I was very honoured to have been awarded the Richard J. Bing Young Investigator Award at the ISHR XVIII World Congress in Brisbane, 2004, for work investigating NADPH oxidase as a source of free radicals in ischaemic preconditioning signalling. I attended medical school at University College, London (UCL), qualifying in 1994 and gaining a Physiology BSc in 1991, from where my interest in academic cardiology originated. Having completed my early medical training, I was fortunate to join Professor Derek Yellon’s laboratory at the Hatter Institute of Cardiovascular Studies at UCL, undertaking a PhD studying nitric oxide signalling in cardiac protection with the financial support of the British Heart Foundation. On completion of my PhD, I moved to the Rayne Institute at St Thomas’ Hospital in 2002 with my current mentors, Professors Michael Shattock and David Hearse. Our laboratory focus is free radical signalling and myocardial protection with an interest in identifying novel targets to induce resilience to ischaemic injury. Within our division, Professor Ajay Shah’s group also has an interest in free-radical signalling, with regard to gp91phox NADPH oxidase in the context of cardiac hypertrophy. Following discussions, we identified what we felt was an interesting parallel between Ajay’s work and our interest in cardiac protection that we felt we could quickly investigate at the start of my project at the Rayne Institute. This study, which started as one of those quick, “two week investigations”, has turned out to be more involved and interesting than we had ever expected!

(continued on page 6)
Dear reader,

On my return from the holiday season, I would like to share some wonderful news with you all: the American and Japanese Sections, like the European Section, have decided to forgo independent Annual Meetings in 2007 and instead to hold their Section meetings and activities during the World Congress in Italy. This will indeed strengthen our Congress and for this I would like to thank the European, American and Japanese Sections tremendously.

This brings me to some initiatives for my term as President that I would like to share with you:

My highest priority will be the World Congress of the ISHR.
I believe that the visibility of our Society depends a lot on the World Congress. It is the time when we all get together, when the Council meetings are conducted, and when our Society receives the greatest international exposure. Therefore, I believe that every possible effort should be made to ensure the scientific and organisational excellence of future congresses. In my opinion, any congress relies on four things for success: a fantastic scientific programme, an enthusiastic organising team, sufficient funding, and an attractive location. I would like to propose a series of recommendations – maybe rules – for future congress organisation. I would strongly recommend that during the year of the World Congress we do not hold the annual Section meetings. As an alternative, each Section should be allocated space and time during the World Congress, or immediately before or after it. This will keep the delegates together, and enable them to mingle. I fully support the proposal to use a significant part of the Society’s income towards the costs of excellent symposia, and not to make economical support available to the organising team without having control of such support. I would encourage each organising team to establish a credible and active scientific committee, with significant input from the Council or its appointed Scientific Programme Committee, to ensure an excellent scientific programme. Also, the organising team should have an obligation to provide yearly reports to the Council on the progress of the Congress organisation, in particular the evolution of the scientific programme. I would encourage greater liaison between our Congress and other cardiovascular societies, to organise joint sessions/meetings/etc. through which the ISHR will gain greater visibility.

Another goal will be to do my utmost in order to promote the success of our Journal, which is already doing very well. In fact, I believe that the Journal is a key strength of our Society. I will liaise with the Editor and assist him in publishing special issues and in recruiting the excellent scientists in our Society to write articles for the Journal.

My third goal will be to liaise with other societies and enterprises involved in cardiovascular research. I will encourage each of the Sections to liaise and possibly arrange joint annual meetings with other cardiovascular societies, such as the recent, highly successful meeting of the European Section in conjunction with the Working Group on Heart Failure of the European Society of Cardiology, in Strasbourg. I believe that by doing so our Society will benefit. The other societies can see the value of what we are able to offer them. We can be visible and often we can share the costs.

Fourth and finally, I will do my utmost to convince industries and institutions to provide financial support for our Society, and to establish some communal research projects on specific points of interest.

To achieve my objectives, I will need the enthusiastic support of the Council and the ISHR membership, whom I wish to assure of my personal enthusiasm and dedication.

Roberto Ferrari
BEGIN with an apology. In my past contributions to Heart News and Views I have avoided references to my own scientific past, because I felt that this would be tactless and presumptuous. In science, facts speak for themselves while in history, individuals have the stage. But there are experiences in life which, because of their general relevance, can be communicated without the danger of appearing self-serving. To find an audience for these experiences is therefore pardonable.

