sympathetic nerve chain stimulation were not inhibited by benextramine but responses to intra-arterial injections of noradrenaline were greatly reduced. As before, these experiments demonstrated a difference between the responses to nerve stimulation and to exogenous noradrenaline.

There are at least two explanations for the experimental findings:- (1) the postsynaptic receptors for noradrenaline are different from alpha-adrenoceptors or, (2) the transmitter noradrenaline is co-released with ATP, with the latter responsible (at least in part) for the electrical and contractile responses resistant to alpha blocking drugs.

Cardiac sympathetic transmission in normotensive and genetic hypertensive rats

A. Dyke, J. Angus.

Enhanced sympathetic nervous system activity may play a role in development of some types of experimental and human hypertension. In collaboration with Biochemical Pharmacology, we have started a programme to learn about the properties of the cardiac sympathetic nerve terminal in normotensive rats and in rats with spontaneous hypertension. Most work to date has been done in normal rats. The isolated right atrium of the rat is suspended between platinum field electrodes to depolarise sympathetic nerves. Responses measured include the release of transmitter noradrenaline by tracer labelling (^3H) of the noradrenaline stores and the effector response — tachycardia. Our results show that the rise in rate is mediated through beta-adrenoceptors, with minimal contribution through alpha-adrenoceptors. Neuronal uptake of exogenous noradrenaline appeared to be of less importance in the rat than in the guinea pig heart. In contrast, the uptake inhibitors such as cocaine, desipramine and Ly139603 caused enhanced responses to nerve stimulation. A significant population of beta-adrenoceptors may occur extra-junctionally in the rat heart.

Renal Laboratory

Head: Dr. W.P. Anderson

Projects

Reversal of angiotensin II mediated events in renal artery stenosis

The autonomic nervous system in renal hypertension — an ultrastructural study

Functional morphology of the macula densa

Renal and vascular actions of atrial natriuretic peptide

Renal actions of vasopressin

Morphology of renal-wrap hypertension

Renal nerves in renal hypertension

Morphology of renin synthesis

Glomerular actions of angiotensin II

Summary

The glomerulus of the kidney is a fine 'filter', which holds back the plasma protein and allows water and electrolytes to pass into the tubules as the first step in urine formation. Its capillaries are unique compared to those in other organs; their perfusion rate with blood is high and they are highly permeable which allows them to filter the plasma at a high rate. In turn, this fast filtration plays an important role in the long-term regulation of blood pressure.

It has long been believed that the rate of filtration of plasma in the glomerulus is determined by the vascular tone of the arterioles entering and leaving the glomerulus, called respectively the afferent and efferent arterioles. Changes in the tone of these vessels affect filtration rate by altering the pressure and flow of plasma within the glomerulus. We have been examining the role of another possible mechanism regulating glomerular filtration rate which specifically affects the permeability of the capillaries. Our hypothesis is that the permeability may be altered by contractions of the mesangial cells of the glomerulus, a type of cell unique to the capillaries of the kidney.

The mesangial cells are attached to the loops of the glomerular capillaries and are known to be contractile in tissue culture, particularly to the hormone angiotensin II. If this also happened in vivo this mechanism could regulate filtration rate by changing capillary size and permeability. Using morphometric and stereological techniques, we have found that angiotensin II, at physiological concentrations, causes contraction of these cells. We believe that this response aids filtration.

The action of angiotensin II on the mesangial cells rather than the efferent arterioles could help explain our previous findings in long-term renal artery stenosis. We had found that angiotensin II was maintaining of glomerular filtration rate in these circumstances, and doing so by maintaining the fraction of plasma filtered in the glomerulus without greatly reducing the

blood flow of the kidney. These findings could be explained if the angiotensin II was producing mesangial cells changes. Whether or not this is the explanation of the results of the stenosis experiments, it seems certain that angiotensin II does cause mesangial contraction in short-term renal artery stenosis. Our results are the first demonstration of mesangial cell contraction *in vivo* and point to a previously unsuspected important role for these cells in the regulation of renal function and blood pressure.

This year we identified a strain of rabbits in our breeding colony, which have glomeruli on the surface of the kidney. This is rare in mammals in which even the outermost glomeruli are usually located at least 1 mm below the surface. The glomeruli on the surface of the kidney are readily accessible to micropuncture, allowing direct pressure measurements in the vessels and analysis of blood and tubular fluids.

It has recently been discovered that the atria of the heart contain a peptide hormone that can stimulate profound loss of sodium through the kidneys. This has caused much speculation that this atrial natriuretic peptide (ANP) may be a key factor in the relationship between salt, blood pressure and the kidneys. We have developed a radioimmunoassay for ANP to examine its role in health and disease. Although ANP can cause a profound natriuresis, the mechanisms involved are not known. We have been studying whether the atrial peptide acts via other intrarenal hormone systems, and whether it has direct effects in the glomerulus itself. We have also started experiments to investigate how ANP lowers blood pressure. Other researchers have shown that cardiac output is reduced by ANP and we are hoping to untangle the direct effects of the peptide from the secondary effects of cardiovascular reflexes.

Projects

Angiotensin II and the glomerulus

W.P. Anderson, D. Alcorn and K.M. Denton

It has been thought that angiotensin II (AII) affects glomerular filtration rate (GFR) of plasma by acting on the afferent and efferent arterioles. We have been examining the hypothesis that AII might act on the mesangial cells in the glomerulus. We have studied the role of AII in renal artery stenosis, where it helps to maintain glomerular function. The kidneys of anaesthetized dogs were perfused fixed with glutaraldehyde following renal artery stenosis with or without inhibition of converting enzyme (captopril). Kidneys with intact renin-angiotensin systems showed marked foldings of the paramesangial basement membranes, an effect mostly absent in captopril treated kidneys. Additionally there was apparent pointing of the capillaries in the mesangial regions and glomerular capillary volume tended to be smaller. These actions are compatible with a contractile action of angiotensin II on mesangial cells which appears to be important in regulating GFR.

Angiotensin II and blood pressure beyond stenosis of the renal artery

W.P. Anderson

When a stenosis is applied to a renal artery to reduce distal pressure, that pressure does not remain at that initial low value but recovers towards pre-stenosis values. This recovery, which occurs over 5-30 minutes, is mediated by angiotensin II acting within the kidney and abolished by giving angiotensin II converting enzyme

before inducing stenosis. However, we reported previously that once it had occurred, the recovery of pressure could not be reversed by administering the converting enzyme inhibitor. In those experiments, we used teprotide to inhibit converting enzyme. This paradoxical result, was reinvestigated using a different converting enzyme inhibitor, captopril. Thirty minutes after inducing stenosis captopril caused post-stenosis pressure to fall close to values obtained immediately after induction of stenosis. The reason for the difference between captopril and teprotide may be the smaller size and greater diffusibility of captopril. This result strengthens our previous hypothesis on the role of AII in the maintenance of post-stenosis pressure.

Macula densa during natriuresis and diuresis

W.P. Anderson, D. Alcorn and G.B. Ryan

The macula densa of each nephron is located in close proximity to the glomerulus and to the afferent and efferent arterioles of that nephron. Changes in tubular fluid at the macula densa are believed to affect glomerular function and renin secretion, but the mechanisms by which the composition of distal tubular fluid is sensed are unknown. We have shown that agents that cause diuresis and natriuresis affect the volume of fluid in intracellular spaces between macula densa cells. At high rates of urine flow, there may be complete closure of the spaces. We suggest that the volume of fluid in these spaces represents changes in fluid fluxes between the tubule and the interstitium of the kidney in this region. We have now identified unique structures containing vesicles and bathed by the fluid of these spaces in each macula densa (see figure 18). These structures are well placed to sense changes in fluid fluxes.

Renal nerves in renal hypertension

K.M. Denton, R.L. Kline and W.P. Anderson

Afferent renal nerves have recently been implicated in the pathogenesis of

some forms of renal hypertension. Renal-wrap hypertension would be a form in which such nerves might be expected to play an especially important role because both renal blood flow and glomerular filtration rate are greatly reduced in this form of hypertension. We have therefore compared the development of renal-wrap hypertension in rabbits with normally innervated kidneys and rabbits in which the kidneys are totally denervated. Denervation of the kidneys, which destroys both afferent and efferent nerves, did not prevent the development of hypertension but the blood pressure elevation was slightly less than in rabbits with normally innervated kidneys.

This probably does not indicate that the afferent nerves play a role in the pathogenesis of renal hypertension, since we observed that renal denervation also caused a small fall in blood pressure in normotensive control rabbits. We suggest that this small effect of denervation on blood pressure is probably due to ablation of the efferent sympathetic nerves to the kidney.

Kidney morphology in renal-wrap hypertension

K.M. Denton, D. Alcorn and W.P. Anderson

Kidney blood flow and glomerular filtration rate are greatly reduced in renal wrap hypertension - an experimental model which may simulate some renal parenchymal diseases. Examination of wrapped kidneys by light and electron microscopy has shown that the capsule of the kidney, thickened more than 100-fold, contains contractile elements (myofibroblasts). In the outer parts of the kidney, there is damage to glomeruli, collapse of many tubules, and a proliferation of interstitial cells. These changes explain the falls in blood flow, filtration rate and PAH extraction ratio that occur as this form of hypertension develops. We are now investigating how angiotensin II helps the kidney maintain its function. There is a marked stimulation of renin synthesis in these kidneys, best seen when the animals are given converting enzyme inhibitor to block the negative feedback of angiotensin II on renin release *(figure 19)*

Cardiac and arterial baroreceptor control of vasopressin and renin release during haemorrhage

A.W. Quail, R.L. Woods and P.I. Korner

We investigated the role of the cardiac and arterial baroreceptor reflexes in the control of release of vasopressin and renin during haemorrhage. We found that vasopressin release is predominantly under the control of cardiac afferent nerves, while renin release is largely independent of both the arterial and cardiac baroreceptors. Furthermore, we showed that it was not until a critical reduction in blood volume of about 25% was reached that vasopressin release was stimulated, adding weight to the idea that vasopressin is important only when the body's other mechanisms for maintaining blood pressure have failed.

Radioimmunoassay of atrial peptide

R.L. Woods, A.M. MacPherson and J.D. Lineham

A radioimmunoassay to measure atrial natriuretic peptide has recently been established using commercial antiserum and tracer. This assay is specific and sensitive enough to measure plasma as well as tissue levels of the peptide. The circulating peptide requires prior extraction from plasma to purify the hormone as cross-reacting, non-specific substances in plasma interfere with the radioimmunosassay measurement. Purification of plasma on small Bondelut chromatographic columns has become the method of choice after testing many different extraction procedures. This method has a good recovery rate and removes interfering substances. Due to the very high cost of commercial antiserum, we have begun growing our own antibodies in rabbits, injected with a conjugate of the atrial natriuretic peptide (28 amino acids, human, synthetic) that is highly antigenic. After 5 months of immunizations, one rabbit has produced highly specific antibodies, with 1:20,000 titre for use in the radioimmunoassay. Similar results to those measured with the commercial antibody have been obtained with our own antibody.

