Adenosine triphosphate-sensitive potassium channels in the cardiovascular system

C. G. NICHOLS AND W. J. LEDERER

Department of Cell Biology and Physiology, Washington University School of Medicine, Washington University Medical Center, St. Louis, Missouri 63110; and Department of Physiology, University of Maryland, Baltimore, Maryland 21201

NICHOLS, C. G., AND W. J. LEDERER. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. Am. J. Physiol. 261 (Heart Circ. Physiol. 30): H1675-H1686, 1991.—ATP is normally available in cells at millimolar concentrations and is “buffered” by intracellular pools of other high-energy phosphates, such as creatine phosphate. Thus intracellular [ATP] ([ATP]i) may seem an unlikely candidate for a regulatory signal inside cells. Recent evidence suggests, however, that [ATP]i regulates the behavior of a class of potassium (KATP) channels that are found throughout the cardiovascular system. KATP channels are present in cardiac, skeletal, and vascular smooth muscle. The channels are inhibited by micromolar [ATP]i, and this inhibition is relieved by micromolar [ADP]i. We present evidence in support of the idea that variations of [ATP]i and [ADP]i, even within normal concentration ranges, may influence cellular function in the heart and vascular system via a direct action on the KATP channel. Furthermore, very specific modulators of KATP channel activity are available. We discuss the mechanism of action of these agents and their interaction with endogenous modulators and consider their potential roles in cardiovascular therapy.

adenosine diphosphate; potassium channel opener; heart; action potential; metabolism; sulfonylurea

ATP, ONE OF THE PRINCIPAL intracellular energy storage chemicals, is normally available in cells at concentrations well above those required to half-maximally activate ion pumps and contractile processes. It is manufactured by the mitochondria and by glycolytic enzymes within the cytoplasm and is “buffered” by intracellular pools of other high-energy phosphates, such as creatine phosphate. Thus there is little reason to suspect that intracellular [ATP] ([ATP]i) is likely to be the signal regulating any important cell function under “normal” conditions.

Recent evidence suggests that [ATP]i regulates the behavior of a class of potassium (KATP) channels that are present in tissues at least as diverse as the heart, skeletal muscle, pancreatic β-cells, smooth muscle, and brain (6, 11). This review presents evidence that leads us to support the idea that variations of [ATP]i and [ADP]i may influence cellular function in the cardiovascular system via a direct action on the KATP channel.

Historical perspective. It has been realized since the 1950s that profound shortening of the cardiac action potential rapidly follows the onset of metabolic inhibition (23, 35, 135). Until 1983, understanding of the underlying mechanisms had not advanced beyond the realization that an increase in a time-independent K+ current was a major determinant of the change in action potential shape (73, 143). An increase in intracellular Ca2+ concentration seemed a plausible, but unproven, trigger to the change in K+ conductance. In 1983, Noma (102) reported the presence of K+-selective ion channels in guinea pig ventricular cells that were inhibited by [ATP]i and other adenine nucleotides. Noma (102) postulated that these KATP channels might be responsible for the action potential shortening that occurs in heart muscle during metabolic block. Ashcroft et al. (8) reported the presence of glucose-inhibitable K+ channels in pancreatic β-cells, which were shown to be KATP channels (30), and which are now strongly implicated in the regulation of insulin secretion in those cells (see Ref. 6). In skeletal muscle, KATP channels may be involved in
**K**^+^ loss during fatigue (27), and, in the brain, K\textsubscript{ATP} channels may be involved in the electrical response to anoxia, or blood-glucose levels (11). In smooth muscle, K\textsubscript{ATP} channels have been identified (127). Nicorandil and similar compounds relax smooth muscle and have been shown to act on K\textsubscript{ATP} channels (see Ref. 115), raising the possibility that the K\textsubscript{ATP} channel may also play an important role in the physiological regulation of smooth muscle electrical and mechanical activity; thus these compounds could be important targets for anti-hypertensive drug therapy.

**Goals of this review.** The K\textsubscript{ATP} channel is closed by ATP with half-maximal closure at [ATP] of 20–100 \mu M in heart (49, 102), skeletal muscle (126), smooth muscle (96), and pancreatic \beta-cells (6). Because [ATP] is normally in the range 5–10 mM, it has been questioned whether the K\textsubscript{ATP} channel is likely to play any role in the physiology or pathophysiology of the heart or vascular system. However, based on available evidence, we think these channels must play an important role in myocardial and vascular function. The purpose of this article is to review the present status of work on the biophysical, physiological, pharmacological, and molecular biological properties of the channel and to integrate this knowledge into a consideration of the relevance of the channel in the physiological control and pathophysiological properties of the cardiovascular system.

**General reviews of K\textsubscript{ATP} channel properties (6, 11),** reviews containing a consideration of the possible role of these channels in cardiac (3) and vascular function (96), and a review of the pharmacology of K\textsubscript{ATP} channels (115) have been published, providing valuable additional material on topics related to this review.

