Pathophysiological Functions of ATP-sensitive K⁺ Channels in Myocardial Ischemia

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SUMMARY

The ATP-sensitive K⁺ channels (K<sub>ATP</sub>) are characterized by strong inhibition by intracellular ATP but their activity is also modulated by various intracellular factors with complicated and undefined mechanism. These factors include a low concentration of ADP (or ATP/ADP ratio), a mildly low pH, G-protein coupled process, adenosine and so on. Intracellular ATP has a ligand action to inhibit the channel activity on the one hand, but on the other ATP is necessary for maintaining the channels in an operative state, probably due to the enzymatic process involving ATP hydrolysis. K<sub>ATP</sub> is inhibited by antidiabetic sulfonylureas and sodium 5-hydroxydecanoate. The channels are activated by the K⁺ channel openers in an ATP-dependent manner, but may have diverse mechanisms of actions depending on different compounds. The K<sub>ATP</sub> channel openings are responsible for shortening the action potential duration (APD) and partial K⁺-efflux during early ischemia. The discrepancy between the high sensitivity of intracellular ATP to inhibit K<sub>ATP</sub> in cell-free, inside-out patches and millimolar orders of myocardial ATP concentration determined by the biochemical techniques may cast some doubts on the actual openings of this channel. It can be explained by the presence of cofactors to stimulate channel opening, heterogeneity or compartmentation of ATP distribution in the cell, the properties and high density of K<sub>ATP</sub>, or a combination of these factors. The opening of K<sub>ATP</sub> during ischemia may contribute to the development and aggravation of serious arrhythmias to some extent, but their opening also protects cellular damage, limits infarct size and improves recovery of cardiac function during reperfusion, acting as a cardioprotection mechanism. K<sub>ATP</sub> opening may mimick the effects of ischemic preconditioning, but its effect may be variable among different animal species and experimental conditions.

Further studies are necessary to clarify the actual role of channel opening and the molecular mechanism. (Jpn Heart J 1997; 38: 297–315)

Key words: Intracellular ATP, ADP, Action potential shortening, Early ischemia, Cardioprotection
Myocardial hypoxia and ischemia are the most common conditions leading to development of serious arrhythmias and to a decrease in cardiac contractility. During myocardial hypoxia and ischemia, action potential shortening is an early and common finding in cardiac membrane potential changes, and would limit the amount of Ca\(^{2+}\)-influx through the Ca\(^{2+}\) channels during activity and decrease the tension development of the myocardium. With respect to the electrical activity observed during myocardial hypoxia and metabolic inhibition, Trautwein et al.\(^1\) were the first to report shortening of the action potential duration (APD) as the most prominent and consistent finding. Subsequently, MacDonald and MacLeod\(^2\) described the restoration of APD shortening by application of high external glucose or by intervention to increase intracellular ATP during hypoxia and metabolic inhibition. This is the first indication that the metabolic state of cardiac cells directly modulates APD possibly through an action on ionic channels. The shortening of APD was then attributed to a decrease in the inward Ca\(^{2+}\) current, increases in the background K\(^+\) currents, or both.\(^3\)

Using the voltage clamp technique in small multicellular preparations, Vleugels, Vereecke and Carmeliet\(^4\) have demonstrated the appearance of the time-independent K\(^+\) current with little decrease in the Ca\(^{2+}\) current during hypoxia, which is thought to be the main ionic mechanism for APD shortening. A similar current could be induced by application of the metabolic inhibitor DNP and cyanide to various cardiac preparations\(^5,6\). Furthermore, intracellular injection of ATP restored shortened APD\(^7\). These findings have received strong support due to the discovery of ATP-sensitive K\(^+\) channels in cardiac cells by Noma\(^8\). In this review, I will discuss the pathophysiological function of this channel opening during myocardial ischemia.

**Properties of Cardiac ATP-sensitive K\(^+\) Channels**

ATP-sensitive K\(^+\) channels (K\(_{\text{ATP}}\)) are ligand-gated channels with a strong inhibition of channel activity by internal ATP and belong to the family of inward rectifier K\(^+\) channels.\(^8-14\) The molecular structure of K\(_{\text{ATP}}\) also belongs to the family of inward rectifier K\(^+\) channels with two transmembrane spanning regions and the channel function can only be expressed by co-assembly with the channel protein (K\(_r\) 6.2) and the sulfonylurea receptor.\(^15\) K\(_{\text{ATP}}\) in the heart is characterized as being highly selective for K\(^+\), having a weak inward rectification and being the second largest single channel conductance among various K\(^+\) channels so far identified\(^10-14\). With symmetrical K\(^+\) concentrations (140 mM) on both sides of the patch membrane, the conductance of the unitary inward current through K\(_{\text{ATP}}\) ranges between 70 and 90 pS, which is smaller than that of Na\(^-\)-activated K\(^+\) channels but larger than those of the inward rectifier, delayed rectifier, acetyl-
choline-activated and transient outward $K^+$ channels. The outward current flow through $K_{ATP}$ is usually smaller than the inward current at comparable voltages, especially at potentials positive to +40 mV, thus displaying a weak inward rectification. The rectification was caused by a voltage-dependent block by internal cations, including $\text{Mg}^{2+}$ and $\text{Na}^+$. The channels are not activated by $\text{Ca}^{2+}$ or voltage in contrast to the $\text{Ca}^{2+}$-activated $K^+$ channels, but $\text{Ca}^{2+}$ and voltage might