Renal actions of atrial peptide

R.L. Woods, W.P. Anderson, D. Ramsay and M. Hume-Cook

Atrial natriuretic peptide is known to markedly enhance salt loss from the body via the kidneys. The mechanism for this action is not understood. Some workers believe that atrial peptide acts directly on renal tubules while others believe the increase in salt excretion is due to changes in renal haemodynamics or that this action may be in part mediated by other hormonal systems. We have found that the salt excreting action of atrial peptide is associated with increases in glomerular filtration rate without changes in renal blood flow. Furthermore, the renin-angiotensin system and the vasopressin antidiuretic system are not involved in the natriuretic effects. These findings suggest that, like angiotensin II, atrial peptide may have a primary action in the glomerulus to increase permeability. The resultant rise in glomerular filtration rate would increase the load of filtered sodium and enhance salt excretion.

Vascular Laboratory

Head: Professor John Ludbrook

Projects

Distribution of blood flow and conductance during exercise in the conscious rabbit.

Role of arterial baroreflexes during spontaneous motor activity in the rabbit.

Opioid receptor mechanisms in the circulatory response to haemorrhage.

Sympatho-adrenal mechanisms in cardiovascular responses to naloxone after haemorrhage.

Blockade of cardiac efferents by intrapericardial instillation of drugs.

Summary

Our previous work on cardiovascular regulation in the rabbit during exercise showed that at the onset of exercise the baroreceptor reflexes that originate from pressure sensitive receptors in the arteries and heart become inoperative. This could be due to suppression within the brain itself of the signals rising from baroreceptors, as part of the animal's response of preparing for exercise. In addition, signals coming from the exercising muscles themselves could help in the suppression of the baroreceptor signals once exercise has begun. The sudden suppression of the baroreceptor reflexes will allow the blood pressure to rise at the beginning of exercise, probably through sudden constriction of non-active vascular beds. Dr. Robert Hales, from the Ian Clunies Ross Animal Research Laboratory at C.S.I.R.O. in Sydney, joined us for a series of experiments involving the use of radioactive microspheres to work out the resulting redistribution of blood flow. We showed that the blood vessels in the gut, skin and kidneys constrict at the onset of exercise, making a greater proportion of the blood flow available for the skeletal muscles at the onset of exercise. This vasoconstriction was entirely accounted for by suppression of the arterial baroreceptor reflex.

This work led us to study the effects of spontaneous exercise in rabbits, such as occurs when the animal is standing up or lying down, or is engaged in grooming and eating. The standard exercise protocol where the rabbit runs on a treadmill involves an element of coercion, whereas the spontaneous exercise was entirely voluntary. We have found that during voluntary activity there is at least partial suppression of the arterial pressoreceptor reflexes, associated with rises of blood pressure and heart rate, just as occurs in treadmill exercise. These findings show that the arterial baroreceptor reflex functions in a much more versatile manner than merely acting as a buffer for *resisting* blood pressure changes.

Another line of work has been performed together with Mr. Peter Rutter, a surgeon from St. Mary's Hospital, London. It has been observed that the drug naloxone, which blocks the action of the opiates (morphine-like substances) that are found naturally in the brain and other parts of the body, causes a marked rise in blood pressure after haemorrhage. This has potential

in combating shock. One theory of its action is that nervous pathways in which opiates act as transmitters are activated during haemorrhage, and that this depresses a number of circulatory functions. This would explain why an opiate antagonist may be of therapeutic value in clinical shock. Thus far, we have confirmed that in conscious rabbits naloxone produces a rise in blood pressure during haemorrhage. We have found that in large dosage it exerts its effect on blood pressure partly by stimulating the sympathetic nerves, and partly by releasing vasoconstrictor catecholamines from the adrenal gland. In doses one- thousand times smaller it appears to act on cardiovascular pathways in the brain itself. The intriguing thing is that in both cases haemorrhage has some sort of priming action, since in normal unbled rabbits naloxone has virtually no effect on the circulation. We are currently engaged in following up an observation by Professor Korner that during the course of severe haemorrhage there is a sudden 'failure' of blood vessels to constrict and maintain blood pressure. We now have some evidence that this 'failure' comes about through the activation of pathways in which opiates act as transmitters.

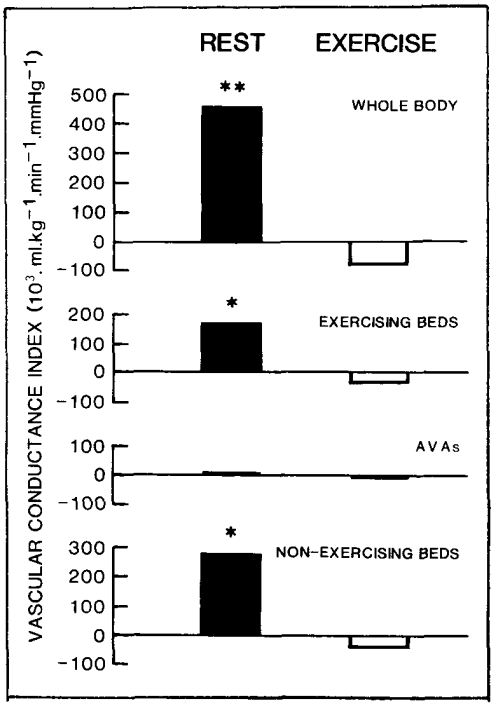


Fig 20
Reflex effects of arterial baroreceptor input on conductance in various vascular beds, at rest and at the onset of exercise.
Exercising vascular beds: skeletal and cardiac musculature.
AVAs: arteriovenous anastomoses, indicated by microsphere lodgement in lungs.
Non-exercising vascular beds: remainder of body, notably splanchnic region, kidneys, skin.
***P<0.01, *P<0.05, for reflex effect differing from zero.*
Note especially that the tonic baroreflex elevation of conductance in non-exercising beds which is present at rest is abolished after 20s treadmill exercise.

Project

Partitioning of systemic vascular conductance and blood flow in exercise

J.R. Hales, J. Ludbrook, S.J. Potocnik

Five instrumented rabbits were studied at rest and 20 s after the onset of exercise, before and after acute denervation of the arterial baroreceptors.

Cardiac output (aortic EMG flowmeter) and arterial pressure (ear artery) were measured, and the distribution of the peripheral blood flow determined by injecting radioactively labelled microspheres through a left atrial catheter. The initial partitioning was into 'exercising' vascular beds (heart, limb musculature, diaphragm, carcass); arteriovenous shunts (lungs); and 'non-

exercising' vascular beds (the remainder of the body). Before denervation of the baroreceptors there was intense vasoconstriction at the onset of exercise in the 'non-exercising' vascular beds (chiefly splanchnic region, skin and kidneys), and in the arteriovenous shunts. After acute baroreceptor denervation vasoconstriction no longer took place in any of these regions at the onset of exercise. Factorial analysis confirmed that the vasoconstriction at the onset of exercise was due to, and could be fully accounted for by, suppression of the reflex effects of arterial baroreceptor input (Fig. 20). Thus in laboratory rabbits exercise is associated with a marked diversion of cardiac output to exercising muscles, which is accounted for by transient suppression of the baroreflex.

Circulatory changes during spontaneous motor activity in rabbits: role of arterial baroreflexes

J. Ludbrook, S. J. Potocnik

Blood pressure and heart rate were continuously measured in rabbits during spontaneous motor activity such as postural change, exploration, grooming and eating; and during interposed periods of inactivity, when blood pressure and heart rate were defined as basal. Six rabbits were observed for 2 hours under each of 3 conditions of arterial baroreceptor afferents: all intact (B_4); one carotid sinus intact (B_1); all interrupted (B_0). In B_4, blood pressure and heart rate were above basal levels 82% of the time; in B_1, 64% and 76% of the time respectively; in B_0, only 21% and 28% of the time respectively. Supplementary experiments in another 6 rabbits established that the activity-associated falls of blood pressure in condition B_0 were independent of heart rate, were not due to engagement of cardiac receptor reflexes, and were not abolished by autonomic ganglion blockade. We concluded that the transient rises of blood pressure and heart rate that occur during everyday activity were associated with partial or complete suppression of the reflex effects of arterial baroreceptor input.

Factors influencing the effects of intravenous naloxone on blood pressure and heart rate after haemorrhage

P.C. Rutter, S.J. Potocnik, J. Ludbrook

The circulatory responses to different intravenous doses of naloxone were studied in conscious rabbits before and after haemorrhage, under different

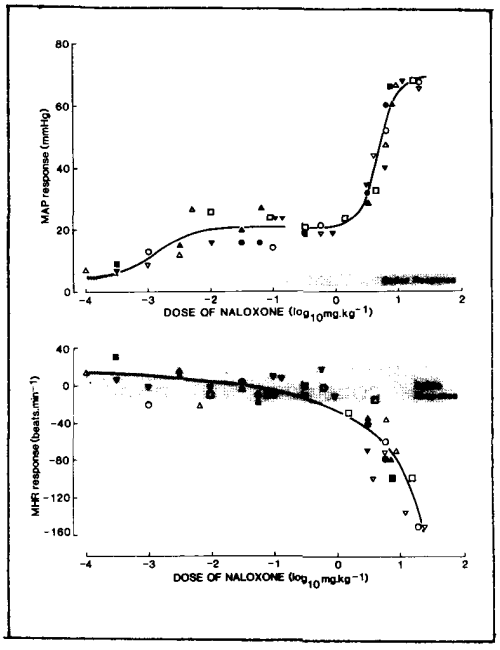


Fig 21
Relationship of intravenous dose of naloxone or saline to responses of arterial pressure (above) and heart rate (below) after a haemorrhage. Naloxone or saline was given 10 min after a haemorrhage which lowered arterial pressure to 40 mmHg. Symbols: data from 8 rabbits. Shaded area: 95% confidence limits for saline responses.

conditions including prior ganglion blockade. Unless there had been blood loss, naloxone elicited no pressor response, even in high dose. After bleeding the rabbit, so that arterial pressure fell to 40 mmHg, the dose-response relationship for naloxone had two components (Fig. 21). Over a low-dose range (threshold $3x10^{-4}$mg/kg) naloxone had a modest pressor effect but did not affect heart rate. Over a much higher dose range (threshold $6x10^{-1}$mg/kg) naloxone caused a marked rise in blood pressure and a profound bradycardia. The highest dose of naloxone examined (2.5x10 mg/kg) caused a rise in blood pressure of 70 mmHg and a reduction in heart rate of 160 beats/min.) The pressor and bradycardic effects of naloxone were the same whether post-haemorrhagic hypotension lasted 5,10,20 or 30 min.

The responses to naloxone in low or high dose depended much more closely on the volume of blood removed than on the level to which blood pressure fell. Even after non-hypotensive haemorrhage a high dose of naloxone had marked pressor and bradycardic effects. Ganglion blockade prior to haemorrhage abolished the pressor response to a low, but not a high, dose of naloxone.

We concluded that prolonged and severe hypotension are not necessary to 'prime' the cardiovascular system to respond to naloxone after haemorrhage. In a high dose its pressor effects are mediated post-ganglionically, but in a low dose it may act within the CNS.

