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ATP-Sensitive Potassium Channels: A Review of their Cardioprotective Pharmacology

Gary J. Grover[1] and Keith D. Garlid[2]

[1] *Cardiovascular and Metabolic Diseases Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, N.J. 08543-4000, USA;* [2] *Department of Biochemistry, and Molecular Biology, Oregon Graduate Institute of Science and Technology, 200000 NW Walker Road, Beaverton, Oregon 97006-8291*

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G. J. Grover and K. A. Garlid. ATP-Sensitive Potassium Channels: A Review of their Cardioprotective Pharmacology. *Journal of Molecular and Cellular Cardiology* (2000) **32**, 677–696. ATP-sensitive potassium channels (K_{ATP}) have been thought to be a mediator of cardioprotection for the last ten years. Significant progress has been made in learning the pharmacology of this channel as well as its molecular regulation with regard to cardioprotection. K_{ATP} openers as a class protect ischemic/reperfused myocardium and appear to do so by conservation of energy. The reduced rate of ATP hydrolysis during ischemia exerted by these openers is not due to a cardioplegic effect and is independent of action potential shortening. Compounds have been synthesized which retain the cardioprotective effects of first generation K_{ATP} openers, but are devoid of vasodilator and cardiac sarcolemmal potassium outward currents. These results suggest receptor or channel subtypes. Recent pharmacologic and molecular biology studies suggest the activation of mitochondrial K_{ATP} as the relevant cardioprotective site. Implications of these results for future drug discovery and preconditioning are discussed.

Key Words: ATP-sensitive potassium channels; Mitochondria; Heart; Myocardial Ischemia, Preconditioning.

Introduction

Recently several lines of investigation concerning ATP-sensitive potassium channels (K_{ATP}) have converged, suggesting that K_{ATP} may be an important therapeutic target for the treatment of acute myocardial ischemia. Pharmacologic studies starting in 1989 showed K_{ATP} openers to exert profound cardioprotective effects in numerous mammalian species. The results of this work were made even more relevant by later findings showing preconditioning to be mediated (at least in part) by K_{ATP} activation. Therefore, pharmacologic K_{ATP} activation would be expected to mimic an endogenous cardioprotective mechanism that also seems to be operative in man. These converging results have created enthusiasm for further studying the therapeutic potential of K_{ATP} for the treatment of acute myocardial ischemia.

Recent studies have further fueled interest in K_{ATP} by suggesting differential importance of K_{ATP} subtypes. These studies show that cardioprotection may not be mediated by sarcolemmal K_{ATP} currents, suggesting an intracellular site of action (mitochondria). Therefore, it is possible to develop agents which can selectively open the K_{ATP} subtype of interest.

With the heightened interest created by the studies described above, it would be useful to review what is presently known about K_{ATP}, particularly the recent insights into the molecular biology and regulation of these channels and how these findings

Please address all correspondence to: Gary J. Grover, Ph.D., Metabolic Diseases Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, USA. Phone: +609 252-5139; Fax: +609 252-6609.

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can help us understand their cardioprotective pharmacology. We will place some emphasis on the newly hypothesized role of mitochondrial channels in cardioprotection. In this review, our heightened knowledge of the nature of the molecular regulation of K_{ATP} will be integrated with pharmacologic and physiologic studies so that the reader can better understand the complex matrix of studies leading to our preset understanding of the role of K_{ATP} and pharmacologic modulators of this channel in myocardial ischemia.

Properties and Molecular Biology of Plasma Membrane K_{ATP}

K_{ATP} were first described by Noma[1] in cardiac ventricular myocytes. These channels are of intermediate conductance and are inhibited by physiologic concentrations of ATP. K_{ATP} were originally termed ATP-dependent potassium channels because ATP was the first modulator studied, although other endogenous modulators have since been found and therefore they are now referred to as "ATP-sensitive" channels. K_{ATP} are inhibited by physiologic levels of ATP and, as ATP falls, channel open probability increases (although the degree of ATP reduction needed would rarely be seen under physiologic conditions; see review by Edwards and Weston[2]). K_{ATP} have been found to be modulated by pH, fatty acids, NO, SH-redox state, various nucleotides, G-proteins and various ligands (adenosine, acetylcholine, benzopyrans, cyanoguanidines, etc.).[2–6] Larsson *et al.*[7] showed that pancreatic K_{ATP} are opened by long-chain acyl CoA esters, and Paucek and Garlid (unpublished results) have observed similar opening of cardiac sarcolemmal channels by acyl CoA esters. K_{ATP} appear to be linked to the metabolic state of the cell and have therefore also been labeled as metabolicaly regulated channels. K_{ATP} are expressed in numerous tissue types including skeletal muscle, brain, kidney, heart, pancreatic β-cells, and smooth muscle.[1,2,8–10] K_{ATP} are thought to serve as a link between metabolism and either secretory activity (insulin, brain) or electro-mechanical coupling in muscle. K_{ATP} are also found in the inner mitochondrial membrane, and these will be described in a later section.

In the case of pancreatic β-cells, insulin secretion is controlled by glucose metabolism. K_{ATP} opening inhibits secretion due to hyperpolarization while reduction of channel activity will increase insulin secretion (see review by Edwards and Weston[2]). Pharmacologic openers and blockers of K_{ATP} will hyperpolarize or depolarize pancreatic β-cells respectively.[11,12] These channels are important for setting membrane potential in pancreatic β-cells and the closure of a few channels (as seen by an increase in extracellular glucose) will depolarize these cells and therefore stimulate insulin secretion.[13,14] Sulfonylurea K_{ATP} blockers such as glibenclamide (glyburide) have utility for treating type II diabetes and increase insulin secretion by depolarizing β-cell membranes. Opening of K_{ATP} in smooth muscle cells would be expected to hyperpolarize sarcolemmal membranes and therefore cause relaxation.[15] Most K_{ATP} openers are potent vasodilators and smooth muscle relaxants and their earliest proposed clinical uses were asthma, hypertension, and urinary incontinence. The role of K_{ATP} in normal myocardium is presently unclear, although a role in ischemic conditions is well known and will be discussed in full detail in this review. Opening cardiac K_{ATP} will, of course, enhance a repolarizing potassium current and therefore cause action potential duration (APD) shortening.[16] K_{ATP} are thought to be involved with the early repolarization seen in ischemic cardiac tissue.[17–19] This early repolarization is associated with the "injury current" of ischemia which is the basis for ST-segment shifts and it is thought that K_{ATP} contribute, at least in part, to this electrophyiologic abnormality.[17,20]

K_{ATP} is a complex of two different proteins.[21–23] One subunit is an inwardly-rectifying potassium channel (Kir) subunit and it is thought that four of these combine to form the channel pore. Two types of Kir (Kir6.1 and Kir6.2) are thought to be associated with K_{ATP} at this time. The sulfonylurea receptor (SUR) is the protein which confers a regulatory role as well as sensitivity of the channel to pharmacologic agents and ATP.[22] SUR is a member of the ATP-binding cassette protein superfamily, also called ABC transporters and is related to CFTR channels (cystic fibrosis transmembrane regulator, also glyburide inhibitable).[24] This ATP transporter, CFTR, releases ATP, which interacts with a purinergic receptor subtype which then opens chloride channels. SUR is a related ATP permeant protein. SUR1 is highly expressed in pancreatic β-cells, while SUR2 is highly expressed in cardiac and skeletal muscle cells.[25] It is unknown how many ways these different Kirs and SURs can interact, but data suggest different combinations in different tissue types. Currently, it is thought that Kir6.2 and SUR2 form cardiac sarcolemmal K_{ATP}.[23] In the study by Inagaki *et al.*,[23] diazoxide did not activate the sarcolemmal cardiac channel while it did open pancreatic K_{ATP}, which is thought to be formed by

Kir6.2 and SUR1.[21] This finding suggests the exciting possibility of tissue selectivity, which has already been strongly suggested on the basis of pharmacologic evidence. The excellent agreement of the pharmacology and molecular biologic data on K_{ATP} (particularly with regard to the pharmacology of diazoxide) will be discussed later in this review.

Much still needs to be learned about the molecular regulation of K_{ATP}, altough we will briefly review what is currently known. ATP inhibits K_{ATP} and this inhibition does not require phosphorylation. Inhibition of K_{ATP} is produced not only by intracellular ATP, but its non-hydrolyzable analogs, suggesting phosphorylation is not critical.[2] ADP reduces the sensitivity of K_{ATP} for ATP and therefore it has been proposed that the channel is modulated by the ratio of these nucleotides. It is thought that ADP competes for the ATP binding site and ADP without the presence of ATP inhibits channel activity, although another ADP site is thought to mediate weak agonist activity. This may be a nucleotide phosphate site which is also stimulated by GDP.

It has been proposed that K_{ATP} openers reduce the affinity of ATP at the regulatory or inhibitory site. In addition to an inhibitory action of ATP, K_{ATP} will "run-down" in the absence of ATP and it is thought that ATP can prime K_{ATP} for opening.[26] Only MgATP (but not free ATP) can reactivate the channel and non-hydrolyzable forms of ATP are ineffective. This is thought to require phosphorylation and therefore, channel "run-down" may be mediated by channel dephosphorylation. It has been proposed that a complex balance of phosphorylation and dephosphorylation of tyrosine and serine/threonine residues can modulate K_{ATP} activity, although much needs to be learned about this regulatory mechanism.[27] It is presently unclear whether dephosphorylation and phosphorylation are involved with the mechanism of run-down and reactivation by MgATP (see review by Hiraoka and Furukawa[28]).

Occupation of the appropriate receptors associated with G-proteins releases GDP and enhances GTP binding. A number of ligands known to interact with G_i increase K_{ATP} open probability and include meditors such as acetylcholine and adenosine. Ligands such as adenosine (through the A_1 receptor subtype) may also activate protein kinase C (PKC) and it is thought that PKC activates K_{ATP} in ventricular myocytes by reducing channel sensitivity to ATP at the inhibitory site.[29] Protein kinase A (PKA) may modulate K_{ATP} activity and data suggest it is involved with K_{ATP} opening induced by calcitonin gene-related peptide, adenosine (adenosine A_2 receptor subtype), prostacyclin and β-adrenoceptor agonists,[6,30,31] although some of these effects could be due to indirect effects of these various agonists.[32] Recent data[33] show that extracellular ATP can enhance K_{ATP} current via a P_2 receptor, through activation of adenylyl cyclase, although this is independent of PKA. It is presently unknown how these data relate to the concept of a secretory role for SUR. While we have listed the potential for PKA or PKC to modulate K_{ATP} activity, this issue is still speculative and not all investigators can show similar actions for these protein kinases.[34]

K_{ATP} openers are compounds of diverse chemotypes that can open this channel in various tissues (see review by Hiraoka and Furukawa[28]) (see structures in Fig. 1). The activation of K_{ATP} is inhibited by increased ATP suggesting an interaction or competition at the ATP binding (inhibitory) site. The K_{ATP} activating effects of the pharmacologic openers are inhibited by glyburide, although this inhibition does not appear to be through displacement from a common receptor site. The effects of openers and blockers of K_{ATP} appear to be modulated by the metabolic state of the cell, with openers being more active under ischemic conditions and blockers such as glyburide being less active during ischemia.[19,35] Recent studies show tissue differences in the activity of K_{ATP} openers and blockers, suggesting K_{ATP} subtypes which is consistent with the recent findings on the molecular biology of these channels.[36,37]

Therefore, the signaling systems involved with K_{ATP} modulation appear complex and as yet, a clear picture has not been elucidated. We have included a schematic diagram for proposed modulatory pathways for K_{ATP} (Fig. 2). This diagram is to be considered speculative, as not all investigators agree on some of the proposed pathways.

