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MINI-REVIEW

ATP-Dependent Potassium Channels of Muscle Cells: Their Properties, Regulation, and Possible Functions

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Abstract

ATP-dependent potassium channels are present at high density in the membranes of heart, skeletal, and smooth muscle and have a low P_{open} at physiological $[ATP]_i$. The unitary conductance is 15–20 pS at physiological $[K^+]_o$, and the channels are highly selective for K^+. Certain sulfonylureas are specific blockers, and some K channel openers may also act through these channels. K_{ATP} channels are probably regulated through the binding of ATP, which may in turn be regulated through changes in the ADP/ATP ratio or in pH_i. There is some evidence for control through G-proteins. The channels have complex kinetics, with multiple open and closed states. The main effect of ATP is to increase occupancy of long-lived closed states. The channels may have a role in the control of excitability and probably act as a route for K^+ loss from muscle during activity. In arterial smooth muscle they may act as targets for vasodilators.

Key Words: potassium channel; potassium current; ATP; skeletal muscle; smooth muscle; vasodilator; metabolic exhaustion.

Introduction

In this review we shall discuss work on a class of potassium channels, recently discovered (Noma, 1983), which bind cytoplasmic ATP. These channels have a very low open state probability (P_{open}) at physiological resting ATP concentration, but P_{open} rises if the concentration of ATP falls (Fig. 1), if the concentration of certain other intracellular constituents changes, or as a consequence of the binding of certain hormones to the cell membrane. We

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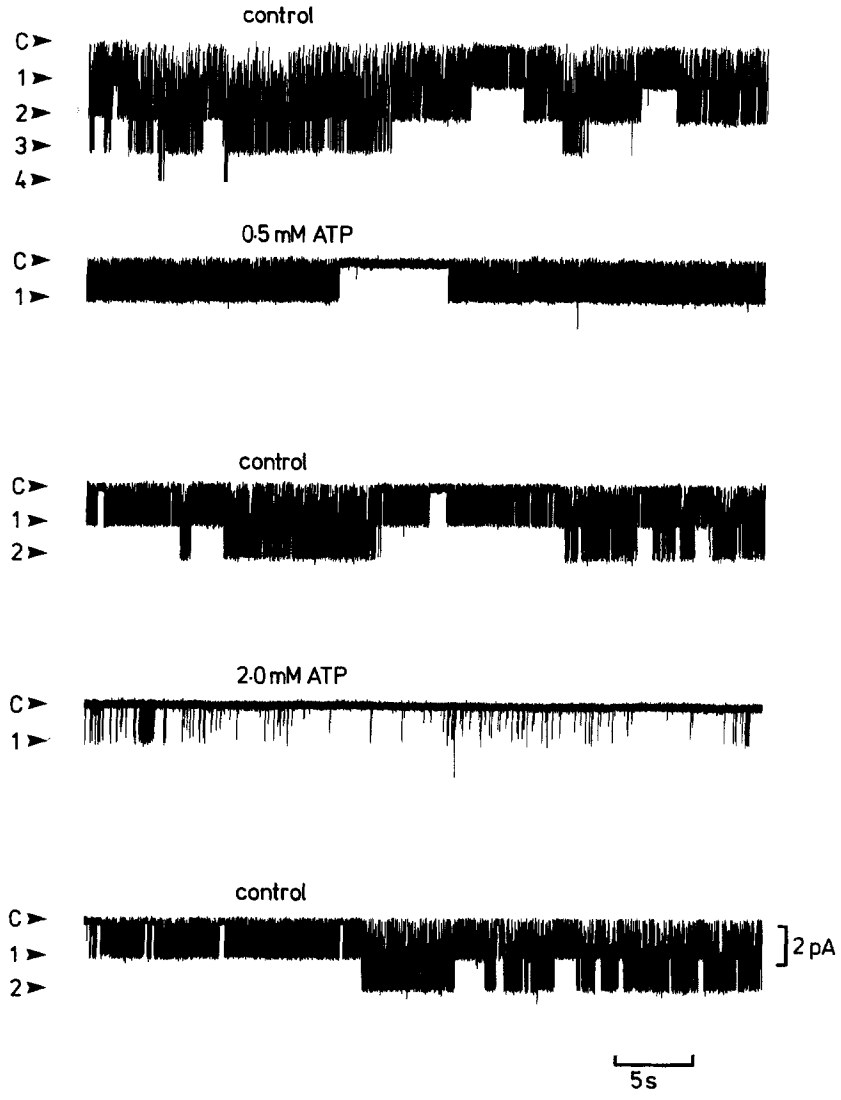


