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Contents

Editorial

Welcome to the July 2001 issue of *The* Bulletin! We are pleased to announce that a co-editor, Dr Helen Maddock has been co-opted by the BSCR Committee to work on *The* Bulletin. Helen is a research fellow at the Hatter Institute, University College London and details for contacting Helen can be found on the facing page. We look forward to working together to produce an interesting Bulletin for BSCR members and as always, we welcome your views and suggestions as to how we can achieve this.

In the Secretary's Column, Dr Gary Baxter highlights the forthcoming election of Committee members and ballot forms are enclosed with this copy of *The* Bulletin. To assist with voting, biographical summaries and statements from the candidates are published within this issue. Please help to select the four candidates who you feel will benefit the Society, by completing the ballot form and returning it to Gary by 17th August.

Our review article for this issue has been written by Dr Derek Terrar of the Department of Pharmacology, University of Oxford. Dr Terrar provides a fascinating overview of the regualation of intracellular calcium release in cardiac muscle.

Drs Richard Heads and Angela Clerk, organisers of the Society's successful Spring Meeting: *'Apoptosis in the heart: Signalling, Mechanisms, Pathology and Protection'*, have provided an account of the proceedings. The report is followed by a selection of abstracts presented at the meeting.

Finally, we bring you the latest details of grants awarded to researchers in the Cardiovascular field, by the British Heart Foundation and the Wellcome Trust.

Nicola Smart & Helen Maddock

Possible Roles For cADPR, NAADP and IP3 In The Regulation Of Calcium Release From Intracellular Stores In Cardiac Muscle

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Introduction

Since the observation by Ringer in 1883 [1] that extracellular calcium is an absolute requirement for contraction of the heart, great strides have been made in elucidating the mechanisms underlying the transient rise in cytoplasmic calcium that couples each cardiac action potential to cell contraction. It is now thought that an initial entry of calcium across the surface membrane (sarcolemma) through L-type calcium channels (and possibly other sources, such as 'reverse mode'sodium/calcium exchange) triggers the release of a more substantial quantity of calcium from the sarcoplasmic reticulum, which acts as a membrane-bound intracellular storage site for calcium [2]. This process of 'calcium-induced calcium release' (CICR) is believed to be mediated by the calcium release channels of the sarcoplasmic reticulum, which have been named ryanodine receptors (RyRs) based on their ability to bind the plant alkaloid ryanodine with high affinity. CICR is thus essential for normal muscle contraction, and may also play a role under pathological conditions in the generation of disturbances of cardiac rhythm.

In the last two decades, much work has been undertaken in a variety of cell types to study the regulation of calcium release from intracellular storage sites, and evidence has accumulated that calcium release may be influenced by a variety of factors [3]. The aim of this article is to briefly review evidence concerning the roles of three substances which might modulate calcium release from intracellular stores in cardiac muscle: cADP-ribose (cADPR), inositol 1,4,5 trisphosphate (IP3) and nicotinic acid adenine dinucleotide phosphate (NAADP).

cADPR and cardiac ryanodine receptors

Since its initial discovery as a calcium mobilising agent in sea urchin eggs, cADPR has been reported to influence calcium release from ryanodine-sensitive intracellular stores in a wide variety of cell types, including neurones, pancreatic β cells, smooth muscle cells, lymphocytes and even plant cells [4-6]. The first evidence that cADPR might influence CICR in the heart was provided by experiments of Meszaros and colleagues, who found that cADPR could promote calcium efflux from microsomes prepared from sarcoplasmic reticulum, and could increase the probability of opening of cardiac ryanodine receptor calcium release channels studied in artificial lipid bilayers [7]. However, other careful experiments failed to detect an effect of cADPR on RyRs [8] or showed effects that were inhibited by concentrations of ATP expected to be present in the cytoplasm of heart cells [9]. The differences in these data are perhaps difficult to reconcile, but may reflect differences in the presence or absence of accessory factors required to confer cADPR sensitivity to RyRs - indeed, it is not yet known whether cADPR binds directly to the RyR, or acts via intermediate proteins.

Effects of cADPR antagonists in intact cardiac myocytes

The first indication that cADPR might play a role in intact cardiac myocytes was provided in experiments using intracellular application of 8-amino-cADPR [10], which had been shown to act as a highly selective competitive antagonist of cADPR in experiments on sea urchin eggs [11]. When 8-amino-cADPR was applied to the cytoplasm of guinea-pig ventricular myocytes, calcium transients and accompanying contractions in cells stimulated to fire action potentials were reduced by approximately 30%. Such inhibitory effects of 8-amino-cADPR were not observed when CICR was suppressed by prior exposure of the cell to ryanodine. These experiments were interpreted in terms of an influence of endogenous cADPR to promote CICR, an action which was competitively antagonised by 8-amino-cADPR. Calcium release from the SR by caffeine was not, however, prevented by 8-amino-

cADPR showing that the calcium release channels were still functional under these conditions. These experiments also showed that the caffeine-releasable store of calcium was not reduced, and even a little increased, in cells in which 8-amino-cADPR had been added to the cytoplasm, perhaps reflecting a greater SR load when the tendency of endogenous cADPR to enhance CICR was reduced by the antagonist.

Similar inhibitory effects of 8-amino-cADPR on calcium transients in cells stimulated to fire action potentials have recently been reported in rat ventricular myocytes [12]. Interestingly, the inhibitory effects of 8-amino-cADPR on calcium transients were greater in rat cells than those observed in guinea-pig myocytes, as might be expected from the greater contribution that SR calcium release makes to the calcium transient in rat, as compared to the guinea-pig.

Effects of cADPR application in intact cardiac cells

Initial experiments to investigate the influence of exogenous cADPR on CICR did not detect any effect of cADPR on calcium transients [13]. However, a subsequent study reported that contractions and calcium transients accompanying action potentials in guinea-pig ventricular cells were increased by exogenous cADPR (applied via a patch pipette) [14]. The effects were concentration-dependent, with 5 μM cADPR causing an increase in contraction of approximately 30%. These actions of exogenous cADPR were prevented by co-application with either of two antagonists, 8-amino-cADPR or 8-Br-cADPR. As expected from their effects on sea-urchin eggs, 8-amino-cADPR appeared to be a more potent antagonist than 8-Br-cADPR in suppressing the effects of exogenous cADPR applied via the patch pipette. The potentiating effect of exogenous cADPR on cell contraction was prevented when SR function was first inhibited using a mixture of ryanodine and thapsigargin, as expected if the effect of exogenous cADPR arose as a consequence of enhancement of CICR. Recent experiments [12] in rat myocytes have also shown effects of exogenous cADPR to increase calcium transients, although in this case cADPR was applied using pressure ejection from a micropipette inserted transiently into the cells. The lack of effect of exogenous cADPR in the work of Guo and colleagues [13] was thought perhaps to reflect the use of room temperature in these experiments, since it has been reported that the effects of exogenous cADPR in guinea-pig ventricular cells were observed at 36°C, but not at room temperature [14]. However, Prakesh and colleagues were able to demonstrate effects of exogenous cADPR on calcium transients in rat myocytes even at room temperature [12].

Flash photolysis of caged cADPR analogues in intact cardiac cells

Another method for application of exogenous cADPR is to photorelease it from a caged analogue applied to the cytosol. Photoreleased cADPR was found to increase calcium transients accompanying action potentials in both guinea-pig and rat ventricular myocytes [15]. The UV light required to cause photolysis did not itself increase calcium transients, and the effects of photoreleased cADPR were suppressed by prior application of the antagonist, 8-amino-cADPR. Interestingly these effects of photoreleased cADPR were not maximal immediately after uncaging but took approximately 15 seconds to develop. We have suggested that this delay may reflect a multimolecular interaction involving other proteins associated with RyRs such as calmodulin (see below) or FK binding protein [3], though further work is necessary to test these possibilities.

Calcium sparks are readily recorded from rat ventricular myocytes, and have provided important insights concerning the organisation of CICR in cardiac cells [16-18]. If cADPR were to enhance the calcium sensitivity of CICR as suggested above, exogenous cADPR would be expected to increase spark frequency. This has indeed been shown to be the case for cADPR photoreleased in rat ventricular cells [15]. The increase in frequency of calcium sparks in this study was not accompanied by any obvious change in their characteristics (amplitude, time to peak, duration, diameter at half amplitude), but was prevented by 8-amino-cADPR. Thus, if sparks are thought to be unitary events underlying the whole-cell calcium transient, the effect of cADPR to increase whole-cell calcium transients described above may reflect an increased number of spark-like events contributing to these transients.

RyR accessory proteins may be required for cADPR-induced calcium release

In sea urchin eggs, calmodulin is required for the action of cADPR on calcium release [19], and a similar requirement for calmodulin has been reported for cADPR-evoked calcium mobilization in pancreatic _ cells [20]. We have investigated whether this might also be the case in guinea-pig ventricular myocytes [21]. The structurally unrelated calmodulin inhibitors W7 and calmidazolium both reduced contraction accompanying

action potentials in guinea-pig ventricular cells. In the presence of either of these calmodulin antagonists, exogenous cADPR (applied via a patch pipette) no longer increased contraction. In order to investigate whether calmodulin-dependent protein kinase might be involved, a peptide inhibitor of this enzyme was added to the patch pipette solution. Under these conditions, exogenous cADPR again failed to increase cell contraction accompanying action potentials. It therefore appears from these experiments that calmodulin is involved in the action of cADPR, and that this might be mediated via a calmodulin-dependent protein kinase. This is supported by other evidence demonstrating a requirement for calmodulin in the actions of cADPR [22].

Role of cADPR in arrhythmogenesis

The release of calcium from the SR is believed to play an important role not only during normal cardiac muscle contraction, but also during abnormal conditions associated with calcium overload and oscillations of cell calcium and membrane potential. These oscillations of intracellular calcium are thought to arise from cyclical release and reuptake of calcium by the SR, and have been suggested to underlie a variety of disturbances in the rhythm of the heart (arrhythmias). There is evidence that CICR under such pathological conditions is also influenced by cADPR. When calcium waves and arrhythmogenic behaviour were provoked in guinea-pig cells by exposure to high concentrations of either a catecholamine or a glycoside, cytosolic application of the antagonist, 8-amino-cADPR, suppressed the oscillations [23]. This is consistent with antagonism by 8-amino-cADPR of the effects of endogenous cADPR to increase the calcium sensitivity of CICR under these pathological conditions, in a manner similar to that observed with calcium transients in healthy cells. In support of this, calcium waves could be initiated by cADPR (applied from a patch pipette) in cells which were exposed to a concentration of glycoside which did not by itself provoke arrhythmogenic activity [23]. Similar actions of cADPR under arrhythmogenic conditions have been reported by Prakash and colleagues [12]: in rat cells exhibiting calcium waves, the frequency of calcium wave occurrence was suppressed by 8-amino-cADPR. Conversely, exogenous cADPR microinjected into rat cells showing spontaneous calcium waves increased the frequency of the waves in a concentration-dependent manner. Taken together, these experiments provide further evidence that the calcium sensitivity of CICR may be enhanced by cADPR under both pathological and 'normal' conditions.

Effects of cADPR in permeabilised cardiac cells

It was mentioned above that attempts to demonstrate calcium releasing actions of cADPR in cell homogenates or subcellular preparations have met with mixed success. One method involving minimal disruption of cellular contents is to use saponin-permeabilised myocytes, which allows direct application of cADPR to a functioning SR. Using this approach, Lukyanenko & Gyorke [24] showed that spark-like activity could be recorded in permeabilised rat myocytes, and that application of exogenous cADPR caused an increase in the frequency of calcium sparks similar to that seen with photoreleased cADPR in intact cells, as described above. In unpublished experiments, we have repeated these experiments and have also seen increases in the frequency of calcium sparks following application of cADPR in the solution superfusing saponin-permeabilised rat cells. This supports the hypothesis that cADPR enhances calcium release from the SR.

Other possible effects of cADPR

In a recent poster presentation to the Biophysical Society [25] evidence was presented that cADPR might increase calcium uptake into the SR by increasing the activity of the SR calcium-ATPase. Increased loading of the SR with calcium as a consequence of increased calcium uptake could contribute to the increased whole-cell calcium transients described above. Although such a mechanism may contribute to the actions of cADPR, several pieces of evidence point to the conclusion that an increased uptake of calcium into the SR cannot be the only effect of cADPR in cardiac cells. In the experiments of Prakash and colleagues [12], microinjection of cADPR in rat cells showing spontaneous calcium waves caused a concentration-dependent increase in the background calcium levels, as well as an increase in the frequency of calcium waves. Similarly, we have observed an increase in resting cytoplasmic calcium in both guinea-pig and rat myocytes following photorelease of cADPR (unpublished observations). These observations are not consistent with a sole effect of cADPR to enhance SR calcium uptake, since this would be expected to decrease rather than increase resting cytosolic calcium levels. Furthermore, if the actions of cADPR and its antagonists were attributable only to actions on the SR calcium-ATPase, it would be expected that these compounds would alter the kinetics of the decay phase

of the calcium transient. However, this appears not to be the case, since several studies have shown that cADPR and 8-amino-cADPR augment and reduce respectively the rising phase of the calcium transient (consistent with actions on SR calcium release), but do not alter the decay of the calcium transient. This is evident in figure 4 of Rakovic *et al.* (1996), in which 8-amino-cADPR does not affect the rate of decay of the calcium transient [10], and also in figure 6 of Iino *et al.* (1997) [14] and figure 1 of Cui *et al.* (1999) which demonstrate no effects of exogenous cADPR (applied through a patch pipette or by photorelease respectively) on the declining phase of the calcium transient [14, 15]. This is supported by the observations of Prakash and colleagues [12].