Effects of Pharmacologic Modulators of K_{ATP} on Myocardial Ischemia

Because of the metabolic regulation of K_{ATP} and its possible contribution to ischemic injury currents, it was natural to presume a role for this channel in the pathogenesis of myocardial ischemia. A good means for determination of the role of K_{ATP} in ischemia was to use selective openers and blockers of this channel in various models of ischemia. Before detailed studies in models of ischemia were completed, two schools of thought existed. In one school, K_{ATP} blockers would be protective, not only in terms of cardioprotection, but also in terms of

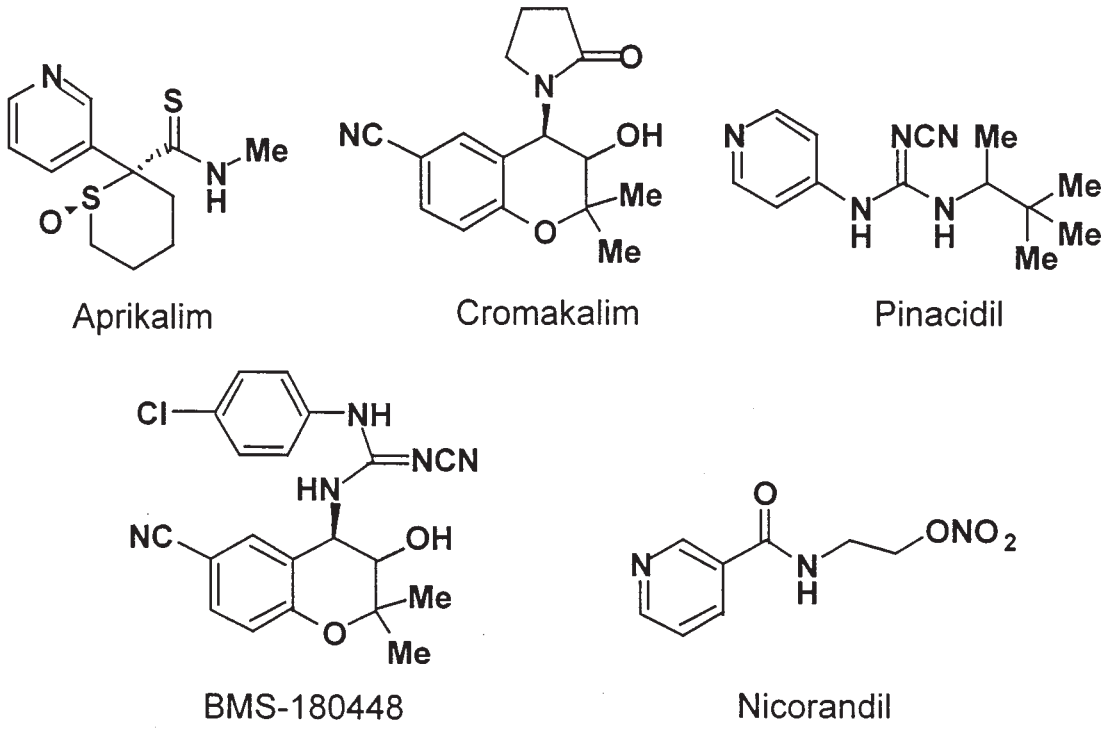

Figure 1 Chemical structures of the major classes of K_{ATP} openers.

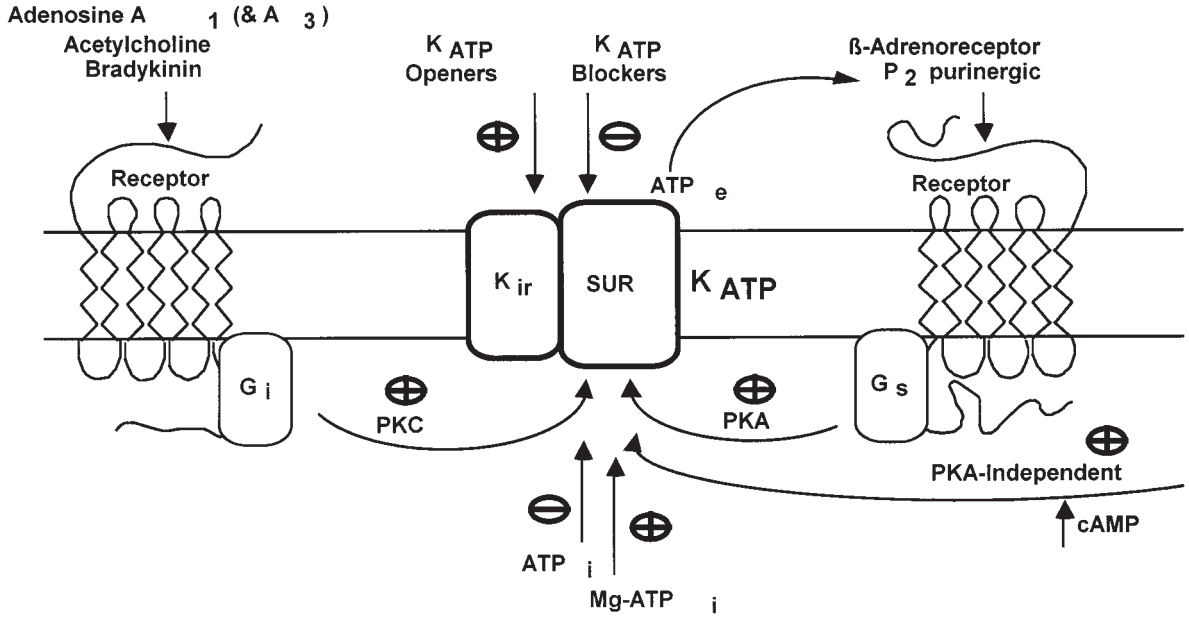

Figure 2 Scheme of proposed control mechanisms for plasma membrane K_{ATP}. Some of these proposed pathways are speculative and for some there are conflicting data (see text).

inhibition of arrhythmogenesis. Another school of thought was that K_{ATP} openers would be protective because of their ability to shorten APD (and also inhibit ischemic depolarization) and therefore reduce calcium entry. This hypothesis presumed that K_{ATP} openers would exert a cardioplegic action and protect by inhibiting energy utilization and contractile function in a manner similar to L-type calcium channel blockers. This hypothesis led many scientists to label K_{ATP} openers as "indirect calcium

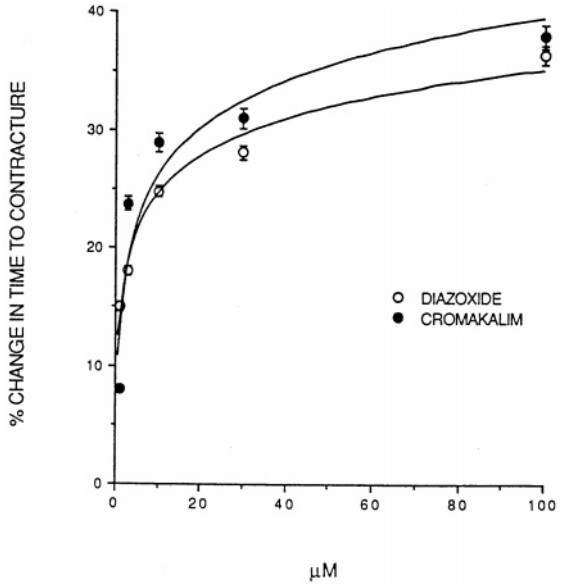

Figure 3 Effect of increasing concentrations of cromakalim or diazoxide on the time to onset of contracture during global ischemia in isolated rat hearts. Time to contracture is defined as the time (min) during ischemia in which a 5 mmHg increase in end diastolic pressure is observed. Figure taken from Garlid *et al.* (1997) with permission. ○ Diazoxide; ● Cromakalim.

channel blockers". As we will see, this hypothesis was inadequate to explain the cardioprotective effects of K_{ATP} openers.

The idea that K_{ATP} might be involved in ischemia was first suggested by studies on nicorandil although the role of K_{ATP} in the protective effects of nicorandil was not fully appreciated at the time (this compound is also an NO donor).[38] As more information on K_{ATP} became available, we wanted to examine the role of K_{ATP} in myocardial ischemia in more detail using more selective pharmacologic agents. When the idea of testing K_{ATP} openers in models of myocardial ischemia was suggested, we had to decide upon the best initial model to test these compounds. While these agents opened cardiac K_{ATP}, they were more potent as vasodilators and we realized that interpretation of the data from systemically treated animals could be difficult. We therefore determined the effect of K_{ATP} openers in an isolated rat heart model of ischemia and reperfusion in which direct cardioprotective activity could be ascertained. We first tested pinacidil and cromakalim and found them both to protect isolated rat hearts subjected to 25 min global ischemia followed by 30 min reperfusion.[39] Protection was denoted by increased time to ischemic contracture, improved post-ischemic recovery of contractile function, reduced reperfusion contracture, and improved reperfusion flow. Figure 3 shows the effect of cromakalim on the time to ischemic contracture in isolated rat hearts. The improved reperfusion flow observed for some openers was not necessary for protection and was most likely secondary to increased metabolic demand in the healthier tissue. In this study, we also found that ischemic depolarization was inhibited by K_{ATP} openers, although it was difficult to know whether this was the mechanism of protection or a result of protection occuring before ischemic depolarization. Since the publication of these results, we have found levcromakalim (showing cardioprotection to be stereoselective), bimakalim, aprikalim, and P-1075 to protect isolated rat hearts with a similar profile of action.[40–42] Interestingly, these agents were protecting hearts at concentrations that did not exert significant negative inotropic effects, therefore distinguishing these agents from calcium channel blockers.

Cole *et al.*[35] published a paper using a perfused guinea-pig ventricle preparation. They found that a high concentration of pinacidil (10 μM) exerted negative inotropic effects and shortened APD, while it enhanced post-ischemic recovery of contractile function. This study was important in further showing cardioprotective effects for a K_{ATP} opener, but also suggested an association between APD shortening and cardioprotection. These data, however, could not definitively prove a connection between cardioprotection and APD shortening. At this concentration (10 μM), pinacidil was certainly opening sarcolemmal K_{ATP}. Also of interest in this study was their finding that pinacidil was more effective in shortening APD during ischemia in myocardium, a finding we were able to confirm later in an *in vivo* model of ischemia in dogs.[16]

Since these early studies, numerous investigators have shown cardioprotective effects for K_{ATP} openers in isolated heart models from several species such as rats, rabbits, ferrets, and guinea-pigs.[44–48] The profile of action of these compounds was similar and in general the cardioprotective concentrations of these compounds were between 300 nM and 10 μM. The cardioprotective effects observed for K_{ATP} openers have been observed as increased post-ischemic functional recovery, increased time to electrical uncoupling, and improved energy status, and reduced necrosis (enzymatic and histologically determined).[35,46,48,49] The study by Tan *et al.*[48] showed that guinea-pig papillary muscles had significantly increased times to electrical uncoupling during hypoxia suggesting protection of gap junctions. Recent studies from Ganote's laboratory have shown K_{ATP} openers to protect hypoxic/reperfused rabbit ventricular myocytes.[50] This study showed that at least

some of the cardioprotective effects of K_{ATP} openers are exerted at the level of myocytes. Liang[51] has also shown K_{ATP} openers to protect isolated chick myocytes. K_{ATP} openers also appear to have some efficacy in human tissue based on studies by Speechly-Dick *et al.*[52] These investigators showed that cromakalim protected hypoxic/reoxygenated human atrial trabecula, suggesting K_{ATP} to be a clinically relevant pharmacologic target.