Fig. 1. Effect of ATP_i on K_{ATP} channels recorded in an excised inside-out patch from adult frog skeletal muscle. The K^+ gradient was $60_o/120_i$ and the membrane potential was -60 mV, so that openings produce inward currents. The arrows at the left of each record correspond to the level at which 0 (C), 1, 2, . . . channels are open. Redrawn with permission from Spruce *et al.* (1985).

shall confine our discussion to the ATP-dependent potassium channels of muscle cells. The nature of these channels in pancreatic B-cells has already been extensively reviewed (Ashcroft, 1988; De Weille *et al.*, 1989; Petersen and Dunne, 1989; Rorsman and Trube, 1990), and the emerging work on neuronal channels has also recently been summarized (Amoroso *et al.*, 1990). In this review we shall call the channels *ATP-dependent*, and use the abbreviation K_{ATP}, because, as we shall attempt to explain, their normal physiological function depends upon ATP (as do certain of their pharmacological responses). We shall avoid the terms *ATP-regulated* (Noma, 1983), which appears to exclude other controlling factors, and *ATP-sensitive* (Noma, 1983; Spruce *et al.*, 1985), which may imply that ATP is peripheral to physiological function.

Discovery of ATP-Dependent K Channels

The discovery of K_{ATP} channels in heart cells by Noma in 1983 (see also Trube and Hescheler, 1983) came as a rapid result of the spread of patch clamp methods (Hamill *et al.*, 1981) and in particular of the use of excised, inside-out membrane patches. It had long been known that metabolic exhaustion resulted in a rise in the resting membrane permeability to K^+ in heart (Trautwein *et al.*, 1954) and in skeletal muscle (Fink and Lüttgau, 1976), as well as in certain other cells, such as neurons (Gottfraind *et al.*, 1970). Most accounts of the phenomenon had attempted an explanation in terms of the types of K^+ permeability known at that time, either suggesting that the properties of certain voltage-dependent K^+ channels were modified by exhaustion (e.g., Fink and Lüttgau, 1976) or that a rise in cytoplasmic $[Ca^{2+}]$, resulting from failure of intracellular sequestering under conditions where internal [ATP] was low, opened Ca^{2+}-dependent K channels (Krnjevic and Lisiewicz, 1972; Fink *et al.*, 1983). While there remains evidence that Ca^{2+}-dependent K channels contribute to the rise in K permeability, the bulk of the increase appears to be due to K_{ATP} channels (Trube and Hescheler, 1984; Carmeliet, 1987; Castle and Haylett, 1987).

Distribution and Permeability Properties of ATP-Dependent K Channels

Distribution

K_{ATP} channels are now known to exist in pancreatic B-cells (Cook and Hales, 1984), and neurons (Ashford *et al.*, 1988; Mourre *et al.*, 1989), as well as in heart muscle, including atrial, ventricular and pacemaking cells (Noma,

1983; Kakei and Noma, 1984), amphibian and mammalian skeletal muscle fibers (Spruce *et al.*, 1984, 1987; Burton *et al.*, 1988), and smooth muscle cells (Standen *et al.*, 1989; Furspan, 1990). There is no reason to suppose that the distribution is at present fully known, and further experiments, examining high-affinity binding of the antidiabetic sulfonylureas such as glibenclamide, a specific blocker of these channels (see below), and using patch clamp recording, are needed to look for additional sites of occurrence. Nor, as has been discussed elsewhere (Stanfield, 1987, 1990), are these the only channel types that depend on ATP binding for their normal function.