Years ago, we became interested in the effects of ischemia of the heart. Later we found that nitric oxide and two prostanoids, prostacyclin and thromboxane, were present in infarcted heart muscle and in coronary sinus blood of patients and experimental animals. Cyclooxygenases (COX) were also found. These are enzymes which catalyze the conversion of arachidonic acid to prostanoids. COX-1 is constitutively produced, while COX-2 is formed during inflammatory processes. Nitric oxide and prostanoids play an important role in the production of growth factor and of angiogenesis. This is beneficial in infarcted heart muscle, but not in colon cancer where COX is also present and where angiogenesis induces spread of the tumor. The presence of prostacyclin and of cyclooxygenases in heart muscle after experimental myocardial infarction led us to a study of drugs which inhibit COX-2 in the infarcted heart. These non-steroidal anti-inflammatory drugs (NSAIDs) interfere with the production of prostacyclin and thromboxane. These two prostanoids have different functions. Prostacyclin inhibits platelet aggregation, is a vasodilator, and prevents ventricular arrhythmias; thromboxane promotes platelet aggregation, is a vasoconstrictor, and initiates ventricular arrhythmias. Aspirin, primarily a COX-1 inhibitor, proportionally diminishes the production of both prostacyclin and thromboxane in infarcted heart muscle, while Celecoxib, a selective COX-2 inhibitor, lowers myocardial prostacyclin while failing to inhibit thromboxane. A selective decline in prostacyclin with a consequent fall in the prostacyclin/thromboxane ratio explains the potential damaging effects of COX-2 inhibitors. This has been the pivotal finding of our studies.

Why were COX-2 inhibitors introduced? COX-2 leads to depletion of prostacyclin in the gastric mucosa and therefore exposes the mucosa to erosion. But the potential of COX-2 inhibitors for disastrous circulatory events is more threatening.

Our findings for the basis for the damaging circulatory effects of COX-2 inhibitors received no attention by government agencies or pharmaceutical companies. Then, suddenly, clinical studies rocked government regulatory agencies, the pharmaceutical industry, and patients who had been placed on COX-2 inhibitors. The reports showed that some COX-2 inhibitors increased the likelihood of catastrophic cardiovascular events. Why were our scientific data on the potential damaging effects of COX-2 inhibitors ignored, although they were published in a variety of journals, including some which deal with clinical cardiology? The answer is that experimental results, not obtained on patients, are often overlooked because of ignorance and of prejudice against laboratory data; they are difficult to understand and to evaluate. Clinical data furnish the final proof and the final answer is based on results on patients, but a knowledge of the fundamental action of drugs contributes to the understanding of its action. Reliable clinical data are not always easy to come by. They are subject to reliable communications between physicians, industry and government agencies, to statistical prowess, and to the courage to look unpleasant facts straight in the eye. At the same time, we as scientists try to be impartial. We are not always willing to enter the slippery slope of public opinion, commercial warfare, and government regulations. We try to remain uninvolved since involvement could be readily interpreted as politically motivated.

What then should we do when we acquire fundamental facts which belong in the public domain? Should we knock on the doors of government offices, the industry, to tell them what we found, should we warn them, or should we publish our results and let it go at that? We have tried to inform the industry and the government agencies of our results, but we have gotten no response.

(continued on page 5)
DESPIE July’s warm sunshine, 27-year-old Joyce Barnes was not having a bright day. Of late, she had developed a dim view of life—the dwindles: easily tired and short of breath at a level of exertion that previously had posed no problem.

Five years had passed since Joyce graduated college and at the top of her class at that! Her career as a computer engineer was off to a quick start having been hired by SmartAssets, a successful Silicon Valley firm whose reputation for a “bottom-line mentality” abrogated any compassion for its employees. Joyce had nonetheless bucked the odds and quickly developed a reputation as an upcoming star: bright, creative and committed to her work. On weekends, she enjoyed tennis and swimming with newfound friends, including Matthew, whom she had been dating for the past year. She and Matthew had enjoyed dining out and dancing in popular night spots. But that was all in the past.

Over the past year, Joyce had gradually noted the appearance of breathlessness with each of these activities. This exertional dyspnea was becoming progressively worse evoked by lesser and now even light workloads. She presently became exhausted walking the level surface from where she parked her car to the elevator in her apartment building—a distance of barely 30 feet. She decided to see her primary care physician, Dr Jennifer Rayborn, whom she visited annually for a routine evaluation and who had prescribed her birth control pills several years ago. Joyce had no history of congenital or acquired heart disease, recurrent sinusitis or bronchitis, and no pertinent family history. Her menstrual cycles were regular and she had never been pregnant. For all intents and purposes, she had been in fine health until a year ago. Dr Rayborn found no pertinent physical findings except for increased intensity of the pulmonic component to Joyce’s second heart sound. Chest x-ray did not reveal evidence of lung disease. She related her essentially negative evaluation to Joyce and suggested a consultation with pulmonologist, Dr Frank Viewmaster.

Joyce saw Dr Viewmaster the following week. His office was crowded with patients using inhalers and/or supplemental oxygen for the ravages of their airway disease. He quickly obtained the above history and recommended office-based pulmonary function studies (PFTs). Within an hour he reported, “Joyce, your lung volumes and air flow rates are normal for your age and gender. There is no evidence for restrictive or obstructive lung disease. Perhaps your breathlessness is secondary to job-related stress,” he suggested.
thrombi may also be found. The causality of PPH remains unknown. Estrogen supplements (birth control pills) have been associated with the appearance of PPH and whether this predisposes to thromboembolic disease is uncertain. Average survival in patients with advanced disease is 3 years after clinical presentation.