Results with K_{ATP} openers in models of myocardial ischemia *in vivo* have been somewhat more variable. This may be due to the fact that most of the K_{ATP} openers used in these studies were not selective for the heart and such activities (including hypotension) could have clouded interpretation of the data. Nevertheless, there have also been numerous *in vivo* studies showing K_{ATP} openers to be cardioprotective. Early into our investigations, we found it difficult to achieve cardioprotection *in vivo* with K_{ATP} openers when given systemically because of profound hypotension. Therefore, we found it necessary to administer the K_{ATP} openers directly into the coronary arteries of dogs in order to observe cardioprotection. We found that both cromakalim and pinacidil significantly reduced infarct size in dogs.[40] Gross's laboratory showed that aprikalim could be given systemically in dogs and a reduction in post-ischemic stunning observed, a result that was confirmed by our laboratory using cromakalim (except it was given into the coronary arterial circulation).[16,53] Later studies by several laboratories have shown K_{ATP} openers to reduce infarct size in diverse species such as dogs, pigs, and rabbits.[54–57] It should be stated that several investigators have shown K_{ATP} openers not to protect ischemic myocardium *in vivo* and it is presently still difficult to reconcile these results.[58,59]

Role of K_{ATP} in Preconditioning

Endogenous protective mechanisms exist in many tissue types. Murry *et al.*[60] described a phenomenon in hearts in which a short bout of ischemia will protect a heart from a subsequent ischemic episode of more prolonged duration. Preconditioning exists in many mammalian species and is found by nearly all investigators. It is not known whether this protective effect evolved specifically to increase resistance to ischemia, or whether protection is a beneficial consequence of triggering a normal physiological response. While all investigators agree on the profound protection conferred by preconditioning, there is less agreement on the molecular mechanism of protection.

Most of the studies aimed at elucidating the mechanism of action of preconditioning have used a pharmacologic approach. This has led to a bewildering array of suggested receptors, signaling pathways and metabolic mechanisms.[61–63] The multiplicity of biochemical systems involved with preconditioning makes it difficult to understand how these various pathways fit together.

A useful organization of these findings divides the process into three components.[64,65] (1) *Triggers* are metabolites and receptor agonists that are released locally during the preconditioning ischemia. These include adenosine, acetylcholine, bradykinin, catecholamines, angiotensin II, and opioids. Receptor activation triggers a cascade that ultimately activates mediators of preconditioning. (2) *Mediators* include, most notably, protein kinase C,[14,66,67] but other kinases may also participate, including tyrosine protein kinase[68] and mitogen-activated protein kinases.[69] There is evidence that a threshold quantity of various triggers must be achieved in order for protein kinase C activation to be sufficient for cardioprotection.[64] The proteins that are phosphorylated have not been identified, but they presumably reside within the final common pathway of protection and may include the end effector itself. (3) *The end effector* of preconditioning is also unknown, but there is a general consensus that this may be the K_{ATP} channel. In view of the results of Garlid *et al.*,[37] mitoK_{ATP} may play this role or may at least be downstream of the previously mentioned mediators (it is currently unknown how mitoK_{ATP} affects protection). In keeping with the scope of this review, we will limit further discussion to aspects involving K_{ATP}.

The studies showing K_{ATP} openers to *mimic* preconditioning, which have already been described, are consistent with the notion that K_{ATP} is crucial to preconditioning, but does not prove it. Further proof required the use of pharmacologic blockers of this channel. Before describing some of this work, it is worthwhile to point out one interesting study showing a role for K_{ATP} in preconditioning using an agonist. Yao and Gross[70] showed that the K_{ATP} opener, bimakalim, significantly lowered the threshold for preconditioning in a canine model of infarction. This was done using a subthreshold preconditioning protocol combined with a subthreshold dose of bimakalim. This clever study strongly suggested that K_{ATP} openers protect ischemic tissue in a manner which is similar to preconditioning.

Gross's laboratory was the first to demonstrate that K_{ATP} blockers abolish preconditioning using glyburide in a canine model.[71] The structurally

distinct blocker 5-HD was also shown to abolish preconditioning in a similar model.[72] K_{ATP} blockers have been shown to abolish preconditioning in rabbits, rats, pigs, and man.[73] The ability of K_{ATP} blockers to abolish preconditioning in rats has been variable and may be model dependent. Thus, neither 5-HD or glyburide abolished preconditioning in isolated rat heart;[73] however, recent studies showed K_{ATP} blockers to abolish preconditioning in rat hearts *in vivo*.[74,75] In addition to animal studies, preconditioning has also been shown to exist in human tissue. Tomai *et al.*[76] showed that glyburide abolished preconditioning in human subjects. They were preconditioned with short periods of ischemia using coronary balloon occlusion and severity of ischemia was deteremined using ST-segment shifts and severity of chest pain. Glyburide abolished this protective effect. These results were confirmed by Yellon's group, showing that human atrial trabelcula could be preconditioned with short durations of hypoxia and that glyburide abolished this protective effect.[52]

It was of great interest that blockers of adenosine A_1 receptors and K_{ATP} completely abolished preconditioning in similar animal models, when given alone. It was difficult to reconcile these results unless these two systems were linked. Studies by Kirsch *et al.*[4] in neonatal rat cardiac myocytes, showed that adenosine A_1 receptor activation also activated K_{ATP} through a G_i coupled pathway. This study prompted us to determine the effect of glyburide on the cardioprotective effect of an adenosine A_1 receptor agonist, R-PIA.[77] We found that R-PIA reduced infarct size in dogs and this effect was completely abolished by glyburide, suggesting adenosine receptor activation to be "upstream" of K_{ATP} activation. Similar studies were performed in pigs showing 5-HD to abolish the protective effect of R-PIA.[78] The cardioprotective effects of adenosine have also been shown to be abolished by glyburide in several species.[79,80] A recent study by Cleveland *et al.*[81] showed that adenosine could protect hypoxic/reoxygenated human atrial trabecula and this effect was abolished by glyburide, suggesting this pathway to be operative in man. Interestingly, rat cerebral preconditioning was abolished by glyburide,[82] suggesting that this phenomenon is important in tissues other than heart. In this study, K_{ATP}-mediated protection appeared to be preceded and activated by adenosine A_1 receptor stimulation. A recent study by Ford *et al.*[83] showed that glyburide failed to abolish the protective effects of adenosine A_1 activation, although rat hearts may not be predictive of human tissue in this regard.

It is important to note that several investigators have suggested that K_{ATP} may be involved with activation of adenosine A_1 receptors. It has been suggested that K_{ATP} openers are protecting ischemic myocardium by increasing adenosine production secondary to activation of ectosolic 5′-nucleotidase.[84] A study in hypoxic rabbit ventricular myocytes showed that the K_{ATP} opener pinacidil was protective and this effect was abolished by the adenosine receptor antagonists SPT and DPCPX.[50] A recent collaborative study between our group and Grosss group showed that the protective effect of bimakalim in dogs was not abolished by DPCPX, suggesting that this "reverse" pathway is not operative in this model.[85] It is difficult to reconcile these differences at the present time.

If one assumes that adenosine receptor activation is "upstream" of K_{ATP} activation during preconditioning, then it becomes important to determine the signaling pathway linking the two systems. Adenosine A_1 receptor stimulation inhibits PKA and can activate PKC, both of which are known to be involved with K_{ATP} gating. PKC activation seems to be involved in the mechanism of preconditioning in several species. PKC has been shown to be involved with activation of sarcolemmal K_{ATP} in patch clamp studies and PKC activation protects ischemic myocardium in a glyburide-reversible manner.[29,52] PKC activation appears to phosphorylate a protein associated with K_{ATP}, resulting in activation. It should be remembered that the work relating potassium currents to PKC activation was done using sarcolemmal channels, and the relevance to the pertinent K_{ATP} (mitochondrial?) is not presently clear. This will be discussed in more detail later in this review.

Finally, it is constructive to review briefly the phenomenon of calcium preconditioning as described by Meldrum *et al.*[86,87] and Miyawaki and Ashraf.[88,89] In calcium preconditioning, a transient increase in intracellular calcium preconditioned rat heart against subsequent global ischemia, and this cardioprotection is associated with activation of protein kinase C.[86,87,90] In a careful study, Kouchi *et al.*[91] have shown K_{ATP} to be a common target of both ischemic and calcium preconditioning. Significantly, these authors showed that glyburide blocked cardioprotection when administered 30 min before preconditioning but not when given 5 min after preconditioning. These data are consistent with data reported previously in rats *in vivo*.[92] The time dependence of glyburide blockade is consistent with the necessity to interact with an intracellular receptor, and emphasizes the importance of consdiering pharmacokinetics in such studies.

Physiologic and Molecular Mechanism of Cardioprotection: Are Sarcolemmal K_{ATP} Involved?

While there appears to be general agreement that K_{ATP} openers are cardioprotective, much less is known about their mechanism of action. Current studies are shedding some light on this subject and we will briefly review what is known. While similar stereoselective cardioprotective activity observed for structurally distinct K_{ATP} openers strongly suggested an involvement of K_{ATP}, further proof was required. Two structurally distinct K_{ATP} blockers, sodium 5-hydroxydecanoate (5-HD) and glyburide were available to further test the hypothesis that K_{ATP} are important in mediating cardioprotection. Both agents have been universally shown to abolish the cardioprotective effects of K_{ATP} openers.[46] In addition, glyburide abolishes the pre-ischemic coronary vasodilator effects of K_{ATP} openers.[93] 5-HD seems to be more selective and was originally thought to be specific for inhibiting the cardioprotective effects of K_{ATP} openers and will not abolish the effects of K_{ATP} openers on non-ischemic cardiac and vascular tissue.[93] This will be discussed in more detail later. It is important to note that most investigators find that K_{ATP} blockers (in reasonable doses) do not further aggravate severity of ischemia, suggesting that K_{ATP} are not sufficiently open during ischemia to contribute to protection, unless the tissue has been preconditioned.[46,71,78] Blockers such as glyburide and 5-HD appear to be specific in blocking the cardioprotective activity of K_{ATP} openers and have been shown to be without effect on cardioprotective agents such as calcium blockers, sodium blockers, Na^+/H^+ exchange inhibitors, and calmodulin inhibitors.[94] These data strongly suggest that K_{ATP} are involved with the protective mechanism observed for K_{ATP} openers. Assuming that K_{ATP} activation is important, it is still unknown how this could confer physiologic protection of ischemic or reperfused myocardium. We will now discuss what is known about the physiologic mechanism of action for cardioprotection for this class of agents.

One question that arose was whether K_{ATP} openers protected during ischemia *per se* or during reperfusion. An increase in the time to onset of contracture during ischemia strongly suggested an effect of K_{ATP} openers during ischemia (see Fig. 3).[46] Pharmacologic studies have shown that K_{ATP} openers work best when given well before the ischemic event, although this does not prove an effect during ischemia.[40] In our hands, most K_{ATP} openers have weak (or non-existent) protective effects when given during reperfusion, but it is possible that insufficient time for adequate drug penetration was allowed in these studies of rapid reperfusion. An interesting study by Gross's laboratory did show significant protection to be observed in a canine infarct size model when bimakalim was given during reperfusion.[95] When we administered a highly amphiphilic K_{ATP} opener, BMS-180448, only during reperfusion, we did show a weak protective effect in rats and ferrets, although bimakalim and cromakalim were without effect.[96] BMS-180448 has been shown to penetrate ischemic tissue more efficiently than some other K_{ATP} openers.[97] Therefore, it appears as if most of the protective effects of K_{ATP} openers occurs during ischemia, although some effects on reperfusion injury cannot be ruled out at the present time.