The general properties of K_{ATP} channels are quite similar from one cell type to another. However, as we shall discuss, there are certain differences in the precise mechanisms of control. There may even be differences in channel regulation between different areas of the membrane of a given cell type. For example, differences appear to exist between channels from the surface and from the T-system membrane (Parent and Coronado, 1989) of skeletal muscle (Neumcke and Weik, 1990).

Channel Density

The density of channels is relatively high and K_{ATP} channels may be among the most common K channels in muscle cell membranes. Most estimates are derived from single-channel recording, where the area of the membrane patch is difficult to assess accurately. In vesicles of skeletal muscle membrane (Standen *et al.*, 1984), all excised patches contain K_{ATP} channels, and most patches contain several. Spruce *et al.* (1985) reported an average of 3.6 channels per patch, roughly equal to the density of voltage-activated, delayed rectifier K channels (Standen *et al.*, 1985), that is, several channels $\cdot\ \mu m^{-2}$. This estimate of channel density is consistent with the observation that the membrane conductance associated with metabolic exhaustion of skeletal muscle (Fink and Lüttgau, 1976) is similar to the maximum conductance associated with voltage-activated K channels under depolarization (Adrian *et al.*, 1970; Stanfield, 1970).

Noma and Shibasaki (1985) used an alternative method, measuring the mean current through K_{ATP} channels in cardiac myocytes dialyzed with ATP-deficient solutions and using this mean current and its variance to estimate the number of channels per myocyte. Their results suggested 2300 per guinea pig ventricular cell (membrane area 4400 μm^2), consistent with the density of K_{ATP} channels in heart being "almost equal to that of inward rectifier K channels," roughly $1/1.8\ \mu m^2$ (Sakmann and Trube, 1984). In skeletal muscle, K_{ATP} channels occur at a much higher density than do inward rectifier channels, and other work on heart, with recordings from excised inside-out patches showing many channels per patch (e.g., Findlay, 1988a, b;

Lederer and Nichols, 1989), may be consistent with a higher density than that suggested by Noma and Shibasaki (1985). In arterial smooth muscle, Standen and his colleagues (Standen *et al.*, 1989) report that many patches do not show openings of K_{ATP} channels, while others contain several channels. They argue that a failure of the maintenance of channel activity, perhaps owing to the loss of channel phosphorylation, rather than the absence of channels, accounts for patches that appear to contain no channels.

Unitary Conductance

The unitary conductances of K_{ATP} channels are similar in cardiac and skeletal muscle, being approximately the same as those of voltage-dependent K channels at least in physiological $[K^+]_o$ (compare, for example, Standen *et al.*, 1985; Spruce *et al.*, 1987). Unitary conductance increases with increasing $[K^+]_o$ (Kakei *et al.*, 1985; Spruce *et al.*, 1987), and in heart muscle is equal to $23.6\,pS \times [K^+]_o^{0.24}$ ($[K^+]$ in mM; experiments at 35–36°C; Kakei *et al.*, 1985). In frog skeletal muscle, the unitary conductance, measured at the reversal potential, rises from 15 pS in physiological $[K^+]_o$ (2.5 mM, $[K^+]_i$ = 120 mM) to 42.3 pS in 60 mM $[K^+]_o$ (measurements at room temperature, approximately 20°C; Spruce *et al.*, 1987), consistent with the conductance increasing with $[K^+]_o^{0.33}$. In each case, the increase is less than that expected if K^+ movements through the channel obeyed the independence principle. For mammalian skeletal muscle, unitary conductances of 60 pS (with symmetrical 140 mM $[K^+]$; Burton *et al.*, 1988) and 74 pS (with 155 mM $[K^+]_o$; Weik and Neumcke, 1989) have been measured at room temperature. In arterial smooth muscle the conductance may show less sign of saturation with increasing $[K^+]_o$ than it does in skeletal muscle or heart. Unitary conductance is approximately 135 pS with 60 mM $[K^+]_o$ (18–22°C; Standen *et al.*, 1989), but is approximately 20 pS with 6 mM $[K^+]_o$ (Nelson *et al.*, 1990a).