\[\text{Figure 1. Effects of ATP on the activity of ATP-sensitive K^+ channels in inside-out patches from guinea-pig ventricular myocytes. (A) shows actual experimental record from an inside-out patch with slow time scale (upper record) and expanded time scale (lower record) at corresponding time (a,b,c). Intracellular side of the patch membrane was first perfused with ATP-free solution and the channels were fully opened (a). Application of 0.5 mM ATP to the intracellular solution markedly suppressed channel activity (b). Addition of 0.1 mM ADP in the presence of Mg$^{2+}$ induced openings of the channels (c). C denotes the closed level of the channel. (B) indicates the dose-response curves for the channel inhibition by ATP. Closed circles are ATP alone and open circles 0.1 mM Mg-ADP. Addition of ADP shifted the curve to the right, indicating a stimulatory effect of 0.1 mM ADP. (Reproduced from 105) by permission}\]
modulate $K_{ATP}$ activity; for example, $Ca^{2+}$ can block and inactivate the channel activity\cite{18,19} and the gating kinetics of the channel depends on electromotive force.\cite{20} The channel gating behaviors are somewhat different at positive and negative voltages relative to the potassium equilibrium potential ($E_K$). Channel opening occurs in clusters in bursts separated by a long closure. At negative potentials to $E_K$ the distribution of open times is fitted to a single exponential, suggesting the presence of a single open state. At positive voltages to $E_K$ two exponentials can give a better fit to the open time distribution, which may indicate the presence of two open states. The closed time distribution is fitted to, at least, two exponentials in the activity of the bursts.\cite{8,10,17,21} The rapid flickering behaviours in the bursts are determined by $K^+$ flux driven by electromotive force for $K^+$ through the channel pore.\cite{20}

The most striking characteristic of $K_{ATP}$ is a strong inhibition by intracellular ATP ($ATP_i$), but not by extracellular ATP. Thus, the channels are opened when $ATP_i$ becomes lower than a critical level.\cite{8-11} In inside-out, cell-free patch membranes, the channels are fully opened in the [ATP]-free condition and sharply inhibited in the presence of ATP in a concentration dependent manner (Figure 1). Half maximal inhibition can be obtained usually at 20–30 $\mu$M or at less than 100 $\mu$M.\cite{12-14,22-24} Since normal cardiac cells contain 5–10 mM of ATP,\cite{25-27} $K_{ATP}$ is closed in the physiological state. The dose-response curve for channel inhibition by ATP is usually fitted to a slope factor of around 2, indicating that there is more than a single binding process between the channel protein or associated unit and ATP molecule,\cite{14,24} although there is an indication that the single time course of the process is ascribed to the channel closure by ATP.\cite{28} The channel inhibition by ATP is produced by a ligand action, and the phosphorylation or hydrolysis is not required for the inhibitory action, since free-forms of ATP are equally effective as Mg-ATP, and non-hydrolyzable ATP analogues can also inhibit $K_{ATP}$.\cite{10,24,29} Other nucleosides can also block the channels but ATP is most potent inhibitor, followed in order by ADP, AMP, CTP and GTP.\cite{12,14,22-24}

The action of $ATP_i$ has dual modes in which ATP not only inhibits the channel activity but also maintains the channel in an operative state. In inside-out patch recordings, channel opening is maximal in the ATP-free condition at first, but the activity gradually decreases with time in a process know as “run-down.”\cite{9,30-33} Similar rundown is induced by the presence of intracellular $Ca^{2+}$ over 10 $\mu$M or $Mg^{2+}$.\cite{16,18,31,34} This rundown process may be regulated by chemical gates located on the cytoplasmic side since treatment of the cytoplasmic side of patch membranes by trypsin can remove or markedly delay the development of rundown.\cite{34} After complete rundown, channel activity can be restored by short exposure to ATP and subsequent wash-out.\cite{12,14,32,33,35} This reactivation of channel activity by ATP can only be achieved by Mg-ATP but not by free-forms of ATP
nor non-hydrolyzable ATP analogues, suggesting phosphorylation or hydrolysis of ATP might be required.\textsuperscript{32,33} A recent study has demonstrated that ATP hydrolysis rather than phosphorylation is necessary for reactivation of rundown channels by Mg-ATP.\textsuperscript{36} It has also been shown that actin cytoskeleton may play an important role in this rundown and reactivation process utilizing ATP hydrolysis energy.\textsuperscript{37}