Most K_{ATP} openers are potent vasodilators and one possible mechanism for their protection was coronary dilation, although this was thought to be unlikely. We showed that the protective effect of aprikalim was still observed even when coronary flow was held constant in isolated rat hearts.[42] *In vivo*, several investigators showed that cardioprotective doses of K_{ATP} openers had no effect on coronary collateral blood flow, although they did enhance reperfusion blood flow.[40,54] The studies by Armstrong *et al.*[50] showed pinacidil to protect isolated cardiac myocytes, which further shows a lack of importance of coronary flow changes. Later development of K_{ATP} openers devoid of vasodilating activity confirmed these earlier results.[36] 5-HD will completely abolish the cardioprotective effects of K_{ATP} openers while being completely devoid of effects on their coronary dilator activity, further proof for a lack of importance for coronary dilator activity in mediating protective effects.[93]

The data showing K_{ATP} openers to increase the time to the onset of ischemic contrcture suggested conservation of ATP during ischemia. Studies from our laboratory[41] and McPherson *et al.*[98] showed K_{ATP} openers to significantly conserve myocardial ATP during ischemia. The degree of ATP conservation was comparable to that seen for calcium channel blockers, although K_{ATP} openers caused little negative inotropic effects, at least in our hands. K_{ATP} openers also enhanced post-ischemic recovery of ATP[99] suggesting that mitochondria are protected and electron microscopy showed this to be true.[49] Efficiency of oxygen utilization was significantly enhanced by K_{ATP} openers, indicating that ischemic/reperfusion uncoupling of mitochondria is inhibited.[99]

The results showing little cardiodepressant effects for K_{ATP} openers, while they conserved ischemic ATP was intriguing as significant APD shortening

should cause reduced contractile function. This suggested the possibility that K_{ATP} openers were not working through a cardioplegic mechanism and possibly not through sarcolemmal channels. A study by Pignac *et al.*[100] showed that aprikalim exerted an additional protective effect over that afforded by depolarizing cardioplegia in isolated rabbit hearts. We confirmed this in isolated rat hearts and found the K_{ATP} openers cromakalim and BMS-180448 to exert additive protective effects over hypothermic or normothermic St Thomas' cardioplegic solution (16 mM K^+).[97] Interestingly, glyburide abolished the additive protective action of K_{ATP} openers and had no effect on the protective activity of the cardioplegic solution alone. These results suggest that K_{ATP} openers work via a mechanism distinct from cardioplegia and since these hearts are arrested in systole, the hearts were electrically quiescent and therefore APD shortening was not likely to be an important mechanism of action for these agents.

The suggestion of a lack of importance of APD shortening was disquieting as it suggested a lack of importance of sarcolemmal K^+ currents in mediating the cardioprotective effects of K_{ATP} openers. At approximately the same time as the cardioplegic studies were published by Pignac *et al.*,[100] Gross and co-workers found that a dose of bimakalim could be used which reduced infarct size in dogs, but was without effect on epicardial monophasic APD.[101] We confirmed these findings by pharmacologically blocking the APD shortening activity of cromakalim (epicardial monophasic APD) with the delayed rectifier blocker dofetilide and found cromakalim to nevertheless reduce infarct size.[102] A recent study by Hamada *et al.*[103] showed that APD shortening is not a prerequisite for cardioprotection induced by pinacidil in a canine model of infarction in anesthetized dogs. While these studies were well executed and represented early evidence for a separation of APD shortening and cardioprotection, measurement of epicardial monophasic action potentials are a relatively crude technique for measuring changes in APD, especially in the critical areas of the ischemic myocardium (below the epicardial surface). It therefore became necessary to undertake more detailed studies using patch clamp and well as intracellular recording techniques.

We have developed pyranyl cyanoguanidine analogs which retain the glyburide-reversible cardioprotective activity of cromakalim while being relatively devoid of vasodilator activity.[36] Interestingly, we also found that many of these agents, including BMS-180448, were poor at opening cardiac sarcolemmal K_{ATP} as measured in single channel patches or by measuring whole myocyte K^+ currents.[47] In guinea-pig papillary muscles, BMS-180448 was devoid of APD shortening activity (intracellular recordings) at concentrations which exerted significant cardioprotective effects, although the cardioprotective effects were abolished by glyburide or 5-HD.[104] These data suggested that compounds which are selective for the cardioprotective site could be developed, although the nature of this site is still not clear. It is evident that some degree of selectivity can be observed for blockers as well. 5-HD efficiently abolishes the cardioprotective effects of all K_{ATP} openers tested, but has little effect on cardiac sarcolemmal K_{ATP}.[37,93] It is also of interest that 5-HD is incapable of blocking the vasodilator effects of K_{ATP} openers.

As discussed earlier, K_{ATP} appear to be involved in mediation of preconditioning and the potential relevance of sarcolemmal K_{ATP} has also been questioned for this phenomenon. Studies have shown preconditioning to cause APD shortening, both during and after preconditioning in some studies, although this does not definitively prove sarcolemmal channels to be essential for preconditioning.[56,105] Recent studies by Much-Ellingsen *et al.*[106] showed that dofetilide has no effect on preconditioning in rabbits. These studies suggest that preconditioning, like pharmacologic K_{ATP} opening, does not depend on activation of sarcolemmal K^+ currents and an intracellular site of action may be relevant. A recent study by Hamada *et al.*[103] confirmed that preconditioning in dogs is independent of APD shortening, further suggesting sarcolemmal K_{ATP} activation is not the primary or sole mediator of preconditioning. A recent study by Linz *et al.*[107] suggested that a pharmacologic blocker which is selective for sarcolemmal K_{ATP} did not abolish preconditioning while glyburide completely abolished preconditioning.

The question is whether sarcolemmal K_{ATP} are critical for the protective effect of K_{ATP} activation (both pharmacologic or preconditioning) or whether intracellular K_{ATP} may be involved. Taken together, the data described above show a poor correlation between sarcolemmal K^+ current activation and cardioprotection for K_{ATP} openers. This is perplexing given the near universal finding of cardioprotection for structurally distinct K_{ATP} openers and their inhibition by K_{ATP} blockers. It is certainly possible that K_{ATP} are not involved with cardioprotection, but this seems improbable. It is also possible that K_{ATP} openers may interact with sarcolemmal K_{ATP} in a manner we do not understand and will affect cardioprotection even without activation of a potassium current. We were hampered

by a lack of knowledge of the relevant cardioprotective binding site for the pharmaologic openers of K_{ATP}. Binding sites for K_{ATP} openers have been found in cardiac, smooth muscle and skeletal muscle cell membranes, but the relationship of these sites to ischemia are not clear.[108,109] It has also been suggested that K_{ATP} openers may interact with an intracellular K_{ATP}. We hypothesized several years ago that mitochondrial K_{ATP} might be the cardioprotective site of action for K_{ATP} openers. In the next section the possible importance of mitochondrial K_{ATP} in mediating cardioprotection will be discussed in detail.

Role of Mitochondrial K_{ATP} in Cardioprotection

In the previous section, we developed the case against the involvement of sarcolemmal K_{ATP} in the cardioprotective effects of K_{ATP} openers. We will now review what is known about the physiology, biophysics, and pharmacology of mitochondrial K_{ATP} (mitoK_{ATP}), then we will discuss evidence for a role of mitoK_{ATP} in mediating cardioprotection.

Physiological role of mitoK_{ATP}

The mitochondrial potassium cycle consists of electrophoretic K^+ uptake and electroneutral K^+ efflux across the inner membrane. K^+ efflux is mediated by the K^+/H^+ antiporter, whose existence was predicted by Mitchell[110,111] and first demonstrated nearly 20 years later.[112] K^+ influx is mediated by the mitochondrial K_{ATP} channel (mitoK_{ATP}) and by inward K^+ leak due to diffusion caused by the high electrochemical gradient favoring inward flux. The redox energy consumed by the K^+ cycle is the cost of regulating matrix volume.[113,114]

A primary role of the regulated K^+/H^+ antiporter is to compensate for unregulated K^+ leak into the matrix, driven by the high voltages required for oxidative phosphorylation. Thus, the K^+/H^+ antiporter is responsible for *volume homeostasis* and is essential for maintaining vesicular integrity in the face of high ionic traffic across the inner membrane. A reconstitutively active K^+/H^+ antiporter from liver and heart mitochondria has been identified as an 82 kD inner membane protein.[115]

MitoK_{ATP} was discovered in 1991. Inoue *et al.*[116] reported evidence from patch clamp studies of fused mitoplasts at the same time that we began describing reconstitution of a highly purified mitoK_{ATP}.[117] Although it possesses unique regulatory properties, mitoK_{ATP} is regulated by every ligand that regulates plasma membrane K_{ATP} channels; consequently, we infer that it belongs to the same gene family.

The sole known function of the mitochondrial K^+ cycle is to regulate matrix volume.[114] The effect of net K^+ movement causes only minor perturbations of matrix pH, because of accompanying movement of weak acids; of matrix K^+ concentration, because of accompanying movement of osmotically obligated water; or of $\Delta\varphi$ (the electrical potential difference), because the fluxes are low relative to proton pumping by the electron transport chain. Thus, the K^+/H^+ antiporter cannot sense changes in either of its substrates. Rather, it is regulated indirectly (by matrix Mg^{2+} and H^+) to sense changes in matrix volume, and consequently, volume must change before K^+/H^+ adjusts to equal the rate of K^+ influx. For example, opening mitoK_{ATP} will transiently shift the balance until K^+/H^+ antiport catches up with the higher rate of K^+ influx. This will cause transient swelling and will result in a higher steady-state volume for as long as mitoK_{ATP} remains open.

Properties and regulation of mitoK_{ATP}

K^+ flux through reconstituted mitoK_{ATP} is highly selective for K^+ and unaffected by Na^+ or tetraethylammonium. MitoK_{ATP} is not voltage-gated, and the flux-voltage dependence is consistent with a channel containing a single energy well near the center of the membrane.[117] The open channel conductance of mitoK_{ATP} is about 16 pS in symmetric 150 mM KCl.[118] MitoK_{ATP} activity has also been studied in intact, respiring mitochondria. To control for the unavoidable coexistence of K^+ diffusion, we compared the effects of ATP on fluxes of K^+ and TEA^+. K^+ flux was inhibited by ATP to the level of TEA^+ flux, whereas TEA^+ flux was unaffected by ATP.[119,120]

MitoK_{ATP} is subject to complex regulation by metabolites and pharmacological agents.[117,120–122] ATP, ADP, and acyl CoA esters are mutually competitive inhibitors of K^+ flux through mitoK_{ATP}. Inhibition by each of these metabolites exhibits an absolute requirement for divalent cations. ATP or palmitoyl CoA have no effect in the absence of Mg^{2+}, and Mg^{2+} has no effect in the absence of ATP or palmitoyl CoA. Since palmitoyl CoA is not a Mg^{2+} chelator, these results imply that Mg^{2+} ions interact independently with mitoK_{ATP}.

The high affinity of mitoK_{ATP} for ATP raised the conundrum of how this channel can be opened

under physiological conditions. We hypothesized that endogenous activators overcome the high affinity for ATP, and we confirmed this hypothesis by showing that guanine nucleotides reverse the inhibition by ATP, ADP or palmitoyl CoA in both mitochondria and proteoliposomes.[122] Guanine nucleotides are competitive with ATP. GTP appears to react with a high affinity (0.2 μM) and a low affinity (15–20 μM) site, whereas GDP appears to react with two low affinity sites (20 μM). ATP is unable to inhibit in the presence of physiological GTP concentrations; for example, 20 μM GTP increased the $K_{1/2}$ for ATP inhibition from 21 μM to 6 mM. On the other hand, the $K_{1/2}$ for GTP activation of palmitoyl CoA inhibition is in the physiological range. Our present thinking is that ATP does not regulate mitoK_{ATP} *in vivo*, and that the open/closed state of the channel is determined by the relative occupancy of the sites by GTP or long-chain acyl CoA esters. We speculate that the two binding sites correspond to the nucleotide binding folds on mitoSUR.

In order to understand the role of mitoK_{ATP} in cellular bioenergetics, it is necessary to know *where* its regulatory sites are located. Do they face the matrix, as suggested by Inoue *et al.*,[116] or do they face the cytosol? Do they all coexist on the same pole of the protein? Experiments in proteoliposomes and mitochondria demonstrate that the mitoK_{ATP} regulatory sites for Mg^{2+}, ATP, GTP and palmitoyl CoA face the *cytosol*, or more precisely, the intermembrane space.[118]

Pharmacological modulators of mitoK_{ATP}

K_{ATP} openers reverse inhibition of mitoK_{ATP} by ATP, ADP, and palmitoyl CoA with $K_{1/2}$ values that are well within the ranges observed for plasma membrane K_{ATP} channels from various tissues.[123] Thus, the $K_{1/2}$ values were 1 μM and 0.4 μM for cromakalim and diazoxide, respectively.[121] Similar values were obtained in intact mitochondria and in liposomes containing reconstituted mitoK_{ATP}.