**K\textsubscript{ATP} CHANNEL BIOPHYSICS AND PHYSIOLOGY**

**Channel ion permeability.** K\textsubscript{ATP} channels are highly selective for K^+ and have negligible permeability to sodium (75, 102). Rubidium substitutes for K^+ with similar permeability (but much lower conductance) and radioactive \textsuperscript{86}Rb has been used as a tracer for flux through the channel (10, 91). K\textsubscript{ATP} channels are essentially time and voltage independent, because channel open- and closed-state distributions do not appear to change substantially with membrane potential (102, 109). Reports indicate that in skeletal muscle, the channel open time is somewhat decreased at hyperpolarized potentials (125). Additionally, there is evidence that, in the heart, the gating of the channel may be linked to the permeation mechanism in that the open probability appears to increase with the electromotive force (136, 154), although the ATP-dependent kinetics are reported to be insensitive to voltage (109). A link between channel gating and permeation would have very important consequences for the detailed molecular nature of these processes, although further study of the possible interactions is needed.

At negative membrane potentials, the channel is blocked by Cs^+ and Ba\textsuperscript{2+} from the outside (110). At positive membrane potentials, the channel is blocked from the inside by divalent and monovalent cations (70). The marked block by Mg\textsuperscript{2+} has even been used to estimate internal [Mg\textsuperscript{2+}] (71). In the absence of open-channel blockers, the channel behaves according to constant-field assumptions at potentials negative to 0 mV (48). However, even in the absence of any internal cations other than K^+, some inward rectification at more positive potentials is still apparent in the open channel current-voltage relationship (48, 62). At potentials of ~0 mV (in symmetrical 140 mM [K^+]), the single channel conductance is ~70 pS in heart (75), and, with physiological ion gradients, the channel conductance over the range of -80 to +20 mV is ~25 pS (48, 97). The conductance properties are similar in skeletal muscle (125) and pancreatic \beta-cells (30). This is approximately twice the single-channel conductance of background inward-rectifier K^+ channels. The ATP-sensitive K^+ channel reported by Standen et al. (127) in vascular smooth muscle has a higher conductance (135 pS with 120 mM internal [K^+] and 60 mM external [K^+]) than the K\textsubscript{ATP} channels in heart and \beta-cells. A K\textsubscript{ATP} channel has recently been described in cortical neurones (12), and this also has a higher conductance than the cardiac and \beta-cell channel. Occasionally, subconductances can be observed \textit{(e.g., Ref. 75)}, although these have not been observed at high enough frequency to warrant a detailed investigation.

**Nucleotide modulation of K\textsubscript{ATP} channel open probability.** K\textsubscript{ATP} channels are, by definition, K^+ channels that are inhibited by [ATP]. There is, however, a second regulatory role of ATP in channel behavior. A marked feature of channel behavior in isolated membrane patches is that activity "runs down" with time after isolation (136). This rundown can be partially reversed by MgATP (52, 92, 104, 131), consistent with phosphorylation being required for a fully functional openable channel (Fig. 1). We will consider the following questions. 1) What is the mechanism by which ATP inhibits the channel? 2) What is the nature of channel rundown and what is the role of ATP in this rundown? 3) What are the effects of K\textsubscript{ATP} channel activity on cellular electrical activity?

![FIG. 1. Sarcolemmal/plasmalemmal receptors (R) can influence K\textsubscript{ATP} channel activity. K\textsubscript{ATP} channel activity may be stimulated by activation of external membrane bound receptors for adenosine (Ado), acetylcholine, etc. (via G proteins (G)), and by phosphorylation (P) at an internal site. Such regulation may take place over a slow time scale (seconds or minutes), and it appears that phosphorylation or G protein stimulation is necessary for channel to open. rundown of channel activity that follows isolation of membrane patches may be due to dephosphorylation and/or loss of G protein stimulation, because presence of GTP or ATP can restore channel activity.]
dependence of channel activity have been made, the dose-
heart, where the most complete studies of the ATP
on the channel (but also see Refs. 98,
Thus it seems that hydrolysis of ATP is not necessary
to cause channel inhibition and that nucleotide inhibi-
Nonhydrolyzable analogues of ATP are also effective
inhibitors of the channel as are AMP, guanosine diphos-
phate, and ADP in the absence of Mg$^{2+}$ (see Ref. 6).
ATP at the intracellular side of the membrane (Fig. 2).
ATP in the absence of Mg$^{2+}$ inhibit the channel, with MgATP possibly inhibiting slightly more effectively (49, 83). The
ability of both forms of ATP to inhibit the channels is a
major difference between the cardiac channel and the β-
cell channel, where there is substantial evidence that
only ATP in the absence of Mg$^{2+}$ can inhibit the channel (9, 39). ATP appears to reduce the channel open time and to increase the channel closed time in most studies (75, 125). However, the problem of channel rundown (136) and the high density of channels (97) make it very difficult to obtain long recordings of single channel events under stable conditions.