Sympatho-adrenal mechanisms in cardiovascular responses to naloxone after haemorrhage

P.C. Rutter, S.J. Potocnik, J. Ludbrook.

Five rabbits were allotted to each of 6 treatments on a matched-individual basis. Treatments were: none; sham; total adrenalectomy with adrenocorticoid replacement; intravenous guanethidine 15 mg/kg/day; adrenalectomy + guanethidine; and adrenal medullectomy. The conscious rabbits were bled 20 ml/kg over 5 min. Naloxone 6 mg/kg was injected intravenously, and the responses of blood pressure, and of plasma adrenaline (A) and noradrenaline (NA) concentrations, were measured and analysed factorially. The action of sympathetic noradrenergic nerves and the adrenal medulla fully accounted for the pressor

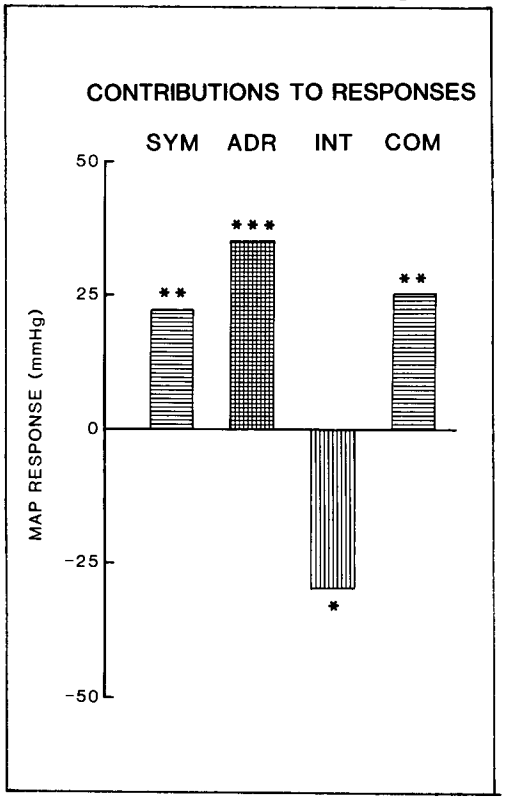


Fig 22
Calculated contributions of noradrenergic sympathetic nerves and adrenal medullary hormones to the rise in arterial pressure caused by naloxone injection after haemorrhage in 5 rabbits.
Sym: independent contribution of sympathetic nerves. Adr: independent contribution of adrenal glands. Int: occlusive interaction between sympathetic and adrenal. Com: effect of sympathetic and adrenal in combination.

response observed in normal and sham-treated rabbits. Acting separately, the sympathetic nerves and the adrenal glands each produced a pressor response, associated respectively with a marked rise of plasma NA or A, but in combination their action on blood pressure was mutually antagonistic (Fig. 22). Prior ganglion blockade does not diminish the pressor response to naloxone 6 mgkg after haemorrhage, so we suggest that endogenous opiates released into the bloodstream during haemorrhage attach to peripheral sympathetic noradrenergic nerves and the chromaffin cells of the adrenal medulla, impairing the release of noradrenaline and adrenaline.

Blockade of cardiac efferent nerves by intrapericardial administration of antagonist drugs

M. J. Lew, J. Ludbrook, J. M. Pavia, A.W. Quail, P.C. Rutter.

Intrapericardial propranolol and atenolol (50 μg/kg) had the same effect on isoprenaline-heart rate dose-response curves and on the sympathetic component of the arterial baroreceptor-heart rate reflex in conscious rabbits as conventional, 5-fold greater, intravenous doses of the drugs. The action of intrapericardial propranolol was attributable to its (-) isomer. Intrapericardial propranolol (50 μg/kg) had little effect on ventricular contractility. Plasma levels of propranolol and atenolol after intrapericardial administration were respectively 7 and 40-fold less than after the usual intravenous doses. Intrapericardial hyoscine methyl bromide (10 μg/kg) abolished baroreflex vagal effects on heart rate as effectively as the conventional 5-fold greater intravenous dose. The duration of receptor blockade by both classes of drugs when given intrapericardially was at least 2 hours. We concluded that β-adrenoceptor and muscarinic cholinergic receptor blocking drugs diffused rapidly to the sinoatrial pacemaker.

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Staff Activities

Professor Korner was an invited speaker at the Primary Hypertension Symposium in Cologne, in February 1985; at the Symposium on Left Ventricular Hypertrophy in Hypertension in Sirmione, Italy in June; at a Symposium on Hypertension organised by the Royal College of Physicians and Surgeons of Canada and the Canadian Society for Clinical Investigation (during this visit he was also Sandoz Visiting Lecturer at the University of Toronto, the Universite de Montreal and the Clinical Research Institute of Montreal); at the 5th International Symposium on SHR and Related Studies in Kyoto in October; at a Symposium on Brain and Blood Pressure Regulation in Tokyo and at a Joint Uehara Foundation and WHO Symposium on the Prevention of Cardiovascular Disease in Tokyo. He also served on the Forward Planning Committee of NH & MRC and the National Scientific and Medical Advisory Committee of the National Heart Foundation of Australia.

Dr. Warwick Anderson was an invited speaker at an international conference on "Angiotensin II in the Kidney" in Venice in September 1985. He lectured to 3rd year medical students at Monash University and to 3rd year Veterinary Science students at the University of Melbourne and was external examiner for the Department of Physiology, University of New England. Dr. Anderson was also appointed Chairman of the National Health and Medical Research Council Animal Experimentation Committee and was also the first convenor of the Committee of Australian Biomedical Societies on Animal Experimentation. He was an invited lecturer at a conference on "Animal Experimentation" at the University of New South Wales.

Professor Ludbrook was Wellcome-Ramaciotti Travelling Research Fellow, St. Mary's Hospital, London, March 1985, and attended the 2nd European Meeting on Hypertension, Milan, Italy. June 1986.

He was also appointed Co-editor (with R.C. Bennett, G.J.A. Clunie) Australian and New Zealand Journal of Surgery. Appointed Co-editor (with V.C. Marshall) of Clinical Science for Surgeons: Basic Surgical Practice. Sydney, Butterworths, Guest Editor, World Journal of Surgery and Chairman, NH & MRC Review Committees for Road Accident Research Unit, University of Adelaide, and Cardiac Transplantation Programme, St. Vincent's Hospital, Sydney.

Dr. Julie Campbell was invited speaker at the 7th Annual European Conference on Vascular Biology held in Bad Buchau near Stuttgart in the Federal Republic of Germany held between the January 8th and 11th, 1986 and also gave lectures in Geneva, Munster, Götenberg and Copenhagen.

She gave the Cell Biology Course to the third year science students at the University of Melbourne. Dr. Campbell was on the management committee for the 7th International Atherosclerosis Conference which was held in Melbourne October 6th - 10th, 1985. She was also a co-chairman of a Workshop at that meeting. Together with Dr. Gordon Campbell she was co-organiser of the Cell Biology of the Artery Wall Satellite Symposium held

on Hamilton Island from October 11th - 16th 1985.

Dr. Murray Esler was convenor of seminar on clinical hypertension to Monash University final year medical students.

Dr. Elspeth McLachlan visited the Department of Physiology, University of Auckland from December 11th - 16th, 1985 and gave a lecture "Functional Differentiation of Sympathetic Neurons". She taught Medical Physiology 311 at Monash University and was appointed to the NH&MRC Ethics Committee and Secretary of the Planning Committee for Bicentennial Meeting, Australian Societies for Experimental Biology.

Dr. Franklin Rosenfeldt presented a paper at the annual meeting of the Society of Thoracic and Cardiovascular Surgeons of Great Britain and Ireland in London and attended the Clinical Congress of the American College of Surgeons in Chicago. He lectured in the Department of Surgery at the University of Hanover, Germany and visited cardiac surgery units and research laboratories in London, Hamburg, Paris and Chicago. He also taught Monash medical students in cardiac surgery and cardiac physiology.

Dr. Marc Rabinov was awarded the Inaugural Alfred Hospital Surgical Research Prize and the David and Geck Award, for a presentation of his trial of the new analgesic, buprenorphine, in patients undergoing cardiac surgery.

Atherosclerosis Unit

Many staff members played a vital role in the conduct of the 7th International Atherosclerosis Symposium held in October at the Regent Hotel. Apart from Dr. Nestel, who was Chairman of the meeting, the Management Committee included Dr. N. Fidge, Dr. Campbell, Dr. Turley and Dr. Billington. Most of the staff in the Atherosclerosis, Lipoprotein and Cell Biology Laboratories played very active, enthusiastic and highly efficient roles in making the conference itself such a success. The attendance of about 1,000 included 800 from overseas.

Dr. Nestel was an invited speaker at a conference in Washington on "Health Effects of Polyunsaturated Fatty Acids in Seafoods", which was sponsored by the National Institutes of Health.

Three Ph.D. scholars, Jeff Bazelmans, Jeff Cohn and Sue Wong completed their courses. Two B.Sc. Honours courses were completed by Barbara Meyer and Jim Apostolopoulos.

Dr. Noel Fidge lectured to Physiology Science students and Biochemistry (Medical) students at Melbourne University. He was also editor of Atherosclerosis VII - proceedings of the 7th International Symposium on Atherosclerosis.

Seminar Programme

Seminars 1985

12 April	Role of endogenous opioid peptides in central cardiovascular actions of clonidine and α methyldopa	Dr G. Head Baker Institute
26 April	Selected aspects of the management of ascites and its complications	Dr. F. Dudley Alfred Hospital
10 May	Autoradiographic localization of adrenoceptors in renal and cardiovascular tissues	Dr. R. Summers University of Melbourne
24 May	Differentiation of functional subgroups of sympathetic ganglion cells	Dr. E. McLachlan Baker Institute
14 June	Approaches to cloning genes for cell surface receptors	Professor J. Goding University of Monash Medical School
25 June	Cardiovascular responses to diving in ducks	Dr. D. Jones University of British Columbia
28 June	Nitroglycerin and nitroglycerin tolerance: Biochemical mechanisms	Dr J. Horowitz Austin Hospital
26 July	High blood pressure and its treatment	Dr. G. Jennings Baker Institute
	Recent advances in cardiology	Dr. A. Broughton Baker Institute
2 August	Recent epidemiological and dietary findings in coronary artery disease	Dr. P. Nestel Baker Institute
	Scope of cardiac surgery in the '80s	Dr. F. Rosenfeldt Baker Institute
23 August	Dopamine innervation of the kidneys: a neurochemical update	Dr. C. Bell University of Melbourne
13 September	Drug design	Professor P. Andrews Victorian College of Pharmacology
27 September	Cardiac transplantation workshop	
4 October	Approaches to controlling parasitic diseases	Dr. G. Mitchell Walter and Eliza Hall Institute