Although the experiments described above might be taken to indicate that the primary action of cADPR is on release rather than sequestration of calcium by the SR, we have investigated the possible influence of cADPR on calcium uptake in microsomes prepared from guinea-pig ventricular muscle. These microsomes take up calcium (measured as a decline in fluo-4 fluorescence in the suspension medium) when provided with ATP and magnesium. This uptake is inhibited by either thapsigargin or cyclopiazonic acid and therefore appears to reflect SERCA pump activity. Ruthenium red (10 μM) was added to suppress any complicating effects of calcium release in these experiments. Under these conditions the microsomes can be challenged with several aliquots of added calcium, each giving a rapid rise of fluo-4 fluorescence followed by a decline reflecting calcium uptake into the vesicles. 10 μM cADPR appeared to be without effect on calcium uptake by these microsomes, in contrast to the observations of Lukyanenko and colleagues [25]. However, it cannot be excluded that factors were missing from this preparation that are required to confer cADPR sensitivity to the calcium uptake mechanism.

Can RyR agonists produce a sustained posititive inotropic action?

There is currently debate regarding the general question as to whether a substance which promotes calcium release from the SR can have a sustained effect on calcium transients and associated contractions. In rat ventricular cells, caffeine has been used as an example of agents which promote calcium release. It has been shown that low concentrations of caffeine lead to an initial enhancement of calcium transients and contractions, but that this potentiating effect does not persist since SR calcium loading falls to compensate for the increased release [26]. Subsequent wash in caffeine-free solution first leads to a reduction in calcium transients and contractions below their initial values, since the augmenting effect of caffeine on release is lost but SR loading with calcium is still reduced; initial conditions are restored as SR loading with calcium re-adjusts to its original value. Conversely, tetracaine has been used as an example of agents which reduce calcium release, and it shows an opposite pattern to that of caffeine: an initial fall in calcium transients and contractions, followed by a restoration of calcium transients and contractions as SR loading with calcium shows a compensating increase [26]. In explaining the complete compensation of SR loading for any change in fractional release of calcium from the SR, calcium balance is emphasised: calcium that enters the myocyte (for example through voltage-gated calcium channels) in one part of the cardiac cycle must in the steady state be lost (for example by extrusion through sodium/calcium exchange) at another part of the cycle. This may be especially relevant in the case of the rat in which rapid calcium entry triggers release, but where the decline in the calcium transient occurs at a potential where calcium-extrusion through sodium/calcium exchange is especially extensive because of the unusually negative plateau of the rat ventricular action potential. Another extreme might occur in a different species if the plateau were sufficiently positive during the decline in the calcium transient: in this case calcium uptake into the SR might occur without the competing influence of sodium/calcium exchange since this will not extrude calcium at a sufficiently depolarised potential, and any increase in fractional release of calcium from the SR can then be accompanied by re-uptake of the additional calcium by the SR calcium-ATPase without contravening the requirement for steady-state balance between influx and efflux. We believe that guinea-pig cells might be closer to this latter case. However, any effect of cADPR to enhance calcium uptake by the SR, in addition to an enhancement of calcium release, would of course permit a sustained effect of cADPR even in a species with a very negative plateau. The observations of Lukyanenko and colleagues [25] may therefore be of relevance here.

The argument that CICR sensitisers cannot maintain a sustained positive inotropic action also relies on the assumption that the relative activities of the SR calcium-ATPase and sodium/calcium exchange do not alter over the course of the cardiac cycle. However, it cannot be excluded that enhanced calcium release in response to a RyR agonist may upregulate calcium

uptake by the SR (relative to calcium extrusion by sodium/calcium exchange), particularly in cells with a prominent positive plateau. This might be due, for example, to phosphorylation of the calcium-ATPase by calmodulin-dependent protein kinase, or depletion of SR calcium following enhanced release. In such a situation, compensation in SR loading would not be complete, and a sustained potentiation of the calcium transient would be expected, albeit at a level lower than the initial peak effect.

An additional point to be considered regarding the actions of an agent which sensitises CICR is the relative effects of that agent on CICR at high and low levels of calcium. Caffeine is known to enhance SR calcium release at diastolic levels of calcium, as well as in response to entry of trigger calcium through L-type calcium channels, and the resulting depletion of the SR during diastole may exaggerate any compensatory reductions in SR loading during application of caffeine. It is possible that some agents may preferentially sensitise CICR to the higher levels of calcium which occur during the entry of trigger calcium (through L-type calcium channels), but not to diastolic levels of calcium. This would tend to reduce the extent of SR calcium depletion in the face of enhanced systolic release.

Control of cADPR synthesis in the heart

If cADPR does play a role in modulating CICR, the question arises whether levels of cADPR might be regulated by hormones or transmitters acting at cell surface receptors. Recent experiments of Higashida and colleagues [27, 28] show that in rat ventricular myocytes, stimulation of β-adrenoceptors and angiotensin II receptors both lead to an increase in the synthesis of cADPR. The increased synthesis results from enhanced activity of an ADP-ribosyl cyclase enzyme which appears to be present in the cell surface membrane and which the authors propose is stimulated by a G protein. In microsomal membrane preparations of guinea-pig ventricle (thought to be made up predominantly of SR), we have shown that catalytic subunits of protein kinase A can enhance the synthesising activity of an ADP-ribosyl cyclase [29]. It is therefore possible that β-adrenoceptor stimulation may enhance the activity of ADP-ribosyl cyclases in both the surface membrane and the SR to increase the synthesis of cADPR, and thus through a sensitising action on CICR contribute to the positive inotropic effects of endogenous catecholamines.

NAADP: a new calcium mobilizing messenger

Another substance which has been shown to influence calcium signals in mammalian cells is NAADP, which can be synthesised by the same enzyme which produces cADPR when the substrate is NADP rather than NAD [6]. Interestingly, this substance is thought to act on calcium stores which are not thapsigargin sensitive [30, 31], and the nature of these stores is still the subject of debate. In the sea urchin egg, there are several pieces of evidence that the calcium release mechanism employed by NAADP is distinct from that of both cADPR and IP3, implying that NAADP-induced calcium release does not occur via RyRs or IP3Rs in this system [6, 32]. However, in some mammalian cells there appears to be complex interplay between NAADP and other molecules with an influence on calcium stores, such as cADPR and IP3 [33].

There are currently few reports in the literature concerning the effects of NAADP on calcium dynamics in cardiac tissue, but recent evidence suggests that NAADP may indeed influence calcium release in the heart. It has been shown that NAADP can be synthesised and degraded in cardiac tissue [34, 35], as perhaps might be expected if the synthesis of NAADP is carried out by the same enzyme that generates cADPR. Specific binding of NAADP to crude rat heart homogenates [35] and rabbit heart microsomes [36] has also been reported, in the latter case to two sites with apparent Kds of 130 pM and 4 nM, providing evidence for the existence of a cardiac NAADP receptor. Furthermore, there is support for a functional role of these receptors. NAADP has been demonstrated to release calcium from rabbit heart microsomes with an EC50 in the region of 320 nM [36]. NAADP (3-10 μM) has also been reported to release calcium from rat heart microsomes [37]; rather intriguingly, this study also demonstrated an effect of NAADP to activate RyRs from dog heart incorporated into planar lipid bilayers, in contrast to evidence in other systems that NAADP acts through mechanisms distinct from the RyR. Whether or not this action of NAADP on the RyR is direct or involves intermediate proteins/factors remains to be established, but an action at the RyR is consistent with recent observations that 10 μM NAADP increases the frequency of spontaneous calcium sparks in saponin-permeabilised rat ventricular myocytes [34, 35].

A role for IP3 in cardiac tissue

IP3 is a molecule generated from a constituent

of the lipid bilayer, phosphatidyl inositol 4,5-bisphosphate, by the action of receptor-coupled phospholipase C, and which is known to cause calcium release from endoplasmic reticulum in a variety of cells. Type II IP3 receptors are known to be present in cardiac myocytes [38], and several membrane receptors have been reported to be coupled to enhanced IP3 synthesis, including α1-adrenergic, endothelin and thrombin receptors. [39, 40]. However, despite these observations, it has previously been thought that IP3 does not play a major role in calcium signalling in cardiac myocytes. Recent experiments of Lipp *et al.* (2000) [41] show that this might require re-evaulation. Particularly in atrial myocytes, Lipp and colleagues have provided evidence that type II IP3 receptors are present and appear to be localised just beneath the surface membrane. Transverse tubules are thought to be lacking, or at least less extensive in atrial cells compared with ventricular cells. Application of a membrane permeant form of IP3 provoked calcium spark-like events and increased calcium transients in response to electrical stimulation. It is possible that cross talk between IP3 receptors and ryanodine receptors (which are found in bands extending deep into the myocytes despite the relative lack of transverse tubules) might aid propagation of calcium signals from the periphery to the centre of atrial myocytes.

Conclusions

The work summarised above shows that CICR, which is normally triggered by calcium entry during the action potential, can be modulated by a variety of intracellular mediators. In the case of cADPR, it appears that this substance can regulate the calcium sensitivity of CICR and that levels may be under hormonal control. Contraction could be enhanced by increased levels of cADPR, though arrhythmogenic effects could arise if these became excessive. The possible role of NAADP remains intriguing, particularly if as in other cells it were to act on a different calcium store other than the SR which is not thapsigargin-sensitive. Possible functional interactions between different intracellular calcium stores open up exciting possibilities. Interactions between IP3-sensitive stores and ryanodine-sensitive stores are also becoming well established in many mammalian cells, and this also may be functionally important in the heart.

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BSCR Autumn Workshop 2001

Mitochondria and myocardial protection

- from single cells to cardiac surgery

Bristol Heart Institute, Bristol Royal Infirmary

Tuesday 25th September 2001

Speakers will include: Kieran Clarke, Martin Crompton, Mike Duchen, Manuel Galiñanes, Elinor Griffiths, Andrew Halestrap, Helen Maddock, Brian O'Rourke, Anne-Marie Seymour, Nick Standen.

Topics: Mitochondrial K_{ATP} channels and ischaemic preconditioning; Mitochondrial Ca^{2+}, membrane potential, and the permeability transition pore; Mitochondrial metabolism; Clinical applications.

Registration: Free to members, £40 to non-members.
Organisers: Elinor Griffiths and M.-Saadeh Suleiman, Tel 0117 9287573; Fax 017 9288274;
Email: Elinor.Griffiths@bristol.ac.uk

Secretary's Column

The end of this year will bring changes to the composition of the Committee. I am taking this opportunity to inform members of these pending changes and to encourage your participation in democratically determining the composition of the Committee.

Both the Treasurer, Dr Michael Curtis, and I will have served three years in our officer positions. I will be retiring but I am delighted to inform members that Dr Barbara McDermott was unanimously elected by the Committee at its April meeting to succeed me as Secretary for the period 2002-2004. Barbara has already served for nearly three years as an ordinary member of the Committee and last year organised the extremely successful Autumn meeting of the Society in Belfast. I am confident that Barbara will bring her enviable organisational skills and flair to the running of the Secretary's office from 1 January 2002. Michael Curtis indicated his willingness to serve another term as Treasurer and was unanimously re-elected to serve in that post. I know all members of the Committee are grateful to Michael for his generosity of spirit. Michael and his assistant Tony Cavalheiro have been vigilant of the Society's finances during the last three years and we are grateful to have this continuity of officership.

Several ordinary members of the Committee are due to retire after three years' service. These are Dr Paul Barton, Dr Saadeh Suleiman and Dr Ian Zachary. These retirements, coupled with Barbara McDermott's move to the Secretary's office mean that there will be four vacancies on the Committee. Nominations were invited in the last Bulletin and six nominations were received. We are therefore required to hold a postal ballot and you will find this enclosed with this issue of the Bulletin. Biographies of the candidates are printed in this issue. I urge all members to participate in the important process of electing new Committee members. I write these lines 48 hours before the General Election for which a low poll is anticipated. It would be heartening and encouraging for the Committee to see a good turnout for the BSCR election! Please note that only official stamped ballot papers will be acceptable and these should be returned to me by mail by 17 August. Formal announcement of the result of the ballot will be made at the Annual General Meeting of the Society to be held on the afternoon of Friday 14 September during the Autumn meeting in Oxford.

The Bulletin editor, Dr Nicola Smart, was recently appointed in a new post at the Institute of Child Health, working with Dr Paul Riley and Professor Peter Scambler. We are delighted that Nicola has agreed to remain as Bulletin editor and credit to her hard work and ingenuity that she has managed to produce this issue of the Bulletin single-handed while moving from one side of the Thames to the other. The Committee co-opted Dr Helen Maddock, of the Hatter Institute, UCL, to serve as co-editor and we wish Helen well in this new position.

The Autumn meeting on 14th -15th September will be a joint meeting with the 6th International Symposium on Magnetic Resonance in Cardiovascular Research. The meeting organised by Dr Kieran Clarke and Dr Stefan Neubauer promises to be a large meeting with an international flavour. Registration is free for BSCR members who are encouraged to register as soon as possible. Details are circulated with this issue of the Bulletin and members should note that registration and abstract forms are available electronically at <http://www.bioch.ox.ac.uk/~mrcvr/>.

Astute readers will have noticed the URL for the Society's new website on the front cover of the Bulletin. This is a new acquisition of the BSCR and a project still in an embryonic form. At present, visitors to the website will be find useful information about the Society's past meetings and downloadable forms for membership. Please make use of this facility, let us have your ideas for further development of the website, and encourage colleagues who are not members to visit the site and download an application form.