In heart and skeletal muscle, the current–voltage relation shows some rectification, and in conditions where $[K^+]_o = [K^+]_i$, outward currents are smaller than inward currents. For this reason, the channels have sometimes been described as inward rectifiers (see, for example, Trube and Hescheler, 1984). The channel kinetics do show some dependence on $(V - E_K)$, rather than on voltage alone (Zilberter *et al.*, 1988; Quayle, 1988; see below), a property characteristic of inward rectifiers (Hodgkin and Horowicz, 1959; Hagiwara and Yoshii, 1979; Leech and Stanfield, 1981). However, in physiological $[K^+]_o$, the current–voltage relation shows outward currents that are larger than inward, rather as expected if $[K^+]_o \ll [K^+]_i$ (Spruce *et al.*, 1987). Indeed, in such conditions, current–voltage relations may be fitted with a constant-field expression and a permeability coefficient of $2.6 \times 10^{-13}\,cm^3\,s^{-1}$. Thus, the channels do not show inward rectification under physiological

conditions. The cause of the rectification in higher external K^+ concentrations is a voltage-dependent block by internal Na^+ and Mg^{2+} (Horie *et al.*, 1987; Quayle and Stanfield, 1989; Woll *et al.*, 1989) of a kind that occurs in several K-channel types. Noma (1983) showed that internal Ca^{2+} also blocks, and Findlay (1987) that alkali earth metal ions block in order of effectiveness $Ca^{2+} > Ba^{2+} > Mg^{2+} > Sr^{2+}$. In arterial smooth muscle the unitary current–voltage relations do not show any sign of inward-going rectification in high $[K^+]_o$, even in the presence of 1 mM $[Mg^{2+}]_i$ (Standen *et al.*, 1989). The action of other blocking agents is dealt with below.

Selectivity

The channels are highly selective for K^+, the estimate for P_{Na}/P_K being 0.015 in skeletal muscle (Spruce *et al.*, 1987). In heart muscle, there is little sign of any deviation from Nernst, even down to the approximately physiological concentration of 5.4 mM, and the reversal potential changes 58 mV for a tenfold change in $[K^+]_o$ (Kakei *et al.*, 1985). Rb^+ also permeates, with P_{Rb}/P_K being 0.76 in skeletal muscle (Spruce *et al.*, 1987) as measured from the change in reversal potential (replacement of external K^+ by Rb^+). Rb currents are, however, much smaller than K currents ($\leqslant 10\%$), indicating that, though Rb^+ enters the channel nearly as readily as does K^+, it traverses the channel much more slowly (Spruce *et al.*, 1987). Further, in mixtures of K^+ and Rb^+ the unitary conductance goes through a minimum, and when $[Rb^+]_o/[K^+]_o = 5$ it is lower than in the presence of either Rb^+ or K^+ alone (Spruce *et al.*, 1986). Such an anomalous mole fraction effect is associated with the more permeant ion interfering with passage of the less permeant one and is characteristic of a multi-ion pore (see, for example, Hille, 1984). Associated with the ion staying longer in the channel, channel openings are prolonged when Rb^+ permeates (Spruce *et al.*, 1986; see also Spruce *et al.*, 1989).

As with other K-channel types, Cs^+ fails to permeate but produces a voltage-dependent block of K currents with an effective valence of 1.22 (Quayle *et al.*, 1988), that is to say, the affinity for the blocking ion increases under hyperpolarization *e*-fold for a 20.5 mV change in voltage. An effective valence greater than unity indicates that the channel contains more than one ion at a time and that permeant K^+ must be moved under the influence of voltage as the blocking Cs^+ is moved to its binding site (Hille, 1984): it provides additional evidence that K_{ATP} channels are multi-ion channels. The block appears flickery under single-channel recording, and the transition rates are rapid.

The channel selectivity has not been much more fully investigated, though the evidence so far, summarized above, is that it is similar in its selectivity to other K-channel types (see Hille, 1984).