Various models have been proposed to explain the
gating of the channel by ATP. Given the steep Hill coefficient, it has been suggested that there should be at least two binding sites for ATP (see Fig. 3; 49, 83, 102). The nucleotide-dependent kinetics may now be studied using the “concentration-jump” technique (108) in which the tip of a microelectrode (although not the recessed inside-out membrane, see Refs. 22, 108) can be instantaneously exposed to a new solution by passing through oil between one solution and another. Using jumps from high to low [ATP], Qin et al. (109) have demonstrated an apparently monoeponential increase in channel-opening probability after a variable lag time during which channels remain silent. Qin et al. (109) have sought to explain this behavior on the basis of a single binding site, the steepness in the dose-response curve being obtained by ATP dependence of the unbinding reaction. They propose that some unknown intrinsic process must precede the unbinding to explain the lag time on lowering [ATP]. Our own experiments (22) suggest that the lag time may be due to problems of diffusion of ATP between the tip of the pipette and the recessed membrane, and we favor a multisite model to explain the kinetics (99).

In on-cell patches, where the cell has been permeabil-
ized by exposure to saponin (“open-cell attached” patch), the $k_i$ for ATP appears to be much higher (500 μM, see Ref. 75) than in inside-out membrane patches (100 μM, see Ref. 102). It has been argued from such results that some agent that modulates the $k_i$ in vivo, or some effect perhaps of the cytoskeleton, is lost on isolation of the patch. By adding creatine phosphate (to dissipate gradients of [ADP] and [ATP]), the ATP dependence in the “open-cell attached” patch is shown to be similar to that in the inside-out patch (97), the difference resulting from the development of gradients in the “open cell attached” patch. It has also been inferred that the $k_i$ in intact cells is higher than that measured in isolated inside-out mem-
brane patches (103), although again this may be due to the presence of gradients of ATP and ADP within cells. Intracellular divalent cations reduce channel activity, whereas intracellular MgADP increases the $k_i$ (50, 83), possibly by competitive binding in place of ATP. Many possible intracellular modulators, e.g., other nucleotides, lactate, phosphate, and H$^+$ have little or no effect on cardiac $K_{ATP}$ channel activity (83), although a marked stimulation of channel activity in acidic conditions has been reported in skeletal muscle (32), and a marked inhibition of activity at low pH has been reported in pancreatic β-cells (93). In the additional presence of physiological Mg$^{2+}$ and [ADP]$, k_i$, may be shifted to 2- to 10-fold higher [ATP] (97, 100). The relevant $k_{i, ATP}$ during ischemia is likely to be 100–200 μM.
Maintenance of channel activity and modulation of [ATP] sensitivity. Estimates of the ATP dependence of channel activity, as discussed above, have generally been made rapidly after patch isolation. With time after isolation, channel activity spontaneously "runs down", i.e., at a given concentration of ATP, channel activity declines, and the channels spend increasingly long times in the closed state (136). This rundown, at least in heart cells, occurs at all [ATP] and is associated with a shift in the \( k_i \) to lower [ATP] (134; C. G. Nichols and C. Ripoll, unpublished observations). There is now increasing evidence that rundown of channel activity can be, at least partially, prevented or reversed by exposure to ATP in the presence, but not in the absence, of Mg\(^{2+}\) (52, 92, 104, 131). Nonhydrolyzable analogues of ATP such as 5'-adenylylimidodiphosphate and adenyl methylene diphosphate do not substitute for ATP in reactivation from rundown (104, 131), so that phosphorylation of the channel appears to be a mechanism by which rundown is prevented (Fig. 1). This scenario is complicated by the observation that MgADP can also facilitate recovery from rundown (140), where phosphorylation is presumably not the underlying mechanism. In pancreatic \( \beta \) cells and in skeletal muscle channels incorporated into bilayers, reactivation from rundown has also implicated a role for G proteins in regulation of channel activity (38, 106), either in the presence or absence of MgATP (79).

It appears therefore that phosphorylation and/or G protein stimulation of channel activity is normally present in cells and that the properties of the channel immediately after isolation are determined by the preexisting level of such modulation. Because the sensitivity of the channel to inhibition by ATP could be modulated by phosphorylation or G protein stimulation, it is then clearly important to know to what extent such modulation is possible and how much is normally occurring in vivo. It is possible that physiological stimuli might, by increasing phosphorylation or by G protein stimulation, lead to enhanced channel activity under normal physiological conditions. A few interesting possibilities have been presented thus far. Adenosine 3',5'-cyclic monophosphate-stimulation of channel activity has been reported by Tseng and Hoffmann (138), who showed that isoproterenol treatment enhanced \( K_{ATP} \) channel activity in ventricular myocytes under whole cell clamp. These experiments require careful interpretation, however, because whole cell voltage-clamped preparations may be susceptible to energy depletion (16, 103), and isoproterenol may well accelerate energy depletion by stimulating metabolism. Kirsch et al. (79) have provided mechanistic evidence for G protein stimulation of \( K_{ATP} \) channel activity in cultured neonatal rat heart cells. They demonstrated that a G protein, specifically \( G_i \), reduced the channel sensitivity to ATP, and they provided further evidence that adenosine could stimulate channel activity in a guanosine 5'-triphosphate (GTP)-dependent manner, consistent with adenosine being a physiological agonist responsible for G protein stimulation (Fig. 1). In adult rat ventricular myocytes, no stimulatory effects of GTP (only channel blockade) have been observed (83), although stimulation of channel activity by GTP in the presence of adenosine at the outside of the membrane has been reported by Tung and Kurachi (139). In pancreatic \( \beta \)-cells, there is also growing evidence that various gastrointestinal peptides, including somatostatin and galanin (36, 55), activate \( K_{ATP} \) channels by stimulation of a G protein. In vascular smooth muscle, Standen et al. (127) and Nelson et al. (95, 96) have also provided evidence that \( K_{ATP} \) channels may be activated by multiple G protein activators. These include calcitonin-gene related peptide, vasoactive intestinal peptide, and acetylcholine, possibly acting through endothelium-derived relaxing factor or endothelium-derived hyperpolarizing factor.

