The phenomenon of ischaemic preconditioning, in which a period of sublethal ischemia can profoundly protect the cell from subsequent lethal ischemic insult, has without doubt been responsible for an enormous amount of research over the last 15 years. Since the original publication in 1986 by Murry et al. [1] there have been in excess of 2000 publications directly attributed to this phenomenon. Furthermore it is virtually impossible to find a publication that fails to demonstrate the ability of sublethal ischemia to protect the myocardium from a lethal ischemic insult thus attesting to the power of the phenomenon of ischemic preconditioning. This early form of protection has become known as “classic or early preconditioning”. This was followed in 1993 by the first description of the “Second Window of Protection” or delayed preconditioning (sometimes referred to as late preconditioning) by Marber et al. [2] in which a second period of protection was observed 24 h after a sublethal ischemic insult. For those interested in statistics it may be noted that the original article in which this phenomenon was first described in 1986 is the most cited article that Circulation has ever published, with that describing the delayed or second window as the 15th most cited paper by that journal.

That a myocardial cell has an innate ability to protect itself from severe lethal ischemia based upon what it can “learn” from a prior sublethal ischemic insult has caught the imagination of basic and clinical scientists all over the world. What is believed to be so unique about this phenomenon is that it tells us how a cell tries to survive a severe ischemic insult. It is therefore important to try and understand the myriad of pathways that are associated with this survival mechanism. From the initial receptor that triggers or initiates the complex and elaborate signalling pathways to the incompletely understood end-effectors that finally produce the profound protection, all attest to the complexity of interrelated biological pathways. There is no doubt that since the initial discovery of preconditioning we have learnt a huge amount with regard to the cellular physiology/molecular biology that was not appreciated before.

With ischemic preconditioning, nature has shown us that cardioprotection is indeed possible. Over the last two decades there has been a concerted goal of trying to understand the mechanism of this adaptive phenomenon. If we can grasp how cells are protected, as a consequence of ischemic preconditioning, then the possibility of developing pharmacological mimics that can be used clinically to help patients at risk of having a severe myocardial infarction, will be a major development. For the first time we are gaining a real understanding of how a cell requires to protect itself from injury and based on this understanding we may be truly at the brink of developing agents capable of directly limiting cellular injury. What is amazing however is how we can know so much about this system and still be so ignorant of the fundamental mechanism as to how preconditioning actually protects the heart. That lack of understanding probably stems from our not knowing what the actual lethal event is in ischemic cell death. Research must continue until a complete understanding of the preconditioning phenomenon has been achieved.

This spotlight issue has been designed to both highlight the amazing progress that has been made, to focus on new and important discoveries that are taking place, and to spotlight the challenges that still lie ahead in this remarkably exciting time.

This spotlight issue begins with four reviews each of which highlights new areas of interest that relate to the potential mechanisms associated with ischemic preconditioning. Since the discovery that the mitochondrial \( K_{\text{ATP}} \) channel is pivotal to the mechanism of ischemic preconditioning, controversy has existed as to whether this channel is a trigger, a mediator, or the end effector of precondition-
The first of these reviews by Oldenburg et al. [3] addresses that question. Their review is followed by a focus on the importance and role of apoptosis in preconditioning by Zhao and Vinten-Johansen [4]. It is well known that necrosis plays the major role in myocyte cell death and that preconditioning clearly delays such necrotic injury, however it is only relatively recently that apoptotic cell death, as a consequence of ischemia and reperfusion, has been studied in the context of acute myocardial injury. Here the authors focus on the role of apoptosis in the extension of lethal myocyte injury and discuss the potential mechanisms involved in reducing apoptosis by ischemic preconditioning specifically in the early or classic preconditioning.

The next review by Laude et al. [6] examines the importance of gap junction mediated intercellular communication in ischemic preconditioning. Cell-to-cell communication via gap junctions can modulate cell death in different tissues. Furthermore during ischemia/reperfusion gap junction communication may promote cell-to-cell spread of hypercontracture and cell death. They discuss how intracellular signal transduction modulates communication through gap junctions and hypothesise that the closure of gap junctions could be end effectors of preconditioning contributing to its protective effect on cell death.

The next review by Garcia Dorado et al. [5] examines the importance of gap junction mediated intercellular communication in preconditioning and the vasculature. This underinvestigated area is discussed in relation to both early and delayed preconditioning and they highlight the potentially important role of the endothelium. They also discuss mediators of endothelial protection such as adenosine, bradykinin, nitric oxide and free radicals. Furthermore the importance of protein kinase C signalling and the role of the K$_{ATP}$ channel are also discussed.

Smith et al. [7] review new work that has begun to explore the role of innate immune systems in intrinsic cardioprotection. They discuss the role of cytokines such as TNF alpha and leukemia inhibitory factor and how they have been shown to mimic ischemic preconditioning. As the immune system functions via a diverse array of non-G protein-coupled receptors, the study of this immunological system in the heart may provide new insight into mechanisms driving and promoting ischemic preconditioning.

The next three reviews provide extensive discussion of the potential mechanisms associated with the second window of protection. Baxter [8] highlights the importance of adenosine as a trigger of this form of protection and discusses the temporal nature of second window of protection. Further discussion on the ability of adenosine receptor agonists to activate complex protein kinase signalling cascades with subsequent activation of gene transcription and post-translational regulation of several proteins including mitochondrial manganese superoxide dismutase and the 27 kDa heat shock protein ensues. This finally leads to a discussion of the K$_{ATP}$ channel as a potential end effector of the second window.