11 October	SATELLITE MEETING — INTERNATIONAL SYMPOSIUM ON ATHEROSCLEROSIS Lipoprotein metabolism: Kinetics of lipids and apoproteins	
25 October	Hyperlipidemia of experimental renal disease	Dr. J. Marsh University of Pennsylvania
1 November	Role of reticular formation and amygdala for the integration of somatomotor and visceral systems	Dr. P. Langhorst Freie Universität Berlin
15 November	Joint Baker/Florey Institutes Seminar	
	Studies on regulation of gene transfer techniques	Dr. R. Richards Florey Institute
	The HDL receptor	Dr. N. Fidge Baker Institute
	Physiological studies on atrial natiuretic factor	Dr. J. McDougall Florey Institute
	Endothelium derived relaxing factor	Dr. J. Angus Baker Institute
	Neuroanatomical and neurochemical aspects of fluid and electrolyte excretion	Dr. M. McKinlay Florey Institute
	Clinical approaches to hypertension	Dr. G. Jennings Baker Institute
22 November	Does behaviour affect coronary artery pathology?	Dr. D. Copolov Mental Health Research Institute of Victoria
29 November	Special Seminar	
	Atherosclerosis — the modern view	Dr. P. Nestel Baker Institute
	Plasma proteins which affect exchange of lipids	Dr. P. Barter Flinders Medical Centre
	The metabolism of lipoproteins containing apo B forms	Dr. J. Marsh University of Philadelphia
	The involvement of E apolipoproteins in lipid metabolism	Dr. E. Janus St. Vincent's Hospital
	The artery wall and atherosclerosis	Dr. J. Campbell Baker Institute
	Apolipoprotein and the HDL receptor	Dr. N. Fidge Baker Institute

Financial Report

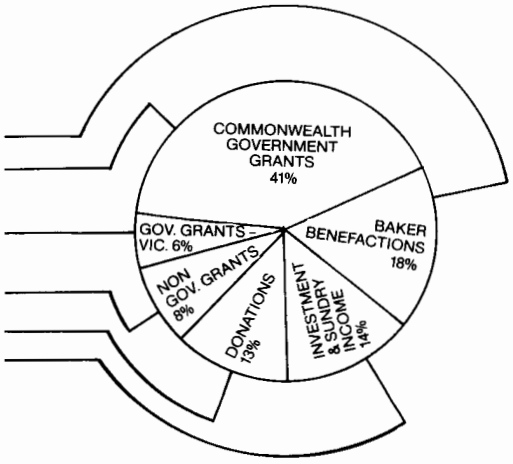
Baker Medical Research Institute

Year ended 31 December 1985

Income and Expenditure at a glance

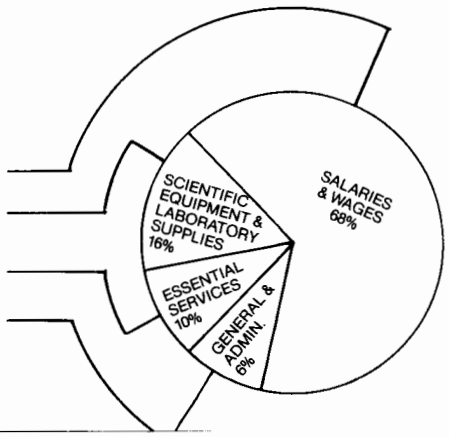
Income Derived from the Following Sources:

1985 000s	%		1984 000s	%
617	18	Baker Benefactions	562	16
1399	41	Government Grants — Commonwealth	1439	42
201	6	Government Grants — Victorian	183	5
277	8	Non-Government Grants	253	8
460	13	Donations	546	16
486	14	Interest from Investments and Sundry Income	449	13
3440	100		3432	100



Expenditure Distributed as Follows:

1985 000s	%		1984 000s	%
2380	68	Salaries & Wages	2166	62
555	16	Scientific Equipment and Laboratory Supplies	832	24
353	10	Essential Services	346	10
190	6	General & Administration	128	4
3478	100		3472	100



Baker Medical Research Institute

Balance Sheet at 31 December 1985

1985	ACCUMULATED FUNDS AND LIABILITIES	1984
	OPERATING FUND	
(382,300)	Accumulated (deficit)	(343,543)
279,106	Bank overdraft	199,634
96,588	Sundry creditors & Accrued Expenses	61,401
221,204	Provision for leave entitlement	249,703
214,598		$167,195
	ENDOWMENT FUND	
1,566,586	Accumulated fund — Page 111	1,580,610
$1,566,586		$1,580,610
	RESEARCH SCHOLARSHIPS AND OTHER FUNDS	
74,356	Research Fund — Page 113	—
424,875	Before 2000 Heart Appeal Fund — Page 113	—
49,847	Restricted Fund — Page 113	116,461
122,093	Edgar Rouse Memorial Fellowship Fund — Page 112	101,589
4,344	Laura Nyulasy Scholarship Fund — Page 112	3,828
39,241	William Buckland Research Fund — Page 112	38,456
4,852	Lang Research Scholarship Fund	4,852
$719,608	Liabilities	$265,186
17,328	Moneys due to Operating Fund	—
$736,936		$265,186

These accounts should be read in conjunction with the notes on page 114.

1985	ASSETS	1984
	OPERATING FUND ASSETS	
400	Cash on hand	**300**
138,149	Sundry debtors & prepayments	**70,955**
58,721	Short term deposits held by the Institute	**95,940**
17,328	Monies due from other funds	—
$214,598		**$167,195**
	ENDOWMENT FUND ASSETS	
	Investments (at cost)	
	Held by the Institute	
77,550	Govt & semi-government stock	202,550
385,024	Shares & debentures in companies	345,064
379,767	Short term deposits	259,114
64,400	Mortgage loans	118,200
906,741		924,928
	Held by ANZ Executors & Trustee Co. Ltd.	
15,065	Govt & semi-government stock	25,581
73,409	Shares & debentures in companies	76,304
360,485	Trust units	340,824
4,294	Short term deposits	10,461
202,500	Mortgage loans	202,500
655,753		655,670
4,092	Cash at bank	12
$1,566,586		**$1,580,610**
	RESEARCH SCHOLARSHIPS AND OTHER FUND ASSETS	
	Investments (at cost)	
	Held by the Institute	
4,852	Shares in companies	4,852
679,243	Short term deposits	219,868
684,095		**224,720**
	Held by ANZ Executors & Trustee Co. Ltd.	
8,253	Shares in companies	8,253
33,375	Trust units	32,928
1,957	Short term deposits	1,103
43,585		42,284
9,256	Cash at bank	(1,818)
$736,936		**$265,186**

Baker Medical Research Institute

Year Ending 31 December 1985

1985	STATEMENT OF MOVEMENT IN ACCUMULATED FUNDS	1984
	OPERATING FUND — EXPENDITURE	
2,379,947	Salaries & Wages	2,165,745
298,444	Laboratory supplies & isotopes	281,571
148,651	Additional equipment & building costs	421,942
37,427	Library maintenance	43,137
45,057	Postage & telephone	18,847
44,335	Printing & stationery	37,252
99,182	Light & power	84,973
46,296	Insurance	45,879
81,992	Repairs & renewals	62,351
10,000	Animal house contribution	10,032
50,000	Collaborative grant — NH & MRC programme	50,000
48,069	Travelling expenses	70,091
13,300	Public relations	11,257
122,870	Fundraising expenses	117,014
36,682	Sundries	32,563
16,388	Clinical services	16,250
960	Data processing	2,874
—	Surplus for year	—
$3,479,600		$3,471,778
(38,757)	Surplus/(Deficit) for year	(40,049)
(343,543)	Accumulated deficit as at 1 January	(303,494)
(382,300)	Accumulated deficit as at 31 December	(343,543)

1985		1984
	DONATIONS FROM BAKER BENEFACTIONS	
11,569	Statutory amount	11,569
605,000	Transfers from Endowment Fund	550,000
616,569		561,569
	GRANTS-IN-AID OF RESEARCH PROJECTS	
—	Life Insurance Medical Research Fund of Australia and New Zealand	17,000
1,398,905	National Health & Medical Research Council	1,439,359
139,952	National Heart Foundation of Australia	110,463
100,385	Alfred Hospital	80,045
1,639,242		1,646,867
	OTHER GRANTS	
9,500	The James & Elsie Borrowman Research Trust	9,500
4,000	The William Buckland Research Fund	4,000
201,500	Victorian State Government	183,500
125	The Laura Nyulasy Research Scholarship Fund	125
23,529	Clive & Vera Ramaciotti Foundation	30,621
238,654		227,746
	INCOME FROM INVESTMENTS	
5,097	Held by the Trustees of the Baker Institute Grant Trust	5,097
294,888	Other investment income	274,546
299,985		279,643
	OTHER INCOME	
116,851	Sundry sales, recoveries & refunds	119,959
69,222	Clinical services	49,454
186,073		169,413
(499,077)	Deficit	(586,540)
	PUBLIC & CORPORATE DONATIONS	
460,320	(Net of Transfers)	546,491
(38,757)	Deficit for year	(40,049)
$3,479,600		$3,471,778

Baker Medical Research Institute

Year Ending 31 December 1985

1985	STATEMENT OF MOVEMENT IN ACCUMULATED FUNDS	1984
	ENDOWMENT FUND	
1,580,610	Balance at 1 January	**1,483,376**
631,153	Donations — Baker Benefactions	593,605
832	T.E. & A. recovery	2,269
10	Interest	33
84	Profit on sale of shares & redemption of stocks	101,274
—	Sale of motor vehicle	5,500
632,079		**702,681**
2,212,689		**2,186,057**
37	Bank charges	30
605,000	Transfer to Operating Fund	550,000
—	Building maintenance & renovations	29,673
41,066	Siemens Switchboard	—
—	Cost of motor vehicles	24,306
—	Brokerage fees	1,438
646,103		**605,447**
1,566,586	Balance as at 31 December	**1,580,610**
	RESTRICTED FUND	
116,461	Balance at 1 January	**220,246**
11,569	Baker Benefactions — Statutory amount	11,569
48,005	Donations	119,500
6,462	Investment income & bank interest	11,112
—	Transfer from Operating Fund	11,452
—	NH & MRC Grant — Turley 1984	22,333
66,036		**175,966**
182,497		**396,212**
	Transfer to Operating Fund	
11,569	Baker Benefactions — Statutory amount	11,569
33,565	Donations, Grants & other income	129,842
87,402	Payments	138,227
114	Bank charges & Federal Tax	113
132,650		**279,751**
$49,847	Balance at 31 December	**$116,461**

NOTE: Income received from Endowment Fund investments is credited direct to the Operating Fund.