Effects of channel activity on cellular electrical activity. The activation of \( K_{ATP} \) channels causes marked shortening of the cardiac action potential (136) and hyperpolarization of vascular smooth muscle (127). At the single cell level, channel opening and the subsequent action potential shortening will, by decreasing Ca\(^{2+}\) entry, have a depressive effect on contractility in the heart and in smooth muscle, which in turn will decrease ATP consumption and thereby tend to oppose further action potential shortening. At the organ level, the effects on cardiac electrical activity may be profound (see below). When, and to what extent, will these effects occur under "physiological" conditions? There is strong evidence that \( K_{ATP} \) channels are opened in metabolic blockade and substrate-free anoxia (17, 75, 84, 129), in ischemia (150, 57) and in the presence of the K\(^+\) channel opening drugs (41, 118). Glibenclamide has even been reported to cause action potential lengthening in control conditions (45), without prior pharmacological activation of \( K_{ATP} \) channels. It has been demonstrated that channel opening in metabolic blockade can be reversed by injection of ATP (84, 103, 139) consistent with a decline of [ATP], being responsible for channel opening in these conditions.

However, as alluded to above, \( K_{ATP} \) channels are inhibited by [ATP] in the submillimolar range, whereas the measured [ATP], at the time of cardiac action potential shortening, in metabolic blockade or ischemia, is in the millimolar range, close to normal levels (2, 40, 68, 86, 151). This consideration has thus far provided a major obstacle in assigning a role for \( K_{ATP} \) channels in the clinical and pathophysiological manifestations of metabolic blockade and ischemia. By measuring the density of \( K_{ATP} \) conductance in the membrane, one can predict, either by using computer models (97), injecting simulated current into current-clamped cells (100), or by direct comparison of action potential duration and voltage-clamped currents in the same cell (51), how much of the available conductance would be necessary to cause a given amount of shortening. These experiments suggest that \( \approx 1 \) nS/cell, or \(< 1\% \) of the available conductance (200–1,000 nS/cell), would be sufficient to shorten the action potential by 50%. In rat or guinea pig ventricle, given the measured ATP dependence of channel activity, this would occur at 1–2 mM ATP for spatially uniform [ATP]. From this approach, we expect that measurable action potential shortening will occur if [ATP] falls at all below normal levels. Similar arguments have been made by Carmeliet et al. (24), Findlay et al. (51), and Nelson et al. (96) for smooth muscle and by Cook and Hales (30) for pancreatic \( \beta \)-cells.
Quantitative calculations do not, however, fully predict the extent of cardiac action potential shortening that is observed (97, 100). At the organ level, spatial or temporal inhomogeneities of [ATP] may help to explain the remaining discrepancy between the [ATP] at which action potential shortening is observed and the ATP dependence of channel activity. Phasic decreases of [ATP] and activation of $K_{ATP}$, in each heart beat, with consequent action potential shortening, might occur after depletion of creatine phosphate (60, 103). Intracellular [ATP] may not be homogeneous between cells in the heart, especially under conditions of ischemia or hypoxia (3). The importance of spatial inhomogeneity can be illustrated by the following limiting-case example (see Ref. 97). Assume that cells are fully coupled electrically, but are poorly coupled chemically, so that [ATP] is different between cells. If the [ATP] in 10% of the cells fell to zero while remaining at control levels in the other 90% of cells, the mean increase in $K_{ATP}$ conductance (to 20–100 nS/cell) would then be sufficient to completely abolish excitability, even though the mean fall in [ATP] would, of course, be only 10% and barely detectable by nuclear magnetic resonance or chemical assay.

Finally, it is possible that the cell membrane senses a different pool of ATP than the rest of the cytoplasm and that near-membrane [ATP] may fall more quickly than the rest of the cytoplasm and be only 10% and barely detectable by nuclear magnetic resonance or chemical assay.