\section*{Modulation of \(K_{\text{ATP}}\) Channel Activity by Intracellular and Extracellular Factors}

In addition to \([\text{ATP}]_i\), various intracellular factors can modulate the channel activity. ADP in the absence of ATP can inhibit \(K_{\text{ATP}}\), but in its presence ADP stimulates channel activity (Figure 1). This action becomes evident only at low (<250 \(\mu\text{M}\)) ADP with Mg\textsuperscript{2+} or the ADP/ATP ratio may determine the stimulatory activity.\textsuperscript{23,24,30,31,38,39} There are two ATP binding sites on the channel protein, one of which has a high binding affinity for ADP than ATP, although the ATP-bound form exhibits stronger channel inhibition than the ADP-bound form. Thus, channel inhibition by ATP is decreased because of decreased ATP binding and the potency order for the channel inhibition by ATP over ADP\textsuperscript{12,14,23,24}; ADP antagonizes the ATP inhibition of channel activity. There may be an additional mechanism involved in ADP stimulation. The second action by ADP is to restore channel activity, and this action is commonly shared by other nucleoside diphosphates such as UDP, GDP, CDP, and IDP.\textsuperscript{23,39} In the absence of ATP, nucleoside diphosphates can stimulate channel activity, or even after rundown, channel activity recovers with nucleoside diphosphates and Mg\textsuperscript{2+}. After restoration of channel activity by nucleoside diphosphates, \(K_{\text{ATP}}\) can be inhibited by ATP, as is the case during spontaneous opening. Thus, ADP-restored channel activity has no antagonism against ATP inhibition. There is some evidence that activation of \(K_{\text{ATP}}\) is coupled to a G-protein mediated process.\textsuperscript{40} Adenosine, which is increased in tissue and coronary blood during myocardial ischemia binds to the A1-receptor of the myocardial membrane. Activation of the A1-receptor by adenosine has been shown to activate \(K_{\text{ATP}}\) via an inhibitory G-protein (G\textsubscript{i}) coupled mechanism at the single channel recording in the cell-free condition, possibly through antagonizing ATP-induced inhibition.\textsuperscript{41-43} This may suggest that \(K_{\text{ATP}}\) is prone to be opened during ischemia by increased adenosine, but its actual channel opening has not yet been demonstrated under experimental conditions comparable to ischemia. Another factor which modifies the opening of \(K_{\text{ATP}}\) is mild acidosis. When the pH is lowered to 6.0–6.8 from the normal level of 7.2–7.4, the opening of \(K_{\text{ATP}}\) is enhanced with a slight decrease in channel conductance.\textsuperscript{19,44-46} The increased activity stems from the increased channel
open probability due to $H^+$. Increased ischemic metabolites such as lactates are thought to activate $K_{ATP}$ channel opening, but this effect is controversial and no definite conclusion has been achieved.

Cardiac $K_{ATP}$ channels are blocked by glibenclamide and other antidiabetic sulfonylureas. The affinity of this class of drugs for cardiac $K_{ATP}$ is lower than the affinity found in pancreatic $\beta$- and insulinoma cells. Channel inhibition by sulfonylureas is not competitive with that by ATP, suggesting different binding sites. ADP has been shown to relieve inhibition by glibenclamide, which is opposite to that seen in pancreatic $\beta$-cells. The access of sulfonylureas to the binding site is from both the external and internal sides of the membrane, and therefore, the binding site appears to be located at the cytoplasmic side of the membrane outside of the electrical field because the drug action is voltage-inde-