Dr G F Baxter

Nominations for Membership of the BSCR Executive Committee

HUGH MONTGOMERY

Dr Montgomery studied at the Middlesex Hospital Medical School. He obtained First Class Honours in his BSc (1984) in Circulatory and Respiratory Physiology, before qualifying in 1987. Since then, he has obtained his Certificate of Higher Medical Training in Cardiology and general medicine, and has trained in Intensive Care medicine. He is due to take up an appointment as Consultant in Intensive care at UCL Hospitals in May 2001. In 1997 he was awarded an MD for his research into paracrine renin-angiotensin system (RAS) in cardiovascular disease. These data led to the award of the British Cardiac Society Young Investigators Award. Since then, and as a Senior Lecturer, he has led an expanding research group in the Department of Cardiovascular Genetics at UCL. His work has focused on the role of paracrine RAS in myocardial and skeletal muscle function. He is additionally leading work investigating the control of myocardial metabolic efficiency, and the roles of inflammation in the investigation of human (patho)physiology. Hugh is anxious that British research in cardiovascular medicine should be strongly collaborative, and that it should also be enjoyable. He would view his committee role as one to facilitate these aims. He also has concerns over the damage to clinical/ basic science collaborative work which has resulted from the recent changes to UK clinical training. He would hope to promote a closer alliance between clinicians and scientists.

Joined Society: 2001 **Proposed by**: Michael Marber **Seconded by**: Metin Avkiran

GILLIAN GRAY

My personal interest in CV research was fostered whilst a PhD student at the University of Strathclyde, a period of which I have very happy (if now distant!) memories. This was followed by several immensely enjoyable years 'post-doc'ing at the Université Louis Pasteur in Strasbourg, France working on NO in septic shock then at Hoffmann-la Roche in Basel, Switzerland working on development of the first endothelin receptor antagonist. My interest in factors endothelial continued when I returned to the University of Edinburgh in 1994 with a BHF fellowship and still continues, although as a Senior Lecturer. I now find my research time being diluted with teaching and inevitable administration. Over my years as a 'research gypsy' I learnt the value of making contacts in organisations such as the BSCR and this lead to many useful collaborations and friendships that continue today. Scotland currently has a very active CV research community covering a diverse range of interests. Since I joined the BSCR in 1995, my research group have benefited from participation in meetings and workshops. As a member of the BSCR committee, I would hope to encourage increased participation of other Scottish CV scientists in BSCR activities, both in attending and hosting events, and to encourage promotion by the BSCR of UK and Europe wide collaborations that are necessary to carry out high quality research in today's high-tech and often expensive research environment.

Joined Society: 1995 **Proposed by**: Cherry Wainwright **Seconded by**: Kathy Kane

GAVIN BROOKS

I qualified in Pharmacy in 1984 from The School of Pharmacy, London before registering as a Pharmaceutical Chemist in 1985. My PhD (1985-1988) was in the field of signal transduction, cellular proliferation and tumour promotion and was followed by a post-doctoral fellowship at the ICRF Laboratories in London. In 1992, I joined David Hearse's Cardiovascular Research Department at the Rayne Institute, St Thomas' Hospital, London where I developed my current research interests in cell cycle control mechanisms that modulate cardiac myocyte proliferation and hypertrophy. During this time I was the recipient of a BHF Intermediate Fellowship and various research grants from the BHF and Special Trustees for St Thomas' Hospital. In July 1997, I joined the cell cycle biotechnology company, Prolifix Ltd., as head of their cardiovascular research programme and here I extended my interests into cell cycle control of vascular smooth muscle cell growth. In January 1999, I returned to academia to pursue my research career at the Endowments Research Fund of the University of Reading and currently am Reader in Cardiovascular Biology. In the past, I have served as an Associate Editor of *Cardiovascular Research* (1991-1995) and as members of the BSCR (1997) and British Association of Cancer Research (BACR) (1992-1995) executive Committees. Currently, I am Chairman of the International Pharmacy Federation Special Interest Group on Pharmaceutical Biotechnology and am Fellow of the AHA. Previously, I have co-organised a BSCR workshop entitled "Signal Transduction mechanisms and growth control in the cardiovascular system" (September, 1998). I now am in the process of co-organising the Spring 2002 meeting of the BSCR that will be held at the University of Reading. I would welcome the opportunity to serve on the BSCR Executive Committee and would strive to maintain the high standards of excellence that current and previous members have achieved. I wish to increase the overall awareness of our Society, to encourage the interaction between basic scientists and clinicians and to promote the Society as one that welcomes young and dynamic cardiovascular researchers. I believe that my experiences as members of the BSCR and BACR Committees would enable me to make a significant contribution to the BSCR and its members.

Joined Society: 1992 **Proposed by**: Michael Shattock **Seconded by**: Gary Baxter

M. SAADEH SULEIMAN

I am currently a Reader in cardiac cellular Physiology and head of the myocardial protection group at the Bristol Heart Institute, University of Bristol. Current research involves investigating cellular mechanisms that may help protecting the heart against cardiac insults with special attention to open heart surgery. Main research projects are: the expression and regulation of amino acid transporters in the heart; anti-apoptotic action of Insulin-like Growth Factors; the mitochondria as targets for myocardial protection and the efficacy of different cardioplegic techniques during myocardial development. The research involves extensive collaboration within the Heart Institute in both clinical and non-clinical units. I am putting my name forward for re-election as a BSCR committee member as I fould the participation in committee affairs to be very interesting and provocative. The committee has undertaken the hard task of steering and promoting cardiovascular research in the UK. In particular, it works hard to improve collaboration between clinical and basic scientists. This has been a personal endeavour in Bristol. I hope to continue to use the expertise gained in Bristol in order to continue to contribute to the activities of the BSCR committee.

Joined Society: 1991 **Proposed by**: Metin Avkiran **Seconded by**: Gary Baxter

AJAY SHAH

My current post is as British Heart Foundation Professor of Cardiology at King's College London. My main research interests are in the mechanisms and pathophysiological roles of endothelial dysfunction and oxidative stress in cardiovascular disease, work that spans basic science and clinical research. I am also an active "card-carrying" clinical cardiologist. I graduated form the University of Wales College of Medicine in 1982, and undertook doctoral training with Professor Dirk Brutsaert in Antwerp (Belgium) and Professor Andrew Henderson in Cardiff as BHF Junior Research Fellow studying the endocardial regulation of myocardial function. This work was recognized by award of the British Cardiac Society Investigator Prize in 1991. My subsequent academic training included a BHF Intermediate Research Fellowship, which incorporated a period at the NIH in the USA with Professor Edward Lakatta, and an MRC Clinical Senior Fellowship. I am committed to working to achieve better integration of basic science and clinical research in the cardiovascular field, with a view to improving the translation of research advances into the clinical arena. I would like to see the BSCR consolidate and strengthen its position as the natural home for all those interested in cardiovascular integrative biology. I would also like to see it raise its profile and contribution to national clinical meetings such as the British Cardiac Society, and to establish more concrete links and interactions with similar societies in Europe.

Joined Society: 1989 **Proposed by**: Michael Marber **Seconded by**: Metin Avkiran

PETER WEINBERG

After graduating from Cambridge in 1979 with a Natural Sciences degree, I moved to Imperial College where I completed an MSc and then a PhD. Following the award of a Lady Davis fellowship to study in Israel and further postdoctoral work at Imperial College, I was appointed Lecturer in the School of Animal and Microbial Sciences at the University of Reading, where I remain. For my PhD I investigated why some regions of arterial wall are prone to atherosclerosis whilst others are resistant, an issue that remains my major interest. The long-term aim is to reduce disease by inducing in susceptible areas of the wall the key properties found in protected regions. We have shown that variations in the permeabillity of the arterial wall can explain the pattern of adult human disease, and that these variations are determined by nitric oxide synthesis and blood flow. I head the Vascular Permeability and Atherosclerosis Group at Reading, which has accommodated 16 staff and students and has received funding form the BHF, Wellcome Trust, MRC and Royal Society and smaller charities. I am also Visiting Senior Research Fellow at King's College London and an active member of the British Atherosclerosis Society and the Physiological Society (for whom I recently organised a symposium on Vascular Cells in Health and Disease); I have served as a committee member for the London Microcirculation group. Dr M J Lever and I are hoping to organise what would be the first international meeting on vascular permeability. I am keen to serve on the BSCR Committee because it would facilitate the organisation of this proposed meeting; to increase representation of Universities which have not traditionally been regarded as cardiovascular centres but which now have nationally significant cardiovascular groupings (Reading's is described in Bulletin vol 13, No 1); and to promote links with researchers in the field of atherogenesis who are not currently involved in the BSCR.

Joined Society: 1995 **Proposed by**: Jeremy Pearson **Seconded by**: Giovanni Mann

Spring 2001 BSCR meeting: Apoptosis in the Heart. Signalling, Mechanisms, Pathology and Protection

22-23 March 2001, The Governor's Hall, St Thomas' Hospital

The significance and mechanisms of apoptosis in relation to cardiovascular disease is becoming increasingly apparent. Apoptosis is under intense investigation in many cell types, but it is not yet clear whether the mechanisms are universal in all cell types. Given the specific characteristics of cardiovascular cells, particularly terminally-differentiated cardiac myocytes, it is probable that at least some aspects will be cell specific. Because of the potential benefits which may be associated with modulating apoptosis in the cardiovascular system, the role and mechanisms of apoptosis in cardiovascular biology has become a rapidly growing area of research which is attracting an increasing number of scientists and clinicians. It will prove of substantial importance to elucidate the mechanisms involved, although as with any emerging area of research, many of the studies in the literature may appear confusing or contradictory. The aim of this meeting was to bring together leading national and international experts in the field to present their recent work, and to provide a forum to discuss the significance of apoptosis and the mechanisms involved. Poster presentations were invited to facilitate discussion of recent research, and some abstracts were selected for oral presentation. There were a total of 20 presentations, 6 of which were from international speakers, and 4 of which were selected from submitted abstracts. The additional 11 posters were viewed through the coffee breaks and lunches, during which time there was also an opportunity to visit the trade exhibition. The meeting was attended by 106 delegates (including speakers) of which 14 were non-members (including speakers) and 92 were members of the BSCR. There were 12 new memberships at the meeting.

The meeting was opened by Dr. Metin Avkiran, as chairman of the BSCR, who took the opportunity to announce that Aventis has agreed to sponsor the BSCR for the coming year. Dr. Richard Heads (King's College, London) gave the welcome speech and introduced the topic of the meeting. The meeting started with Dr. Richard Kitsis (Albert Einstein College, New York, USA) who gave the British Cardiac Society Lecture. His talk on the pathophysiological role of myocyte apoptosis in ischemic injury and heart failure was not only an excellent introduction to the mechanisms which are involved in apoptosis, but illustrated how transgenic animals can provide insights into the specific mechanisms in the heart. In addition to mechanistic studies on the role of Fas and Bid in cardiac apoptosis, his data clearly showed that apoptosis has a causal role in pathologies associated with myocardial infarction and that this can be rescued, at least in part, by caspase inhibition.

Following his lecture, Dr. Kitsis took the chair for the first session on apoptosis in cardiac pathophysiology. Dr. Lisa-Maria Voipio-Pulkki (University of Turku, Finland) provided insights into the role of apoptosis in the human heart, suggesting that increased apoptosis is particularly apparent in fibrotic areas. Dr Marcel Borgers (Jansen Research Foundation, Beeres, Belgium) spoke on the role of apoptosis in the hibernating myocardium and left us with thoughts on whether the dedifferentiation seen in hibernating myocardium may reflect a "programmed cell survival" or "programmed cell death". Also in this session, Dr. Martin Bennett (University of Cambridge, UK) reminded us that the cardiovascular system consists of more than the cardiac myocyte and showed that apoptosis is a significant factor in human vascular smooth muscle cells, and may lead to vascular remodelling and atherosclerosis. Increased apoptosis in these cells may reflect, in part, a reduction in the cytoprotective effects of insulin-like growth factor 1 (IGF1) due to downregulation of the receptors.

Dr. J. Burniston (John Moores University, Liverpool) closed the session by presenting data which indicate that *in vivo* administration of isoprenaline can induce apoptosis and necrosis in cardiac myocytes and skeletal muscle. The second session (chaired by Dr. Rudiger von Harsdorf) focused on the role of apoptosis in growth and development. Dr. Deborah Henderson (Institute of Child Health, London) provided us with an excellent insight into embryonic development of the

heart and how apoptosis is necessary for sculpting of the organ. She presented elegant data which showed that by overlaying serial images of embryo sections at different time points it can be seen that a large amount of apoptosis takes place in the endocardial cushions over time. Dr. Katrina Goodge (University of Reading) closed the session and the afternoon with her talk of phosphatases in cardiac development and hypertrophy. Following this very full programme, the scientific discussion continued into the evening at the wine and cheese reception.