RESEARCH, SCHOLARSHIP & OTHER FUNDS

EDGAR ROUSE MEMORIAL FELLOWSHIP FUND

1985		1984
101,589	Balance at 1 January	88,713
4,685	Donations	8,798
15,497	Investment income & bank interest	11,113
324	Trustees Executives & Agency Company recovery	883
20,506		20,794
122,095		109,507
—	Payments	7,913
2	Federal Tax	5
2		7,918
$122,093	Balance at 31 December	$101,589

LAURA NYULASY SCHOLARSHIP FUND

3,828	Balance at 1 January	3,489
592	Investment income	499
79	TE&A recovery	215
671		714
126	Transfer to Operating Fund	125
29	Payments	25
—	Adjustment	225
155		375
$4,344	Balance at 31 December	$3,828

WILLIAM BUCKLAND RESEARCH FUND

38,456	Balance at 1 January	38,458
5,037	Investment income	4,208
4,000	Trustees Executives & Agency Company recovery	4,000
252	Payments	210
4,252		4,210
$39,241	Balance at 31 December	$38,456

RESEARCH FUND

1985		1984
Nil	Balance at 1 January	Nil
464,171	Donations	—
11,840	Investment income	—
476,011		—
390,274	Transfer to Operating Fund	—
10,879	Payments	—
502	Financial Institutions Duty & Bank Fees	—
401,655		—
$74,356	Balance at 31 December	Nil

BEFORE 2000 HEART APPEAL FUND

Nil	Balance at 1 January	Nil
424,870	Donations	—
21,822	Investment income	—
446,692		—
21,804	Transfer to Operating Fund	—
13	Financial Institutions Duty	—
21,817		—
$424,875	Balance at 31 December	Nil

Baker Medical Research Institute

NOTES TO AND FORMING PART OF THE ACCOUNTS FOR THE YEAR ENDED 31 DECEMBER 1985

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 Institute at book value.

2. Statement of Accounting Policies

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

(a) Historial 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 of non-current assets.

(b) Institute Funds, Income and Expenditure

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

Income from 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 1985 was approximately $7,500,000.

3. Investments – Endowment Fund

The market value of shares in companies listed on the Australian Stock Exchange at 31 December 1985 was $879,249 (1984 $608,567).

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 action 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.

DONATIONS

Victorian State Government	201,500
Ruby Wallace Estate	71,000
AMP Society	50,000
Emily E.E. Stewart Estate	37,170
Mary Stella Geraci Estate	30,518
Allan Williams Trust Fund	11,000
Astra Pharmaceuticals	10,000
Windermere Hospital Foundation Ltd	10,000
R.M. Sykes	6,200
William Angliss Vic Charitable Trust	5,000
Elisabeth Murdoch	5,000
The G.W. Vowell Foundation	5,000
Estate of H.P. Blaich	4,000
William Buckland Research Fund	4,000
Dr. Victor Chang	4,000
Nevett Coutts & Wilson	4,000
D.N. Gibbs & J.H. Anderson	4,000
Roche Products	4,000
Richard Edward Tonkin Estate	4,000
Ramaciotti Foundation	3,529
Goodman Cannington	3,456
Estate of E.H. Flack	3,450
ACI Australia	3,000
Trustees of the Late Edward Wilson	3,000
B.P. Australia	2,500
The Eirene Lucas Foundation	2,500
Bell Charitable Fund	2,000
Bongiorno & Co	2,000
Miss M.L. Dillion	2,000
ICI Australia Operations	2,000
Westpac Banking Corp	2,000
V.P.S.A.	1,721
Anonymous	1,302
George Frederick Little Settlement	1,170
B.C. Fitzpatrick	1,100
Medical Research Council of Canada	1,034
Aetna Life & Casualty	1,000
Comalco Ltd	1,000
Commonwealth Bank	1,000
CRA Services Ltd	1,000
David Syme & Co	1,000
Harwood Pincott	1,000
J.F. Wren	1,000
Estate of Edward Naylor	946
Miss Dorothy A. Parker Estate	874
E.L. & C. Baillieu	750
Australian Guarantee Corp	700
Moukles	600
F. & M. Alfredson	500
G. Ansell	500
H.P. Atchison	500
Australian Nutrition Foundation	500
Construction Engineering (Aust) Pty Ltd	500
Crawford Productions	500
Dalgety Aust Operations	500
R.G. De Nardo	500
Mr. M. Donnelly	500
A. & B.K. Douglas	500
K & O Eisner	500
H. Glaser	500
Gordon & Gotch Ltd	500
James Hardie & Co	500
William Heath Estate	500
Rev. F.S. Imnay	500
N.A. Jackson	500
Jennings Industries Ltd	500
Kooper Australia Pty Ltd	500
Linfox Transport (Aust) Pty Ltd	500
The Miller Foundation	500
M. Murdoch	500
T.M. Prynor	500
Rayson Industries	500
Mrs. Beth Smith	500
Mr. A. W. Spooner	500
Sir Laurence & Lady Muir	450
K.R. Ansell	400
A. Muir	400
Coopers Lybrand Pty Ltd	350
Pfeiffer Engineering	350
M.O. McDonald	320
J.S. Collingwood	300
K.M. Cunningham	300
F. & V. Hannemann	300
A.P. Kennedy	300
A.K. Lowe	300
Macquarie Charitable Foundation Ltd.	300

J.D. Moir	300
D.S. Ryzman	300
A.S. Stickland	300
Anonymous	250
The Ballarat Courier	250
Mr. E. Boland	250
Bunge Australia Pty Ltd	250
Colgate-Palmolive Pty Ltd	250
M.A. Cuming	250
B.S. Dyson	250
Mr. Fusspots	250
Glo-Weave Consolidated	250
Ibis D.H. & S. Victoria	250
Kraft Foods Ltd	250
Mee Family Trust	250
F.J. Murphy	250
Reserve Bank of Australia	250
Dr. Rovier & Mrs. M. Skinner	250
Sally Browne Pty Ltd	250
H.E. Towers	250
H. Watkins	250
Wiretainers Pty Ltd	250
T. & B. Wood	250
Wormald International	250
E.D. Service	230
J.W. & V. Engelbert	220
Air International	200
Anonymous	200
Arthur Robinson & Co	200
P.F. Ayers	200
A.L. Bottomley	200
A. Buel	200
K.S. Bull	200
E.J. Collings	200
Mrs. I. R. Collingwood	200
L.R. Crochland	200
F.D. Culley	200
Cypress & Sons Pty Ltd	200
A. Dickason	200
A. Edwards	200
Mr. V. Flynn	200
E.R. Gaylard	200
H.D. Glascodine	200
Glaxo Australia Pty Ltd	200
J.S. Grey	200
Mr. Ham	200
V.P. Joel	200
Julius Marlow Ltd	200
J.P. Keahy	200
H.J. Kohane	200
Mrs. E. Korner	200
N.R. Korner	200
B.M. Lees	200
N. Lees	200
A.D. Leitch	200
J.H. Little	200
A.L. Lowe	200
Mallesons	200
D.M. Martin	200
S. Meyer	200
G. Moir	200
D.S. Moore	200
J.L. Murray	200
I.F. Phipps	200
The Pierce Armstrong Foundation	200
E. Pitman	200
Plessey Australia Pty Ltd	200
Press Fast Industries	200
D. Ray	200
D. & J. Ray	200
T.S. Santos	200
Spionkop Pty Ltd	200
W. Tope	200
Witchery Pty Ltd	200
World Services	200
E.W. Wortley	200
Mr. M.J. O'Neill	156
Mr. J.M. Greystaetter	152
M.A. Dickman	150
T.M. Hoult	150
P.M. Hudson	150
Mrs. E.G. Hunter	150
Mr. F.T. Ireland	150
James Hardie Paper & Packaging	150
P.K. & J.B. Marsh	150
T.G. & M.G. Pickford	150
F.R. Townsend	150
H.E. Vivian	150
EAC Wade	150
G.C. Warland	140
J.C. Habersberger	125
H. Allan	120
L.M. Francome	120
J.V. Perry	120
Ballarat Co-op	110

265 donations of $100 and many other donations to a total of $701,167.

BEFORE 2000 APPEAL

Kasebta Pty Ltd	200,000
Lustig & Moar Group	50,000
Percy Baxter Trust	15,000
Esso Australia	12,000
ACI Australia Ltd	10,000
Kodak Australasia Pty Ltd	10,000
Sir Laurence Muir	10,000
Elisabeth J. Murdoch	10,000
Perpetual Trustees	8,000
Anonymous	5,000
The Besen Charitable Foundation	5,000
BHP Ltd	5,000
G.J. Coles & Co Ltd	5,000
Concrete Construction (NSW) Pty Ltd	5,000
Gandel Charitable Foundation	5,000
J. Liberman	5,000
Sir James McNeill, CBE	5,000
R.M. Sykes	5,000
E.B. Myer Charity Fund	3,000
William Paxton Trust	3,000
CRA Limited	2,500
National Australia Bank Ltd	2,500
Anonymous	2,000
P. & I. Arnhold	2,000
Entrad Corp Ltd	2,000
Ernst & Whinney	2,000
The Myer Emporium Ltd	2,000
North Broken Hill Pty Ltd	2,000
Oliver - Affleck Trust	2,000
H.P. Williams Trust Fund	2,000
Mr. L.J. Fitzgerald	1,500
Robert Bosch (Aust) Pty Ltd	1,200
APM Ltd	1,000
Blue Circle Southern Cement Ltd	1,000
Boral Ltd	1,000
Chamber of Manufacturers Insurance Ltd	1,000
David Syme & Co Pty Ltd	1,000
M.G. & M.I. Downes	1,000
Dunlop Olympic Ltd	1,000
Mr. Leon Fryde	1,000
George Western Foods Ltd	1,000
Joseph Brown Pty Ltd	1,000
Maddock Lonie & Chisholm	1,000
Mayne Nickless Ltd	1,000
Monier Ltd	1,000
S.B. Myer	1,000
Nestle Australia Ltd	1,000
Mr. B.H. Pascoe	1,000
Potter Partners	1,000
Royal Insurance Ltd	1,000
Shell Co of Australia Ltd	1,000
Mr. A.S. Stickland	1,000
J.B. Were & Son	1,000
Yabba Pty Ltd	1,000
Young Presidents Organisation (Brisbane)	1,000
J.C. Habersberger	750
P.M. Hudson	750
Rev. J.P. Leahy	600
Uncla Pty Ltd	600
Mr. F.K. Alfredson	500
Anonymous	500
Mrs. Hazel Appel	500
Brambles Industries Ltd	500
CIG Ltd	500
Commercial Union Insurance Ltd	500
The Herald & Weekly Times	500
John Holland Holdings Ltd	500
Joubert & Joubert Pty Ltd	500
Dr. H.B. Kay	500
McCaughan Dyson & Co	500
Mr. J.D. Moir	500
Moore Business Systems Aust Ltd	500
Nicholas Kiwi Ltd	500
W.D. McPherson	500
McPhersons Ltd	500
Mrs. W.J. Wark	500
Hendersons Industries Ltd	350
Mrs. G. Ansell	300
V.V. Bower	300
Cadbury Schweppes Ltd	300
Miss Wilma Johnson	300
Mr. L.J. King	300
Mr. A.A. Paige	300
Miss A.P. Kennedy	300
Dulux Australia Ltd	250
Mr. E.N.M. Finnie	250
Leighton Holding Ltd	250
Lloyds International Ltd	250