**Figure 2.** Effects of pinacidil (A) and nicorandil (B) on ATP-sensitive $K^+$ channel currents recorded with inside-out patches from guinea pig ventricular myocytes. The membrane potential was held at $+60$ mV under nearly symmetrical $K^+$ concentrations ($140$ mM). In A,a, [ATP] is free and channel activity is fully opened. In the presence of 2 mM [ATP], channel is completely closed (A,b). Application of pinacidil in the presence of 2 mM ATP, channel activity appears without significant change in single channel conductance (A,c). Application of glibenclamide (A,d) completely blocks the pinacidil-induced channel activity. In B,a, [ATP] is free and channel is fully opened. In B,b, addition of 0.5 mM [ATP] partially inhibits channel activity. Application of nicorandil induces channel activity without changes in current amplitude (B,c) and glibenclamide blocks the nicorandil-induced channel activity (B,d). (Reproduced from 59 and 60 with permission).
pendent. The sensitivity of K_{ATP} to glibenclamide is markedly reduced in profound inhibition of cardiac cell metabolism.\(^{32}\) Another agent, sodium 5-hydroxydecanoate, with a chemical structure different from sulfonylureas, has also been shown to block K_{ATP}.\(^{53}\)

Certain groups of vasodilating drugs or so-called “K+ channel openers” can activate K_{ATP}.\(^{21,54-57}\) The activating action of the K+ channel openers (KCO) on cardiac K_{ATP} is much lower than those used to open pancreatic K_{ATP}, but is one to two orders of magnitude higher than those used in vascular smooth muscle cells.\(^{23,24}\) KCOs include cromakalim, pinacidil and nicorandil,\(^{23,57}\) all of which activate the time-independent K+ current through the opening of K_{ATP} with ATP_i-dependent manner; the lower the ATP_i, the stronger the activation of the channel by the KCO. The activation induced by KCO is antagonized or inhibited by increasing ATP, (Figure 2).\(^{21,23,54-56,58-60}\) Since the KCOs applied to the internal face of the membrane can activate the channels and their actions are not voltage dependent, the drug binding site seems to be located on the cytosolic side of the membrane. At the single channel level, KCOs increase the channel open probability without changing the conductance.\(^{54-56,58-61}\) They prolong the burst durations and shorten the interburst intervals without affecting the fast flickering behaviour in the bursts\(^{21,60}\) the effects of which are seemingly opposite to increased ATP. The competitive manner of the drug effect against ATP-inhibition of the K+ current led to the suggestion that these K+ channel opening drugs may compete with ATP for the channel binding sites.\(^{54,56,58}\) However, close observation of the dose-response curves at the single channel level reveal that pinacidil does not act in a simple 1:1 competitive manner to the [ATP]-inhibition.\(^{61}\) Therefore, at least pinacidil does not seem to compete with ATP in a 1:1 fashion at its binding site, although its action site may be located close to the ATP binding site for inhibition. It has been reported, however, that certain types of KCO can open K_{ATP} by competing with ATP.\(^{23}\) Therefore, these drugs may have diverse mechanisms of action to open the channels.

**Hypoxic or Anoxic Conditions could Induce Activation of the ATP-sensitive K+ Channels in Cardiac Myocytes**

Hypoxic interventions and metabolic inhibitions could cause activation of the time-independent K+ current,\(^{4-7}\) which would cause shortening of APD, K+-efflux and extracellular K+ accumulation in early ischemia.\(^{62}\) The next question is whether hypoxic and anoxic interventions could induce activation of a specific K+ channel or K+ transport mechanism. If the former is the case, the opening of K_{ATP} exclusively or other types of K+ channel, as well, is involved in the increase in the outward current. There are other types of K+ channels besides K_{ATP}, which
might open under ischemic conditions. They are the internal Na\(^+\) activated K\(^+\) channel\(^{63}\) and two types of K\(^+\) channels activated by arachidonic acid,\(^{64}\) since ischemic conditions could cause the accumulation of intracellular Na\(^+\) and arachidonic acid metabolites. In multicellular preparations and single isolated myocytes, ischemic or hypoxic interventions could produce shortening of APD and accumulation of K\(^+\) in the extracellular space without a concomitant increase in co- or counter-transported ions. These effects were abolished by glibenclamide, a specific blocker of K\(_{\text{ATP}}\), suggesting the K\(_{\text{ATP}}\) channel might be a main contributor to these changes.\(^{65-70}\) The activated current is a potassium current which has properties similar to K\(_{\text{ATP}}\) in terms of conductance, kinetics and pharmacological responses to sulfonylurea compounds.\(^{57-70}\) There have been no reports thus for describing the opening or activation of other types of K\(^+\) channels besides K\(_{\text{ATP}}\) under these experimental settings. Benndorf et al,\(^{71}\) using a specific chamber to expose myocytes to anoxia (PO\(_2\) < 0.1 Torr), demonstrated that only K\(_{\text{ATP}}\) was opened under this condition and that the channel opening was caused by a sufficient decrease in submembrane ATP levels. We also ob-

![Figure 3](image-url)