Patients with PPH most often are women between the ages of 20 and 40 who present to their physician only after symptoms compromise quality of life. Their most frequent presenting symptom is exertional dyspnea. In more advanced disease, near syncope or even syncope may appear during or immediately following physical activity. Other symptoms include: easy fatigability, retrosternal chest pressure with exertion; and palpitations. On examination, an increased intensity to the pulmonic component of the second heart sound may be the only finding. In summary, PPH appears in previously healthy young women without many physical findings.

Chest x-ray may reveal enlargement of the pulmonary artery conus, dilatation of major pulmonary arteries and truncated distal vessels while lung fields are clear. ECG may be normal or it may demonstrate evidence of right axis deviation and RV hypertrophy. Routine PFTs are normal with the only abnormality a reduction in the diffusing capacity for carbon dioxide (DLCO), indicative of a ventilation:perfusion mismatch. Because of this mismatch, arterial hypoxemia may appear with exertion to stimulate chemoreceptors and exaggerate minute ventilation—a relative hyperventilation to exercise.

The pathophysiology of PPH is based on abnormal structure and impaired vasodilator reserve of pulmonary resistance vessels. A 50% reduction in the pulmonary vasculature (i.e., the equivalent of a pneumonectomy) is required before PA pressure begins to rise. Elevations in pulmonary vascular resistance (PVR) can reach levels comparable to the systemic circulation. This pressure overload on the right heart leads to RV hypertrophy and dilatation, right atrial enlargement and a shift of the interventricular septum toward the LV that, in turn, alters the configuration and compliance of the LV. Inadequate LV filling can impair cardiac output. In the face of exercise-induced vasodilatation of systemic arterioles in working skeletal muscle, a failure of cardiac output to rise can lead to impaired cerebral perfusion with near syncope or syncope the result.

Management of PPH is generally unsatisfactory. The vasomotor reactivity of diseased pulmonary arterioles is severely compromised. Hence, vasodilator agents are often ineffective in reducing elevations in PVR created by alterations in vascular structure. There is also the risk of reducing systemic vasculature resistance with these agents, which can lead to a marked fall in arterial pressure if cardiac output fails to rise. More recently, it has been suggested prostacyclin, inhaled nitric oxide and endothelin receptor antagonism may hold promise for these unfortunate patients. A frustrated Dr Rayborn mused that in order to diagnose these patients earlier, we need a blood pressure cuff for the pulmonary artery.

Karl T. Weber, M.D.
The role of reactive oxygen species (ROS) in the recruitment of preconditioning induced protection is not a new observation; Chris Baines working in Jim Downey’s laboratory in 1997 demonstrated that modest, single-cycle, ischaemic preconditioning stimuli in rabbit could be readily blocked by the co-administration of ROS scavengers. An interesting additional finding from this work was that more robust preconditioning stimuli (four-cycle) was not attenuated by free radical scavenging, implicating the existence of at least two signalling pathways: a free-radical dependent, and a free-radical independent pathway. Mike Cohen has revisited these pathways more recently, demonstrating that a wide variety of pharmacological triggers of preconditioning appear to signal through this ROS-dependent pathway, with the notable exception of adenosine A₁ receptor agonists that appear to signal through the ROS-independent pathway.

The source of the ROS that result in myocardial protection remains unclear. Ajay Shah’s group has recently demonstrated that ROS-generating gp91phox-containing NADPH oxidase is present in the heart, and that the activity of the NADPH oxidase was linked to the pathophysiology of cardiac hypertrophy. Interestingly, the known pharmacological activators of NADPH oxidase in the myocardium were also known ‘preconditioning-mimetics’ (Fig. 1B) and moreover, all these preconditioning-mimetics were known to trigger myocardial protection via a free radical based mechanism. We therefore hypothesised that gp91phox-containing NADPH oxidase would be essential in the triggering of ischaemic preconditioning. We also hypothesised that the phenotype of hearts isolated from the gp91phox knockout mouse would match that of wild type hearts treated with free-radical scavengers, in that more robust preconditioning triggers or adenosine A₁ receptor agonists would result in protection independently of NADPH oxidase and ROS.

To remove the potentially confounding non-cardiac sources of NADPH oxidase derived ROS, we chose a mouse heart Langendorff model of ischaemia/reperfusion to make use of Ajay Shah’s colony of gp91phox wild type and knockout mice. The study protocol comprised of 20 minutes stabilisation, a preconditioning regimen followed by injurious 35 minutes ischaemia and 30 minutes reperfusion, prior to deter–
mining infarct size by TTC staining. The preconditioning protocol consisted of 2 or 4 cycles of 5 minutes ischaemia followed by 5 minutes reperfusion or 10 minute infusion of the adenosine A$_1$ receptor agonist 2-chloro-N(6)cyclopentyl adenosine (CCPA) followed by 10 minutes wash out. In addition to the infarct end-point, further hearts were subjected to the preconditioning protocol, but prior to entering injurious ischaemia, were snap frozen for later lucigenin-enhanced chemoluminescence assessment of ROS generation.