PHARMACOLOGICAL REGULATION OF CHANNEL ACTIVITY

$K_{ATP}$ channels are blocked by many of the less-specific organic $K^+$ channel blockers such as tetraethylammonium ions, quinine, and quindine (33, 65, 75, 141), but they appear to be specifically blocked by the sulfonylureas (6, 137). Knowledge of the antidiabetic effect of sulfonylurea compounds on pancreatic $\beta$-cells has led to the unravelling of their action as potent and specific inhibitors of $K_{ATP}$ channels in that cell type (120, 155, 156). Several broadly related smooth muscle relaxants have been suggested to act as $K_{ATP}$ channel openers (PCOs) in the heart and in smooth muscle. Of relevance to our topic of consideration are the studies investigating the two classes of drugs in cardiac and smooth muscle, results of which are somewhat confusing, and the large number of pharmacological studies that have been made in an attempt to define the action of PCOs in smooth muscle.

Action of sulfonylureas on $K_{ATP}$ channels. It has been known since World War II that the sulfonylureas, such as tolbutamide, are clinically effective in the treatment of noninsulin-dependent diabetes mellitus. More recently, single channel recordings have revealed that these agents inhibit $K_{ATP}$ channel activity in pancreatic $\beta$-cells (130, 137). The $K_{ATP}$ channel inhibitory effect occurs over the same [drug] range as that required for binding to isolated membranes (1, 130). This suggests that sulfonylureas may act by binding to a specific site on the $K_{ATP}$ channel or to a very closely associated protein. These drugs can inhibit the channel from inside or outside (114, 137, 157), consistent with the suggestion that the binding site is reached through the lipid phase of the membrane (137). In the heart, a similar order of binding efficacy has been noted to that seen in $\beta$-cells (54), and although there have been several qualitative reports of sulfonylurea inhibition of cardiac $K_{ATP}$ channels, the only quantitative determinations of the dose-response properties of channel activity in heart (16) have suggested that tolbutamide is ~50 times less potent in heart cells than in $\beta$-cells (54). Binding studies show that glibenclamide binding to heart cells as well as to pancreatic $\beta$-cells can be competed for by ADP (101). Mechanistically, it is becoming harder to present a qualitative scheme that can accommodate all of the regulatory mechanisms that have been identified and
still keep the explanation within a consistent framework. However, results presented so far seem consistent with

glibenclamide and ATP binding to separate sites, the

binding properties of each one being affected by binding

of MgADP at a third site (see Fig. 3).

The action of PCOs on $K_{ATP}$ channels. PCOs include
diazoxide, nicorandil, minoxidil, pinacidil, cromakalim
(BRL 34915), and RP 49356 (89). The chemistry and
tissue selectivity of these compounds have been recently
reviewed (115). The demonstration that $K_{ATP}$ channels

do in fact exist in arterial smooth muscle (127) has led
to the demonstration that these agents do also activate
$K_{ATP}$ channels in smooth muscle. In 1986, Kakei et al.
(75) demonstrated that the effect of nicorandil in guinea
pig ventricular cells was to increase the K$^+$ conductance,
and it has since been demonstrated that this does indeed
result from the activation of $K_{ATP}$ channels (69).

Pinacidil was developed in the mid-1970s by Leo Phar-
maceuticals (Copenhagen, Denmark) from a series of
thioalkylureas and was recognized as a potent vasodilator
(122) first described its action as a K$^+$ channel opener.
Cromakalim and RP 49356 (Rhone-Poulenc Sante,
Paris, France) are structurally unrelated to pinacidil and
to one another (115) but have apparently indistinguish-
able effects on smooth muscle and cardiac muscle $K_{ATP}$
channel activity (41, 42, 127). It must be borne in mind
that effects of these compounds on other channel types
have been reported. There is evidence that cromakalim
can block the background K$^+$ channels in cardiac muscle
(29). Importantly, cromakalim has been shown to act on
smooth muscle Ca$^{2+}$-activated K$^+$ ($K_{ca}$) channels in iso-
lated membrane patches (61) and in channels reincor-
porated into lipid bilayers (58), by shifting the voltage
dependency of channel opening. However, neither apo-
minal nor charybdotoxin (potent blockers of $K_{ca}$ channels)
block the currents activated by cromakalim in smooth
muscle, suggesting that vasoactive PCOs may not act through $K_{ca}$ channels in vivo. Furthermore, experiments on
$K_{ca}$ channels from vascular smooth muscle have
generally failed to demonstrate modulation by ATP or
PCOs (15, 82).

The best mechanistic evidence concerning the mech-
anism of action of PCOs on $K_{ATP}$ channels has been
obtained from experiments on cardiac $K_{ATP}$ channels.
Much qualitative evidence (4, 5, 41, 42, 51, 94, 118) and
recent quantitative evidence (46, 47, 113, 134) have
shown that these agents decrease $K_{ATP}$ channel sensitiv-
ity to ATP. They act by shifting the $k_+$ for channel
inhibition by ATP to higher ATP, with little or no change in
the steepness of the dose-response curve. This demon-
stration of an apparent competition between the PCOs
and ATP may imply that the two agents act at a common
binding site (see Fig. 3). However, the recent detailed
study of Fan et al. (16) presents evidence that the effect
of pinacidil on the $K_{ATP}$ saturates at high [pinacidil],
consistent with a true competition between ATP and
pinacidil. It remains to be shown whether displacement
of PCOs by ATP, and vice versa, can be demonstrated
biochemically.