**Figure 3.** Openings of the ATP-sensitive K\(^+\) channels in hypoxia, glucose-free with-deoxyglucose solution in a cell-attached patch. A): Single channel record demonstrating channel opening 26 minutes (arrow) after the start of hypoxic intervention. The type of the channel activity was not seen in the control. B): Expanded time scale of current records after the opening of channels at two voltages (-50 mV, the upper and -70 mV, the lower trace). C): Amplitude histograms. D): I-V relation of the channel current. The extrapolated reversal potential was about 0 mV. (Reproduced from 105) with permission).
served similar channel opening in cell-attached patch recordings from guinea-pig ventricular myocytes subjected to hypoxia without glucose plus 2-deoxyglucose (5 mM) to block glycolysis (Figure 3). Therefore, K<sub>ATP</sub> is the main and only K<sup+</sup> channel to be opened during hypoxic and anoxic conditions in living cells.

Despite accumulating evidence indicating the opening of K<sub>ATP</sub> during early ischemia or at the time when APD is shortened under these conditions, the mechanism by which the current is activated is a matter of controversy since the measured [ATP]<sub>i</sub> is still at or near normal levels of around 5 mM, which is two orders higher than the IC<sub>50</sub> value of the K<sub>ATP</sub> closure obtained in inside-out patch recordings. Several explanations have been proposed to account for the discrepancy between [ATP]<sub>i</sub> and the channel opening:

1. In living cells, the sensitivity of the channel closure to the ATP inhibition is lower than that of excised patches because of the presence of co-factors, lost during patch excision, to modulate the channel openings. These co-factors include ADP, G-protein, H<sup+</sup> and extracellular adenosine.

2. Distribution of intracellular ATP may be compartmentalized or heterogeneous and, therefore, submembrane ATP content which is located close to the channels is lower than the total [ATP]<sub>i</sub>. (3) Because of the high density of the channel distribution (about 3000 channels per ventricular cell) and the large conductance of K<sub>ATP</sub>, a channel open probability of < 1% could activate a current sufficient to shorten APD to less than 50% of the control.

4. There is a large variability in the IC<sub>50</sub> value of [ATP]<sub>i</sub> for channel closure among different patches, about 50-fold, suggesting heterogeneous sensitivity among K<sub>ATP</sub> channels. Most likely all of these factors contribute to the opening of K<sub>ATP</sub>, and the presence of co-factors may play a particularly key role in the actual opening of K<sub>ATP</sub>. For measuring the ATP inhibition in inside-out patches, rundown of channel activity often hinders an accurate estimation. We have found that tryptic treatment of the internal face of patch membranes can greatly remove the rundown in inside-out patches. Using this preparation, the channel open probability in the presence of a millimolar order of [ATP]<sub>i</sub> in the presence of ADP and with a low pH was examined. It was observed that the open probability of K<sub>ATP</sub> at 2 mM-ATP with ADP and pH 6.8 was > 1%, which is well above the level to activate the current causing APD shortening to 50% of the control, whereas the channel open probability in the patches without tryptic treatment was nearly zero at 2 mM ATP and pH 7.2. In advanced stages of ischemia, intrinsic properties of K<sub>ATP</sub> regarding the closure by ATP, might be altered due to Ca<sup2+</sup>-dependent process giving persistent channel activity in the presence of ATP.

The APD shortening observed during early ischemia and hypoxia appears to be caused mostly by the opening of K<sub>ATP</sub>, because of the magnitude of the current activation and the sensitivity to glibenclamide. As for the mecha-
nism of $K^+$ loss from ischemic or hypoxic myocardium, however, the evidence supporting $K_{ATP}$ channel opening as the main factor is somewhat controversial. Pretreatment with $K_{ATP}$ channel blockers reduces $K^+$ loss in hypoxia or from ischemic myocardium,65-67,70,75,76, and therefore, extracellular $K^+$ accumulation in early ischemia is attenuated but not completely attenuated depending on the experimental design.62,76 In the presence of $K^+$ channel openers, the rate of increase in $[K^+]_o$ is not enhanced,77-79, see also 62) suggesting that the opening of $K_{ATP}$ is not a major factor responsible for increased $K^+$ efflux. There may be additional factor(s) involved in $K^+$ efflux and external $K^+$ accumulation in ischemic myocardium.