**Results of gp91$^{phox}$ Deletion**

We found that although wild type hearts could be protected with 2 cycles of ischaemic reconditioning, this protection was entirely absent in the gp91$^{phox}$ knockout hearts (Fig. 2A - solid bars). Moreover, on measuring ROS production in these hearts, in contrast to the wild type, there was no increase of ROS generation, thus supporting the hypothesis that gp91$^{phox}$ NADPH oxidase dependent ROS generation is indeed required for ischaemic preconditioning (Fig. 2A - hatched bars). Furthermore, the gp91$^{phox}$ phenotype was found to be similar to the ROS-scavenged wild type heart, in that both 4 cycles of preconditioning and adenosine A$_1$ receptor activation would trigger protection in both N-2-mercaptopropionylglycine (MPG) ROS-scavenged wild type hearts and gp91$^{phox}$ knock outs. That adenosine A$_1$ receptor activation with CCPA triggers protection independently of ROS has further support from the measurement of NADPH oxidase activity; CCPA failed to result in a significant increase of ROS synthesis in isolated heart.

**Conclusions and Further Investigation**

We propose that gp91$^{phox}$ containing NADPH oxidase is indeed an essential component of the ischaemic preconditioning trigger, generating a necessary burst of ROS that leads to the induction of a subsequent signalling cascade - which can effectively be bypassed by CCPA (Fig. 2B). These data do pose further questions that we intend to investigate, including the mechanisms of NADPH oxidase recruitment, and the identity of the downstream redox sensor - might this be the mitochondria, as a number of investigators have suggested? Identifying these signalling components may provide novel therapeutic targets that may prove useful for triggering a pharmacological cardioprotective phenotype for the management of patients with acute coronary syndromes.

**Figure 2.** (A) Figure shows the effect of preconditioning in wild type (WT) and gp91$^{phox}$ knockout (KO) mouse hearts: solid bars are infarct size, whereas the hatched bars are NADPH oxidase activity. In WT hearts, there was a significant attenuation of infarct size (* p<0.05 vs control, n=6-8 per group), that was entirely absent in the KO hearts subjected to two-cycles of preconditioning. Moreover, whereas WT hearts displayed a significant increase of NADPH oxidase activity (§ p<0.05 vs control, n=10 per group), this increase was entirely absent in KO hearts.

(B) The cartoon shows a proposed preconditioning pathway that involves an upstream recruitment of an isoform of PKC, with activation of NADPH oxidase. The resultant generation of ROS leads to activation of protective downstream pathways. This preconditioning pathway can be bypassed using ROS-independent signalling triggers, such as the adenosine analogue, CCPA.

**References**

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The Autumn of 2004 saw a blue sky and sea as well as lush greenery in Weihai, where the VIII Annual Meeting of the Chinese Section Meeting was held on September 18-21, 2004. The meeting was sponsored by the Cardiovascular Section of the Chinese Association of Pathophysiology, and organized by the Medical Association of Weihai City, the Geriatric Cardiology Department of the PLA General Hospital, and the Cardiovascular Research Institute of the No. 3 Hospital of Beijing University. Eight foreign speakers had been invited, including Drs Naranjan S. Dhalla (Canada), Paul M. Vanhoutte (Hong Kong) and Lindsay Brown (Australia). More than 30 Chinese experts in basic and clinical cardiovascular research were invited to give lectures. Among them were Professors Qide Han (director of the Medical Department of Beijing University) and Depei Liu (president of the Chinese Academy of Medical Sciences). Professors Xing Lu, Youyi Zhang were the other members of the Organizing Committee. Representatives of Weihai City and Shandong Province attended the opening ceremony.

During the plenary sessions 45 papers were read, and there were 52 posters on display throughout the meeting. Of the more than 380 papers presented, 343 had their abstracts published in the Chinese Journal of Pathophysiology 20(13), 2004.

The topics of the nine symposia included: coronary heart disease and atherosclerosis, hypertension, ischemia/reperfusion injury and myocardial protection, arrhythmias and electrophysiology, heart failure and function, coagulative and fibrinolytic systems, vascular and microvascular metabolic disorders, and cardiovascular drug therapy.

Yali Zhao (Department of Physiology and Pathophysiology of Beijing University) was the winner of the first prize for the best oral presentation. The title of her paper was ‘The PKC pathway of VSMC proliferation induced by AngII’. The first prize for the best poster presentation went to Qian Li.

The Chinese Section held an election of officers during the meeting. The following officers were re-elected to the Fourth Executive Committee: Qide Han (President), Qi Chen (Vice-President), Xiaoying Li (Secretary), and Liling Wu (Treasurer).

Xiaoying Li, M.D.
Beijing, China
R. JOHN SOLARO has been Professor and Head of the Department of Physiology and Biophysics in the College of Medicine at the University of Illinois at Chicago since 1988. Dr Solaro is also Co-Director of the UIC Center for Cardiovascular Sciences. In 1998, he was appointed Distinguished University Professor at the University of Illinois.

Dr Solaro graduated from the University of Pittsburgh, College of Medicine with a PhD degree in 1971. In the same year, he was appointed to the faculty at the Medical College of Virginia. In 1975-76, Dr Solaro was a British-American Heart Fellow in Birmingham, England. In 1977, he joined the faculty at the University of Cincinnati, where he was supported by an NIH Research Career Development Award and was offered an AHA Established Investigator award. In 1987, he was a Fogarty International Fellow at University College London.