G.C. Schofield	250
Vealls Securities & Finance Ltd	250
King W. Whittel (Minet Partners)	250
Dorothy L.P. Chong	220
Estate of the Late George Adams	200
Ansett Transport Industries Ltd	200
John Bailey	200
CIBC Ltd	200
Mr. F.J. Collings	200
A. Edwards	200
Mr. D. Hogarth	200
R.B. & A.M.M. Johnstone	200
Lanes Motors (Holdings) Ltd	200
Mr. E. Newman	200
I.R. & A.R. Parmenter	200
I.T. & M.H. Perkins	200
Mr. E. A. Richards	200
H. Schreiber	200
M.A. Simpson	200
Trade Credits Ltd	200
H. Allan	120
Mrs. S.G. Alley	120
D.E. & W.C. Chambers	120
Mrs. M. Fink	120
Jessie L.M. Gadsden	120
James M. Gardiner	120
V.G. & S.W. Gardner	120
Miss H.D. Glascodine	120
Mrs. M. McDonald	120
M.O. McDonald	120
B.G. Ashley	100
Mr. R.J. Barcham	100
Goulden Nominees Pty Ltd	100
Humes Ltd	100
Dr. S.J. Iceton	100
Dr. K. Oppenheimer	100
Mrs. M.A. Rose	100
Prof. G.B. Ryan	100
Mr. A.I. Sinclair	100
R & R Solnordan	100
Mr. Theo Stephens	100
Mr. H.E. Vivian	100
Mr. K. Wilks	100
Witchery Pty Ltd	100
W.J. & D.J. Bailey	80
K.M. Cunningham	60
B.L. Samuel	60
Emeritus Prof. R.R. Andrew	50
BT Australia Ltd	50
H.P. & H.M. Blakiston	50
Flax Sportswear	50
A.G. Goode	50
J. Harris	50
Mr. A.W. McDonald	50
Peninsular Suspensions	50
Ms. Deborah S. Ryzman	50
F.J. & H. Upton	50
James D. Wall	50
Western Mining Corp Ltd	50
B.L. & P.A. Young	40
Mr. R. Croft	30
R.H. Cunningham	30
F.R. Gaylard	30
Virman Nominees Pty Ltd	30
P.J. & P.M. Craig	25
Mrs. J. Freemantle	25
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Alfred Hospital/ Baker Institute

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Clinical Research Unit

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PROJECTS

Effects of regular exercise on cardiovascular risk factors in normal subjects.

Effects of regular exercise in patients with primary hypertension.

Prevalence of left ventricular hypertrophy in essential hypertension.

Clinical pharmacology of milrinone.

Sympathetic function in congestive heart failure.

Summary

The Hospital

The year has seen the departure to Adelaide of Dr. Paul Nestel, who has been Deputy Director of CRU for the last 9 years (see Baker Institute Report). The new Deputy Director is Dr. Garry Jennings who has worked in the unit for a slightly longer period with great distinction. Dr. Nestel's successor as Deputy Director of the Baker Institute is Dr. Philip Barter, who becomes Associate Director of CRU. These appointments ensure that our overall clinical research activities will flourish. In addition Dr. Alex Bobik our Chief non-medical Scientist has been made Associate Director (Laboratories). These are run jointly with the Baker Institute and have been modernized as a result of the recent Appeal.

At the time of writing this report we have almost 200 beds closed in the Alfred Hospital. Patients are only admitted for acute emergencies and most of the other work of a large public hospital in investigating and caring for disease at earlier and more reversible stages has ceased with the curtailment of arranged (elective) admissions. These events have far reaching ramifications and the effects could last long beyond the present crisis and are part of a long lasting malaise of the Victorian public hospital system. There are numerous underlying problems, quite apart from the shortage of nursing staff. There is a feeling of alienation between health care professionals of all categories and the increasingly ponderous administrative apparatus both in the hospital and in Collins Street. At present there is every indication that things will get worse before they get better. Undergraduate and postgraduate medical education is suffering, particulary from the viewpoint of practical experience. The latter is gained in an apprenticeship in public hospitals. It only works if the resident staff are guided by a highly skilled consultant staff and encounter a wide range of medical problems, investigations and procedures. Today the consultants and specialists gravitate more and more towards private hospitals. What is needed is a rekindling of total sense of commitment to the hospital and to serve the sick.

Medical research also suffers from the present disarray. It can only be carried out in public institutions and relies on the ready availability of a wide spectrum of patients. It is obvious that any diminution in the nation's capacity to perform research, will glue us into existing methods of medical treatment and will limit Australia's capacity to find new forms of treatment and disease prevention. Private hospitals in the medium longterm will also deteriorate unless there are rapid improvements in the public hospital sector. The latter must provide highly specialised and expensive services which are not economic and need to be subsidized.

The natural response to the disarray by hospital administrators is to formulate more rules to put matters right. In the same way the long suffering public want community involvement. The discontent of the nursing staff is part of the same feeling of alienation and a long period of neglect. With all wishing to restore the system we must not neglect the unique situation of practising doctors. Doctors make the clinical decisions and collectively are in the best position to decide which clinical services require greater or lesser emphasis in our hospital. This input is needed if limited financial resources are to be put to the best possible use. The organisation within the medical staff needs to be improved and there must be the capacity for more rapid and better

decision making, with more commitment to the institution. Appointments of Chiefs of Medicine and Surgery who are distinguished professionally, could be valuable at this point in time.

The Clinical Research Unit has been less affected than other medical departments by the reduction in hospital services. Our major tasks - the prevention of atherosclerosis and treatment of hypertension - are best addressed at an early stage of each disorder before clinical catastrophies occur. Our outpatient services have been able to expand in the face of inpatient bed closures. We have now amalgamated the Lipid Clinic and Hypertension Clinics to broaden our approach to reducing cardiovascular 'risk'. The Risk Evaluation Service which Dr. Nestel has conducted at the Baker Institute with the support of the National Heart Foundation will continue and may move to the hospital later in the year. Our inpatient work as a general medical unit has been restricted, but one advantage of clinical research training is that it encourages the search for new ways around difficulties. Thus, many of our clinical laboratory procedures including most of our invasive studies which were formerly performed only after admission to hospital have now been adapted to outpatients. It is particularly unfortunate that we are not able to function at maximum capacity at a time when we have requests from around the world from doctors seeking research experience here.

Summary

Last year we outlined exciting new findings on the beneficial longterm effect of moderate exercise on cardiovascular risk factors in normal subjects. The blood pressure fell substantially after 3 bouts of exercise weekly, each of 40 minutes duration. This year we have examined the effects of a similar exercise programme in patients with moderate primary hypertension. Sixteen patients were studied after normal sedentary activity after 3 times weekly exercise and after daily exercise; each period lasted one month; the order of the three levels of activity was randomised, to avoid bias due to training. As in normal subjects, there was a substantial fall in blood pressure after both exercise periods, sufficient to achieve normal levels of blood pressure in most patients. The fall was associated with a reduction in vascular resistance and in the amount of noradrenaline in the blood, suggesting reduction of sympathetic nervous activity. We currently believe that physical exercise may have a greater place in the management of hypertension than previoulsy suspected.

We are also testing the hypothesis that non-drug treatment of hypertension may have wider applications and that a proportion of patients with severe hypertension may be managed without drugs. Based on experimental studies in animals we have developed a hypothesis that the amplifying properties of the heart and blood vessels contribute more to the maintenance of high blood pressure than the basic underlying cause. After successful reversal of the hypertrophy by antihypertensive drugs it may be possible to maintain normal blood pressure by non-drug methods much as in mild/moderate hypertension.

This appears to have been substantiated from results obtained in three groups of patients with moderate/severe hypertension in whom blood pressure was controlled for (i) 1 month, (ii) 1 year, (iii) 15 months to 5 years. The rate of subsequent redevelopment of hypertension between the series on stopping drug therapy was inversely

related to the duration of therapy, which was mostly with beta-blockers and diuretics. With these drugs substantial reversal of vascular hypertrophy was achieved in series (ii) and (iii), whilst reversal of left ventricular hypertrophy was achieved in only a proportion of patients in series (iii), where there was an inverse relationship between LV mass and duration of therapy. Redevelopment of hypertension was slowest after obtaining regression of both vascular hypertrophy and left ventricular hypertrophy. We need to develop new methods for establishing more clearly the relationship of 'faults' in sympathetic nervous and other body functions to establish the cause(s) of hypertension in different patients.

Our findings have suggested a new therapeutic strategy, where the effectiveness of non-pharmacological methods of controlling blood presure is enhanced by first causing regression of cardiovascular hypertrophy by drug treatment followed by a non-drug phase of maintaining blood pressure normal. Moderate regular exercise has turned out to be one of the most effective methods of lowering blood pressure. Our numbers are still small but we have maintained about 75% of patients with mild/moderate hypertension without drugs at any time for over a year and about 40% of patients with severe hypertension. In the latter group this followed an initial period of drug therapy.

Projects

Effects of exercise on cardiovascular risk factors in normal subjects

G. Jennings, L. Nelson, P. Nestel, M. Esler, P. Korner

The effects of four levels of activity on heart rate, blood pressure, cardiac index (CI), total peripheral resistance index, norepinephrine spillover rate, (a measure of sympathetic nervous activity), insulin sensitivity, lipids and some hormones, were studied in 12 normal subjects. The 4 week periods were (i) below sedentary activity, (ii) sedentary activity, (iii) 40 minutes bicycling 3 times per week, (iv) similar bicycling, 7 times per week. They were administered according to a LATIN SQUARE experimental design.

Exercise 3 times/week reduced resting blood pressure by 107 mmHg ($P < 0.01$) and by 127 mmHg after 7 times/week (both $P < 0.01$). This was associated with reduction in peripheral resistance, increase in cardiac output and cardiac slowing. At the highest level of activity norepinephrine spillover rate was of the sedentary value ($P < 0.001$) in 8/10 subjects. In 2 other subjects spillover rate rose, although BP and TPRI were reduced. Metabolic changes included lowering of total cholesterol, but not HDL. Insulin sensitivity rose by 27% after 3 times/week, but declined to sedentary levels with 7 times/week exercise. Maximum oxygen uptake increased linearly with activity. Exercise performed 3 times/week lowers BP and should reduce cardiovascular risk. The same exercise 7 times/week enhances physical performance with little further reduction in cardiovascular risk factors. A moderate exercise programme has important beneficial effects on cardiovascular risk factors.

A study of the effects of exercise training in patients with primary hypertension.

L. Nelson, G. Jennings, M. Esler, P. Korner

We have tested whether exercise exerts long term effect on blood pressure

in a randomised study of 3 levels of activity in 13 untreated patients with mild essential hypertension. Following a 6 week 'run-in' phase, the levels of activity studied were (i) sedentary activity, (ii) 45 mins bicycling (at 60-70% maximum work capacity (Wmax)) 3 times per week, (iii) 45 mins bicycling 7 times per week, each for a duration of 4 weeks. The order of performing these levels of activity differed between subjects in accordance with a Latin square design.

Supine blood pressure, measured 48 hours after the end of each phase, averaged 148/99 during the 'run-in' phase and 143/96 in the sedentary phase. Blood pressure fell below the latter value by 11/9 with 3/week exercise, and by 16/11 with 7/week exercise (both $P < 0.001$, $n = 13$). Standing BP fell by similar amounts during the exercise periods. As in normal subjects, the fall in BP with increasing activity was associated with a reduction in total peripheral resistance and an increase in cardiac output. Plasma noradrenaline concentration fell below sedentary values by 21% after 3/week exercise and by 33% after 7/week exercise. Body weight and 24 hour sodium excretion remained constant throughout the study.