The keynote lecture on day 2 was given by Dr. Peter Kang (Harvard Medical School, Boston, USA) who spoke on apoptosis in adult cardiac myocytes induced by hypoxia/reoxygenation. In this model, oxidative stress activates the mitochondrial cell death pathway requiring release of cytochrome c, formation of the apoptosome and activation of caspase 9. However, the pathway is also modulated by signalling through mitogen-activated protein kinase (MAPK) cascades and Bcl-2 family proteins. Overexpression of Bcl-2 protects against apoptosis during reoxygenation and preserves cardiac function in transgenic mice. Dr. Kang's data led into the first session of the day which was specifically on the role of mitochondria and Bcl-2 family proteins in apoptosis. The session was chaired by Prof. Andrew Halestrap (University of Bristol) and Prof. Martin Crompton (University College London), both of whom provided an excellent background to the specifics of mitochondrial function in relation to the mitochondrial permeability transition pore (MPTP) in addition to examining its role in ischaemic damage in the heart. The MPTP is a non-specific pore that opens under conditions of elevated Ca^{2+} in the mitochondrial matrix particularly under oxidative stress, conditions which occur during reperfusion following cardiac ischaemia. Prof. Halestrap presented data to show that minimising MPTP opening protects Langendorff and working hearts from reperfusion injury. Also in this session, Dr. Nicola Smart (King's College London) presented data to show that the cytoprotective effects of interleukin 6 may be due to its effects on the regulation of mitochondrial calcium handling. The Bcl-2 family were explored in more detail by Dr. Angela Clerk (Imperial College, London), who showed that oxidative stress-induced apoptosis in cardiac myocytes is associated with clear changes in Bcl-2 proteins and mitochondrial depolarization, and that phosphorylation of the pro-apoptotic Bcl-2 family member Bad through the ERK cascade and cAMP pathway may be cytoprotective.

The afternoon sessions were chaired by Dr. Richard Heads and Dr. Angela Clerk. The first session examined the signalling pathways which regulate injury and protection. Dr. Lorrie Kirshenbaum (University of Manitoba, Winnipeg, Canada) spoke on the role of NFκB in cardiac myocyte apoptosis and presented exciting data to show that some of the protective effects of Bcl-2 may be mediated through the activation of NFκB. The roles of the JAK/STAT pathway and MAPK pathways in apoptosis were explored by Dr. Anistasis Stephanou (Institute of Child Health, London) and Dr. Richard Heads who demonstrated the protective roles of the p42/p44-MAPK (ERK) and phosphoinositide-3-kinase pathways following ischaemic preconditioning and interleukin-6-induced protection in cardiomyocytes. The role of the IGF-1 pathway in promoting cytoprotection against ceramide-induced cell death was discussed by Dr. Claire Stewart (Bristol Royal Infirmary).

The final session was designed to explore the emerging theme of how cell cycle progression can influence apoptosis. A general overview of the cell cycle, particularly in relation to the terminally-differentiated state of the cardiac myocyte was given by Dr. Gavin Brooks (University of Reading). This provided a background for Dr. Rudiger von Harsdorf's talk on the role of cyclin dependent kinase (CDK) inhibitors, p21CIP1 and p27Kip1 may serve to keep CDKs in check and prevent caspase activation. Unregulated CDK activity may promote apoptosis. The final two presentations of the meeting were selected from the submitted abstracts. Dr. Jane Harper (University of Reading and King's College, London) spoke on the role of T-type calcium channels in cell cycle arrest. Finally, the role of urocortin in protecting cardiac myocytes from ischemic damage was discussed Dr. Dick Knight. With the large body of data presented at the meeting, it was probably inevitable that the meeting would be a little behind schedule, but despite a 6 pm finish, many delegates stayed on to the end.

In addition to financial support from the British Cardiac Society and the BSCR, the meeting was supported by grants from the British Heart Foundation and the Wellcome Trust. In addition, several companies (keen to market their apoptosis products) formed a trade exhibition which was well-attended throughout the meeting. We are also grateful to these companies (AD Instruments, Amersham International Biotech Ltd., BioRad, CN Biosciences, New England Biolabs, Promega UK, TCS Biologicals) for their financial support

of the meeting. As organisers of the meeting, we very much appreciated the opportunities offered by this meeting to bring together a range of experts to discuss the many aspects of apoptosis in cardiovascular disease. We found the meeting highly stimulating and very enjoyable and hope that the same was felt by everyone who attended. We are grateful to the BSCR for offering this opportunity and to the speakers, particularly those from abroad, who took the time to attend and who were willing to share what was often novel unpublished data.

Dr Angela Clerk is a lecturer at the Division of Biomedical Sciences (Molecular Pathology), Imperial College School of Medicine, London.

Dr Richard Heads is a lecturer at the Department of Cardiology, Guy's, King's and St Thomas's School of Medicine, Dentistry and Biomedical Sciences, London.

Spring 2001 BSCR meeting: abstracts

BCL-2 FAMILY PROTEINS IN THE HEART. Angela Clerk, Imperial College School of Medicine, London. a.clerk@ic.ac.uk

The Bcl-2 family proteins are key regulators of apoptosis in many cell types, and are comprised of pro-apoptotic proteins (e.g. Bcl-2, Bcl-xL) and anti-apoptotic proteins (e.g. Bad, Bax). Our recent studies have focused on the expression of Bcl-2 family proteins in the heart. Bcl-2, Bcl-xL, Bad and Bax were all detected in extracts from neonatal rat hearts using Western blot analysis. Although all proteins were still detected in adult heart, levels of anti-apoptotic Bcl-2 and Bcl-xL remained high relative to total protein, but pro-apoptotic Bad and Bax were downregulated.Consistent with studies in other cells, Bcl-2 and Bcl-xL were located predominantly in the mitochondrial fraction of neonatal cardiac myocytes, whereas Bad and Bax were detected in both the cytosol and the mitochondria. The mechanisms by which Bcl-2 family proteins exert their effects on the cell death pathway are not known, but the Bcl-2 proteins appear to act as dimers. One current theory suggests that the formation of Bax homodimers drives apoptotis and Bcl-2/Bcl-xL prevent this by forming heterodimers with Bax thus preventing Bax homodimerization. In its dephosphorylated state, Bad dimerizes with Bcl-2 preventing interaction with Bax, thus inducing apoptosis. H_2O_2, an example of oxidative stress, induces cardiac myocyte apoptosis. In myocytes exposed to H2O2, there was rapid (<5 min) translocation of Bad to the mitochondria which preceded release of cytochrome c (5 - 15 min), suggesting that Bad translocation may be a trigger event in the apoptotic response. Following release of cytochrome c, Bcl-2, Bcl-xL, Bad and Bax were all downregulated. Although Bad promotes apoptosis, in its phosphorylated state it associates with 14-3-3 proteins and is retained in the cytoplasm away from the mitochondria. Thus, the protein kinase pathways which lead to phosphorylation of Bad (on Ser-112, Ser-136 and Ser-155) may be considered as cytoprotective. Phenylephrine (PE), endothelin-1 (ET-1) and PMA all promote cardiac myocyte hypertrophy in the short term, but over a longer period of time, PE also appears to protect myocytes from cell death, whereas cell death is increased in cells treated with ET-1 or PMA. We therefore examined the effects of PE (100 μM) on the phosphorylation of Bad in neonatal cardiac myocytes by immunoblotting with antibodies selective for each of the phosphorylation sites. We have as yet been unsuccessful in detecting phospho-Bad(Ser-136), but PE induced phosphorylation of both Ser-112 and Ser-155. This phosphorylation was rapid (detected within 5 min) and maximal at approximately 1h. The phosphorylation of Bad(Ser-112) and Bad(Ser-155) induced by PE was sustained over 48h, whereas the phosphorylation induced by ET-1 or PMA declined rapidly. Using receptor antagonists, we found that PE stimulation of Bad(Ser-155) was mediated the β-adrenoceptor, whereas stimulation of Bad(Ser-112) required both the α-and β-adrenoceptors. Consistent with this, protein kinase inhibitors indicated that phosphorylation of Bad(Ser-155) required cAMP-dependent protein kinase (PKA), and phosphorylation of Bad(Ser-112) was dependent on both PKA and the extracellular signal-regulated kinase (ERK) cascade. Purification of kinases by Mono Q and Mono S FPLC showed that the kinases which phosphorylated Bad(Ser-155) and Bad(Ser-112) were PKA and the ERK-activated p90-ribosomal S6 kinases (RSKs), respectively. It therefore appears that although p90RSKs phosphorylate Bad(Ser-112), efficient co-phosphorylation of Bad(Ser-155) may be required to stabilize the Ser-112 phosphorylation. CPT-cAMP also promoted phosphorylation of Bad(Ser-155) and Bad(Ser-112) and both phosphorylationw were inhibited by the PKA inhibitor H89, indicating the primacy of the Ser-155 phosphorylation over the Ser-112 phosphorylation. In conclusion, co-activation of two independent intracellular signalling pathways (ERK/p90RSKs and cAMP/PKA) by PE may provide a concerted cytoprotective effect in cardiac myocytes by increasing phosphorylation of Bad on Ser-112 and Ser-155.

APOPTOSIS AND CELL CYCLE IN THE HEART. Rudiger v. Harsdorf, Franz-Volhard-Klinik, Universitatsklinikum Charité, Humboldt-Universitat, Berlin, Germany

Progressive impairment of left ventricular function is a characteristic process in the failing heart. There appears sufficient evidence that heart failure of both ischemic and nonischemic origin results, at least in part, from ongoing apoptotic loss of cardiomyocytes. Since the progression into

late G1 phase favors the induction of apoptosis, a close functional relationship between apoptosis and cell cycle can be assumed. Hypophosphorylated, active retinoblastoma protein (pRb) blocks the cell cycle in G1 state by inhibiting E2F-mediated G1/S phase transition. While arresting the cell cycle, pRb elicits a protective role in response to genotoxic stress. Cyclin-dependent kinases (cdks) are among the important regulators of cell division. They form quarternary protein complexes consisting of a cdk, a cyclin, proliferating cell nuclear antigen (PCNA) and a cdk inhibitor. Cdk inhibitors comprise two functionally distinct groups: proteins of the INK4 and the CIP/KIP family. INK4 family members like p16INK4 bind specifically to cdk4 and cdk6. In contrast, CIP/KIP proteins, including p21CIP1, p27KIP1, p57KIP2, bind to a variety of cyclin/cdk complexes, e.g. cyclin E/cdk2 and cyclin A/cdk2. In cardiomyocytes, p21CIP1and p27KIP1 are key factors that maintain cell cycle arrest. Both are supposed to prevent cardiac pRb from inactivating hyperphosphorylation and the myocyte from subsequent E2F-mediated entry into S phase. Growing evidence suggests that specific cdk2 activation is a crucial event in distinct pathways of apoptosis. Recently, our group could show that adenoviral delivery of p21CIP1 rescued neonatal cardiomyocytes from E2F-1-induced apoptosis and furthermore blocked E2F-mediated S phase entry. To test the potentially anti-apoptotic effects of p21CIP1 and p27KIP1 in primary rat cardiomyocytes, we applied two established apoptotic models, DNA damage by Actinomycin D (ActD) and hypoxia by Desferrioxamine (DFX). We identified both DNA damage and hypoxia leading to apoptosis by downregulation of p21CIP1 and p27KIP1, sustained activation of cdk2, and increasing caspase-3 activity. In p21CIP1 or p27KIP1-overexpressing cardiomyocytes, cdk2 phospho-transferase activity and proteolytic activity of caspase-3 were completely inhibited. Importantly, overexpression of p21CIP1 and P27KIP1 efficiently rescued cardiomyocytes from programmed cell death induced by DNA damage or hypoxia. In conclusion, these data provide evidence for a pivotal role of cell cycle factors in apoptotic signaling in cardiomyocytes outlining the potential of cell cycle inhibitors in controling cardiomyocyte apoptosis.

ROLE OF NFkB IN APOPTOSIS OF VENTRICULAR MYOCYTES. Lorrie A. Kirshenbaum, University of Manitoba, Faculty of Medicine, Winnipeg Manitoba Canada R2H2A6

NFkB is a ubiquitously expressed transcription factor whose function is regulated by the cytoplasmic inhibitor protein IkBα. Biological agents such as TNFα that activate NFkB result in the rapid degradation of IkBα. A role for NFkB in the regulation of apoptosis has recently been suggested. Staining of ventricular myocytes with vital dyes revealed that TNFα was not cytotoxic to myocytes and alone did not provoke apoptosis. Adenoviral mediated gene transfer of Bcl-2 prevents apoptosis of neonatal ventricular myocytes induced by TNFα plus the protein synthesis inhibitor cycloheximide (CHX). Stimulation of myocytes with TNFα resulted in a 2.1 fold increase ($p<0.001$) in NFkB dependent gene transcription and nuclear DNA binding. Similarly, a 1.9 fold increase ($p<0.0002$) in NFkB-dependent gene transcription was observed in myocytes expressing Bcl-2. Nuclear DNA binding activity of NFkB was significantly increased in myocytes expressing Bcl-2, with a concomitant reduction in IkBα protein level. The Bcl-2 -mediated loss of IkBα could be prevented by the proteasome inhibitor lactacystin, consistent with the notion that the targeted degradation of IkBα consequent to over expression of Bcl-2 utilizes the ubiquitin-proteasome pathway. However, transfection of cells with an N-terminal deletion mutant DBH4 (amino acids 10-30) or point substitution mutants of the Bcl-2 BH4 domain, had no effect on IkBα activity ($p=0.31$). Consistent with these findings was the observation that the Bcl-2 BH4 domain mutants were equivalently defective for directing NFkB dependent DNA binding compared to wild type Bcl-2. Furthermore, adenovirus mediated delivery of an IkBα mutant to prevent NFkB activation, impaired Bcl-2's ability to suppress apoptosis provoked by TNFα-CHX in ventricular myocytes. The data provide the first direct evidence for the regulation of IkBα by Bcl-2 through a mechanism that requires the conserved N-terminal BH4 domain and further suggests the importance of an intact NFkB signaling pathway for suppression of apoptosis in ventricular myocytes.