Hoshida et al. [9] then proceed to provide extensive evidence for the role of manganese superoxide dismutase (MnSOD) in delayed preconditioning, initially discussing the time course of induction followed by the many experiments used to demonstrate the importance of MnSOD. Finally they discuss how cytokines and reactive oxygen species are involved in the signal transduction leading to the upregulation of this protein in the preconditioned heart.

Bolli et al. [10] review the importance of cyclooxygenase-2 (COX-2) as a cardioprotective protein and how it could mediate the second window. Extensive studies in their laboratory recently demonstrated an obligatory role of COX-2 in the second window of protection produced not only by ischemia but also as a consequence of δ opioid agonists and exercise which helps to support the view that this protein may play a central mechanistic role. They argue that their results challenge the widely held view that COX-2 activity is purely detrimental and that the clinical use of COX-2 inhibitors in cardiac patients needs to be examined.

The final two invited reviews deal specifically with therapeutic targets and preconditioning in the clinical arena. Fryer et al. [11] discuss the former and focus on target receptors that have been shown to have potential to mimic the cardioprotective effect of ischemic preconditioning. They discuss the evidence supporting the possibility of manipulating several of the G protein-coupled receptors as potential therapeutic targets including, among others, the adenosine A1, the bradykinin B2 and the δ opioid receptors. Kloner et al. [12] take this further in the clinical setting by reviewing the clinical models and scenarios in which conventional ischemic preconditioning and/or pharmacological preconditioning mimetics have shown promise in eliciting cardioprotection. They also outline for each of the various clinical settings a proposed design for the multicenter trials that will be needed to prospectively test the clinical utility of preconditioning-based interventions.

The series of original research contributions which follows the reviews reveals an abundance of interesting and exciting studies which this fascinating area continues to spawn. The first by Hausenloy et al. [13] describes a new paradigm for preconditioning which for the first time implicates the mitochondrial transition pore in the mechanism of the protection observed. Oldenburg et al. [14] describe how one preconditioning mimetic, acetylcholine, induces free radical generation involving a muscarinic surface receptor, a pertussis toxin-sensitive G protein, PI3 kinase, at least one tyrosine kinase and a 5HD-dependant K$_{ATP}$ channel. Smith et al. [15] demonstrate that classic, but not pharmacological preconditioning, is abrogated following genetic ablation of the TNFa gene suggesting that diverse signalling pathways converge at the level of mitochondrial K$_{ATP}$ channel activation to mediate cardiop-
rotection. There follows a study by Domenech et al. [16] examining the role of exercise to induce both early and delayed preconditioning which appears to be mediated through the mitochondrial K\textsubscript{ATP} channel. The mitochondrial K\textsubscript{ATP} channel is again implicated, this time in a high altitude hypoxia study in rats, by Neckar et al. [17].

The next three articles investigate the role of what has been termed remote preconditioning and its ability to protect the myocardium. The first of these by Wang et al. [18] use rats in which the mesenteric artery was occluded for 25 min prior to subjecting the myocardium to a lethal ischemic insult. They demonstrate protection that was correlated with both protein kinase C and the mitochondrial K\textsubscript{ATP}. Wolfrum et al. [19] in a similar model relate remote preconditioning protection to the role of protein kinase C epsilon. Weinbrenner et al. [20] also undertake a study investigating remote preconditioning and try to ascertain whether it is transduced by either neuronal or humoral factors in an in situ model of infrarenal occlusion of the aorta in rats.

Strohm et al. [21] use actinomycin-D a known RNA-synthesis inhibitor to investigate whether a modification of transcriptional events are related to the cardioprotection observed using the pig heart. Joyeux-Faure et al. [22] investigate the involvement of endocannabinoids in the infarct size reducing effect conferred by heat stress preconditioning in isolated rat hearts. This is then followed by a study by Gres et al. [23] investigating the involvement of prostaglandins in preconditioning. Two studies follow in which sheep are used to study the mechanisms associated with preconditioning. Firstly Tanoue et al. [24] examine ventricular energetics in an in vivo sheep model and then Del Valle et al. [25] use a conscious diabetic sheep model to demonstrate that preconditioning can protect against stunning in normal sheep but not in diabetics. The next report is an evaluation of the role of the chloride channel by Batthish et al. [26] with the conclusion that they are downstream effectors of preconditioning.

The final series of three papers all relate to the importance of the signalling pathway in preconditioning in the mouse, rat and pig, respectively. In the mouse Saurin et al. [27] using targeted disruption of the protein kinase C epsilon gene demonstrate the importance of this isoform for the reduction of infarction but not functional recovery. Using the rat heart Yue et al. [28] implicate both reactive oxygen species generation and p38MAPK in preconditioning’s protection and that the mitochondrial K\textsubscript{ATP} channel is upstream of mitochondrial ROS generation. Finally Schultz et al. [29], using the anaesthetized pig, provide evidence to demonstrate that p38MAPK is a mediator of ischemic preconditioning in this species.

The reviews and original articles that comprise this spotlight issue highlight the enormous and ongoing wealth of information that is forthcoming, all as a consequence of single pivotal paper published in 1986. It has been a pleasure for us to edit this focused edition. Let us point out that all the original articles were reviewed by independent reviewers arranged by the editorial staff at Cardiovascular Research as is normally the case. The journal staff also made the final acceptance decisions. We remain optimistic that the knowledge obtained from the many outstanding laboratories and clinics around the world will eventually result in a thorough understanding of the preconditioning phenomenon. That knowledge will without doubt directly contribute to improved clinical care and decreased mortality from coronary artery disease.

References