**ROLE OF $K_{ATP}$ OPENING IN ISCHEMIA/REPERFUSION**

It is well known that there are various types of arrhythmias developed during myocardial ischemia. The types and mechanism of these ischemia-induced arrhythmias differ depending on time after onset of ischemia, and differ in different experimental models and species used.80 During early ischemia within 30 minutes of its onset, ventricular fibrillation and ventricular tachycardias are major arrhythmias and causes of fatal events. These fatal arrhythmias are due to reentry.80 It has also been suggested that premature beats during early ischemia are mostly caused by the reverse,81) The opening of $K_{ATP}$ during an ischemic period could lead to a shortening of APD and partly increased extracellular $K^+$ accumulation. The former condition can be beneficial, on the one hand, for cardiac function since the shortened APD limits the Ca$^{2+}$-influx during activity to decrease tension development and thereby to decrease energy consumption. On the other hand, shortening of APD and extracellular $K^+$ accumulation leading to a shortened refractory period, development of slowing in conduction and block are the main factors predisposing reentry and serious arrhythmias in the early phase of ischemia,82,83 since epicardial cells are more susceptible than endocardial cells for APD shortening and $K_{ATP}$ in the former is more sensitive to metabolic inhibition for its opening than that in the latter,82) the heterogenous appearance of these conditions enable easy development of reentry. Pretreatment with $K_{ATP}$ blockers prevents APD shortening, attenuates $K^+$ accumulation to some extent, and decreases or improves conduction slowing and block. As a result, the development or incidence of serious arrhythmias during early ischemia can be reduced by the application of sulfonylurea drugs.65,66,72,75-78,83) Additional activation of $K_{ATP}$ in acute ischemia may enhance the rate of APD shortening and adversely affect the incidence of serious ventricular arrhythmias.62,83 In addition to these direct electrophysiological effects, $K_{ATP}$ channel modulation can affect several factors that influence the development of arrhythmias. Application of KCOs delays the
onset of ischemic contracture, postpones the occurrence of electrical uncoupling and abbreviates irreversible cell injury.\(^7,8^{4-89}\) Eventually, infarct size is reduced by application of KCOs in dogs,\(^85,88-91\) although conflicting results have been reported.\(^93,94\) Glibenclamide, a K\(_{\text{ATP}}\) blocker, shortened the development of ischemic contracture and irreversible damage,\(^84-92\) which supports the cardioprotective effect of K\(_{\text{ATP}}\) channel activation, while conflicting results have also been presented depending on the experimental conditions and animal models used (Table).

Recent evidence supports the notion that the opening of K\(_{\text{ATP}}\) mimicks the effect of ischemic preconditioning; a brief ischemic event protects cellular damage and reduces infarct size in subsequent, more severe ischemic insult.\(^95\) The cardioprotection by the K\(_{\text{ATP}}\) opening is provided by abolition of the effects of ischemic preconditioning by pretreatment with glibenclamide or 5-hydroxydecanoate, and by tolerance to subsequent ischemia achieved with the application of KCOs.\(^96-99\) It is hypothesized, therefore, that the activation of K\(_{\text{ATP}}\) is involved in mediating ischemic preconditioning, although somewhat conflicting results against this cardioprotective effect by channel activation have been presented in different experimental models.\(^100-102\) Cardioprotection by KCOs appears to be independent from their peripheral vasodilatory action and depends on a direct action on the myocardium. Despite their diverse chemical structures, the cardioprotective effects of KCOs are uniformly observed. Different types of K\(_{\text{ATP}}\) blockers can equally abolish this cardioprotection, which supports the involvement of K\(_{\text{ATP}}\) opening in this process. The exact mechanism of cardioprotection afforded by K\(_{\text{ATP}}\) activation is not known. One explanation is that opening the channels during the preceding ischemia causes shortening of APD and limits Ca\(^{2+}\)-influx during activity, which decreases tension development, abbreviates energy consumption, and results in elimination of Ca\(^{2+}\)-overload in myocardial cells. It has been shown that the effects of preconditioning associated with K\(_{\text{ATP}}\) activation are associated with energy preservation during ischemia and reperfusion,\(^103\) while again an unfavourable result without increased high energy phosphate content is presented in rat hearts.\(^104\) Most of these supporting data for K\(_{\text{ATP}}\) opening in cardioprotection have shown improved mechanical function on reperfusion, reduced infarct size and cell damage. As to the beneficial or inhibiting effects on reperfusion arrhythmias, studies are limited,\(^87,92,103,105\) and poor results have been reported.\(^104,106-108\) The major supporting, opposing and inconclusive data with respect to the involvement of K\(_{\text{ATP}}\) activation in various types of cardioprotection with different models depending on animal species are listed in the Table.