THE ROLE OF APOPTOSIS IN VASCULAR REMODELLING AND ATHEROSCLEROSIS. Professor Martin R Bennett, University of Cambridge, UK

Vascular smooth muscle cell (VSMC) apoptosis occurs at increased rates in human atherosclerotic plaques compared with normal vessels and in unstable vs. stable lesions, suggesting that apoptosis may promote plaque instability. VSMC apoptosis also occurs after arterial injury, and profound changes in vessel calibre in remodelling are achieved by coodinated VSMC apoptosis and cell proliferation. These studies have identified a number of gene products that either protect or induce VSMC apoptosis in animal models. However, the regulation of apoptosis in human VSMCs differs from animal cells, and increasing evidence suggests that human VSMCs from diseased arteries show different regulation to those from normal vessels. We have examined regulation of human VSMC apoptosis from both atherosclerotic and normal arteries. Human primary plaque VSMCs show increased apoptosis in culture compared with VSMCs from normal vessels. This increased apoptosis is due in part to increased sensitivity to death by failure of endogenous protection through Insulin-like growth factor-1 (IGF-1). Lack of IGF-1 signalling is due to reduced IGF-1 receptor expression seen in vivo and in vitro. In addition to increased endogenous sensitivity to apoptosis, plaque VSMCs undergo apoptosis induced by human monocyte/macrophages, via pathways that include Nitric Oxide and death receptors. Macrophage priming of VSMCs is required for VSMCs to respond, and monocyte maturation into macrophages is also necessary for effective killing. Thus, the local plaque microenvironment also promotes VSMC apoptosis. Recent studies have also identified consequences of VSMC apoptosis in atherosclerosis including a local and systemic pro-coagulant state, inflammation via monocyte recruitment, and calcification. We have found that human VSMCs undergoing apoptosis and apoptotic bodies accumulate calcium and phosphate, acting as a focus for calcification. Calcification and calcium / phosphate uptake can be inhibited by blocking apoptosis. Thus, VSMC apoptosis in the vessel wall may promote secondary calcification. In summary, human VSMC from atherosclerotic plaques show an increased sensitivity to apoptosis, and are also exposed to

a plaque microenvironment that induces apoptosis. In contrast to the prevailing idea that apoptosis is silent, we demonstrate that apoptosis in atherosclerosis may have profound clinical consequences.

MITOCHONDRIA AND CARDIOMYOCYTE CELL DEATH. Andrew Halestrap, Samantha Clarke, Gavin McStay, Sabzali Javadov and Kelvin Lim, Department of Biochemistry and The Bristol Heart Institute, University of Bristol, Bristol, BS8 1TD, U.K.

Mitochondria are now known to play a critical role in initiating both apoptotic and necrotic cell death. A major player in this process is the Mitochondrial Permeability Transition Pore (MPTP), a non-specific pore that opens in the inner mitochondrial membrane under conditions of elevated matrix [Ca^{2+}], especially when this is accompanied by oxidative stress and depleted adenine nucleotides. These are exactly the conditions that occur during reperfusion of the heart following a period of ischaemia and lead to ìreperfusion injury. Data from this and other laboratories have led to the proposal that the MPTP is formed through a calcium-mediated conformational change of the adenine nucleotide translocase (ANT), facilitated by bound cyclophilin-D (CyP-D). Some workers believe that outer membrane components such as porin and Bcl-2 may be associated with the MPTP. CyP-D binds tightly to the ANT following modification of critical thiol groups by oxidative stress which also cause adenine nucleotides to dissociate from their binding sites on the ANT. We have confirmed this model by showing that the MPTP can be reconstituted into proteoliposomes using purified ANT and CyP-D. The extent of MPTP opening in the Langendorff perfused heart has been determined using mitochondrial entrapment of [3H]-2-deoxyglucose. The MPTP does not open in the ischaemic heart, but does do so during subsequent reperfusion. Later in reperfusion it can close again and the extent of closure correlates with functional recovery of the heart. The implications of this for necrotic versus apoptotic cell death in reperfusion injury will be discussed. A range of protocols that minimise opening of the MPTP are shown to protect both Langendorff and working hearts from reperfusion injury; these include addition of cyclosporin A, propofol and pyruvate to the perfusion medium prior to ischaemia. Recently we have successfully extended these studies into an open-chested pig model that closely mimics normal surgical procedures. The role of mitochondria in ischaemic preconditioning is also under investigation, and data will be presented that implicate the mitochondria in this response, but not through the action of mitochondrial KATP channels.

THE ROLE OF PROTEIN KINASES IN CELL DEATH AND CYTOPROTECTION IN CARDIOMYOCYTES. Richard Heads, The Rayne Institute, Kings College London, St Thomas' Hospital, London, UK.

We have compared the activation patterns of MAPK and SAPK pathways in different models of simulated ischaemia (SI) and following cytokine (IL-6) or adenosine A3 receptor agonist stimulation in primary neonatal rat ventricular myocytes (NCMs). SI consisted of modified Krebs-Henseleit buffer; 20mM Na lactate; 16mM KCl, 10mM 2-deoxyglucose, pH 6.8 further supplemented with either the oxygen scavenger Na dithionite [1mM: severe metabolic SI (smSI)] or hypoxia [hypoxic SI (hSI)]. Preconditioning (PC) of NCMs with 1 hour of smSI lead to the transient activation of p42/p44-MAPK and p38-MAPK as determined by Western blotting with dual phospho-specific antibodies. Simulated 'reperfusion'in normal maintenance medium following PC again caused transient activation of p38-MAPK which was then suppressed below baseline within 40-60 minutes. This p38-MAPK suppression by PC lasted 8 hours before returning to baseline. However, both p42/p44-MAPK and PKB showed sustained activation during 'reperfusion' lasting up to 4 hours. PC of NCMs with smSI lead to protection against a lethal SI (LSI) insult (smSI for 3 hours) 24 hours later. This protection was blocked by the MEK1 inhibitor PD98059 (50μM) or the Src tyrosine kinase inhibitor PP2 (10μM) when given during PC and early 'reperfusion', but not PC alone, indicating roles for 'reperfusion'-associated activation of SrcTK and p42/p44-MAPK in protection. Furthermore, transfection of NCMs with a constitutively active Src mutant lead to constitutive p42/p44-MAPK activation and protection. However, neither the p38-MAPK inhibitor SB203580 (10μM) nor the PI3K/PKB inhibitor wortmanin (100 nM) blocked protection. Interestingly, SB203580 alone given prior to LSI was able to protect against injury, suggesting that p38-MAPK activation may exacerbate injury during ischaemia. This was further supported by the fact that PC suppressed p38-MAPK activation and that transfection of NCMs with a dominant negative p38-MAPKa mutant also protected against LSI. Pretreatment of NCMs with interleukin-6 (IL-6) or the adenosine A_3-receptor agonist N^6-benzyl-NECA caused sustained activation of p42/p44-MAPK, p46/p54-JNK (SAPK1), p38-MAPK and PKB and lead to delayed protection against either 3 hours of smLSI or 6 hours of hypoxic LSI given 24 hours later. In the case of hypoxic LSI protection was abolished by both PD98059 and wortmanin, indicating a role for both p42/p44-MAPK and PI3K/PKB in protection against hypoxic LSI. We tested the hypothesis that PI3K/PKB involvement was due to the presence of apoptosis in the hypoxic but not the smSI model, due to a slower rate of oxygen and thus ATP depletion in hLSI as compared to smLSI. In the smLSI model, neither caspase activation, nor cytochrome C release were observed. In the hLSI model neither caspase activation nor changes in FAS or FLIP expression were observed. Furthermore, neither model showed any morphological evidence of apoptosis as determined by electron microscopy. The large amounts of creatine phosphokinase (CPK) release observed following either metabolic or hypoxic LSI are consistent with the mode of cell death being predominantly necrosis in both models. However, caspase activation as evidenced by pro-caspase-3 and PKCδ cleavage was observed after 24 hours when the NCMs were treated under conditions of mild SI with buffer in the absence of dithionite or hypoxia, suggesting that apoptosis only occurs in NCMs when ATP is depleted very slowly.

ROLE OF THE CELL CYCLE IN CARDIAC MYOCYTE GROWTH AND HYPERTROPHY. Gavin Brooks, Cardiovascular Research Group, AMS, University of Reading, PO Box 228, Whiteknights, Reading, Berkshire. RG6 6AJ

The adult myocardium is unable to regenerate following injury e.g. as a result of myocardial infarction (MI) since the ability of the cardiac myocyte to divide is lost at, or shortly after, birth. Although they cannot divide, adult cardiac myocytes

undergo cell enlargement (hypertrophy) in response to stress or injury in an effort to maintain normal cardiac pump function (compensatory hypertrophy). However, if the heart suffers severe or prolonged stress, the surviving myocytes are unable to grow sufficiently and this leads to scar formation, compromised heart function and heart failure (decompensatory hypertrophy). In an effort to understand more clearly the mechanisms responsible for the loss in proliferative capacity of the post-natal myocyte, we have investigated how the cell cycle machinery is regulated in these cells, both during normal development and during the development of hypertrophy. Our results previously have shown that the mRNA and protein expressions and associated kinase activities of certain positive cell cycle regulators (cyclins and cyclin-dependent kinases [CDKs]) are down-regulated during development and that this is associated with a concomitant increase in the expressions and inhibitory activities of specific CDK inhibitors leading, primarily, to a G0/G1 arrest in adult myocytes. Furthermore, during the development of pressure overload-induced left ventricular hypertrophy (LVH) in rats, there is a partial re-activation of the cell cycle machinery in these cells. Thus, a significant, but transient (~2 weeks), decrease in functional expression of the CDK inhibitors, p21CIP1 and p27KIP1, occurs in myocytes following aortic constrction and this is accompanied by an increase in G1/S phase cyclin/CDK complex formation and activation leading to an accumulation of cells in the G2/M phase of the cycle. Current work is focussing on the E2F transcription factor family, that functions at the G1/S transition, in an effort: (a) to understand the role that these molecules play in hypertrophic growth; (b) to inhibit the progression of myocytes from compensatory hypertrophy to decompensated hypertrophy; and, (c) to attempt to reverse established decompensated hypertrophy to improve cardiac function and prognosis for heart failure patients.

CHARACTERIZATION OF APOPTOSIS DURING HYPOXIA AND REOXYGENATION IN ADULT CARDIAC MYOCYTES. Peter M. Kang, MD and Seigo Izumo, MD, Harvard Medical School, Boston, MA, USA.

After the initial concept of apoptosis was introduced in 1970's, there has been an explosion of knowledge regarding the pathophysiology and the regulation of apoptosis in various diseases. In heart, it is now clear from the data obtained from numerous cardiac models that apoptosis plays an important role in the development of variety of cardiovascular diseases. In adult cardiac myocyte culture, cardiac apoptosis can be induced by various oxidative stresses. In this model system, the main apoptotic pathway utilizes mitochondria, where apoptotic stimuli trigger the release of cytochrome c from the mitochondria. Released cytochrome c, in the presence of dATP and apaf-1, activates caspase-9 which than activates downstream caspases to execute the final morphological and biochemical alterations. This pathway is regulated by MAP kinases (e.g. JNK), Bcl-2 family of proteins (e.g. Bcl-2 and Bcl-xL) and caspase inhibitors (e.g. XIAP). In fact, in hypertrophic adult cardiac myocyte culture, there is modulations of these regulatory factors that predispose these cells to be more sensitive to apoptotic stimulation compare to the normal (non-hypertrophic) adult cardiac myocytes. In contrast, cardiac specific overexpression of Bcl-2 results in phenotypically normal mice at baseline. However, Bcl-2 transgenic mice hearts, in compare to the controls, demonstrate decrease rate of cardiac apoptosis as well as favorable acute functional parameters and myocardial energetics during recovery in response to ischemia/reperfusion. In summary, mitochondria-dependent apoptosis pathway is principally activated during oxidative stress in adult cardiac myocytes. This pathway involves cytochrome c release from the mitochondria, JNK activation and caspases activations. The protective response (or conversely, deleterious response) to oxidative stress can be achieved by modulation of these regulatory factors in adult cardiac myocytes.

PROGRAMMED CELL SURVIVAL VS PROGRAMMED CELL DEATH IN HIBERNATING MYOCARDIUM. Marcel Borgers, Dept.of Molecular Cell Biology, Cardiovascular Research Institute Maastricht, Maastricht University, The Netherlands.

Evidence that apoptosis contributes to cardiomyocyte loss is substantial for some cardiac pathologies such as cardiac infarction and a variety of cardiomyopathies, for others such as chronic hibernating and stunned myocardium its involvement is still debated. Recent studies have indicated that the heart remodels its structure in a rather stereotypic way when subjected to unfavourable conditions such as ischaemia and pressure- and/or volume overload. This stereotypic response is characterized by subcellular adaptations in the cardiomyocytes whereby the cells switch from an adult (functional) to a foetal (survival) phenotype, a process called dedifferentiation. Hallmarks of dedifferentiation are reduction of contractile filaments, abundance of glycogen, dispersion of nuclear heterochromatin, mitochondrial size and shape changes, and loss of T-tubules and sarcoplasmic reticulum. These changes are accompanied by important alterarions in the expression and distrubution of structural proteins in these organelles. Today, there is only circumstantial evidence that cardiomyocyte dedifferentiation is an adaptive and reversible phenomenon instead of a degenerative event leading to apoptotic cell death.Indeed, some research groups consider the switch to a foetal phenotype a rescue reaction and therefore coined the name “programmed cell survival”, whereas others interprete these as an event on the “programmed cell death” pathway. It is obvious that resolving this controversial issue is of direct clinical importance as far as prognosis and therapy is concerned.