Pharmacology

Blockers

Sulfonylureas. Sulfonylureas have been used for many years as anti-diabetic agents, causing a stimulation of insulin secretion. More recently, it has been shown that the primary action of these drugs is to block the K_{ATP} channel of pancreatic B-cells (Sturgess *et al.*, 1985, 1988; Schmid-Antomarchi *et al.*, 1987). Glibenclamide is the most potent of these agents, with a K_i in the range 5–30 nM (see De Weille *et al.*, 1989 and Quast and Cook, 1990 for reviews).

The effects of sulfonylureas have been studied much less extensively in muscle. It is clear that sulfonylureas also block K_{ATP} channels here, though at higher concentrations than in insulin-secreting cells. The current induced by intracellular ATP depletion or metabolic inhibition of cardiac muscle is blocked by tolbutamide with $K_i = 380\,\mu M$ (Belles *et al.*, 1987) and by glibenclamide at 0.3–2 μM (Escande, 1989; Escande *et al.*, 1989; Carmeliet *et al.*, 1990). Varying degrees of block of single K_{ATP} channels of cardiac muscle have been reported with glibenclamide concentrations between 20 nM and 0.3 μM (Fosset *et al.*, 1988; Arena and Kass, 1989b; Escande, 1989) and with 2 mM tolbutamide (Belles *et al.*, 1987). Labelled glibenclamide binds to cardiac muscle, and a range of sulfonylureas inhibits this binding with the same order of potency as seen in insulinoma cells (Fosset *et al.*, 1988), while glibenclamide also binds to membranes of rabbit skeletal muscle (R. I. Norman, unpublished observations). In frog skeletal muscle a component of the K^+ efflux that occurs on metabolic exhaustion is inhibited by glibenclamide (3–100 μM) and tolbutamide (0.3–2 mM) (Castle and Haylett, 1987), and glibenclamide reduces P_{open} in single-channel experiments with a K_d around 3 μM (M. W. Beeston, N. W. Davies and P. R. Stanfield, unpublished observations). Woll *et al.* (1989) have shown that tolbutamide blocks single K_{ATP} channels of rat skeletal muscle mainly by increasing the duration of intervals between bursts of openings, and with a predicted K_i of 60 μM. Single K_{ATP} channels of arterial smooth muscle were substantially inhibited by 20 μM glibenclamide in the presence of cromakalim (Standen *et al.*, 1989). Sulfonylureas block K_{ATP} channels when applied to either side of the membrane, and probably access the channel by way of the membrane lipid (Trube *et al.*, 1986).

The degree to which sulfonylureas are selective for K_{ATP} channels over K^+ channels is important as these agents are often used to implicate K_{ATP} channels in responses to drugs such as K^+ channel openers, and to physiological or pathological changes such as hypoxia and ischemia. To our knowledge, the only report of an effect of sulfonylureas on other K^+ channels in

muscle (or insulin-secreting cells) is a slight reduction in outward (presumably delayed rectifier) K^+ current in portal vein smooth muscle by 50 μM glibenclamide (Beech and Bolton, 1989). In cardiac muscle, sulfonylureas have been found to be ineffective on inward and delayed rectifier K^+ channels (Belles *et al.*, 1987; Escande *et al.*,1988, 1989; Arena and Kass, 1989b; Sanguinetti *et al.*, 1988), while in arterial smooth muscle they do not block BK_{Ca} channels (Langton *et al.*, 1990). Thus, sulfonylureas appear to be selective blockers of K_{ATP} in the sense that they do not block other K^+ channels.

TEA^+ and Other Nonselective Blockers. A number of agents that block several types of K^+ channel have been found to block K_{ATP} to varying extents (see Cook and Quast, 1990 for review). Here we mention only those where block has been reported in muscle.