At UIC, Dr Solaro has received the University Scholar Award and the Faculty of the Year Award. He served as Secretary General of the ISHR, as Scientific Council Chair of the American Heart Association of Metropolitan Chicago, and as a member of the Councils of the ISHR American Section, and AHA Council on Cardiovascular Sciences, and as Chair for the Gordon Research Conference on “Cardiac Regulatory Mechanisms.” Dr Solaro was a full member of the NIH Physiology Study Section and Chair of the Cardiovascular Sciences Study Section. He is past-president of the Cardiac Muscle Society and the Association of Chairs of Departments of Physiology. He has served as an Associate Editor of the American Journal of Physiology, and on the Editorial Boards of Circulation Research, Journal of Molecular and Cellular Cardiology, and The Journal of Clinical Investigation. He is a guest editor at Circulation Research of a thematic series on “Regulatory Signaling by Thin Filament Modulation.”

Dr Solaro has published over 200 papers in the areas of cellular and molecular mechanisms controlling the contraction of the heart and how these mechanisms are altered by pathological conditions and by pharmacological interventions. He has done seminal work on the role of troponin and tropomyosin in switching on contraction, on the role of myofilament protein phosphorylation in the control of cardiac dynamics and in the transition to heart failure, on the unique properties of the embryonic/neonatal isoform of troponin I, and on the enhancement of myofilament activation by pharmacological agents, two of which, Acardi (Pimobendan) and Simdax (Levosimendan), are in clinical use. His current studies focus on multiplex functions of myofilament and Z-disc proteins in contraction and signaling. Dr Solaro is currently the holder of a 10-year NIH Merit Award, and is Principal Investigator on an NIH Program Project Grant, an NIH RO1 Award, and an NIH Training Grant.

Each year, the International Council selects a speaker to deliver the Keith Reimer Distinguished Lecture at the World Congress or speaker’s section meeting. The purpose of this lecture is to honor the memory of Dr Reimer and to recognize his contributions to cardiovascular research. The topic of the lecture must be in the field of ischemia, coronary hemodynamics, cardiac metabolism, or contractile mechanisms.

Previous honored speakers are:

- Roberto Bolli, USA
  (Madison, USA; 2002)
- Gerd Heusch, Germany
  (Strasbourg, France; 2003)

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The 2004 Keith Reimer distinguished lecture
Sarcomeric proteins as a Center of Multiplex Functions in Signaling and Mechano-transduction in the Myocardium honored Speaker: R. John Solaro, Ph.D. (August 2004; Brisbane, Australia)
The 2004 YIA Winner of the American Section

δPKC Mediates Reperfusion Damage-Induced Apoptosis in the Infarcted Myocardium

I am honored to have been presented with the 2004 Young Investigator Award during the XXVI Annual Meeting of the ISHR American Section held in Cancun, Mexico. I am originally from Clinton, Mississippi, but my love for science has led me to vastly different parts of the country. I completed my undergraduate studies at Washington University in St. Louis and will finish my Ph.D. in the Department of Molecular Pharmacology at Stanford University in June, 2005. My goal is to lead an academic research group after postdoctoral training. Since I joined the lab of Professor Daria Mochly-Rosen, much of my research has focused on determining the molecular basis of the effects of the δ isozyme of protein kinase C (δPKC) during the heart’s response to post-ischemic reperfusion-based damage.

Cardiac Reperfusion Damage

Acute myocardial infarctions (AMI), or heart attacks, affect millions of people worldwide and continue to be one of the leading causes of adult mortality. Although current treatment methods aimed at disrupting blocked coronary arteries enzymatically or mechanically are very effective, a majority of patients treated by these methods develop cardiac dysfunction, subsequent MI, heart failure and even death. It has thus been postulated that restoration of blood flow to the heart following ischemia, termed reperfusion, is a major component of cardiac cell damage. However, none of the current available therapeutic strategies effectively target cardiac injury resulting from reperfusion-based damage.

Current research efforts are aimed at discerning the molecular basis of ischemia and reperfusion damage. Since a major outcome of ischemia and reperfusion involves cell injury and death, much research has focused on determining whether the damage that occurs is a consequence of apoptosis or necrosis. While much of the earlier research suggested that necrosis was the major cause of ischemia and reperfusion damage, apoptosis has more recently been shown to result from ischemia and reperfusion as well. To determine the molecular basis of the damage leading to apoptosis and/or necrosis, studies have targeted many different signaling cascades shown to be modulated in response to ischemia and reperfusion. One such signaling cascade involves the activation of the PKC family of related enzymes.