ROLE OF STAT1/3 SIGNALLING IN CARDIAC CELLS IN ISCHAEMIC INJURY. Anastasis Stephanou, Tiziano Scarabelli, B. Brar, Richard Knight & David Latchman, Medical Molecular Biology Unit, Institute of Child Health, London, UK.

Loss of cardiac myocytes by apoptosis is an important process in the pathogensis of heart disease. Recent studies have indicated that cardiac cells exposed to hypoxia/ischaemic (I) insult followed by reoxygenation/reperfusion (R)undergo apoptotic cell death. Signal transducers and activators of transcription (STATs) are transcription factors that are activated by Janus kinases (JAKs) and mitogen activated protein kinases (p42/p44, p38 and JNK) by cytokine signalling. Six STATs have been cloned (STAT1-6), some which exist in different isomeric forms, and all share a high degree of

conservation of their structural domains. STATs have been implicated in both pro- and anti-apoptotic cell death. Cardiomyocytes exposed to simulated I/R in vitro and also in the intact perfused heart ex-vivo show increased expression and phosphorylation (Tyr701 and Ser727) of STAT1. Moreover, STAT1 overexpression in cardiomyocyte were more susceptible to I/R-induced cell death. In contrast, STAT3 overexpression in cardiomyocytes reduced STAT1 mediated apoptotic cell death following exposure to simulated I/R. We have began to identify taget genes for STAT1 and STAT3 including caspase-1, Fas, FasL, Bax, Bcl-2 and Bcl-x. These results have suggested that STAT1 and STAT3 have opposing roles in modulating apoptosis in cardiomyocytes by regulating both pro- and anti-apoptotic genes.

ISOPRENALINE-INDUCED APOPTOSIS AND NECROSIS IN MYOCYTES OF CARDIAC AND SKELETAL MUSCLE.
J.G. Burniston, Y. Ng, W.A Clark, H. Cox, L-B Tan*, J. Colyer & D.F. Goldspink, Research Institute of Sports & Exercise Sciences, Liverpool John Moores University and * Cardiovascular Research and **Department of Biochemistry & Molecular Biology, University of Leeds.**

Higher than normal levels of catecholamines are found in heart failure patients and may be involved in the progressive deterioration of the heart through cumulative damage. In our studies we have observed myocyte death, both as a consequence of necrosis (identified by an anti-myosin antibody *in vivo*) and apoptosis (using an anti-active caspase 3 antibody on tissue sections), in cardiac and skeletal muscles of the rat after administrating a single injection of 5mg of isoprenaline kg-1. Catecholamine-induced damage in skeletal (i.e. soleus) muscle has not previously been reported, even though this muscle was found to be more sensitive than the myocardium to the isoprenaline. Peak apoptosis was observed at 4 hours, whereas peak necrosis occurred at 12 and 18 hours respectively in the soleus and heart. Prior treatment with bisoprolol (a selective β1-AR antagonist), but not ICI-118551 (selective β2-AR antagonist), prevented the isoprenaline-induced myocyte death in the heart, but not the soleus. The reverse was true for the soleus, demonstrating the involvement of different adrenoceptor subtypes in the 2 striated muscles.

EXPRESSION OF CDK ACTIVATING KINASE IN CARDIOMYOCYTE DEVELOPMENT AND HYPERTROPHY.
M. Movassagh, K. A. Goodge and G. Brooks. AMS, University of Reading, Reading, Berkshire RG6 6AJ, UK

Soon after birth, mammalian cardiomyocytes lose the ability to divide and are restricted to hypertrophic growth. Previous studies from our laboratory have shown that the expressions and activities of specific positive regulators of the cell cycle machinery, *viz*. cyclin:cyclin-dependent kinase (CDK) complexes, are correlated with the growth potential of myocytes since levels are down-regulated during normal development, but are partially re-activated during hypertrophic growth. The activities of cyclin:CDK complexes are themselves controlled by phosphorylation events mediated by CDK activating kinase (CAK). We hypothesise that CAK might act as a ìmaster switchî to activate the cell cycle machinery in cardiomyocytes. In this study, we have investigated the expressions of CAK constituents, CDK7, cyclin H and MAT1, in rat myocytes both during normal development and during the development of hypertrophy. During normal development from foetal (E18) to neonate (P2) to adult (Ad), the expressions of all CAK constituents were down-regulated significantly, both at mRNA (MAT1, 13%; cyclin H, 61%; CDK7, 19%, in Ad vs. E18 myocytes) and protein (= 1% of MAT1, cyclin H and CDK7 protein, in Ad vs. E18 myocytes) levels. Interestingly, immunoblot analysis with CDK7 antibody identified two immunoreactive bands at ~34 kDa and 37 kDa. The lower band likely represents the active, hyperphosphorylated form of CDK7 and this is consistent with its high level of expression in dividing foetal myocytes. Hypertrophy was induced in cultured P2 myocytes with foetal calf serum (FCS, 20%) or phenylephrine (PE, 100 mM) for 24 hrs leading to an increase in atrial natiuretic factor (ANF; 1.5-2 fold) and brain natriuretic peptide (BNP; ~3.5 fold) mRNA expressions. FCS treatment led to significant increases in MAT1 (171%), cyclin H (163%) and inactive hypophosphorylated (37 kDa) CDK7 (209%) protein expressions compared to control myocytes. In contrast, 100 mM PE led to a significant up-regulation in MAT1 (173%) and the active, hyperphosphorylated form of CDK7 (>200 %) protein expressions, but a down-regulation in cyclin H protein (53%) compared to control myocytes. Our results suggest that loss of hyperplastic growth potential in adult myocytes might be due to a significant down-regulation of CAK constituents. In addition, CAK might play a role in re-activating the cell cycle machinery during hypertrophic growth.

BLOCKADE OF T-TYPE CALCIUM CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS CAUSES G1/S CELL CYCLE ARREST.
Jane V Harper[1], Linda M McLatchie[2], Edward Perez-Reyes[3], Leanne Cribbs[4], Gavin Brooks[1] and Michael J Shattock[2]. [1]AMS, The University of Reading, Reading, RG6 6AJ, [2]The Rayne Institute, St Thomas' Hospital, London, SE1 7EH, [3]University of Virginia, USA, [4]Loyola University, USA.

Aberrant vascular smooth muscle cell (VSMC) proliferation is a critical component of restenosis and in-stent stenosis. Previous reports have suggested a correlation between T-type Ca channel expression and cardiovascular cell growth. In support of this hypothesis, we previously have demonstrated that transient over-expression of a T-type Ca channel (a1H) increases proliferation in rat A10 VSMCs. In the present study, we show that inhibition of the T-type Ca channel current causes cell cycle arrest at the G1 phase of the cell cycle in A10 VSMCs. Cells were transfected with the a1H and stable clones selected and maintained in G418 (1mg/ml). Cells stably over-expressing a1H had a mean T-type current 21 times greater than vector controls and displayed an increased proliferative capacity of 2.6-fold at 72 hours (cell nos. increased from 1x104 to 3.3x104 and 8.7x104 in controls and a1H clones, resp.). In order to establish where in the cell cycle T-type currents are maximal, cells were treated with either the selective T-channel blocker, mibefradil (8.75mM), that also blocked VSMC proliferation by 50%, or the L-type Ca channel blocker, verapamil (8.75_M), in the presence of nocodazole (40ng/ml, 24 hrs), to favour G2/M blockade. Mibefradil led to an accumulation of cells in G0/G1 (76%), with 5% in S and 19% in G2/M. In contrast, verapamil failed to induce a G1 arrest and

was not significantly different from nocodazole treatment alone (G0/G1, 19%; S, 10%; G2/M, 71%). These results demonstrate that the effect of the T-type calcium channel on VSMC proliferation occurs in a cell cycle-dependent manner. Targeting T-type Ca channel activity might be a useful therapeutic approach for the treatment of vascular proliferative diseases such as in-stent stenosis.

ASCORBATE PROMOTES OXIDISED LOW DENSITY LIPOPROTEIN-INDUCED APOPTOSIS OF MACROPHAGES. L.K.Harris, G.E.Mann* and D.S.Leake, School of Animal and Microbial Sciences, The University of Reading, Reading, UK. *Centre for Cardiovascular Biology and Medicine, GKT School of Biomedical Sciences, King's College London, UK

Macrophage apoptosis may be of importance in the progression of atherosclerotic lesions. Oxidised low density lipoprotein (LDL) is implicated in the pathogenesis of atherosclerosis. We have shown using LDL oxidised to defined extents that LDL containing the maximum level of lipid hydroperoxides (which we term moderately-oxidised LDL), (Siow *et al.*, 1999), can induce apoptosis in J774 mouse macrophages, whereas native LDL does not. Treatment of cells with moderately-oxidised LDL (200 µg protein/ml) induced externalisation of phosphatidylserine to the cell surface, detected by annexin V labelling and confocal microscopy. Mitochondrial dehydrogenase activity was decreased by up to 30% after 24 hours and substantial DNA fragmentation was evident after 48 hours of treatment. Pre-treatment, co-treatment or both pre- and co-treatment of J774 macrophages with ascorbate (100µM) increased levels of apoptosis induced by moderately-oxidised LDL. Macrophages externalised more phosphatidylserine and quantification using flow cytometry demonstrated the increase to be significant (co-treatment $p<0.05$, pre- and co-treatment $p<0.05$). Levels of fragmented DNA within the cells were also increased. Our findings suggest that ascorbate can augment the cytotoxicity of moderately-oxidised LDL and may be pro-apoptotic toward macrophages within atherosclerotic lesions.

Siow, R.C.M., Richards, J.P., Pedley, K.C., Leake, D.S., Mann, G.E. (1999) Arterioscler. Thromb. Vasc. Biol. ***19****, 2387 - 2394.*

DOWN-REGULATED EXPRESSION OF THE ANTI-APOPTOTIC GENE BAG-1 IN HEART OF COXSACKIE VIRUS B3 EXPERIMENTALLY INFECTED MOUSE. T. Peng, T. Sadusky, Y. Li, G.R. Coulton, *P.J. Richardson, H. Zhang & L.C. Archard, Division of Biomedical Sciences, Imperial College School of Medicine, London SW7 2AZ & *Cardiac Department, King's College Hospital, London SE5 9RS.

We used hybridisation to cDNA arrays to identify changes in cardiac gene expression after experimental CVB3 infection of MF-1 mouse and found that expression of the anti-apoptotic gene *Bag-1* was significantly reduced. Semi-quantitative RT-PCR confirmed that *Bag-1* mRNA expression was reduced by up to 30% in mouse heart at 7 days post infection. Cell fractionation and western blot showed that levels of the cytoplasmic p32 isoform of *Bag-1* were selectively reduced in mouse heart at 4 and 7 days after intraperitoneal inoculation with CVB3: this was confirmed in by immunocytochemistry. TUNEL-positive nuclei in cardiomyocytes were increased 3-4 fold compared with controls, suggesting that CVB3 infection induces differential expression of *Bag-1*and consequent apoptosis in mouse heart. This may be important in the pathogenesis of CVB induced heart muscle disease.

INHIBITION OF VASCULAR SMOOTH MUSCLE CELL PROLIFERATION BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS OCCURS VIA G1-DEPENDENT AND -INDEPENDENT CELL CYCLE ARREST. Y.Q. Wang, J.V. Harper, K.A. Goodge, M.J.C. Crabbe, M.J. Shattock*, G. Brooks. School of Animal and Microbial Sciences, University of Reading, Reading, Berks. RG6 6AJ, *Rayne Institute, St Thomas' Hospital, London, SE1 7EH, UK

Abnormal vascular smooth muscle cell (VSMC) proliferation is known to play an important role in the pathogenesis of both atherosclerosis and restenosis. Recent studies suggest that salicylates, in addition to inhibiting cyclooxygenase activity, exert an antiproliferative effect on VSMC growth both *in vitro* and *in vivo*. However, whether all non-steroidal anti-inflammatory drugs (NSAIDs) exert similar antiproliferative effects on VSMCs, and do so via a common mechanism of action, remains to be shown. In the present study, we demonstrate that the NSAIDs, aspirin, ibuprofen and sulindac induce a dose-dependent inhibition of proliferation in rat A10 VSMCs (IC50=1666µM, 937µM and 520µM, resp.). These drugs did not show significant cytotoxic effects as determined by LDH release assay, even at the highest concentrations tested (aspirin, 5000µM; ibuprofen, 2500µM; and sulindac, 1000µM). Flow cytometric analyses showed that a 48 hrs exposure of A10 VSMCs to ibuprofen (1000µM) and sulindac (750µM) led to a significant G0/G1 arrest (from 68.7±2.0% of cells in G1 to 76.6±2.2% and 75.8±2.2%, resp., $p<0.05$). In contrast, aspirin (2500µM) failed to induce a significant G1 arrest (68.1±5.2%). Clearer evidence of a G1 block was obtained by treatment of cells with the mitotic inhibitor, nocodazole (40ng/ml), for the final 24hr of the experiment. Under these conditions, aspirin still failed to induce a G1 arrest (from 25.9±10.9% of cells in G1 to 19.6±2.3%) whereas ibuprofen and sulindac led to a significant accumulation of cells in G1 (51.8±17.2% and 54.1±10.6%, resp., $p<0.05$). These results indicate that ibuprofen and sulindac inhibit VSMC proliferation by arresting the cell cycle in the G1 phase whereas the effect of aspirin appears to be independent of this phase of the cell cycle. Irrespective of mechanism, our results suggest that NSAIDs might be of benefit in the treatment of vascular proliferative disorders.