We have recently succeeded in removing a major barrier to understanding the pharmacology of mitoK_{ATP} inhibitors.[120] Under most conditions, glyburide and 5-HD are ineffective in intact, respiring mitochondria.[119] We have now shown that this phenomenon reflects a particular characteristic of mitoK_{ATP} and is due entirely to the conditions of the experiment. Thus, in the absence of other ligands, the channel is open, but not inhibitable by glyburide or 5-HD. Sensitivity to inhibitors requries the simultaneous presence of Mg^{2+}, ATP, and an opener which may be GTP or a pharmacologic opener. Of course, these ligands are precisely those which would normally be present during *in vivo* experiments. We infer from these and other experiments that binding of glyburide and 5-HD to mitoSUR is conformation-dependent and that mitoSUR conformation is changed by binding of the other ligands. When the appropriate ligands (e.g. Mg^{2+}, ATP, or cromakalim) are present in the assay medium, glyburide and 5-HD are potent, specific blockers of K^+ flux in respiring mitochondria, with K_i values of about 1 μM and 50 μM, repectively.[120]

Molecular structure of mitoK_{ATP}

We have tentatively identified two components of mitoK_{ATP}—a 55 kD channel protein and a 63 kD sulfonylurea receptor (SUR), based on its labeling with bodipy-glyburide (unpublished observation). Thus, it appears that mitoK_{ATP} has a hetero-multimeric structure akin to that of plasma membrane K_{ATP}. Neither subunit has been cloned, but this is the focus of active research.

An interesting observation was made by Suzuki *et al.*,[124] who showed that mitochondria are immunostained with antibodies to KIR6.1, an inward rectifying K^+ channel that is known to be expressed in plasma membranes. Preliminary data show that these antibodies do indeed react with a mitochondrial protein; however, they do not react with any protein in the reconstitutively active purified fraction of mitoK_{ATP}. In view of the fact that the antibodies were raised to a 12 amino acid fragment of KIR6.1, it may be that they recognize a non-K_{ATP} protein with homology in a limited domain.

Differential pharmacology of cardiac sarcolemmal and mitochondrial K_{ATP}

We measured K^+ fluxes in liposomes containing purified K_{ATP} to address the question of receptor subtypes within the cardiac myocyte. These protocols have several advantages, including the ability to compare proteins from different sources using identical assay conditions. Sarcolemmal K_{ATP} and cardiac mitoK_{ATP} were purified from beef heart and assayed for flux. The two preparations shared many properties in common. Flux was inhibited by ATP, and this inhibition was reversed by cromakalim in both preparations. The resulting pharmacological open state was inhibited by glyburide in both preparations.

5-HD exhibited a profoundly differet pharmacology in the two preparations. 5-HD always inhibited mitoK_{ATP} under appropiate conditions, but

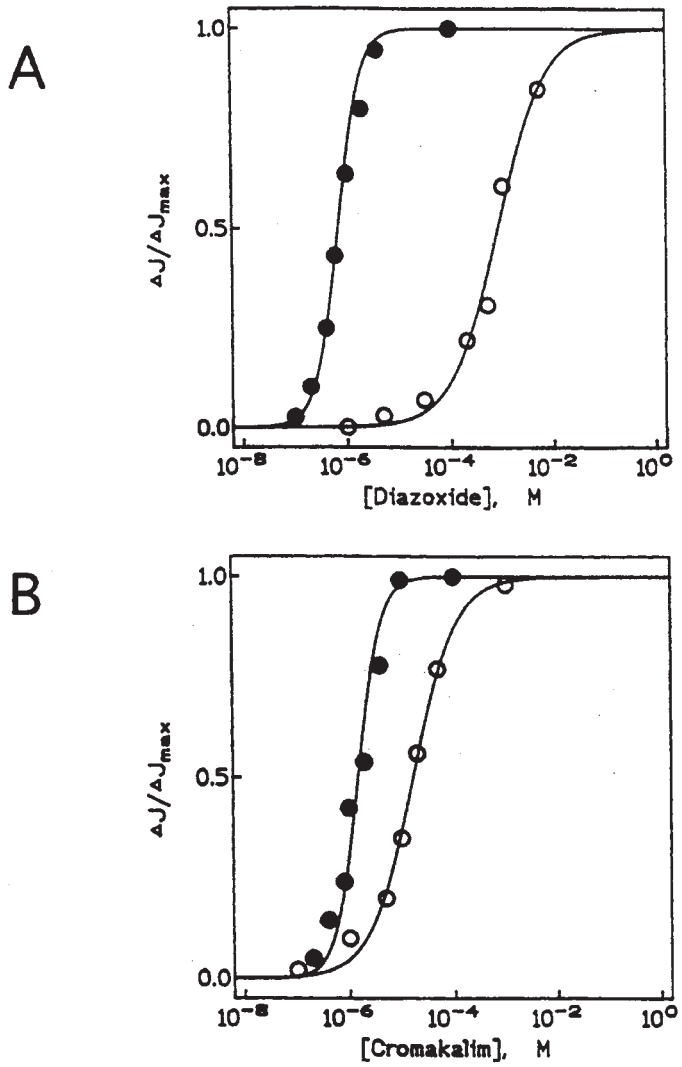

Figure 4 Activation of K^+ flux by diazoxide or cromakalim in K_{ATP} from bovine heart mitochondria and sarcolemma. Relative K^+ flux ($\Delta J/\Delta Jmax$) is plotted *v* concentration of drug added to the assay. The figure shows relative fluxes from cardiac mitochondrial K_{ATP} (solid circles) and sarcolemmal channels (open circles) in response to diazoxide or cromakalim. Observed $K_{1/2}$ values for cromakalim were 1.6 ± 0.1 μM for mitochondrial channels and 18 ± 2 μM for sarcolemmal channels. $K_{1/2}$ values for diazoxide were 0.8 ± 0.03 μM for mitochondrial K_{ATP} and 840 ± 25 μM for sarcolemmal K_{ATP}. Figure taken from Garlid *et al.* (1997) with permission.

never inhibited sarcolemmal K_{ATP} under any conditions. This failure of 5-HD to block sarcolemmal K_{ATP} is consistent with previous observations.[93] It should be pointed out that the blocking activity of 5-HD in sarcolemmal channels has not been studied in any detail.

Diazoxide also exhibited a pronounced differential pharmacology between sarcolemmal K_{ATP} and mitoK_{ATP}, as shown in Figure 4. In this experiment, diazoxide was 1000-times more potent in opening mitoK_{ATP} than in opening sarcolemmal K_{ATP}. We normally observe $K_{1/2}$ values for opening by diazoxide of 0.5–0.8 μM with mitoK_{ATP} and 0.8–1.0 mM with sarcolemmal K_{ATP}.

Are mitoK_{ATP} the sites of cardioprotection?

A number of studies have demonstrated that cardiac sarcolemmal K_{ATP} are relatively insensitive to diazoxide,[23,121] whereas mitoK_{ATP} are sensitive in the micromolar range.[121] Accordingly, we studied the effect of diazoxide in an isolated rat heart model of ischemia and reperfusion. We observed concentration-dependent cardioprotection in the low micromolar range with no correlation to APD shortening (Figs 3, 5 and 6).[37] While diazoxide had little effect on APD, cromakalim did shorten APD markedly within its cardioprotective range. Therefore, diazoxide and cromakalim were found to be-

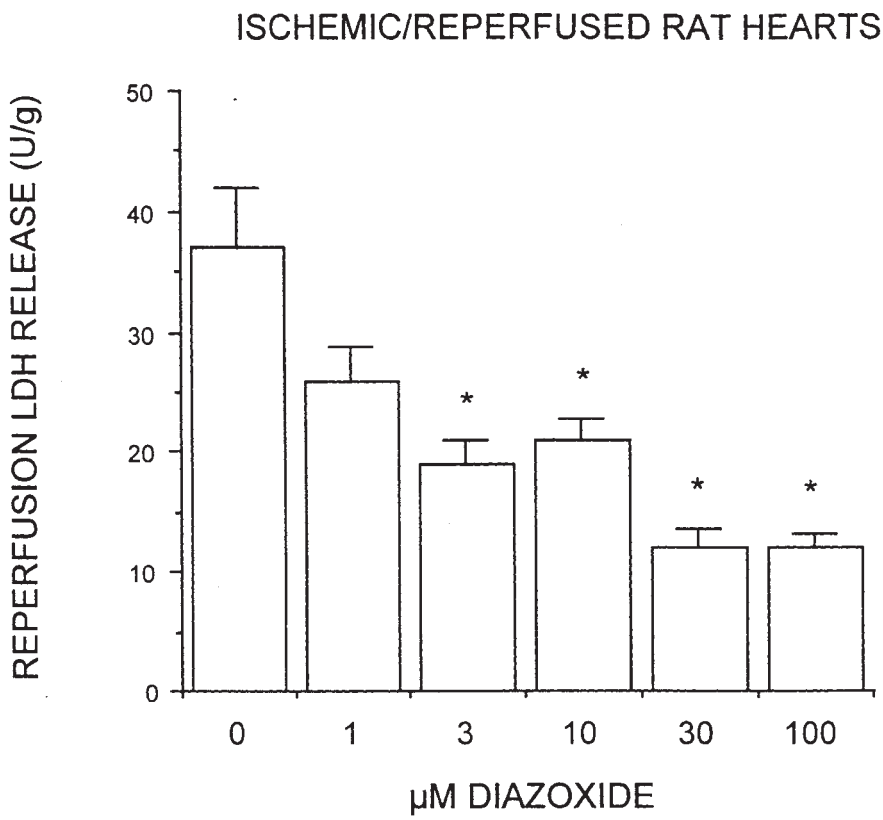

Figure 5 Effect of diazoxide on the release of LDH (lactate dehydrogenase) from ischemic/reperfused rat hearts. The hearts were pre-treated for 10 min with diazoxide and then were rendered totally ischemic for 25 min. This was then followed by 30 min of reperfusion without drug. LDH release was reduced in a concentration-dependent manner by diazoxide. * Denotes significance ($P<0.05$) compared to vehicle. Figure taken from Garlid *et al.* (1997) with permission.

have similarly in terms cardioprotection and in opening mitoK$_{ATP}$. They differed markedly in their effects on APD and on the opening of reconstituted sarcolemmal K$_{ATP}$. Both glyburide and 5-HD completely abolished the protective effects of diazoxide (Fig. 6). These results appear to exclude a role for sarcolemmal K$_{ATP}$ in ischemic protection.

These differential effects of 5-HD and diazoxide are consistent with our hypothesis that mitoK$_{ATP}$ is the receptor for cardioprotection by K$_{ATP}$ openers and for 5-HD blockade of pharmacolgical protection or preconditioning.[37] The caveat, at present, is that the existence of a third myocardial K$_{ATP}$ subtype, with receptor properties similar to mitoK$_{ATP}$, has not yet been ruled out.

Liu *et al.*[125] have recently confirmed diazoxide cardioprotection in the isolated rabbit myocyte model and these authors support the hypothesis that mitoK$_{ATP}$ mediates cardioprotection with pharmacologic K$_{ATP}$ openers. Additional experiments on the same model provide evidence that protein kinase C activation potentiates diazoxide opening of mitoK$_{ATP}$, providing a partial linkage between ischemic preconditioning, PKC activity and mitoK$_{ATP}$.[126] With regard to preconditioning, it is important to note that 5-HD also completely abolishes the protective effect of this phenomenon, further suggesting an involvement of mitoK$_{ATP}$.

K$_{ATP}$: Future Directions

Recent years have seen an explosive growth in interest in the role of K$_{ATP}$ in the pathogenesis of myocardial ischemia. The great majority of investigators find that K$_{ATP}$ openers exert cardioprotective effects in numerous models of myocardial ischemia. This protection appears to be related to energy sparing effects which are not due to reductions in cardiac work. Overall, K$_{ATP}$ openers have an excellent cardioprotective profile which is further enhanced by the possibility that they mimic an endogenous protective mechanism. While an important role for K$_{ATP}$ in ischemia seems to have met with general agreement, there is much that is still unknown.