We conclude that moderate regular exercise produces significant lowering of blood pressure, independent of changes in weight and sodium intake in patients with hypertension and appears an important non-pharmacological method of lowering blood pressure.

Prevalence of left ventricular hypertrophy in essential hypertension

E. Laufer, G. Jennings, E. Dewar, A. McKenzie

The prevalence of left ventricular hypertrophy in human essential hypertension is uncertain. Some previous studies have reported that about 1/2 of patients with hypertension have left ventricular hypertrophy, although hypertrophy is found universally in experimental models of hypertension. Echocardiography was used to calculate left ventricular wall thickness, relative wall thickness (thickness/radius) and left ventricular mass in 52 normal subjects, 30 patients with borderline hypertension and 33 untreated patients with essential hypertension. In 52 normal subjects wall thickness was independent of sex, BP or habitual physical activity, but left ventricular mass was significantly related to BSA and age.

After correction of left ventricular mass for age and BSA, 88% of 33 patients with untreated essential hypertension had left ventricular mass greater than predicted which identified a larger proportion of hypertensives with left ventricular hypertrophy than previous studies suggest. Wall thickness measurement alone also indicated that about 80% of newly diagnosed hypertensives have left ventricular hypertrophy.

(See also Baker Institute Report - Human Autonomic Function).

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Alfred Hospital
Clinical Pharmacology Unit

Clinical Pharmacology Laboratory

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Projects

Hepatic blood flow and extraction of high clearance drugs.

Audit and rationalisation of peptic ulcer therapy - the role of surgical intervention.

Nutrition and oral drug use.

Audit and rationalisation of antibiotic use in surgical prophylaxis.

Therapeutic drug monitoring of aminoglycosides.

Oral interactions of high clearance cardiovascular drugs.

Anticonvulsant drug monitoring.

The department assumed enlarged responsibilities in 1985, including
- responsibility for consultative advice to the Queen Victoria Medical Centre in addition to Prince Henry's and Alfred Hospital.
- consultative services to the Victorian Drug Usage Advisory Committee to the Minister of Health.
- consultative services to the Victorian Postgraduate Medical Foundation.
- examination responsibilities to the Royal Australian College of Obstetricians and Gynaecologists.

Educational services were maintained to the following organisations:-
Monash University Department of Pharmacology (second and third year lectures).
Monash University Department of Medicine (responsibility for fourth and sixth year series in Clinical Pharmacology and Therapeutics).
Monash University Department of Psychological Medicine (Diploma of Psychological Medicine).
Victorian Postgraduate Medical Foundation (General Practitioner and Country Education Programmes).
AMA Branch Postgraduate Programme.
Royal Australian College of Physician Trainee Programmes (Alfred Hospital, Prince Henry's Hospital and Queen Victoria Medical Centre.

Overseas travel involved attendance and presentation at the American Society for Clinical Pharmacology and Therapeutics and the following centres.
Faculty of Health Sciences, University of Washington, Seattle.
Dupont Research Foundation, Wilmington, Del.
State University of New York, Buffalo.
University of Texas Health Sciences Centre, San Antonio, Texas.
ICI Clinical Research Centre, Macclesfield, U.K.
Overseas visitors included:-
Professor Black, Department of Medicine and Pharmacology, School of

Medicine, Temple University, Philadelphia, Pa.

Professor C. Peck, Uniformed Health Services University, Bethesda, Maryland.

Professor A. Melander, University of Lund, Sweden.

Professor D. Lalka, State University of New York at Buffalo, New York.

The Department gratefully acknowledge the active help and collaboration of numerous individuals, Hospital Departments and external colleagues. We wish to specially acknowledge the provision of laboratory space within the Baker Institute and the generous assistance and cooperation of Mr. A. Bons and Staff of the Baker Institute Biological Research Unit.

The laboratory maintains its dual interests in Clinical Pharmacology and Clinical Therapeutics as detailed below in Project Reports.

Summary

One major initiative of the Laboratory in 1985 involved the therapeutics of antianginal medications. The study involved clinical audit followed by analysis of potential interactions, followed by a clinical trial of clinically significant representative drugs. Our audit involved all patients who were prescribed combination antianginal therapy over a 4 month period. The findings which we recognised as important was that clinicians rarely used verapamil in combination with beta blockers, rather nifedipine was almost exclusively used as a combination therapy. Literature review showed us that no distinction was drawn between lipophilic versus hydrophilic beta blockers, accordingly we investigated this consideration as a factor contributing to the interaction. We found in preliminary studies that verapamil caused a doubling of the peak concentration and the concentration - time profile of metroprolol. These findings have been confirmed in follow-up studies in larger groups of patients while preliminary studies indicate that such interactions do not occur with a water-soluble beta blocker.

Another initiative involves aminoglycoside to allow optimisation of clinical drug delivery. Audit of a complete sample of all patients undergoing therapy in the Hospital during a four month period revealed a significant incidence of increased levels of drug and associated increases in serum creatinine as markers of renal toxicity. Conversely, there was a significant incidence of patients with evidence of clinically important infection and suboptimal concentrations in the blood. In an attempt to remedy the problem we are developing a collaborative pharmacokinetic service involving Clinical Microbiology, Pharmacy Department, and Clinical Pharmacology using a model successfully implemented in the United States of America.

Our studies in the area of ulcer therapeutics continue. We have extended our review of relapse rates after therapeutic intervention to include surgical therapies. Relapse rates following highly selective vagotomy are in the order of 0.2% per month compared to relapse rates of > 4% per month for all medical therapies. A number of propositions regarding the strategy of ulcer therapeutics follow:- a) medical therapies cannot be regarded as curative, rather disease-modifying at best, or symptomatic tools at worst, b) given that most patients do not require surgery, peptic ulcer must be a self-limiting disease, c) ulcer therapeutics should be based on a strategy for determining the "high-risk" patient who will not be amenable to medical management and who will

go on to elective surgery. Other patients can then be assigned to therapies which will control symptoms, with or without acceleration of healing, with or without modification of relapse rate.

We have continued studies aimed at elucidation of the pathogenetic role of Campylobacter pylorides infection. This has involved the development of both laboratory screening techniques and quantitative culture techniques to determine tissue mass of organisms in gastric biopsy specimens. Intervention studies aimed at manipulating the number of organisms present (eg antibiotic administration) have been undertaken in parallel with assessment of the presence or absence of peptic ulcer and the presence or absence and grade of antral gastritis.

Studies in epilepsy continue in collaboration with the Neurology Service of patients with resistant epilepsy, as previous intervention studies have been helpful in improving the control of epilepsy in the Neurology Clinic patients.

Our basic research interests continue in hepatic physiology using drug markers. We have made a number of advances involving the application of new theoretical concepts and analytical techniques. A planned initiative involves the use of hormone substrates in our established dog models. We also intend to apply these techniques to the analysis of mechanism of interaction between beta blockers and calcium antagonists referred to above.

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29th Annual Report of Ewen Downie Metabolic Unit

Ewen Downie Metabolic Unit

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J.W. Barlow M.Sc., Ph.D. (on overseas fellowship)

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J. Snider, M.B., B.S.

INTRODUCTION

In 1986 the Ewen Downie Metabolic Unit, founded in 1957 as the Diabetic and Metabolic Unit, approaches the end of its thirtieth year. At its inception the Unit was the first specialised endocrine unit in Australia to have adjacent laboratory and metabolic ward facilities, thereby allowing the development of clinical investigation dependent on a close relationship between ward and laboratory, with staff trained in both spheres. This type of unit has since been developed in other university-affiliated hospitals in Australia.

The Endocrine Society of Australia was founded in 1958, almost at the

same time as the Alfred Hospital Diabetic and Metabolic Unit. The key role of the Unit in the development of the specialty is clear when one appreciates that the first office-holders of the society were Ewen Downie as President and Pincus Taft as Secretary/Treasurer.

The Unit has played a key role in the training of many specialists now active in various aspects of Clinical and Laboratory Endocrinology here and overseas. Notable former trainees in the department before 1973 who have subsequently worked elsewhere include: Henry Burger, Ian Burr, Donald Cameron, Kevin Catt, Peter Davoren, Gordon Ennis, James Evans, Richard Gordon, Ian Martin, Gabriele Medley and Paul Zimmet.

The interests of the Unit have been diverse, but one major theme has been research on aspects of thyroid disease. In the early years of the Unit a prominent figure was that of Dora Winikoff, a graduate of Warsaw University who came to Australia in the late 1930s. Before joining the Unit soon after its inception, she was on the staff of Melbourne University Biochemistry Department. She was the first in Australia to establish a chemical assay (protein-bound iodine) for circulating thyroid hormone in 1954. In 1960 she was coauthor of reports which documented the effectiveness of injected iodized oil as an approach to the treatment of endemic goitre due to iodine deficiency. This type of treatment has subsequently been used on a world-wide scale.

A list of publications traces the development of thyroid research in the Unit from its inception:

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J Clin Endocrinol Metab 58:736-739, 1984.
(continued in list of current publications).

In July 1985, Dr. Richard Arnott took up the appointment of Assistant Endocrinologist following Dr. Lording's appointment as Visiting Physician in succession to Dr. Breidahl. Dr. Jonathan Taft joins the Unit as Clinical Assistant Specialist.

During 1985 we were privileged to have with us Dr. Bai Yao, a senior endocrinologist from the Beijing Union Hospital and Chinese Academy of Medical Sciences Beijing, China, during tenure of a Fellowship awarded by the Australia-China Council. After spending the initial part of his sixteen months stay in Australia at the Medical Research Centre, Prince Henry's Hospital, he spent eight months working in our thyroid research program.

We continue to enjoy helpful and valued collaboration with Monash University Department of Medicine, the Baker Institute and Clinical Research Unit, the Medical Research Centre Prince Henry's Hospital, and the Howard Florey Institute, University of Melbourne.

Financial support, either direct, or in the form of equipment or travel funding from the following sources is gratefully acknowledged:

Estate of the late Vincenza Acton
Estate of the late H. Vistaline
G.M. Rollason Trust
Vivian Hill Trust
Alfred Hospital Whole-time Medical Specialists' Private Practice Fund.
Research Support from the National Health & Medical Research Committee is also acknowledged.

Projects

1. Thyroid hormone pathophysiology
   a. Thyroid hormone changes during recovery from critical illness.
   b. Assessment of factors that determine potency of inhibitors of thyroid hormone plasma binding.
   c. Drug interactions with nuclear sites of triiodothyronine binding.
   d. Studies of familial dysalbuminaemic hyperthyroxinaemia.
   e. Fatty acid effects on thyroid hormone pathophysiology.
2. The expression of mineralocorticoid action in states of hormone resistance and endocrine hypertension.
3. Structure-activity relationships of steroid hormones.

PROJECTS

Effect of therapeutic doses of oral frusemide on serum binding of thyroxine

H.H. Newnham, P.S. Hamblin, F. Long, C-F. Lim, D.J. Topliss, J.R. Stockigt.