The Role of PKC in the Heart’s Response to Myocardial Infarction

PKC isozymes affect various normal and disease-state processes from cell fate determination and differentiation to cell death and apoptosis. In the heart, two PKC isozymes (ε and δPKC) are activated and translocate from the cell cytosol to the membrane fraction during ischemia and reperfusion. Yet these two isozymes have opposing effects on the myocardium—εPKC activation is cardioprotective, while that of δPKC is damaging. Recent evidence from our lab has shown that εPKC participates in protecting the heart from ischemia if activated before or early during ischemia, while δPKC’s effects occur during reperfusion. To determine the effects of ε and δPKC on the myocardium, our lab used specific PKC isozyme-selective peptides that inhibit or activate these two isozymes in a selective manner.

In numerous different cell types, δPKC, in particular, has been shown to be involved in mediating apoptosis in response to various cell damaging stimuli. Therefore, we hypothesized that δPKC activity may mediate reperfusion damage by promoting apoptosis.

Christopher L. Murriel (Stanford, CA) was the winner of the Young Investigator Award at the XXVI American Section Meeting (Cancun, Mexico; May 2-5, 2004).

δPKC Mediates Ischemia and Reperfusion-Induced Apoptosis

To determine whether δPKC mediated ischemia and reperfusion-induced apoptosis, we used a simulated ex vivo rat heart model utilizing Langendorff retrograde perfusion through aortic cannulation. Adult male Wistar rat hearts were subjected to 30 minutes of no-flow global ischemia followed by 60 minutes of reperfusion with (for the first 15 minutes) or without 500 nM of the TAT protein-derived peptide (TAT 47-57)
conjugated δPKC inhibitor, δV1-1 (TAT-δV1-1). After peptide treatment, reperfusion was either stopped or continued for another 45 minutes and Western blot analyses of treated and non-treated ex vivo hearts were carried out for activation of δPKC and changes in the levels and activity of several apoptosis-related proteins.

We found that during reperfusion there was a greater than 2-fold increase in δPKC translocation to the particulate fraction (see Figure above). Analysis of subcellular membrane compartments revealed a significant translocation of δPKC, particularly to mitochondria, that also correlated with a significant increase (>50%) in cytochrome c release when compared with perfused control hearts. When hearts were treated with TAT-δV1-1 during the onset of reperfusion, this increase in mitochondrial δPKC translocation and cytochrome c release were significantly inhibited (p < 0.02). In addition, ischemia and reperfusion also led to cytochrome c release, caspase 3 activation, PARP cleavage, and DNA fragmentation (as measured by an increase in TUNEL staining) and all were inhibited by TAT-δV1-1 treatment. When we examined changes in the levels of pro- and anti-apoptotic Bcl-2 related proteins, we observed that ischemia and reperfusion induced a significant (>60%; p < 0.05) increase in pro-apoptotic BAD protein levels with parallel decreases in anti-apoptotic Bcl-2 (<40%; p < 0.03) and Bcl-x_L (<40%; p < 0.01). Importantly, when δPKC translocation was inhibited using TAT-δV1-1 at the beginning of reperfusion, the increase in BAD protein only was completely inhibited but not the decreases in Bcl-2 or Bcl-x_L. We also investigated whether the activity of another pro-survival protein, Akt, changed in response to ischemia and reperfusion. We observed a decrease in Akt activation and dephosphorylation during reperfusion; however, in hearts treated with TAT-δV1-1 the decrease in Akt phosphorylation was inhibited (<50%; p < 0.005).

Conclusions and Future Research Directions

Although the direct substrate of δPKC activity during reperfusion has not been identified, our data indicate that δPKC activity during reperfusion results in myocardial apoptosis by decreasing mitochondrial stability and increasing the levels of pro-apoptotic BAD while inhibiting the activity of pro-survival Akt proteins. These data illustrate the molecular basis for the cardioprotective effect of the δPKC inhibitor that we have recently reported in a porcine model of acute myocardial infarction, in vivo (Ref. 1). In addition, we also reported that δPKC activity during reperfusion not only alters the levels of pro-apoptotic BAD, but also the phosphorylation of BAD at Ser 136. Finally, δPKC activation during reperfusion also leads to DNA laddering. (This work is now published; Ref. 2). Therefore, targeting δPKC may be a valuable therapeutic means for protecting the myocardium from ischemia and reperfusion damage.

References


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The 2004 YIA Winner of the European Section

Structural and Electrical Integration of Engineered Heart Tissue Leads to Improved Regional Function in Infarcted Rat Hearts

It was a great honor to receive the Young Investigator Award at the 2004 Annual Meeting of the ISHR European Section in Dresden. This work would not have been possible without the excellent contributions from Ivan Melnychenko and the help from experts in cardiac imaging in Erlangen (echocardiography: Dr Wasmeier, Privatdozent Dr Nixdorff; magnetic resonance imaging: Dr Hess) and electrophysiology in Leipzig (Prof. Dhein). I am especially grateful for the guidance and advice from Prof. Scholz and Prof. Eschenhagen throughout my academic career. I received my degrees in medicine and molecular biology at the University Hospital Hamburg-Eppendorf in 1998 and 2001, respectively. After 4 years at the Institute of Experimental and Clinical Pharmacology and Toxicology at the University of Erlangen-Nuremberg, I recently returned to the University Hospital Hamburg-Eppendorf to hold a position as Juniorprofessor for Cardiac Tissue Engineering at the Institute of Experimental and Clinical Pharmacology. Our laboratory has been focusing on cardiac tissue engineering for several years and we have developed a novel technology to construct Engineered Heart Tissue (EHT) with properties of native myocardium. Consequently, we aim at utilizing EHT as an in vitro heart muscle model and for cardiac regeneration in vivo.