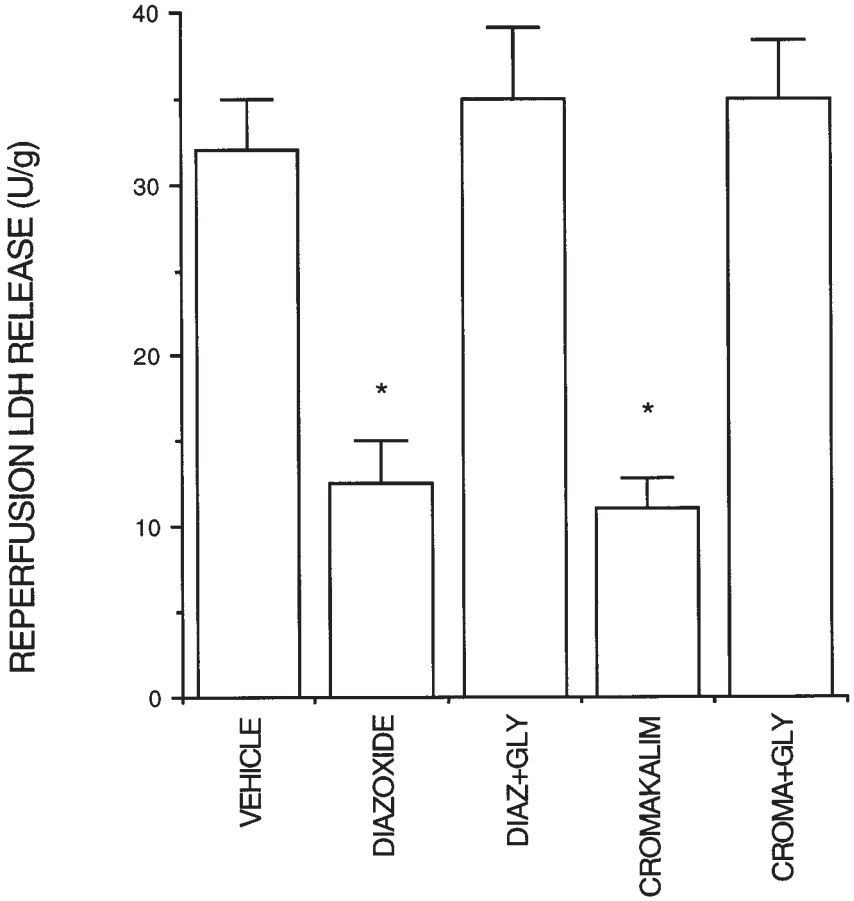

Figure 6 The effect of diazoxide or cromakalim with or without concomitant glyburide on release of LDH (lactate dehydrogenase) from ischemic/reperfused rat hearts. Both cromakalim and diazoxide significantly (* $P<0.05$) reduced reperfusion LDH release and this effect was abolished by glyburide. Figure taken from Garlid *et al.* (1997) with permission.

There are two major mechanistic issues: current information suggests that mitoK_{ATP} may be the end effectors of cardioprotection, but further work is essential to assure ourselves that mitoK_{ATP} is indeed the protective site of action of both pharmacologic agonists and preconditioning. Secondly, it is not clear how opening mitoK_{ATP} will exert ATP-sparing effects. It is critical to determine the molecular mechanism of cardioprotection provided by activation of K_{ATP}. Only when this mechanism is understood can we begin to understand the complex signaling pathways important in preconditioning. Understanding this mechanism may also be important in developing novel therapeutics for treating myocardial ischemia.

The therapeutic issue is that existing K_{ATP} openers may not be optimal for treating acute myocardial ischemia. Most K_{ATP} openers have little tissue selectivity, and they also have the propensity to cause hypotension and APD shortening, which may limit their use. Agents have been reported which retain cardioprotective effects while being devoid of vasodilator activity,[36] suggesting that tissue selectivity is possible. In this series are found many agents that are devoid of vasodilator and APD shortening activities, making them more selective for treating ischemia without the potential for increasing reentrant arrhythmias, as might be seen for agents activating sarcolemmal currents.[127] The results of studies designed to assess pro-arrhythmic potential of non-selective K_{ATP} openers have been variable,[128–133] but nevertheless any drug discovery effort should take this potential toxicity seriously and attempt to minimize the effects of these agents on APD or refractoriness. Despite the absence of APD shortening or vasodilator activity, the cardioprotective effects of the selective compounds described by Atwal *et al.*[36] are abolished by glyburide and 5-HD. It is presently unknown whether these compounds are selective for mitoK_{ATP}. Although these agents are more selective for cardioprotection,

little improvements in cardioprotective potency have been seen.

A convincing demonstration that mitoK_{ATP} are the cardioprotective site, together with cloning of this channel, would set the stage for setting up high throughput screening. Such a model would be conducive to breakthroughs in terms of increasing potency and selectivity while simultaneously reducing the prospects for toxicity.

While much of the work described in this review revolves around the heart, K_{ATP} exists in other tissues. K_{ATP} has been shown to be involved in protection in cerebral ischemia.[82] Interestingly, K_{ATP} activation in CNS may be modulated by adenosine. Recent studies by Pang's laboratory[134–136] show that K_{ATP} openers are protective in models of skeletal muscle ischemia. K_{ATP} also seems to be involved in the mechanism of skeletal muscle preconditioning and, as in heart and brain, adenosine is involved in the modulation of this channel. It would be of interest to determine the effect of K_{ATP} openers in models of hepatic ischemia, and it is possible that K_{ATP} openers may have general protective effects in numerous tissue types. It should be noted that as we learn more about tissue differences among K_{ATP}, other utilities may become apparent. Even antidiabetic sulfonylureas may be improved if selectivity towards pancreatic β-cell K_{ATP} could be achieved.

Future research will address the possibility that mitoK_{ATP} is the end effector of protection in other tissues. To date, mitoK_{ATP} has been identified in heart, liver and brown adipose tissue mitochondria.[117,121] Because mitoK_{ATP} plays an important role in regulating mitochondrial volume, it is anticipated that it will be expressed in all tissues. Another issue which needs to be addressed is the role of sarcolemmal K_{ATP} in ischemia or normal cardiac function. This channel appears to have a role in outward currents during ischemia as well as under normal conditions (see review by Wilde and Janse, 1994).[131] While current data suggest a lack of importance for sarcolemmal K_{ATP} in mediating cardioprotection, a protective hyperpolarizing cardioplegic effect cannot be completely ruled out for high doses of pharmacologic openers, although such high doses would probably be outside of the therapeutic range of these agents.

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References

1. Noma A. ATP-regulated K^+ channels in cardiac muscle. *Nature* 1983; **305**: 147–148.
2. Edwards G, Weston A. The pharmacology of ATP-sensitive potassium channels. *Annu Rev Pharmacol Toxicol* 1993; **33**: 597–637.
3. Kim D, Clapham D. Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. *Science* 1989; **244**: 1174–1176.
4. Kirsch G, Codina J, Birnbaumer L, Brown A. Coupling of ATP-sensitive K^+ channels to A_1 receptors by G proteins in rat ventricular myocytes. *Am J Physiol* 1990; **259**: H820–H826.
5. Coetzee W, Nakamura T, Faivre J. Effects of thiol-modifying agents on K_{ATP} channels in guinea pig ventricular cells. *Am J Physiol* 1995; **269**: H1625–1633.
6. Ming Z, Parent R, Lavallee M. Beta 2-adrenergic dilation of resistance coronary vessels involves K_{ATP} channels and nitric oxide in conscious dogs. *Circulation* 1997; **95**: 1568–1576.
7. Larsson O, Deeney TJ, Branstrom R, Berggren PO, Corkey BE. Activation of the ATP-sensitive K+ channel by long chain acyl-CoA. *J Biol Chem* 1996; **271**: 10623–10626.
8. Spruce A, Standen N, Stanfield P. Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* 1985; **316**: 736–738.
9. de Weille J, Schmid-Antomarchi H, Fosset M, Lazdunski M. ATP-sensitive K^+ channels that are blocked by hypoglycemia-inducing sulfonylureas in insulin-secreting cells are activated by galanin, a hyperglycemia-inducing hormone. *Proc Natl Acad Sci USA* 1998; **85**: 1312–1316.
10. Treherne J, Ashford M. The regional distribution of sulphonylurea binding sites in rat brain. *Neuroscience* 1991; **40**: 523–531.
11. Sturgess NC, Ashford MJL, Cook DL, Hales CN. The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* 1985; **2**: 474–475.
12. Dunne MJ, Illot MC, Petersen OH. Interaction of diazoxide, tolbutamide and STP on nucleotide-dependent K+ channels in an insulin-secreting cell line. *J Membr Bio* 1987; **99**: 215–224.
13. Ashcroft FM, Harrison DE, Ashcroft SJH. Glucose induces closure of single potassium channels in isolated rat pancreatic &bgr;-cells. *Nature* 1984; **312**: 446–448.
14. Mitchell MB, Meng X, Ao L, Brown JM, Harken AH, Banerjee A. Preconditioning of isolated rat heart is mediated by protein kinase C. *Circ Res* 1995; **76**: 73–81.
15. Quast U, Cook NS. Moving together: K^+ channel openers and ATP-sensitive K^+ channels. *Trends Pharmacol Sci* 1989; **10**: 431–435.
16. D'Alonzo A, Darbenzio R, Parham C, Grover G. Effects of intracoronary cromakalim on post-ischaemic contractile function and action potential duration. *Cardiovasc Res* 1992; **26**: 1046–1053.
17. Shaw RM, Rudy Y. Electrophyiosologic effects of acute myocardial ischemia: a theoretical study of altered cell excitability and action potential duration. *Cardiovasc Res* 1997; **35**: 256–272.