We have shown that the diuretic frusemide is a potent inhibitor of serum binding of thyroxine, acting by competition for binding sites on thyroxine binding globulin (TBG), prealbumin and albumin. High dose intravenous treatment with frusemide has been associated with transient hypothyrox-

inaemia, but the effect of standard therapeutic doses in the absence of severe renal impairment has not so far been established. Thyroid status is often difficult to assess clinically in patients with severe congestive cardiac failure because tachycardia, atrial fibrillation, muscle weakness and weight loss occur in both hyperthyroidism and in severe heart failure of any cause. In order to rule out hyperthyroidism in such patients one relies heavily on laboratory assays, making it crucial to fully assess the effect of drugs that alter the interpretation of commonly used tests.

Informed consent for serial blood sampling was given by 34 hospital inpatients who were receiving oral frusemide for congestive cardiac failure; in most cases the studies were conducted just prior to discharge from hospital at a time when the patients' condition was relatively stable. Those taking other medications known to affect assessment of thyroid hormone levels, and those with severe renal impairment were not studied.

A statistically-significant reduction in total T_4, elevation in T_3 resin uptake and increase in free T_4 index was seen 1-3 hours after 250 mg, 120 mg and 80 mg oral doses of frusemide. In four of the eight patients taking 250 mg doses, the free T_4 index rose to above the normal range. In those taking 80 mg or 120 mg doses, the changes, while statistically-significant, were within the normal range. Time-course studies demonstrated maximum inhibition of binding about 120 min after drug ingestion, consistent with drug absorption. Changes in serum thyroid stimulating hormone and triiodothyronine were not significant. Displacement of T_4 from serum binding sites was confirmed by equilibrium dialysis and by an estimate of free T_4 using a commerical analogue assay.

Hence, frusemide should be added to the extensive list of drugs that may affect biochemical assessment of thyroid function. The fall in serum T_4 is consistent with enhanced clearance due to displacement of thyroid hormone from serum binding sites. The influence of chronic frusemide administration on thyroid hormone action at the tissue level remains to be further defined. It is possible that repeated drug-induced increments of free T_4 may have tissue effects, both peripherally and at the pituitary, thereby influencing the feedback relationships of thyroid hormones. Because these questions will not easily be accessible to study in humans, the possibility of developing an animal model has been explored in preliminary studies. Both rats and rabbits are unsuitable for such studies because thyroid hormones are carried predominantly in association with transthyretin (prealbumin) in these species. While frusemide also displaces T_4 from this protein in vitro, the effect is much less potent at therapeutic, or experimental, concentrations. The complexity of these interactions is further demonstrated by our recent finding that frusemide also interacts with nuclear thyroid hormone receptors in isolated rat hepatocytes. Thus, this drug has the potential to interact with thyroid hormones by displacement from plasma binding, by altering feedback effect and by direct binding to a site where tissue actions of thyroid hormone are initiated, i.e. the cell nucleus.

Euthyroid hypothyroxinaemia: a common finding in Australian aborigines

*V.S. Mohr, D.J. Topliss, *K. O'Dea, J.R. Stockigt, J.W. Barlow.*

**Department of Medicine, Repatriation General Hospital, Heidelberg.*

Numerous plasma protein abnormalities can cause total hormone levels in affected euthyroid subjects to fall well outside the normal reference ranges. Such abnormalities include familial dysalbuminaemic hyperthyroxinaemia, familial increase in thyroxine binding to prealbumin, and inherited absence, deficiency, or excess of thyroxine binding globulin (TBG). It has recently been recognized that euthyroid hypothyroxinaemia due to TBG deficiency occurs in up to 40% of pure-blood Australian aborigines. Clinical thyroid status is normal and serum levels of TSH are identical in subjects with low T_4 and normal T_4. All groups who have investigated this variant agree that the level of TBG is subnormal, as demonstrated by a reduced immunoreactive concentration and a diminished T_4 binding capacity. However, it has until recently been uncertain whether this variant is due to reduced rate of secretion of structurally-normal TBG, or whether an abnormal molecule is produced, the lower concentration perhaps being due to accelerated breakdown. The latter possibility is supported by the recent demonstration of increased heat lability of TBG in aborigines with subnormal concentrations of this protein (Refetoff et al 1985).

Detailed studies of T_4 and T_3 binding kinetics were undertaken to further elucidate this variant. Samples were obtained from 100 aborigines from the Kimberly region of Western Australia as part of a population survey of environmental influences on carbohydrate tolerance, conducted by Dr. Kerin O'Dea. There was no apparent link between abnormalities of thyroid hormone binding and disturbances of carbohydrate tolerance.

These studies have shown a clear difference in T_4 binding capacity between the two groups of aborigines (normal T_4 v. low T_4, p(0.001). Difference in affinity, i.e. slope of the Scatchard plot, were less obvious and between-subject variation was considerable, but the difference in mean slope was significant ($p < 0.05$), consistent with the existence of a structural TBG variant in aborigines with low T_4 levels. Direct studies of TBG turnover may not be feasible, so that the presumptive evidence for a variant circulating protein will probably continue to be based on such indirect evidence. From the practical point of view, the diagnosis of primary hypothyroidism should not be made in Australian aborigines without demonstrating TSH excess. It is still uncertain whether this hereditary binding abnormality is associated with any alteration in thyroid hormone action, or any physical advantage or disadvantage.

Evaluation of oleic acid, arachidonic acid, various drugs and endogenous competitors for serum binding of thyroxine

J.R. Stockigt, C-F. Lim, Y. Bai, K.N. Wynne, S. A. Dyer, D.J. Topliss

Numerous drugs can displace thyroid hormones from plasma protein binding and it has been suggested that substances of endogenous origin can also inhibit binding, particularly in severe nonthyroidal illness. The potency of a competitor is influenced by at least three factors: its total circulating concentration, its free fraction in undiluted serum, and relative affinity for T_4 binding sites. From these three factors a prediction of inhibitory potency can be made. This prediction has been tested by measuring the effect of circulating concentrations of potential inhibitors on ^{125}I T_4 binding in undiluated serum at 37°C. We examined these

predictions for furosemide, various nonsteroidal anti-inflammatory drugs, and several free fatty acids that appear to be promising candidates as thyroid hormone binding inhibitors. Recent studies suggest that oleic acid may be especially important in this respect. We examined the effect of serum dilution on the apparent inhibitory potency of added fatty acids in order to evaluate effects previously described in competitive ligand binding assays.

The free fraction of drug competitors was established by equilibrium dialysis at 37°C of undiluted serum either using ^{14}C preparations, or by spectrophotometric measurements of drug transit from dialysate to serum. For long chain ^{14}C free fatty acids, their free fraction was measured by partition between heptane and the aqueous serum phase. Competition for T_4 binding was measured using serum diluted 1;10,000. Relative inhibitory potency was predicated from the product:

Circulating Concentration x Free Fraction x Relative Affinity.

Equilibrium dialysis of ^{125}I T_4 in undiluted serum was used to directly examine the effect of various competitors.

At pathophysiological concentrations of oleic and arachidonic acids in undiluted serum both the prediction and direct dialysis at 37°C showed little inhibitory activity. A substantial increase in total concentration and/or free fraction would be required to achieve an inhibitory effect. Increments of oleic acid, added to a normal serum pool, on free fraction of ^{125}I T_4 in undiluted serum at 37°C showed only a slight increase in T_4 free fraction with addition of 1 and 2 mM oleic acid and a 2-3 fold increase at 5 mM. However, there was a marked influence of serum dilution on the increase in ^{125}I T_4 free fraction produced by particular increments of added oleic acid. The effect of 5mM oleic acid added to undiluted serum was reproduced by 0.5 mM addition to serum diluted 1:10, indicating that conclusions as to inhibitory potency must be interpreted cautiously if diluted serum is used as the detection system. Such studies will generally over-estimate potency if interpreted without regard to dilution of the test serum, because the free fraction of competitor is disproportionately high and the concentration of T_4 binding sites reduced by dilution.

In serial studies of some patients admitted to hospital with diabetic ketoacidosis, a condition where free fatty acids are increased, we have been able to demonstrate an extractable inhibitor of T_4 binding in normal serum. The relationship of this substance to known fatty acids is currently under investigation.

Meetings and Conferences

Dr. Shane Hamblin, Dr. Jim Stockigt and Ms. Virginia Mohr each presented papers at the 67th Meeting of the US Endocrine Society in Baltimore in June 1985. Virginia Mohr attended this meeting during her tenure of an Alfred Hospital Whole-time Medical Specialists' Travelling Scholarship for technical staff. She also visited laboratories in Brussels, London, Boston, Minneapolis and Los Angeles. Shane Hamblin was at that time en route to his Fellowship position with Dr. Jack Oppenheimer in Minneapolis. Dr. Stockigt was an invited speaker at the International Symposium on Thyroid Hormone Methodology in Berlin in May, and attended the IX International Thyroid Congress in Sao Paulo, Brazil in September, serving also on the Program Organizing Committee for that meeting.

At the annual meeting of the Endocrine Society of Australia in Adelaide

in August 1985, papers were presented by Drs. Duncan Topliss and Harvey Newnham, and Mr. Chen-Fee Lim. Dr. Topliss was again a member of the Programme Organizing Committee for the meeting.

During 1984 members of the Unit gave lectures and presentations in numerous country centres as part of continuing education and family medicine programmes, under auspices of the Victorian Medical Postgraduate Foundation, the College of General Practitioners and College of Physicians.

Teaching and Seminars

Drs. Breidahl, Taft, Lording, Topliss and Stockigt gave seminars and lectures in the 6th year clinical teaching programmes and participated in the teaching of general medicine, diabetes and endocrinology for 4th, 5th and 6th year students.

Postgraduate teaching sessions were held weekly during the first half of 1985 for candidates for Part 1 FRACP. In addition, clinical lunchtime seminars were held twice monthly throughout the year.

The following seminar topics were presented in 1985:

Pituitary
Acromegaly, management and complications
Cushing's disease
Recovery of pituitary-adrenal axis after unilateral adrenalectomy for Cushing's disease

Diabetes
Pathophysiology of diabetic ketoacidosis
Cerebral oedema in diabetic ketoacidosis
Hyperosmolar coma

Thyroid
Amiodarone and the thyroid
Thyrotoxic periodic paralysis
Muscular abnormalities in hypothyroidism

Adrenal
Primary hyperaldosteronism due to adrenal carcinoma
Phaeochromocytoma - diagnosis and management

Gonads
Hypogonadism in middle-aged men
Testicular tumours

Calcium and bone disease
Elusive parathyroid glands
Osteomalacia

Grand Rounds 1985:
Hypercalcaemia and malignancy
Diagnosis of Cushing's disease

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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 efforts of one sector of the community, either scientists, governments, companies or individuals, but by the combined effort of 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.

Gifts are exempt from tax

For further information contact:-
The Director
Baker Medical Research Institute
Commercial Road
P.O. Box 348
Prahran Victoria, 3181
Telephone (03) 522 4313

Suggested form of bequest:-

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 $..... free of all duties, for which the written acknowledgement of the Senior Executive Officer shall be sufficient discharge.

To preserve the value of the bequest it can be an advantage to express the gift as a percentage of the value of the estate