Cardiac Tissue Engineering: Implications

Pharmacologic treatment can slow the progression of heart failure after myocardial infarction; however, true disease reversal cannot be achieved by classical treatment regimens. For patients with end-stage heart failure heart transplantation remains the only causal treatment option. Unfortunately, donor organ shortage imposes a significant limitation to the broad application of heart transplantation. In this context, cardiac tissue engineering aims at providing alternative surrogate cardiac tissues including myocardium, valves, and vessels. In general, tissue constructs are assembled in vitro and must display key features of native heart tissues to be applicable in vivo. So far, the concept of tissue engineering based cardiac muscle repair has not been proven. Thus, our goals were first, to construct artificial heart muscle with structural and functional properties of native myocardium and second, to utilize engineered cardiac muscle constructs to repair diseased hearts in a rat model of myocardial infarction.

Construction of Engineered Heart Tissue

We constructed Engineered Heart Tissue (EHT) by mixing heart cells from neonatal rats with solubilized collagen type I, extracellular basement membrane proteins (Matrigel), and horse serum- and chick embryo extract-containing culture medium. This liquid reconstitution mixture facilitates the spontaneous organization of heart cells into a 3D heart muscle syncytium. Conditioning of EHT by mechanical loading resulted in further improvement of its contractile function and morphology (see Figure on p. 13). Essentially, EHTs gain a remarkably high degree of differentiation and display contractile properties of native myocardium. The latter includes preload-dependent force development (Frank-Starling mechanism) as well as organotypic inotropic, chronotropic, and lusitropic responses to calcium and isoprenaline stimulation.

Grafting of Engineered Heart Tissue

Given the heart muscle-like properties of EHTs in vitro, we hypothesized that EHTs could be applied as muscle grafts to replace diseased myocardium in vivo. We tested this hypothesis in a rat model of myocardial infarction. Infarctions were generated by ligation of the left descending coronary artery (LAD) in a first surgery. After two weeks, infarct size was evaluated by echocardiography to exclude animals without significantly reduced left ventricular function post LAD ligation. Subsequently, we grafted EHTs on infarcted hearts with a fractional area shortening below 40% (fractional area shortening in healthy Wolfram Zimmermann (Hamburg, Germany) was the winner of the Young Investigator Award at the XXIV European Section Meeting (Dresden, Germany; June 2-5, 2004).
Engineered Heart Tissues exhibit properties of native myocardium. A crucial step in EHT generation is mechanical loading during culture (a: phasic stretch). After 14 days in culture EHTs display structural properties of native myocardium. Cardiac myocytes in EHT organize in muscle bundles (d; HE-staining) and display histomorphological features of adult myocardium (b: neonatal myocardium; c: adult myocardium; HE-staining). This includes sarcomere organization in registry (e: confocal scanning microscopy: myomesin in green and f-actin in red). Essentially, EHTs demonstrate positive introphic responses to calcium (f; single contraction amplitudes; Ctr.: 0.4 mmol/l calcium) as well as positive inotropic and lusitropic responses to isoprenaline (g). The latter can be antagonized by carbachol indicating that important cardiac signaling mechanisms, G-protein mediated activation of the adenyl cyclase through Gs as well as its inhibition through Gi, are conserved in EHT.

Bars: 10 mm in (a) and 30 µm in (b)-(e).

controls: ~60%). Four weeks after EHT grafting or Sham surgery, cardiac function was analyzed by echo-cardiography, magnetic resonance imaging, and left heart catheterization. Notably, EHT grafting prevented left ventricular enlargement, reduced left ventricular end-diastolic pressure, enhanced systolic thickening of the infarcted anterior wall, and improved left ventricular relaxation when compared to Sham operated controls. In addition, epicardial mapping studies revealed that EHT grafts coupled electrically to the recipients’ hearts.

Conclusion and Future Perspectives

We conclude that EHT grafting can improve myocardial performance in a rat model of myocardial infarction. Naturally, primary heart cells are not available for a clinical application of our tissue engineering concept. Thus, our group is focusing on identifying a cell source that may be used for human cardiac tissue engineering and clinically relevant cardiac regeneration.

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DAVID KASS has been a Professor of Medicine and Professor of Biomedical Engineering at the Johns Hopkins University Medical Institutions since 1998. He graduated from Harvard University in 1975 where he majored in Applied Physics and Engineering, and then received his medical degree from Yale University in 1980. Following postgraduate training in Internal Medicine at George Washington University, he joined the Cardiology Division at Johns Hopkins where he has remained since.