18. Shigamatsu S, Arita M. Anoxia-induced activation of ATP-sensitive K^+ channels in guinea pig ventricular cells and its modulation by glycolysis. *Cardiovasc Res* 1997; **35**: 273–282.
19. Venkatesh N, Lamp ST, Weiss JN. Sulfonylureas, ATP-sensitive K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ Res* 1991; **74**: 623–629.
20. Kondo T, Kubota I, Tachibana H, Yamaki M, Tomoike H. Glibenclamide attenuates peaked T wave in early phase of myocardial ischemia. *Cardiovasc Res* 1996; **31**: 683–687.
21. Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J. Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor [see comments]. *Science* 1995; **270**: 1166–1170.
22. Ashcroft F. Fresh insights into the interactions of drugs with ATP-sensitive K^+ channels. *ID Research Alert* 1996; **2**: 43–37.
23. Inagaki N, Gonoi T, Clement J, Wang C, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* 1996; **16**: 1011–1017.
24. Philipson L, Steiner D. Pas de deux or more: the sulfonylurea receptor and K^+ channels [comment]. *Science* 1995; **268**: 372–373.
25. Wellman G, Quayle J. ATP-sensitive potassium channels: molecular structure and therapeutic potential in smooth muscle. *ID Research Alert* 1997; **2**: 3–5.
26. Spruce A, Standen N, Stanfield P. Studies of the unitary properties of adenosine-5′-triphosphate-regulated potassium channels of frog skeletal muscle. *J Physiol (Lond)* 1987; **382**: 213–236.
27. Kwak Y, Park S, Cho K, Chae S. Reciprocal modulation of ATP-sensitive K^+ channel activity in rat ventricular myocytes by phosphorylation of tyrosine and serine/threonine residues. *Life Sci* 1996; **58**: 897–904.
28. Hiraoka M, Furukawa T. Functional modulation of cardiac ATP-sensitive K^+ channels. *News Physiol Sci* 1998; **13**: 131–137.
29. Hu K, Duan D, Li G, Nattel S. Protein kinase C activates ATP-sensitive K^+ current in human and rabbit ventricular myocytes. *Circ Res* 1996; **78**: 492–498.
30. Jackson W, Konig A, Dambacher T, Busse R. Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. *Am J Physiol* 1993; **264**: H238–243.
31. Kleppisch T, Nelson M. Adenosine activates ATP-sensitive potassium channels in arterial myocytes via A_2 receptors and cAMP-dependent protein kinase. *Proc Natl Acad Sci USA* 1995; **92**: 12441–12445.
32. Schackow TE, Ten Eick RE. Enhancement of ATP-sensitive potassium current in cat ventricular myocytes by &bgr-adrenergic stimulation. *J Physiol* 1995; **474**: 131–145.
33. Babenko A, Vassort G. Enhancement of the ATP-sensitive K^+ current by extracellular ATP in rat ventricular myocytes. Involvement of adenylyl cyclase-induced subsarcolemmal ATP depletion. *Circ Res* 1997; **80**: 589–600.
34. Wollheim CB, Dunne MJ, Peter-Reisch B, Bruzzone R, Pozzan T, Petersen OH. Activators of protein kinase C depolarize insulin-secreting cells by closing potassium channels. *EMBO J* 1988; **7**: 2443–2449.
35. Cole W, McPherson C, Sontag D. ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res* 1991; **69**: 571–581.
36. Atwal K, Grover G, Ahmed S, Ferrara F, Harper T, Kim K, Sleph P, Dzwonczyk S, Russell A, Moreland S. Cardioselective anti-ischemic ATP-sensitive potassium channel openers. *J Med Chem* 1993; **36**: 3971–3974.
37. Garlid KD, Paucek P, Yarov-Yarovoy B, Murray HNM, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, Grover GJ. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive potassium channels: possible mechanism of cardioprotection. *Circ Res* 1997; **81**: 1072–1082.
38. Lamping K, Gross GJ. Improved recovery of myocardial segmental function following a short coronary occlusion in dogs by nicorandil, a potential new antianginal agent, and nifedipine. *J Cardiovasc Pharmacol* 1985; **7**: 158–166.
39. Grover GJ, McCullough JR, Henry DE, Conder ML, Sleph PG. Anti-ischemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with the potassium channel blocker glyburide. *J Pharmacol Exp Ther* 1989; **251**: 98–104.
40. Grover GJ, Dzwonczyk S, Parham C, Sleph P. The protective effects of cromakalim and pinacidil on reperfusion function and infarct size in isolated perfused rat hearts and anesthetized dogs. *Cardiovasc Drugs Ther* 1990; **4**: 465–474.
41. Grover G, Newburger J, Sleph P, Dzwonczyk S, Taylor S, Ahmed S, Atwal K. Cardioprotective effects of the potassium channel opener cromakalim: stereoselectivity and effects on myocardial adenine nucleotides. *J Pharmacol Exp Ther* 1991; **257**: 156–162.
42. Grover GJ, Dzwonczyk S, Sleph PG. Reduction of ischemic damage in isolated rat hearts by the potassium channel opener RP 52891. *Eur J Pharmacol* 1990; **191**: 11–19.
43. Sargent CA, Dzwonczyk S, Sleph PG, Normandin DE, Antonaccio MJ, Grover GJ. Cardioprotective effects of the cyanoguanidine potassium channel opener P-1075. *J Cardiovasc Pharmacol* 1993; **22**: 564–570.
44. Ohta H, Jinno Y, Harada K, Ogawa N, Fukushima H, Nishikori K. Cardioprotective effects of KRN2391 and nicorandil on ischemic dysfunction in perfused rat heart. *Eur J Pharmacol* 1991; **204**: 171–177.
45. Galinanes M, Shattock M, Hearse D. Effects of potassium channel modulation during global ischaemia in isolated rat heart with and without cardioplegia. *Cardiovasc Res* 1992; **26**: 1063–1068.
46. Grover G. Protective effects of ATP-sensitive potassium-channel openers in experimental myocardial ischemia. *J Cardiovasc Pharmacol* 1994; **24** Suppl 4: S18–27.
47. Grover G, Atwal KS. BMS-180448, a glyburide-reversible cardioprotective agent with minimal

vasodilator activity. *Cardiovasc Drug Rev* 1995; **13**: 123–136.
48. Tan H, Mazon P, Verberne H, Sleeswijk M, Coronel R, Opthof T, Janse M. Ischaemic preconditioning delays ischaemia induced cellular electrical uncoupling in rabbit myocardium by activation of ATP sensitive potassium channels. *Cardiovasc Res* 1993; **27**: 644–651.
49. Monticello T, Sargent C, McGill J, Barton D, Grover G. Amelioration of ischemia/reperfusion injury in isolated rats hearts by the ATP-sensitive potassium channel opener BMS-180448. *Cardiovasc Res* 1996; **31**: 93–101.
50. Armstrong S, Liu G, Downey J, Ganote C. Potassium channels and preconditioning of isolated rabbit cardiomyocytes: effects of glyburide and pinacidil. *J Mol Cell Cardiol* 1995; **27**: 1765–1774.
51. Liang BT. Direct preconditioning of cardiac ventricular myocytes via adenosine A_1 receptor and K_{ATP} channel. *Am J Physiol* 1966; **271**: H1769–H1777.
52. Speechly-Dick M, Grover G, Yellon D. Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ channel? Studies of contractile function after simulated ischemia in an atrial in vitro model. *Circ Res* 1995; **77**: 1030–1035.
53. Auchampach J, Maruyama M, Cavero I, Gross G. Pharmacological evidence for a role of ATP-dependent potassium channels in myocardial stunning. *Circulation* 1992; **86**: 311–319.
54. Auchampach JA, Maruyama M, Cavero I, Gross GJ. The new K^+ channel opener aprikalim (RP 52891) reduces experimental infarct size in dogs in the absence of hemodynamic changes. *J Pharmacol Exp Ther* 1991; **259**: 961–967.
55. Rohmann S, Weygandt H, Schelling P, Kie Soei L, Verdouw P, Lues I. Involvement of ATP-sensitive potassium channels in preconditioning protection. *Basic Res Cardiol* 1994; **89**: 563–576.
56. Schulz R, Rose J, Heusch G. Involvement of activation of ATP-dependent potassium channels in ischemic preconditioning in swine. *Am J Physiol* 1994; **267**: H1341–1352.
57. Toombs CF, Norman NR, Groppi VE, Lee KS, Gadwood RC, Shebuski RJ. Limitation of myocardial injury with the potassium channel opener cromakalim and the nonvasoactive analog U-89,232: vascular vs. cardiac actions in vitro and in vivo. *J Pharmacol Exp Ther* 1992; **263**: 1261–1268.
58. Imai N, Liang C, Stone C, Sakamoto S, Hood WB. Comparative effects of nitroprusside and pinacidil on myocardial blood flow and infarct size in awake dogs with acute myocardial infarction. *Circulation* 1988; **77**: 705–711.
59. Kitzen J, McCallum J, Harvey C, Morin M, Oshiro G, Colatsky T. Potassium channel activators cromakalim and celikalim (WAY-120,491) fail to decrease myocardial infarct size in the anesthetized canine. *Pharmacology* 1992; **45**: 71–82.
60. Murry C, Richard V, Reimer K, Jennings R. Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. *Circ Res* 1990; **66**: 913–931.
61. Gho BCG, Eskildsen-Helmond YEG, de Zeeuw S, Lamers JMJ, Verdouw PD. Does protein kinase C play a pivotal role in the mechanisms of ischemic preconditioning. *Cardiovasc Drugs Ther* 1996; **10**: 775–786.
62. Vuorinen K, Ylitalo K, Peuhkurinen K, Raatikainen P, Ala-Rami A, Hassinen IE. Mechanisms of ischemic preconditioning in rat myocardium. Roles of adenosine, celular energy state and mitochondrial F_1F_0-ATPase. *Circulation* 1995; **91**: 2810–2818.
63. Dekker LRC. Toward the heart of preconditioning. *Cardiovasc Res* 1998; **37**: 14–20.
64. Goto M, Liu Y, Xi-Ming Y, Ardell JL, Cohen MV, Downey JM. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. *Circ Res* 1995; **77**: 611–621.
65. Yellon DM, Baxter GF, Garcia-Dorado D, Heusch G, Sumeray MS. Ischaemic preconditioning: present position and future directions. *Cardiovasc Res* 1998; **37**: 21–33.
66. Ytrehus K, Liu Y, Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. *Am J Physiol* 1993; **266**: H1145–H1152.
67. Downey JM, Cohen MV. Signal transduction in ischemic preconditioning. *Z Kardiol* 1995; **4**: 77–86.
68. Baines CP, Cohen MV, Downey JM. Protein tyrosine kinase inhibitor, genistein, blocks preconditioning in isolated rabbit hearts. *Circulation* 1996; **94** (Suppl. 1): I–661 (abstract).
69. Maulik N, Watanabe M, Zu YL, Huang CK, Cordis GA, Schley JA, Das DK. Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts. *FEBS Lett* 1996; **396**: 233–237.
70. Yao Z, Gross GJ. Activation of ATP-sensitive potassium channels lowers threshold for ischemic preconditioning in dogs. *Am J Physiol* 1994; **267**: H1888–H1894.
71. Gross G, Auchampach J. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res* 1992; **70**: 223–233.
72. Auchampach JA, Grover GJ, Gross GJ. Blockade of ischemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc Res* 1992; **26**: 1054–1062.
73. Grover GJ. Role of the K_{ATP} channel in ischemic preconditioning. In *Ischemia: Preconditioning and Adaptation* (Marber MS, Yellon DM, Eds). Bios Scientific Publishers, Oxford, UK. 1996; pp. 35–58.
74. Schultz J-E, Yao Qian YZ, Gross GJ, Kukreja RC. The ischemia-selective channel antagonist, 5-hydroxydecanoate, blocks ischemic preconditioning in the rat heart. *J Mol Cell Cardiol* 1997; **29**: 1055–1060.
75. Bugge E, Jonassen AK, Ytrehus K. Ischaemic preconditioning against infarction is dependent on activation of ATP-sensitive K^+ channels in rat heart. *Circulation* 1997; **96** (Suppl.): I–689, (Abstract).
76. Tomai F, Crea F, Gaspardone A, Versaci F, De Paulis R, Penta de Peppo A, Chiariello L, Gioffre P. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker. *Circulation* 1994; **90**: 700–705.
77. Grover G, Sleph P, Dzwonczyk S. Role of myocardial ATP-sensitive potassium channels in me-