Tetraethylammonium ions (TEA^+) block K_{ATP} of skeletal muscle from either side of the membrane (Spruce *et al.*, 1987; Davies *et al.*, 1989a). External TEA^+ is a fairly weak blocker, with a K_d of 6.7 mM for reduction in unitary current amplitude and rapid kinetics. Block also prevents channel closure, so that external TEA^+ will be even less effective at blocking macroscopic currents; the degree to which prolongation of bursts compensates for reduction in mean unitary current depends on the channel open-state probability (P_{open}), so that $K_{i(macroscopic)} = K_{d(unitary)}/P_{open}$ (Davies *et al.*, 1989). In agreement with this, Castle and Haylett (1987) found a K_i of 27 mM for TEA^+ block of the K^+ efflux from exhausted muscle, which is thought to flow mainly through K_{ATP} channels, while K^+ efflux from ATP-depleted heart cells was blocked with K_i = 10.7 mM (Haworth *et al.*, 1989).

Internal TEA^+ is more effective, blocking with slower kinetics and a K_d of 1.4 mM at 0 mV in skeletal muscle, where there is also a faster lower-affinity block (Davies *et al.*, 1989). It produces almost complete block in ventricular cells at 1 mM (Kakei *et al.*, 1985).

4-Aminopyridine has been reported to block K_{ATP} channels and K^+ efflux of heart at millimolar concentrations (Kakei *et al.*, 1985; Haworth *et al.*, 1989). 4-Aminopyridine was reported to be effective in exhausted skeletal muscle, where the local anesthetics tetracaine and lignocaine (at 1 mM) reduced K^+ efflux (Castle and Haylett, 1987). Other agents reported to block K^+ efflux from ATP-depleted cardiac muscle are the antiarrhythmics quinidine, verapamil, and amiodarone, and the antipsychotic haloperidol, all of which act in the range 2–30 μM (Haworth *et al.*, 1989; Escande, 1989); a new antiarrhythmic, sodium 5-hydroxydecanoate, also blocks K_{ATP} in heart (Notsu *et al.*, 1989).

A number of cations also block K_{ATP} of muscle, either from inside or outside the membrane, and some of these have been discussed above, in the context of channel permeability properties. From the outside Ba^{2+} is a fairly

effective blocker of K_{ATP}, blocking single channels with $K_d = 0.1$ mM at -60 mV in skeletal muscle (Quayle *et al.*, 1988) and blocking K^+ efflux at similar concentrations (Castle and Haylett, 1987), while 1 mM external Ba^{2+} produced complete block in atrioventricular node cells (Kakei and Noma, 1984).

Openers

In recent years several chemically diverse compounds, including cromakalim, pinacidil, minoxidil, diazoxide, and RP 49356 (Rhone-Poulenc) have been proposed to open K^+ channels in smooth muscle, and thus to cause membrane hyperpolarization and relaxation (Hamilton *et al.*, 1986; Quast and Cook, 1989a). There is evidence, discussed below, these these K^+ channel openers activate K_{ATP} channels in muscle as well as other tissues, though they have also been shown to activate Ca^{2+}-dependent K^+ channels under some circumstances (Gelband *et al.*, 1989; Klöckner *et al.*, 1989). It is not clear at present whether the target K^+ channel is the same in all tissues or for all the compounds, though the widespread sensitivity of their effects to sulfonylureas suggests that K_{ATP} channels are involved in many cases (see also Quast and Cook, 1989a; Weston, 1989).

The experimental evidence that K^+ channel openers activate K_{ATP} channels falls into two broad groups. First, there are a number of direct demonstrations of activation of single K_{ATP} channels. Second, there are many reports in which the activation of K_{ATP} is inferred indirectly from sensitivity of the effects of the openers to sulfonylureas.

Direct demonstrations of K_{ATP} activation at the single channel level have used inside-out excised patches, so that ATP can be applied to the cytoplasmic face, and K^+ channel openers have also been applied to this face. Channels have usually also been characterized as K_{ATP} by their conductance, and, in addition, block by glibenclamide has often been shown. Thus cromakalim, pinacidil, SR 44866 (Sanofi), and RP 49356 at concentrations of 10–300 μM activate cardiac K_{ATP} channels (Escande *et al.*, 1988, 1989; Arena and Kass, 1989b; Findlay *et al.*, 1989; Fan *et al.*, 1990), while 1 μM cromakalim activates in smooth muscle (Standen *et al.*, 1989). As would be expected, if these agents are to act on channels in intact cells, ATP sensitivity is reduced. Subsequent addition of higher ATP concentrations have been shown to inhibit channels after their activation by RP 49356, pinacidil, and SR 44866 in cardiac myocytes and after activation by SR 44866 in frog skeletal muscle (Arena and Kass, 1989b; Thuringer and Escande, 1989; Findlay *et al.*, 1989; Fan *et al.*, 1990; Ecault *et al.*, 1990). Thuringer and Escande (1989) have shown that the ATP concentration dependence of K_{ATP} channels from cardiac cells is shifted to 10-fold higher ATP concentrations by 30 μM RP 49356 without a change in Hill coefficient, and have suggested that RP 49356 may