DIFFERENTIAL REGULATION OF E2F FAMILY MEMBERS DURING NORMAL CARDIAC DEVELOPMENT AND MYOCYTE HYPERTROPHY. D. J. Vara, K.A Goodge, G. Brooks. AMS, University of Reading, Reading, Berkshire RG6 6AJ, UK

E2F comprises a family of 6 transcription factors that are instrumental in controlling the G1 to S transition in mammalian cells. It previously has been reported that E2F expression is downregulated during cardiac development and this is coincident with the loss in proliferative capacity of the cardiomyocyte during this period. However, it remains unclear whether all E2F family members are expressed in developing cardiac tissue and whether they all follow this pattern of downregulation. In addition, a role for E2F in the development of cardiac hypertrophy still remains to be determined. In the

present study, we have investigated the mRNA and protein expressions of E2F 1, 2, 3, 4 and 5, DP-1, DP-2, cyclin E and ANF during normal rat cardiac development and during the development of hypertrophy in cultured neonatal myocytes. Our results show that during development, protein levels of E2F 1, 2, 3 and 4, DP-1 and DP-2 are downregulated from E18 to adult (2.5, 3, 1.5, 3.5, 3 and 2.5-fold, respectively). In contrast E2F 5 is upregulated 9.33-fold during this period. Induction of hypertrophy in cultured 3d-old neonatal rat myocytes (20% FCS, 24 hrs) caused a significant up-regulation of E2F 1, 2, 3, 4 & DP-1 (2.5, 0.5, 3.0, 2-fold respectively), and downregulation in E2F-5 (5.6 fold), compared to control cultures (0% serum). That hypertrophy had been induced was confirmed by measuring ANF levels mRNA that were increased 1.9 fold. Finally, expression of the E2F-regulated gene product, cyclin E, was shown to increase significantly during the development of hypertrophy suggesting that E2F transcriptional activity is elevated during this process. Thus, we have shown that E2F family members are expressed differentially during cardiac development and hypertrophy. A greater understanding of E2F in cardiac growth might enable it to be used as a target for the treatment of diseases such as heart failure.

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Basic Science Renewal

Dr P R Kemp, University of Cambridge. "Transcriptional control of the vascular smooth muscle cell phenotype and its role in atherosclerosis" £238,569

Project Grants Committee, March 2001
DEFERRED APPLICATIONS AWARDED

Dr K A Dellow et al National Heart & Lung Institute, London. "Cloning and characterisation of novel, cardiac-restricted transcription factors" 2 years, £90,551

Dr K Suzuki et al Harefield Hospital. "The role of IL-1 inhibitors in cell transplantation to the heart" 2 years, £138,149

Dr K S Authi King's College London., "Expression and role of inositol phosphate receptors and HTRP proteins in human platelets and related cells" 2 years, £195,400

Professor N S Peters & Dr D W Davies St Mary's Hospital, London. "Non-uniformity of ventricular activation in the arrhythmic infarcted human heart" 2 years £138,282

Drs P R Kemp & C W J Smith University of Cambridge. "Smooth muscle-specific alternative splicing in vivo: analysis and application to the temporal control of gene expression in transgenic mice" 3 years £181,364

NEW APPLICATIONS AWARDED

Dr J Gavrilovic & Prof N D Hastie University of East Anglia, Norwich. "The role of the WT1 tumour suppresser gene in epicardial development" 1 year £38,919

Dr M G Dunckley & Prof F Muntoni ICSM, Hammersmith Hospital, London. "The role of 5' muscle-specific sequences in regulating dystrophin gene expression in healthy & dystrophic cardiac muscle" 3 years £154,016

Dr M-S Suleiman et al Bristol Heart Institute. "The effect of "hot shot" blood cardioplegia on clinical outcome, myocardial metabolism and reperfusion injury during aortic valve surgery" 2 years £64,794

Dr F R Green Wellcome Trust Centre for Human Genetics, Oxford. "An investigation of allele specific transactivation by a liver-enriched factor that explains inter-individual differences in plasma fibrinogen level" 2 years £87,106

Prof D J Field et al University Hospitals of Leicester. "Pilot investigation of hypothermia in neonates receiving extracorporeal membrane oxygenation (ECMO)" 6 months £18,721

Dr R Motterlini & Dr R Foresti Northwick Park Inst for Med Research, Harrow. "Counteracting oxidative and nitrosative stress in the ischaemic myocardium: role of endogenously generated bilirubin" (3 years) £168,176

Dr S J Fuller National Heart & Lung Institute, London. "Mechanisms regulating expression of the mkp-1 gene in cardiac myocytes" 3 years £118,441

Dr N Thorogood et al London School of Hygiene & Tropical Medicine. " The social and psychological impact on relatives of a family tracing a programme for familial hypercholesterolaemia" 1 year £23,568

Prof P Durrington et al Manchester Royal Infirmary. " Development of a convenient method for the determination of small-dense LDL in large numbers of samples" 1 year £46,270

Dr J Wharton & Prof M R Wilkins ICSM, Hammersmith Hospital, London. "Regulatory roles of bone morphogenetic proteins in human pulmonary vascular cells" 3 years £146,593

Dr M I Polkey et al Royal Brompton Hospital, London. "Do respiratory muscles limit exercise in congestive heart failure" 3 years £150,742

Dr S Neubauer et al John Radcliffe Hospital, Oxford. "Development and application of 3D-echocardiographic techniques for phenotype characterisation in mice" 1 year £61,121

Dr J E Preston King's College London. " Glucocorticoid transport into brain in a growth restricted, hypertensive rat model" 2 years £78,584

Dr J E Cartwright et al St George's Hospital Medical School, London. "Trophoblast invasion of spiral arteries: a novel in vitro model" 2 years £93,990

Mr M Caputo et al Bristol Royal Infirmary. "A prospective randomised study of the effects of normothermic and hypothermic cardiopulmonary bypass in children undergoing open heart surgery" 2 years £121,535

Prof N J Severs National Heart and Lung Institute, London. "Gap junctions, connexin expression and susceptibility to post-operative atrial fibrillation" 2 years £98,826

Prof M Bennett & Dr N J McCarthy Addenbrooke's Hospital, Cambridge. "Regulation of CD95/Fas localisation and turnover in vascular smooth muscle cells" 3 years £120,464

Prof P Durrington et al Manchester Royal Infirmary. "Further comparison of antecedents of coronary heart disease between migrant British Gujaratis and Gujaratis in the migrant's original villages in India" 1 year £84,432

Dr A H Gershlick et al Glenfield Hospital, Leicester. "Localising thrombolytic agents by targeting recently formed thrombus: in-vitro studies to develop effective antigen directed conjugates" 2 years £76,318

Prof J Ayres et al Birmingham Heartlands Hospital. "The effect of exposure to common air pollutants on cardiac autonomic control and inflammatory markers in patients with heart disease" 2½ years £180,275

Dr A Townsend-Nicholson University College London. "Molecular cloning and characterisation of novel nucleotide receptors in the cardiovascular system" 2 years £99,477

Prof D Chamberlain et al Lansdowne Hospital, Cardiff. "To blow or not to blow…. a comparison of two telephone CPR instruction sets with unassisted bystander CPR in simulated cardiac arrest" 6 months £17,417

Dr M P Gilbey Royal Free Campus, London. "Control of sympathetic activity regulating skeletal muscle circulation: sympathetic rhythms and their possible involvement in

dynamic responses" 3 years £115,909

Dr S Y Ho National Heart & Lung Institute, London. "Ventricular structure in adults with congenital heart disease: complete versus congenitally corrected transposition" 2 years £71,908

Dr M Crompton University College London. "Does the opening of a mitochondrial pore contribute to apoptotic cell death following myocardial ischaemia?" 1½ years £61,236

Dr I L Megson & Dr I Rahman University of Edinburgh. "An investigation of the molecular mechanism underlying altered glutathione metabolism in atherosclerosis: implications for disease progression" 2 years £80,776

Fellowships Committee, January 2001

Intermediate Research Fellowships

Dr S H Abedi & Prof Sugden, Imperial College, London. "The role of the ephrin/Eph families in endothelial cell biology and apoptosis, and the contribution of VEGF receptor-mediated signalling" 3 years. £127,043

Dr I P Salt & Prof G G Gould, University of Glasgow. "The regulation of endothelial nitric oxide synthase by insulin" 3 years £112,613

Junior Research Fellowships

Mr R C Baker & Dr W T McBride, Queen's University of Belfast. "The renal effects of immuno-modulatory interventions in a porcine hind-limb ischaemia-reperfusion model" 1 year £39,400

Mr N Moorjani & Prof P H Sugden, National Heart & Lung Inst, London. "The effect of pressure overload on caridmyocyte apoptosis and its genetic regulators" 1 year £45,236

Dr S Yusuf & Prof A J Camm, St George's Hospital Medical School, London. "The role of various 5HT4 receptor isoforms in the initiation and maintenance of atrial fibrillation" 2 years £90,084

Dr L Crowe & Dr D N Firmin, Royal Brompton Hospital, London. "Development of zonal magnetic resonance imaging techniques for ultra-fast imaging of vessel structure and flow" 2 years £72,543

Dr N Johnston & Prof H J Dargie, Western Infirmary, Glasgow. "The redefinition of uraemic cardiomyopathy using magnetic resonance" 2 years £76,790

Mr D di Bernardo & Prof D Noble, University of Oxford. "Reconstructing the heterogeneity of electrical activity in the heart: a whole organ modelling study" 2 years £61,841

Dr R Thaman St & Dr P M Elliott, George's Hospital Medical School, London. "Reversal of inappropriate vasodilatation during exercise and lower body negative pressure in hypertrophic cardiomyopathy" 2 years £84,433

PhD Studentships

Ms C A Risebro & Dr P R Riley, Institute of Child Health, London. "Investigating the role of *Hand1* in cell movement and migration during cardiac morphogenesis" 3 years £66,051

Miss G Ellison & Prof D F Goldspink, Liverpool John Moores University. "Do sustained high levels of catecholamines impair the regeneration of necrotic and apoptotic myocytes by satellite cells in skeletal muscles?" 3 years £61,378

Unnamed & Dr S J George Bristol Royal Infirmary. "The role of cadherins in vascular smooth muscle cell apoptosis" 3 years £60,898

Unnamed & Dr D Bell, The Queen's University of Belfast. "Adrenomedullin: potential defence mechanism against development of ventricular cell hypertrophy and transition to heart failure in a rat model of hypertension" 3 years £60,827

Unnamed & Prof D A Lane, Hammersmith Hospital, London. "High level expression of soluble EPCR for structural and function studies" 3 years £65,652

Unnamed & Dr J Ohanian, Manchester Royal Infirmary. "The role of lipid rafts in noradrenaline-induced vascular smooth muscle contraction" 3 years £60,885

Mr P J Dean & Dr J Chamberlain, Northern General Hospital, Sheffield. "An investigation into the relationship between the mannose-6-phosphate receptor, thrombospondin-1 and active transforming growth factor- in arterial wound healing" 3 years £60,945

Mr D O Kellett & Prof D Jordan, Royal Free & University College Medical School. "Role of central 5-hydroxtryptamine containing pathways in the control of vagal outflow to the heart" 3 years £66,501

Unnamed & Dr A Baker, University of Glasgow. "Development and evaluation of genetically retargeted viral vectors for vascular gene therapy" 3 years £61,591

Mr M Crabtree & Prof G A A Ferns, University of Surrey, Guildford. "The effect of dietary copper supplementation on apoptosis in atherogenesis: the role of nitric oxide superoxide and the extracellular matrix" 3 years £61,874

Mrs J Bostock & Dr A M Carter, Leeds General Infirmary. "CCAAT/Enhancer binding protein genetic variants and gene regulation: implications for haemostasis and thrombosis" 3 years £61,558

Mr J Wentworth & Dr A W Poole, University of Bristol. "Cross-talk between tyrosine and serine/threonine kinase signalling pathways in platelet activation: the role of PKC isoforms" 3 years £60,960

Unnamed & Dr N A Booth, University of Aberdeen. "Expression of transglutaminase activity in cells of the vessel walls" 3 years £61,448

Unnamed & Dr V Ellis, Univeristy of East Anglia, Norwich. "Reciprocal interactions between the hepstocyte growth factor/scatter factor and plasminogen activation systems in vascular smooth muscle cells" 3 years £60,960

Ms S Muzaffar & Prof G D Angelini, Bristol Royal Infirmary. "Superoxide in the aetiology of pulmonary hypertension in sepsis-induced adult respiratory distress syndrome" 19 months £37,208

PhD Studentships (Clinical)

Dr J A Byrne & Professor Shah, Kings College, London. "Role of phagocyte-type NADPH oxidase in pathophysiology of progressive cardiac hypertrophy" 2 years £104,313

Dr A M Kabir & Prof M S Marber, St Thomas' Hospital, London. "The interaction between mito KATP channels and signals initiating ischaemic preconditioning" 3 years £138,666

Dr S Dhamrait & Dr V Mohamed-Ali, University College,

London. "Metabolic efficiency and sketal muscle uncoupling protein: modulation by the renin-angiotensin and sympathetic nervous system" 3 years £120,802