The other major hypothesis attributed to the involvement in ischemic preconditioning is activation of A1 adenosine receptors as an initiating mechanism
### Table. K<sub>ATP</sub> Channel Activation Involved in Cardioprotection

A. Supporting data

<table>
<thead>
<tr>
<th>Authors</th>
<th>Animal models (drugs)</th>
<th>Results</th>
<th>Ref. No.</th>
</tr>
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<tr>
<td>1) Grover et al.</td>
<td>rat, global ischemia (pinacidil, cromakalim, glib.)</td>
<td>Improved reperf. function decreased LDH release</td>
<td>(84)</td>
</tr>
<tr>
<td>2) Grover et al.</td>
<td>rat, global ischemia, dog coronary occl. &amp; reperf. (pinacidil, cromakalim)</td>
<td>Improved reperf. function Reduced infarct size</td>
<td>(85)</td>
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<tr>
<td>3) McCullough et al.</td>
<td>rat, global ischemia (cromakalim, 5-HD, glib.)</td>
<td>Improved reperf. function decreased LDH release</td>
<td>(86)</td>
</tr>
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<td>4) Cole et al.</td>
<td>guinea pig, arterial perfused RV (glib, pinacidil)</td>
<td>Improved electromechanical function</td>
<td>(87)</td>
</tr>
<tr>
<td>5) Auchampach et al.</td>
<td>dog, coronary occl. &amp; reperf. (RP 52891, glib)</td>
<td>Reduced infarct size</td>
<td>(88)</td>
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<tr>
<td>6) Auchampach et al.</td>
<td>dog, coronary occl. &amp; reperf. (nicorandil, glib.)</td>
<td>Improved reperf. function</td>
<td>(89)</td>
</tr>
<tr>
<td>7) Gross et al.</td>
<td>dog &amp; rat, coronary occl. &amp; reperf. (nicorandil)</td>
<td>Attenuates stunning</td>
<td>(90)</td>
</tr>
<tr>
<td>8) Yao &amp; Gross.</td>
<td>dog, coronary occl. &amp; reperf. (glib, KCO, A&lt;sub&gt;1&lt;/sub&gt;-receptor agonists &amp; antagonists)</td>
<td>Attenuates stunning via A&lt;sub&gt;1&lt;/sub&gt;-receptor activation</td>
<td>(91)</td>
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<tr>
<td>9) Tan et al.</td>
<td>rabbit, arterial perfused papillary muscle (glib, cromakalim)</td>
<td>Delayed electrical uncoupling</td>
<td>(92)</td>
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<td>10) Gross &amp; Auchampach</td>
<td>dog, 5 min PC followed by isch. &amp; reperf. (glib, RP52891)</td>
<td>Mediates PC effects</td>
<td>(96)</td>
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<tr>
<td>11) Auchampach &amp; Gross</td>
<td>(5-HD)</td>
<td></td>
<td>(97)</td>
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<tr>
<td>12) Yao et al.</td>
<td>dog, brief repetitive isch. (glib, aprikalim, sotalol)</td>
<td>Protects functional damage</td>
<td>(98)</td>
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<td>13) Yao &amp; Gross</td>
<td>dog, 10 min PC followed by isch. &amp; reperf.</td>
<td>Lower threshold for PC through APD shortening</td>
<td>(99)</td>
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<tr>
<td>14) McPherson et al.</td>
<td>guinea pig, arterial perfused RV (pinacidil, glib.)</td>
<td>Improved electro-mechanical function with preserved high energy phosphates</td>
<td>(103)</td>
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<tr>
<td>15) Hiraoka et al.</td>
<td>rabbit, global hypoxia &amp; reperf. (pinacidil, glib.)</td>
<td>Decreased incidences in VF during reperf.</td>
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<td>16) Grover et al.</td>
<td>5 min PC followed by isch. &amp; reperf. (R-PIA, glib.)</td>
<td>Mediates PC via A&lt;sub&gt;1&lt;/sub&gt; -receptor activation</td>
<td>(112)</td>
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<tr>
<td>17) Yao &amp; Gross</td>
<td>dog, 10 min PC followed by isch. &amp; reperf. (N-NMMA, L-NAME, 5-HD, Ach)</td>
<td>Mediates PC by Ach activation but not by NO synthesis</td>
<td>(113)</td>
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<tr>
<td>18) Van Winkle et al.</td>
<td>pig, 10 min PC followed by isch. &amp; reperf. (Ado, R-PIA, 5-HD)</td>
<td>Mediates PC by A&lt;sub&gt;1&lt;/sub&gt;-receptor activation</td>
<td>(114)</td>
</tr>
<tr>
<td>19) Yao &amp; Gross</td>
<td>dog, 10 min PC followed by isch. &amp; reperf. (Ado, Ach, glib., 5-HD)</td>
<td>Mediates PC by A&lt;sub&gt;1&lt;/sub&gt; receptor activation</td>
<td>(115)</td>
</tr>
<tr>
<td>20) Speechly-Dick et al.</td>
<td>human RA trabeculae, simulated isch. PC (cromakalim, DOG, Glib.)</td>
<td>Mediates PC by PKC activation (end effector of PC)</td>
<td>(116)</td>
</tr>
</tbody>
</table>
or directly mediating factor. This hypothesis is based on the observations that the blockade of cell-surface adenosine receptors with adenosine antagonists resulted in the loss of ischemic preconditioning and that administration of adenosine A1 receptor agonists produces cardioprotective effects comparable to ischemic preconditioning in rabbit hearts. However, it is not clear whether adenosine and A1-receptor activation is an initiating factor for preconditioning or if it is a direct mediating factor for its development. The effects of adenosine or its analogues appear to vary depending on the animal model and species. Furthermore, the application of different types of K<sub>ATP</sub> blockers abolishes the effects of ischemic preconditioning afforded by adenosine and A1-receptor activation, suggesting the involvement of K<sub>ATP</sub> activation in the adenosine-induced process. In the patch clamp study, adenosine and A1 receptor activation have been