Whatever the underlying mechanism of the shift in
the ATP dependence of channel activity on exposure to
PCOs, the demonstration of this shift is important in
considering the actions of PCOs on intact tissue. A shift
in the ATP dependence of $K_{ATP}$ channels in the presence
of PCOs may explain a number of observations. First, PCO
effects on action potential duration are more readily
observed at 37°C (41, 42, 118). In isolated cells, dialyzed
by a patch pipette, intracellular [ATP] apparently de-
pletes spontaneously (16), and it seems possible that
with higher [ATP] consumption, intracellular [ATP]
could be lower at 37°C than at lower temperatures.
Second, the use-dependent increase in the response to
pinacidil in dialyzed cells (42) might also be expected if
intracellular [ATP] falls continually (16). Third, cro-
makalim provides cardioprotection in metabolic blockade
without affecting control action potential or resting po-
tential (63). It should be possible to find a dose of PCO
that causes only a marginal increase in $K_{ATP}$ channel
activity at high (several millimolar) [ATP], but that
significantly speeds up the onset of activation of channels
as [ATP] falls in conditions of metabolic compromise
(113). This potentially provides a logic to the use of
PCOs as drugs to specifically act only at sites suffering
from metabolic compromise.

MOLECULAR ASPECTS OF $K_{ATP}$ CHANNELS

In this review, we have developed a model of a K$^+$
selective channel whose background level of activation
is set by G protein stimulation and by phosphorylation.
The channel gating is directly modulated intrinsically by
nucleotides and pharmacologically by inhibitory sulfo-
ylureas and stimulatory channel openers. It seems that
the sulfonylurea binding site should be distant from the
ATP-binding site, given the accessibility of the sulfonyl-
urea-binding site from either side of the membrane.
There is evidence that the stimulatory PCOs either bind
at the ATP-binding site or at least modify the properties
of this site for binding of ATP. Weik and Neumcke (144)
have made an interesting study of the nature of the ATP-
binding site, by analogy with experiments on the Na-K-
ATPase. They have shown that sulfhydryl-modifying
agents can cause an irreversible inhibition of channel
activity, suggesting that an important -SH group may be
located at, or near to, the ATP-binding site.

So far, with the use of oligonucleotide probes derived
from the Shaker gene of Drosophila, the genes coding for
a number of voltage-dependent delayed-rectifier chan-
nels have been cloned and sequenced (13, 133). Such
approaches have not yet resulted in the cloning of
the $K_{ATP}$ channel gene. However, there are at least three
routes to cloning that provide promise, and all are cur-
rently being actively followed. 1) By taking consensus
sequences of ATP-binding sites or sequences derived from
the K$^+$ conducting pore region of other cloned K$^+$
channels, it may be possible to isolate the $K_{ATP}$ channel
by heterologous screening of a cDNA library. Because
the PCOs and, in some cases, ATP (61) may modulate
the activity of $K_{ca}$ channels, the $K_{ca}$ channel and the
$K_{ATP}$ channel may then share structural homology, which
may be exploited in cloning the $K_{ATP}$ channel. At the
time of writing this study, several groups appear to be
close to cloning the $K_{ca}$ channel gene using classical
the sulfonylurea receptor from pancreatic β-cells (1). It is unknown whether the receptor is the same molecule as the K\(_{\text{ATP}}\) channel. If it is not, then it can presumably associate with the channel, and so copurification of the receptor and the channel may then be a feasible approach to isolate the channel. 3) There is one report of expression of K\(_{\text{ATP}}\) channels in Xenopus oocytes after injection of β-cell mRNA (7), and so it may prove possible to isolate the gene from a library by "expression cloning". This approach, which involves splitting a pool of recombinants into smaller and smaller pools while assaying for the gene of interest by expression, has been successfully used to clone a delayed-rectifier K\(^+\) channel (DRK-1) from brain cells (56).

It is an exciting possibility that the structure of the channel will soon be available and that it will then be possible to probe directly into the nature of the regulatory sites on the channel.

**POTENTIAL INVOLVEMENT OF K\(_{\text{ATP}}\) CHANNELS IN NORMAL AND DISEASED CARDIOVASCULAR SYSTEM**

In preceding sections, we have detailed the relevant physiological and biophysical properties of K\(_{\text{ATP}}\) channels that should allow one to predict the role that channel activity will play in the function of a tissue. To predict the effects in the whole animal is necessarily more complex. Broadly, we would expect that in the heart, channel activity will be minimal under normal circumstances but may become evident in periods of ischemia or other metabolic insult. Similarly, in fatigued muscle it seems likely that vasodilation will result from K\(_{\text{ATP}}\) channel-induced hyperpolarization, channel activity being stimulated perhaps by a fall of [ATP] in the smooth muscle itself, as well as by factors released from the fatigued muscle and stimulated endothelium.