diating preconditioning in the dog heart and their possible interaction with adenosine A_1-receptors. *Circulation* 1992; **86**: 1310–1316.
78. Van Winkle D, Chien G, Wolff R, Soifer B, Kuzume K, Davis R. Cardioprotection provided by adenosine receptor activation is abolished by blockade of the K_{ATP} channel. *Am J Physiol* 1994; **266**: H829–839.
79. Auchampach J, Gross G. Adenosine A_1 receptors, K_{ATP} channels, and ischemic preconditioning in dogs. *Am J Physiol* 1993; **264**: H1327–1336.
80. Toombs CF, McGee DS, Johnston WE, Vinten-Johansen J. Protection from ischaemic-reperfusion injury with adenosine treatment is reversed by inhibition of ATP-sensitive potassium channels. *Cardiovasc Res* 1993; **27**: 623–629.
81. Cleveland JC, Meldrum D, Rowland R, Banerjee A, Harken A. Adenosine preconditioning of human myocardium is dependent upon the ATP-sensitive K^+ channel. *J Mol Cell Cardiol* 1997; **29**: 175–182.
82. Heurteaux C, Lauritzen I, Widmann C, Lazdunski M. Essential role of adenosine, adenosine A_1 receptors, and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. *Proc Natl Acad Sci USA* 1995; **92**: 4666–4670.
83. Ford WR, Lopaschuk GD, Schulz R, Clanahan AS. K_{ATP} channel activation: effects on myocardial recovery from ischaemia and role in the cardioprotective response to adenosine A_1 receptor stimulation. *Br J Pharmacol* 1998; **124**: 639–646.
84. Kitakaze M, Hori M, Morioka T, Minamino T, Takashima S, Sato H, Shinozaki Y, Chujo M, Mori H, Inoue M. Infarct size-limiting effect of ischemic preconditioning is blunted by inhibition of 5′-nucleotidase activity and attenuation of adenosine release. *Circulation* 1994; **89**: 1237–1246.
85. Gross G, Mei D, Sleph P, Grover G. Adenosine A_1 receptor blockade does not abolish the cardioprotective effects of the adenosine triphosphate-sensitive potassium channel opener bimakalim. *J Pharmacol Exp Ther* 1997; **280**: 533–540.
86. Meldrum DR, Cleveland JC, Mitchell MB, Sheridan BC, Gamboni-Robertson F, Harken AJ, Banerjee A. Protein kinase C mediates Ca2+-induced cardioadaptation to ischemia-reperfusion injury. *Am J Physiol* 1996; **271**: R718–R726.
87. Meldrum DR, Cleveland JC, Sheridan BC, Rowland RT, Banerjee A, Harken AH. Cardiac preconditioning with calcium: clinically accessible myocardial protection. *J Thorac Surgery* 1996; **112**: 778–786.
88. Miyawaki H, Ashraf M. Ca^{2+} as a mediator of ischemic preconditioning. *Circ Res* 1997; **80**: 790–799.
89. Miyawaki H, Ashraf M. Isoproterenol mimics calcium preconditioning-induced protection against ischemia. *Am J Physiol* 1997; **272**: H927–H936.
90. Miyawaki H, Zhou HB, Ashraf M. Calcium preconditioning elicits strong protection against ischemic injury via protein kinase C signalling pathway. *Circ Res* 1996; **79**: 137–146.
91. Kouchi I, Murakami T, Nawada R, Akao M, Sasayama S. KATP channels are common mediators of ischemic and calcium preconditioning in rabbits. *Am J Physiol* 1998; **274**: H1106–H1112.
92. Schultz J-E, Yao Z, Cavero I, Gross GJ. Glibenclamide-induced blockade of ischemic preconditioning is time dependent in intact rat heart. *Am J Physiol* 1997; **267**: H2607–H2615.
93. McCullough J, Normandin D, Conder M, Sleph P, Dzwonczyk S, Grover G. Specific block of the anti-ischemic actions of cromakalim by sodium 5-hydroxydecanoate. *Circ Res* 1991; **69**: 949–958.
94. Sargent CA, Smith MA, Dzwonczyk S, Sleph PG, Grover GJ. Effect of potassium channel blockade on the anti-ischemic actions of mechanistically diverse agents. *J Pharmacol Exp Ther* 1993; **259**: 97–103.
95. Mizumura T, Nithipatikam K, Gross GJ. Bimakalim, an ATP-sensitive potassium channel opener, mimics the effects of ischemic preconditioning to reduce infarct size, adenosine release, and neutrophil function in dogs. *Circulation* 1995; **92**: 1236–1245.
96. Gomoll AW, Roth RA, Swillo RE, Baird AJ, Sargent CS, Behling RW, Malone HJ, Grover GJ. Effect of timing of treatment on the glyburide-reversible cardioprotective effects of BMS-180448. *J Pharmacol Exp Ther* 1997; **281**: 24–33.
97. Grover G, Sleph P. Protective effect of K_{ATP} openers in ischemic rat hearts treated with a potassium cardioplegic solution. *J Cardiovasc Pharmacol* 1995; **26**: 698–706.
98. McPherson C, Pierce G, Cole W. Ischemic cardioprotection by ATP-sensitive K^+ channels involves high-energy phosphate preservation. *Am J Physiol* 1993; **265**: H1809–1818.
99. Grover GJ, Dzwonczyk S, Sleph PG, Malone H, Behling RW. Cardioprotective effects of the ATP-sensitive potassium channel opener BMS-180448: functional and energetic considerations. *J Cardiovasc Pharmacol* 1997; **29**: 28–38.
100. Pignac J, Bourgouin J, Dumont L. Cold cardioplegia and the K^+ channel modulator aprikalim (RP 52891): improved cardioprotection in isolated ischemic rabbit hearts. *Can J Physiol Pharmacol* 1994; **72**: 126–132.
101. Yao Z, Gross G. Effects of the K_{ATP} channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs. *Circulation* 1994; **89**: 1769–1775.
102. Grover G, D'Alonzo A, Parham C, Darbenzio R. Cardioprotection with the K_{ATP} opener cromakalim is not correlated with ischemic myocardial action potential duration. *J Cardiovasc Pharmacol* 1995; **26**: 145–152.
103. Hamada K, Yamazaki J, Nagao T. Shortening of action potential duration is not a prerequisite for cardiac protection by ischemic preconditioning or a K_{ATP} channel opener. *J Mol Cell Cardiol* 1998; **30**: 1369–1379.
104. Grover GJ, D'Alonzo A, Hess T, Sleph P, Darbenzio R. Glyburide-reversible cardioprotective effect of BMS-180448 is independent of action potential shortening. *Cardiovasc Res* 1995; **30**: 731–738.
105. Perchenet L, Kreher P. Mechanical and electrophysiological effects of preconditioning in isolated ischemic/reperfused rat hearts. *J Cardiovasc Pharmacol* 1995; **26**: 831–840.
106. Much-Ellingsen J, Bugge E, Lokebo JE, Ytrehus K. Potassium channel blocker dofetilide does not abolish ischaemic preconditioning. *Scand J Clin Lab Invest* 1997; **57**: 13–20.

107. Linz W, Jung O, Jung W, Scholkens BA, Englert HC. Different effects of K_{ATP} channel blockers on ischemic preconditioning. *J Mol Cell Cardiol* 1998; **30**: A18.
108. Atwal K, Grover G, Lodge NJ, Normandin DE, Traeger SC, Sleph PG, Cohen RB, Bryson CC, Dickinson KEJ. Binding of ATP-sensitive potassium channel openers to cardiac membranes: correlation of binding affinities with cardioprotective and smooth muscle relaxing potencies. *J Med Chem* 1998; **41**: 271–275.
109. Bray KM, Quast U. A specific binding site for K_{ATP} channel openers in rat aorta. *J Biol Chem* 1992; **267**: 11689–11693.
110. Mitchell P. Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature* 1961; **191**: 144–148.
111. Mitchell P. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol Rev* 1966; **41**: 445–502.
112. Garlid KD. Unmasking the mitochondrial K^+/H^+ exchanger: swelling-induced K^+-loss. *Biochem Biophys Res Comm* 1978; **83**: 1450–1455.
113. Garlid KD. On the mechanism of regulation of the mitochondrial K^+/H^+ exchanger. *J Biol Chem* 1980; **255**: 11273–11279.
114. Garlid KD. Mitochondrial Volume Control. In *Integration of Mitochondrial Function* (Lemasters JJ, Hackenbrock CR, Thurman RG, Westerhoff HV, Eds). Plenum Publ. Corp., New York. 1998: pp. 257–276.
115. Li X, Hegazy MG, Mahdi F, Jezek P, Lane RD, Garlid KD. Purification of a reconstitutively active K^+/H^+ antiporter from rat liver mitochondria. *J Biol Chem* 1990; **265**: 15316–15322.
116. Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K^+ channel in the mitochondrial inner membrane. *Nature* 1991; **352**: 244–247.
117. Paucek P, Mironova G, Mahdi F, Beavis AD, Woldegiorgis G, Garlid KD. Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K^+ channel from rat liver and beef heart mitochondria. *J Biol Chem* 1992; **267**: 26062–26069.
118. Yarov-Yarovoy V, Paucek P, Jaburek M, Garlid KD. The nucleotide regulatory sites on the mitochondrial K_{ATP} channel face the cytosol. *Biochim Biophys Acta* 1997; **1321**: 128–136.
119. Beavis AD, Lu Y, Garlid KD. On the regulation of K^+ uniport in intact mitochondria by adenine nucleotides and nucleotide analogs. *J Biol Chem* 1993; **268**: 997–1004.
120. Jaburek M, Yarov-Yarovoy V, Paucek P, Garlid KD. State-dependent inhibition of the mitochondrial K_{ATP} channel by glyburide and 5-hydroxydecanoate. *J Biol Chem* in press.
121. Garlid KD, Paucek P, Yarov-Yarovoy V, Sun X, Schindler PA. The mitochondrial K_{ATP} channel as a receptor for potassium channel openers. *J Biol Chem* 1996; **271**: 8796–8799.
122. Paucek P, Yarov-Yarovoy V, Sun X, Garlid KD. Inhibition of the mitochondrial K_{ATP} channel by long-chain acyl-CoA esters and activation by guanine nucleotides. *J Biol Chem* 1996; **271**: 32084–32088.
123. Cook NS, Quast U. Potassium channel pharmacology. In *Potassium Channels* (Cook NS, Ed.). Ellis Harwood Ltd. 1990; pp. 181–231.
124. Suzuki M, Kotake K, Fujikura K, Inagaki N, Suzuki T, Gonoi T, Seino S, Takata K. Kir6.1: a possible subunit of ATP-sensitive K^+ channels in mitochondria. *Biochem Biophys Res Commun* 1997; **241**: 693–697.
125. Liu Y, Sato T, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* 1998; **97**: 2463–2469.
126. Sato T, O'Rourke B, Marban E. Modulation of mitochondrial ATP-dependent K^+ channels by protein kinase. *Circ Res* 1998; **83**: 110–114.
127. Chi L, Uprichard ACG, Lucchesi BR. Profibrillatory actions of pinacidil in a conscious canine model of sudden coronary death. *J Cardiovasc Pharmacol* 1990; **15**: 452–464.
128. de La Coussaye JE, Eldedjam J-J, Bruelle P. Electrophysiologic and arrhythmogenic effects of the potassium channel agonist BRL 38227 in anesthetized dogs. *J Cardiovasc Pharmacol* 1993; **22**: 722–730.
129. D'Alonzo AJ, Darbenzio R, Hess TA, Zhu JL, Parham CS, Grove GJ. Effects of BMS-191095, a nonvasodilating KATP opener, on hemodynamics, electrophysiology, cardioprotection, and arrhythmias in naive and myocardial infarcted dogs. *J Mol Cell Cardiol* 1998; **30**: 415–423.
130. Vegh A, Papp JG, Gyorgy K, Kaszala K, Parratt JR. Does the opening of ATP-sensitive K^+ channels modify ischaemia-induced ventricular arrhythmias in anaesthetized dogs? *Eur J Pharmacol* 1997; **333**: 33–38.
131. Wilde AAM, Janse MJ. Electrophysiological effects of ATP senstivie potassium channel modulation: implications for arrhythmogenesis. *Cardiovasc Res* 1994; **28**: 16–24.
132. Le Grand B, Hatem S, LeHeuzey J-Y, Deroubaix E, Benitah J-P, Coraboeuf E. Pro-arrhythmic effect of nicorandil in isolated rabbit atria and its suppression by tolbutamide and quinidine. *Eur J Pharmacol* 1992; **229**: 91–96.
133. Walleben CD, Sanguinetti MC, Siegl PKS. Influence of ATP-sensitive potassium channel modulators on ischemia-induced fibrillation in isolated rat hearts. *J Mol Cell Cardiol* 1989; **21**: 783–788.
134. Forrest CR, Neligan P, Zhong A, He W, Yang RZ, Pang CY. Acute adenosine treatment is effective in augmentation of ischemic tolerance in muscle flaps in the pig. *Plast Reconst Surg* 1997; **99**: 172–182.
135. Pang CY, Neligan P, Zhong A, Xu H, Forrest CR. Effector mechanism of adenosine in acute ischemic preconditioning of skeletal muscle against infarction. *Am J Physiol* 1997; **273**: R887–R895.
136. Pang CY, Neligan P, Xu H, Zhong A, Hopper R, Forrest CR. Role of ATP-sensitive K^+ channels in ischemic preconditioning of skeletal muscle against infarction. *Am J Physiol* 1978; **273**: H44–H51.