Dr Kass’s research career began during medical school, where he joined a laboratory at Harvard to study circadian control of renal/cardiovascular reflex mechanisms, work for which he was awarded the Young Investigator Prize in Renal Physiology from the American Physiologic Society. In 1983, he joined the laboratory of the late Kiichi Sagawa at Johns Hopkins, where his research focused on ventricular mechanics and heart failure pathophysiology. He received an NIH Clinician Scientist Award in 1986 and was recipient of an AHA Established Investigator Award shortly thereafter. His pioneering work melding pressure-volume relation analysis to clinical pathophysiology investigations led to his being awarded the first Melvin Marcus Award of the American Heart Association in 1990. He has been a member of the American Society of Clinical Investigation since 1994, an editorial board member for Circulation since 1996, and Associated Editor of Circulation Research since 1999. He is past president of the Cardiovascular Systems Dynamics Society, Fellow of the AHA and member of the AHA National Research Committee. He was awarded the Professor’s Award for Distinction in Teaching for both Basic and Preclinical Sciences from Johns Hopkins University in 2001, and the Johns Hopkins Mentorship Award in 2003. He directs the Training Grant in Cardiovascular Science in the Cardiology Division at Johns Hopkins.

Throughout his career, Dr Kass has melded both clinical and basic science interests pursuing each with similar levels of commitment. He has published over 165 papers primary research papers in leading journals, and authored numerous book chapters, review articles, and invited editorial/reviews. His clinical research has focused on elucidating the pathophysiology of cardiac failure and hypertrophy, as well as testing novel treatments for these disorders. His recent landmark work on cardiac resynchronization therapy played a major role in the rapid development of this new clinical treatment. His basic research has spanned inquiries from the molecular/cellular level to intact organ-integrated systems. Current projects are investigating molecular signaling coupling load-induced hypertrophy to cyclic GMP metabolism/catabolism, endothelial mechano-signaling and the mechanisms coupling reduced compliance to increased vascular risk, molecular-cellular and organ pathophysiology of cardiac dyssynchrony and resynchronization, structure-function relations of sarcomeric protein mutations, and novel pharmacologic treatments of heart failure based on nitric oxygen-related species.

His work has been extensively supported by the National Institutes of Health, and the American Heart Association, including clinical and basic research grants. He also works closely with industry to develop novel clinical treatments and diagnostics for the treatment of heart failure and vascular aging. He is a co-founder in Robin Medical Inc., a start-up company developing novel MRI-based tracking systems for use in real-time position sensing and catheter-based therapies, and holds several patents for bioengineering-based diagnostics, and novel pharmaceuticals and/or applications. In his spare time - he looses miserably to his 8 year old son in video games, but seems to have greater success as a chamber music performer on the clarinet.
In October 2004, the ISHR International Council created a new distinguished lecture, named The President’s Lecture, which will be a highlight of ISHR World Congresses and Section meetings and reflects the continuing growth of the ISHR as a professional society.

The President’s Lecture will be held at each World Congress of the ISHR and, in non-Congress years, at the meeting of the Section to which the selected speaker belongs. For example, if the selected speaker belongs to the Japanese Section, the Lecture will be held at the Japanese meeting; if he/she belongs to the Australasian Section, it will be held at the Australasian meeting, etc. This lecture is intended to be a high profile event and will be scheduled as a keynote plenary lecture. The International Council will select the speaker.

The topic of the lecture will be in the field of molecular biology, genetics, genomics or proteomics, with a content that is of broad interest to the cardiovascular community. The speaker will be reimbursed for travel expenses, and will receive a plaque and a $1,000 honorarium. A photograph and biosketch of the speaker will be published in the Journal of Molecular and Cellular Cardiology, and in Heart News and Views, and will be posted in the ISHR website.

The President’s Lecture will enhance the content of the ISHR scientific meetings by providing a high-quality presentation that complements the areas that are covered by other themed award lectures (The Keith Reimer Distinguished Lecture, which covers the fields of ischemia, coronary hemo-dynamics, cardiac metabolism and contractile mechanisms, and The Janice Pfeffer Achievement Award, who declined to collect the monetary prize associated with the Award and requested that it be used for this purpose.

This President’s Lecture is funded by a generous donation from Roberto Bolli, MD, Winner of the ISHR 2004 Research Achievement Award, who declined to collect the monetary prize associated with the Award and requested that it be used for this purpose.

Metin Avkiran, PhD DSc
Secretary General
HEART NEWS AND VIEWS is published thanks to an educational grant from Servier

Servier supports a number of important projects in the field of cardiology, such as the Education and Training Programs of the European Society of Cardiology.

Servier is also the founding father of The European Cardiologist Journal by Fax and Dialogues in Cardiovascular Medicine, a quarterly publication with a worldwide circulation edited by Roberto Ferrari and David J. Hearse. Dialogues discusses in a comprehensive way issues from the cutting edge of basic research and clinical cardiology.

The forthcoming issue, devoted to HYPERTENSION & LEFT VENTRICULAR HYPERTROPHY will feature articles by:

E. Agabiti-Rosei and M. L. Muiesan;
A. Ganau and G. Talanas; J. Diez;
B. M. W. Schmidt and R. E. Schmieder

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HEART NEWS AND VIEWS is the official News Bulletin of the International Society for Heart Research and is published every fourth month.

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