In the heart, ischemia typically results in a very rapid decline of contractile function, and regional ischemia similarly leads to regional loss of contractility. This is not always accompanied by marked action potential shortening as would have to be the case if K\(_{\text{ATP}}\) channel activation were responsible. However, action potential shortening and extracellular K\(^+\) accumulation does typically proceed over the first few minutes after global ischemia (26, 81). In interpreting experiments using glibenclamide, it may be an oversimplification to assume that all effects are due to K\(_{\text{ATP}}\) channel inhibition, because there are reports of glibenclamide having an additional effect on cellular metabolism (e.g., see Ref. 25). Nevertheless, there are many reports that the cellular K\(^+\) loss is partially inhibited by glibenclamide (14, 77, 150), and it seems reasonable to conclude from these experiments that the K\(^+\) loss results at least in part from the activation of K\(_{\text{ATP}}\) channels. Action potential shortening may well be cardioprotective (124). By shortening the action potential, Ca\(^{2+}\) entry will be reduced, contractile energy consumption will be reduced, and the drastic effects of Ca\(^{2+}\) loading in the ischemic period on subsequent recovery from ischemia will be reduced. Evidence that this is in fact the case has been presented by Grover et al. (63), who showed that contractile function (as measured by first derivative of left ventricular pressure), 30 min after reperfusion of an isolated Langendorff perfused heart, was improved by the prior administration of 1 μM pinacidil, although this dose had no effect on precontracture contractility. Grover et al. (62) showed that intracoronary administration of cromakalim and pinacidil resulted in decreased ischemia-induced fibrillation and infarct size. These experiments were performed with global ischemia, but in experiments with regional ischemia, the direct effects of K\(_{\text{ATP}}\) channel modulators on the cardiac muscle could be complicated by effects on coronary vessels, increasing or possibly reducing collateral flow into the ischemic region. A somewhat confusing and potentially very important consideration is the role of K\(_{\text{ATP}}\) channel activity in cardiac arrhythmia activity. Under conditions where afterdepolarizations may result in arrhythmias, administration of K\(_{\text{ATP}}\) channel openers has been shown to have antiarrhythmic effects (72, 85, 128). This action is expected from the hyperpolarizing effect of channel activation. However, in conditions such as regional ischemia, where reentrant arrhythmias may result, the administration of PCOs has been shown to be proarrhythmic (28), presumably because of decreased refractoriness of the tissue, whereas glibenclamide has been shown to be markedly antiarrhythmic, (77, 152).

Thus, although activation of K\(_{\text{ATP}}\) channels might be expected to oppose spontaneous activity due to increased automaticity (e.g., early or late afterdepolarizations), it may exacerbate arrhythmias due to conduction block (e.g., reentrant arrhythmias). This suggests that therapeutic interventions involving K\(_{\text{ATP}}\) channels should be used cautiously and with careful cardiac monitoring.

It remains to be clearly demonstrated whether or not K\(_{\text{ATP}}\) channel activity has any role to play in normal cardiovascular function, but there is now substantial evidence that K\(_{\text{ATP}}\) channel activation is a feature of cardiovascular disease processes. The therapeutic value of K\(_{\text{ATP}}\) channel manipulation in myocardial ischemia is not yet proven, but this may not be the case for the use of PCOs to open smooth muscle (presumably) K\(_{\text{ATP}}\) channels in hypertensive patients. Pinacidil and cromakalim are now being examined in the cardiovascular context in clinical trials (64, 115). The higher sensitivity of vascular smooth muscle to K\(^+\) channel opening drugs, than of other tissues, makes practical use of these drugs possible. At doses that have no effect on normal cardiac or pancreatic function, profound reversal of norepinephrine-induced vasoconstriction can be achieved in vitro (127), and clinically, twice-daily administration of pinacidil alone or pinacidil plus hydrochlorothiazide, was an effective treatment of mild to moderate hypertension (115). The side effects of reflex tachycardia, headache, flushing, and edema could be attenuated by coadministration of β-blockers or diuretics (20). Potentially, given the smooth muscle specificity (over cardiac specificity) of the K\(^+\) channel opening drugs, there may be a real
value in the use of these compounds to treat hypertension.

Local control of blood flow by \([\text{ATP}]_i\) and the \(K_{\text{ATP}}\) channel: "adenosine hypothesis" or "\(\text{ATP} \) hypothesis?"