Overseas Visiting Fellowship

Dr Haugen & Prof Hanson, Princess Anne Hospital, Southampton. "The role of fetal cardiovascular adaptations and liver development in programming the risk of adult coronary heart disease" 6 months £44,521

Project Grants Committee, January 2001

DEFERRED APPLICATIONS AWARDED

Dr D Singer & Dr A H Chester, Harefield Hospital, Middlesex. "Role of c-type natriuretic peptide in human vascular smooth muscle cells" 3 years £138,013

NEW APPLICATIONS AWARDED

Dr N Morrell, University of Cambridge. "Cross - talk between the IGF-I axis and the TGF- super family in pulmonary vascular cells" 3 years £106,303

Prof D Beech & Dr I Wood, University of Leeds. "KCNx gene function in arteriolar smooth muscle cells" 3 years £115,273

Dr A Clerk, Imperial College, London. "The role of the Fas receptor (CD95) in cardiac myocyte apoptosis and hypertrophy" 3 years £123,434

Dr H Colhoun et al,University College London. "Defining the association of candidate genes with coronary artery calcification and other atherosclerosis related phenotypes" 6 months £30,836

Prof D Yellon & Prof D Latchman, University College London. "The importance of HSP27 and its phosphorylation state in the mechanism of early and delayed preconditioning" 3 years £52,685

Prof D Lane & Dr H Philippou, ICSM, Hammersmith Hospital, London. "Functional domains on thrombin required for factor XIII activation" 3 years £160,839

Prof H Markus et al, St George's Hospital Medical School. "Cytokine polymorphisms and host environment interactions in the pathogenesis of carotid atherosclerosis" 2 years £96,484

Dr R Heads & Prof M Marber, St Thomas's Hospital, London. "IL-6 and IL-10 cytokines as mediators of delayed preconditioning in cardiomyocytes: role of adenosine receptors and induciable nitric oxide synthase" 3 years £261,982

Prof D J Webb et al, Western General Hospital. "Oxidative stress, erythrocyte anion channel damage, and antioxidant function in healthy lowlanders at high altitude" 1 year £10,525

Dr D E Newby et al, Royal Infirmary, Edinburgh. "Effects of inflammatory cytokines on endothelial vasomotor and fibrinolytic function: role of interleukin-1 and interleukin-6 and tumour necrosis factor " 1½ years £81,935

Prof DI Wilson, Southampton General Hospital. "An clinical genetics investigation of children with hypoplastic left heart syndrome" 1½ years £71,775

Mr D P Taggart & Prof P Matthews, John Radcliffe Hospital. "The role of microemboli, detected by transcranial doppler imaging during cardiac surgery on cerebral injury defined by structural and functional MRI imaging" 1 year £94,085

Dr K M O'Shaughnessy, Addenbrooke's Hospital, Cambridge. "Investigation of the molecular regulation of thiazide-sensitive sodium transport in the distal nephron" 2 years £86,733

Dr A Kaumann et al, University of Cambridge. "Heterogeneous interactions of -blockers with recombinant 1 adrenoceptors" 2 years £90,762

Dr A K Soutar & Dr R Naoumova, ICSM, Hammersmith Hospital, London. "Identification of a gene on chromosome 1p36-35 that is defective in atypical homozygous familial hypercholesterolaemia" 3 years £146,732

Prof D A Lane et al, ICSM, Hammersmith Hospital, London. " In vivo analysis of the human EPCR gene promoter- a transgentic approach" 1 year £68,597

Dr R W Brown et al, Western General Hospital, Edinburgh. "Elucidation by study of mineralocorticoid excess of sodium retention and pressure natriuresis pathways maintaining sodium balance and blood pressure" 3 years £159,166

Dr I B Wilkinson, Addenbrooke's Hospital, Cambridge. "Arterial stiffness and isolated systolic hypertension" 2 years £124,651

Dr A S Izzard & Prof A Heagerty, Manchester Royal Infirmary. "Flow dilation in rat distal mesenteric arteries, the role of an EDHF mechanism involving K+ channels and gap junctions" 1½ years £20,765

Prof E J Johns, The Medical School, Birmingham. "The role of the brain renin-angiotensin system in the neural control of the kidney" 3 years £180,620

Dr J McCarron, University of Glasgow. "Generation of spontaneous transient outward currents by ryanodine receptor activity and suppression by InsP3 receptor activity" 3 years £126,462

Dr M Peckham et al, University of Leeds. "Familial hypertrophic cardiomyopathy; effects of mutations in ß-cardiac myosin on force in vivo and in vitro" 2 years £96,832

Dr R C Saumarez, Papworth Hospital. "Prospective evaluation of paced electrogram fractionation as a predictor of sudden death in hypertrophic cardiomyopathy" 3 years £201,112

Dr C Peers & Prof S Ball, University of Leeds. "Structural requirement for oxygen sensing by a human L-type Ca2+ channel" 3 years £96,986

Submission Deadlines for *The* Bulletin:

Volume	*Date*	*Deadline*
14(4)	*Oct. 2001*	*Sept. 1st*
15(1)	*Jan. 2002*	*Dec. 1st[t]*
15(2)	*April 2002*	*March 1st*
15(3)	*July 2002*	*June 1st*

Cardiovascular Related Wellcome Trust Grants

December 2000 to May 2001

Programme Grants

Prof Peter S Mortimer, Division of Psychological Medicine, Dept of Medicine, St George's Hospital Medical School, London. Mechanisms underlying breast cancer related lymphoedema of the arm (BCRL). 5 years £697,382

Project Grants

Prof M McKee, Health Services Research Unit, London School of Hygiene & Tropical Medicine, London. A baseline survey of the risk factors for and prevalence of type 2 diabetes mellitus in Tirana, Albania. 1 year £34,390

Dr Hilary J Powers, Centre for Human Nutrition, Northern General Hospital, Sheffield. Hyperhomocysteinaemia and vascular damage in children with IBD. 15 months £40,943

Prof David S Latchman, Institute of Child Health, London. Role of caspase-8 and caspase-9 in ischaemia/reperfusion-induced injury in the intact heart. 3 years £277,670

Dr Margaret D Brown, School of Sport & Exercise Sciences, University of Birmingham. Haemodynamics and remodelling in the microcirculation of skeletal muscle after chronic flow reduction. 1 year £61,966

Dr C M P Rees, Nuffield Dept of Obstetrics & Gynaecology, John Radcliffe Hospital, University of Oxford. Adrenomedullin, calcitonin receptor-like, receptor activity modifying proteins and endometrial angiogenesis. 3 years £150,215

Prof L G Fine, Rayne Institute, UCL. The stepped care blood pressure control project. 15 months £91,590

Prof M R Duchen, Dept of Physiology, University College London. Mechanisms of cardioprotection by mitochondrial ATP-dependent K+ channel. 3 years £14,865

Dr B Therese Kinsella, Dept of Biochemistry, University College Dublin, Eire. Investigation of the differential roles of alpha and beta isoforms of the human Thromboxane A_2 Receptor in prostacyclin and nitric oxide (NO) regulated vascular hemostasis. 3 years £166,110

Prof P R Unwin, Dept of Chemistry, University of Warwick. Characterisation of endothelial cell glycocalyx and its disturbances in the diabetic state using high resolution electrochemical imaging techniques. 2 years £90,210

Prof Angela C Shore, Dept of Vascular Medicine, University of Exeter. Characterisation of endothelial cell glycocalyx and its disturbances in the diabetic state using high resolution electrochemical imaging techniques. 2 years £74,690

Biomedical Research Collaboration Grants

Dr F W Flitney, School of Biomedical Sciences, Bute Medical Building, University of St Andrews. Studies of a novel cell-matrix attachment in the mechanotransduction of flow by endothelial cells with particular reference to activation of the NF-kB nuclear transcription factor pathway. 3 years £11,750

Research Training Fellowships in Mathematical Biology

Mr E Crampin, Laboratory of Physiology, University of Oxford, Oxford. Mathematical and computational modelling of mycardial ischaemia. 3 years £110,237

Entry Level Fellowships For Medical & Dental Graduates

Dr Joanna K Lovett, Dept of Clinical Neurology, Radcliffe Infirmary, University of Oxford. Gender differences in the anatomy, the distribution of atherosclerotic plaque surface morphology at the carotid bifurication. 1 year £37,626

Dr Yasmeen Ahmad, ARC Epidemiology Research Unit, University of Manchester. Manchester. The genetic contribution to risk of premature atherosclerosis in women with systemic lupus erythematosis. 1 year £49,649

Four Year Phd Studentships

Miss Emma L Taylor, Dept of Medical Sciences, Western General Hospital, University of Edinburgh, Scotland. Induction of human inflammatory cell apoptosis and inhibition of adhesion by nitric oxide donor drugs: mechanisms of action and complications for cardiovascular disease. 3 years £28,360

Mr David O'Regan, Dept of Medical Sciences, Western General Hospital, University of Edinburgh. WT CVRI 3 years £44,990

Research Training Fellowships In Clinical Epidemiology

Dr Una Fallon, Dept of Social Medicine, University of Bristol. The association between the methylene tetrahydrofolate reductase gene mutation, neural tube defects and artherosclerosis in men and women. 4 months £14,305

Dr Ursula G Schulz, Dept of Clinical Neurology, Radcliffe Infirmary, University of Oxford. Prevention of ischaemic stroke distal to asymptomatic carotic stenosis: asymptomatic carotid stenosis study and carotid endarterectomy trialists's collaboration. 3 years £133,754

International Prize Travelling Research Fellowships

Dr S A Cook, Cardiovascular Research Centre, Massachusetts General Hospital, Charlestown, USA. The control of BAD function by phosphorylation and the interaction of BAD with the 14-3-3 family proteins: implications for cardiac myocyte hypotrophy and apoptosis. 20 months £1,875

Dr Sarah L Withington, Victor Chang Cardiac Research Inst, Darlinghurst, Australia. Defining tissue interactions and CITED2 gene function associated with cardiovascular development. 3 years £48,666

International Research Development Awards

Dr S Kantachuvesiri, Dept of Medical & Radiological Science, Medical School, University of Edinburgh, Scotland. Fine mapping of congenic regions in rats and characterisation of genetic polymorphisms of the rat Ace gene that contributes to malignant hypertension. 3 years £74,240

Symposia

Dr Angela Clerk, Division of Biomedical Sciences, Imperial College School of Medicine, London. A contribution towards a meeting on "Apoptosis in the Heart: Signalling, mechanisms, pathology and protection. £3,000

Dr K Clarke, Dept of Biochemistry, University of Oxford. A contribution towards a meeting on "Magnetic resonance in cardiovascular research". £4,000

THE BRITISH SOCIETY FOR CARDIOVASCULAR RESEARCH

Joint Meeting

BSCR Autumn Meeting 2001

and

Sixth International Symposium on Magnetic Resonance in Cardiovascular Research

Dates: 14th and 15th September, 2001
Venue: Department of Biochemistry and Laboratory of Physiology, University of Oxford
Organisers: Kieran Clarke and Stefan Neubauer

Invited Speakers will include: Sir George Radda (London), Joanne Ingwall (Boston), Theo Wallimann (Zurich), Matthias Spindler (Würzburg), Renée Ventura-Clapier, (Chatenay-Malabry), Aarnt Herschap (Nijmegen), Hugh Watkins (Oxford), Charles Springer (New York, USA), Dudley Pennell (London), Matthias Friedrich (Berlin), Frank Rademakers (Leuven), U Sechtem (Stuttgart), G. Allan Johnson (Durham, USA), Robert Weiss (Baltimore), Frank Wiesmann (Wurzburg), Ulrich Decking (Dusseldorf), Paul Bottomley (Baltimore), Gerald Pohost (Birmingham), Frank Kober (Marseille), Meinrad Beer (Wurzburg), Jorn Sandstede (Wurzburg), Hildo Lamb(Leiden), Wulf-Ingo Jung (Buehl), Gustav von Schulthess (Zurich), Robert Balaban (Bethedsa), Jurgen Schrader (Dusseldorf), Cees van Echteld (Utrecht), Pamela Garlick (London), Keith Channon (Oxford), Zahi Fayad (Philadelphia), Paul Hockings, (GlaxoSmithKline), Markus von Kienlin (Basel), David Firmin (London), Thomas Voigtlaender (Mainz), Juergen Hennig (Freiburg), Axel Haase (Wurzburg), Christine Lorenz (London).

Communications: Part of this meeting will be devoted to the presentation of posters. Abstracts, on any relevant topic, are welcomed. **Abstract deadline: 1st June 2001.**

Travel & Accommodation: The Oxford Information Centre has information on getting to Oxford and accommodation in Oxford (http://www.visitoxford.org, e-mail: tic@oxford.gov.uk). Oxlink also has accommodation information (http://www.oxfordcity.co.uk/accom/index.html). For airport express bus information, see the Oxford Bus Company website (http://www.oxfordbus.co.uk).

Registration: Free to BSCR members, £50 for non-members. Registration and abstract forms will be available on the website **http://www.bioch.ox.ac.uk/~mrcvr/.** For further information contact: Yvonne Green, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU. Tel 01865-275272, Fax: 01865 275194, e-mail: ysg@bioch.ox.ac.uk.

Bursaries: The Society will consider awarding travel grants of up to £150 to *bona fide* PhD students. Application forms are available from Dr Gary Baxter at the address below.

Applications for membership and student bursaries are available from Dr Gary Baxter, Secretary of the BSCR, The Hatter Institute for Cardiovascular Studies, University College Hospital, Grafton Way, London WC1E 6DB.