<table>
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<th>Authors</th>
<th>Animal models (drugs)</th>
<th>Results</th>
<th>Ref. No.</th>
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<tr>
<td>Imai et al.</td>
<td>dog, 4hr coronary occl. &amp; 20hr reperf. (pinacidil)</td>
<td>No effects on infarct size</td>
<td>(93)</td>
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<td>Kitzen et al.</td>
<td>dog, 90 min coronary occl. &amp; reperf. (cromakalim, celikalim)</td>
<td></td>
<td>(94)</td>
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<tr>
<td>Grover et al.</td>
<td>rat, 5 min PC followed by isch. &amp; reperf. (glib.)</td>
<td>Glibenclamide failed to abolish PC</td>
<td>(100)</td>
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<tr>
<td>Thornton et al.</td>
<td>rabbit, 5 min PC followed by isch. &amp; reperf. (glib. pinacidil)</td>
<td>Inhibition and activation of K&lt;sub&gt;ATP&lt;/sub&gt; did not modify PC</td>
<td>(101)</td>
</tr>
<tr>
<td>Fralix et al.</td>
<td>rat, 5 min PC followed by isch. &amp; reperf. (glib.)</td>
<td>Did not prevent PC, although K&lt;sub&gt;ATP&lt;/sub&gt; inhibition accentuated energy reduction</td>
<td>(102)</td>
</tr>
<tr>
<td>Tosaki &amp; Hellegouarch</td>
<td>guinea pig, various duration of isch. &amp; reperf. (glib., cromakalim)</td>
<td>No improvement in recovery function &amp; Vf incidence</td>
<td>(104)</td>
</tr>
<tr>
<td>Vegh et al.</td>
<td>dogs, 5 min PC followed by isch. &amp; reperf. (glib.)</td>
<td>Reduced infarct size but no changes in Vf incidence</td>
<td>(106)</td>
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<tr>
<td>Tosaki et al.</td>
<td>rat, 30 min global isch. &amp; reperf. (glib., cromakalim)</td>
<td>No depression in reperfusion arrhythmias</td>
<td>(107)</td>
</tr>
<tr>
<td>Bellemín-Baurreau et al.</td>
<td>rabbit, coronary occl. (BRL-38227, glib.)</td>
<td>Accentuates shortening of ERP and increases Vf incidence</td>
<td>(108)</td>
</tr>
</tbody>
</table>

occl. = occlusion; reperf. = reperfusion; PC = ischemic preconditioning; glib. = glibenclamide; isch. = ischemia; Vf = ventricular fibrillation; APD = action potential duration; 5-HD = sodium 5-hydroxydecanoate; Ach = acetylcholine; PKC = protein kinase C; ERP = effective refractory period; KCO = K<sup>+</sup> channel openers; L-NMMA = N-monomethyl-L-arginine; L-NAME = N-nitro-L-arginine methyl ester; R-PIA = [N]-N<sup>6</sup>-(1-methyl-2-penylethyl) adenosine.
shown to activate $K_{\text{ATP}}$ via a G-protein coupled mechanism.\(^{41-43}\) A link between $A_1$ receptor activation and $K_{\text{ATP}}$ channel opening in ischemic preconditioning has been suggested. Whether the two factors are located in the same line of the process involved in ischemic preconditioning or not has not been clarified. Additional factors such as muscarinic receptors, G-protein coupled mechanism, activation of protein kinase C and others have been implicated in ischemic preconditioning using different experimental approaches, models and animal species.\(^{111}\) It is not known whether their actual roles and the interrelations among these factors involved in initiation and development, or if $K_{\text{ATP}}$ activation is serving as an end effector of ischemic preconditioning.\(^{116}\) Further studies are necessary to elucidate the actual mechanism, including at the molecular levels, involved in this very important cardioprotective action.

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