How is it that regional blood flow within muscle tissue can be regulated? An answer to this question has been suggested by the "adenosine hypothesis" (18, 37, 105, 123; see Fig. 4). This hypothesis suggests that tissue-consuming oxygen can "signal" smooth muscle in blood vessels to relax, by an action of adenosine released from the muscle. Adenosine, which can cross cell membranes, is generated in increasing amounts as a shift in the distribution of adenosine-containing chemicals occurs (ATP \(\rightarrow\) ADP \(\rightarrow\) AMP \(\rightarrow\) adenosine) when ATP regeneration is prevented by inadequate oxygen supply. The adenosine then diffuses from the tissue source to the precapillary sphincter and neighboring smooth muscle. Adenosine receptors in the smooth muscle membrane then signal the muscle to relax, increasing blood flow to the tissue, thus increasing the oxygen supply, reversing the process, and reducing the adenosine concentration (Fig. 4). This hypothesis is supported by evidence that adenosine antagonists and adenosine deaminase (ADA) treatment can reduce coronary metabolic vasodilation (67, 91, 119). The unknown transducing mechanism could be the activation of \(K_{\text{ATP}}\) channels through a G protein-coupled mechanism (79, 139). The recent demonstration that coronary vasodilation in myocardial ischemia can be blocked by glibenclamide (31) is strong support for the notion that activation of \(K_{\text{ATP}}\) channels does indeed underlie coronary metabolic vasodilation. However, an objection to the adenosine hypothesis providing a complete or exclusive explanation of metabolic vasodilation are the many examples where coronary flow responses to metabolic compromise are not purely dependent on adenosine efflux (67, 91, 119) or cannot be blocked by ADA treatment (34, 66, 80). The following alternate "\(\text{ATP} \) hypothesis," arising from the arguments presented in previous sections, may help to explain the vasodilation in such conditions. We propose that one of the signals transmitted from the metabolically impaired muscle to the vascular smooth muscle is oxygen concentration itself (Fig. 4). The cardiac or skeletal muscle, with a higher rate of oxygen consumption than the vascular smooth muscle, will reduce \([\text{O}_2]\) levels in the microenvironment of the precapillary sphincter, so that the smooth muscle ATP-generating capacity is impaired. Thus \([\text{ATP}]\) within the smooth muscle itself will fall, with consequent activation of \(K_{\text{ATP}}\) channels in the smooth muscle membrane. As discussed for the cardiac action potential sensitivity to ATP and ADP (see above), the activation of very few \(K_{\text{ATP}}\) channels will cause marked hyperpolarization and relaxation of vascular smooth muscle (96). Because the smooth muscle \(K_{\text{ATP}}\) channel sensitivity to ATP may be shifted to higher [ATP] than cardiac channel sensitivity (49, 83, 96, 102), smooth muscle \(K_{\text{ATP}}\) channels may respond more quickly to tissue metabolic stress than neighboring skeletal or cardiac channels. We propose therefore that \(K_{\text{ATP}}\) channels may contribute to the normal regulation of blood flow in microregions of a tissue as a response to alterations in the [ATP] and [ADP] in the smooth muscle cells themselves.

Because the same tissue conditions are needed for the "adenosine" mechanism or the "\(\text{ATP} \)" mechanism (namely a redistribution of adenosine compounds from ATP to ADP and adenosine), it would seem that the two hypothetical mechanisms would complement one another. It should be noted that smooth muscle cells can vary in expressed features such as drug sensitivity and vasomotion properties (87), depending on their tissue placement, and position in the vascular tree (e.g., large artery vs. capillary or vein) and thus may vary with respect to \(K_{\text{ATP}}\) channel properties and density. Additional evidence is needed to further characterize the precapillary sphincter at the cellular level. Such work...
will provide evidence on the relative importance of the ATP hypothesis in accounting for the nonadenosine-mediated components of vasoregulation in different tissues.

PERSPECTIVES

We have reviewed recent reports on the biophysical and physiological regulation of K\textsubscript{ATP} channels, with special reference to these channels in the cardiovascular system. With this body of information, we have attempted to develop a coherent picture of how this important channel works in the cardiovascular system under normal and pathophysiological conditions.

It is a little over a decade since the first generation of K\textsuperscript{+} channel opening drugs appeared. It is only seven years since K\textsubscript{ATP} channels were discovered. Over the next five years, a relatively small number of investigators pursued the biophysical regulation of these channels. The pace of research involving K\textsubscript{ATP} channels has recently accelerated enormously, however, as it has become clear that the subject of both lines of investigation is the same. Future work at the single channel level must seek to further elucidate the mechanisms of channel regulation by endogenous substrates as well as by the pharmacological modulators. The interaction of channel-gating mechanisms with its permeation properties needs to be clarified. These studies will be propelled by insights into the molecular structure of the channel protein, as well as by biochemical elucidation of ligand binding properties. At the present time, there are no major obstacles to the development of K\textsuperscript{+} channel opening drugs as rivals or complements to Ca\textsuperscript{2+} channel blockers in the treatment of hypertension, and as effort is devoted to developing more tissue-specific drugs, we believe that their use in the treatment of cardiovascular disease will become increasingly important and widespread.

We acknowledge our collaborators, Drs. G. L. Smith, C. Ripoll, and M. R. Cannell, who were involved with many aspects of the experimental work.

Our own experimental work described in this review was carried out with the support of a grant-in-aid from the American Heart Association, Maryland Affiliate, to C. G. Nichols, and by National Institutes of Health grants to C. G. Nichols and to W. J. Lederer.

Address for reprint requests: C. G. Nichols, Dept. of Cell Biol, and Physiol., Box 8228, Washington Univ. School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110.

REFERENCES


The potassium channel opener cromakalim (BRL 34915) activates ATP-sensitive K+ channels in guinea-pig cardiac muscle (Abstract).


94. NAKAYAMA, K., Z. FAN, F. MARUMO, AND M. HIROAKA. Interre-