compete with ATP for a binding site, while Fan *et al.* (1990) showed that pinacidil and ATP had essentially opposite effects on channel kinetics.

There are many recent reports showing that the effects of various K^+ channel openers may be antagonized by sulfonylureas, usually glibenclamide (see reviews by Quast and Cook, 1989a and Cook and Quast, 1990). In cardiac muscle, whole cell currents or action potential shortening induced by cromakalim, pinacidil, RP 49356, and nicorandil are blocked by glibenclamide (Sanguinetti *et al.*, 1988; Arena and Kass, 1989a; Escande *et al.*, 1989; Fan *et al.*, 1990). The effects of these agents on intact cells have been reported to occur at 35–37°C, but not at room temperature (Sanguinetti *et al.*, 1988; Escande *et al.*, 1989), though Arena and Kass (1989a) found that pinacidil did activate current at room temperature. Findlay *et al.* (1989) report that SR 44866 was >3 times more effective at 34°C than 24°C in shortening cardiac action potentials. In human skeletal muscle, cromakalim restored the resting potential of depolarized fibers; this effect was blocked by tolbutamide (Spuler *et al.*, 1989). In smooth muscle, the effects of K^+ channel openers on whole-cell current, K^+ permeability, tension, and cardiovascular effects have all been reported to be antagonized by sulfonylureas (Beech and Bolton, 1989; Buckingham *et al.*, 1989; Cavero *et al.*, 1989; Eltze, 1989; Quast and Cook, 1989b; Standen *et al.*, 1989; Wilson, 1989; Winquist *et al.*, 1989).

Binding of ATP

K_{ATP} channels have a very low P_{open} at physiological ATP concentrations, but P_{open} rises in excised membrane patches if the ATP concentration is reduced. The general assumption is that ATP binds to the channel protein and that channels with ATP bound are closed. Although further work is needed to elucidate the molecular nature of the binding site or sites for ATP, there seems little reason to doubt the general view. It is less certain whether a change of ATP concentration is the primary controlling factor, particularly in muscle cells, or whether changes in the concentration of other intracellular constituents and, in arterial smooth muscle, the action of hormones on channels otherwise held closed by ATP are not more important (see below).

Figure 1 and Table I summarize some of the results concerning the ATP dependence of channel P_{open}. Though there is a broad agreement that channel P_{open} is reduced by rather low concentrations of ATP, there is a wide spread in the reported values for the half-saturating concentration of the nucleotide and some variation in the value for the Hill coefficient from 1 in skeletal muscle (Spruce *et al.*, 1987) to 3 or 4 in heart (Kakei *et al.*, 1985). It is not clear whether differences in experimental conditions account for some of the variations, though a Hill coefficient greater than unity has not been reported

Table I. Dependence of ATP Concentration of Channel P_{open}

Preparation	K_i (μM)	Hill coefficient	Reference
Heart muscle			
Guinea pig and rabbit	100	ca. 3[a]	Noma (1983)
Guinea pig ventricle	500	3–4	Kakei *et al.* (1985)
rat ventricle	30	2.0	Findlay (1988b)
rat ventricle	25	2.0	Lederer and Nichols (1989)
Skeletal muscle			
Frog	135	1.0	Spruce *et al.* (1987)
Frog	17	1.0	N. W. Davies (unpublished observations)

[a]Value calculated from Fig. 3 of Noma (1983).

for skeletal muscle and the coefficient is consistently higher in heart. It seems certain that there is cooperativity in the action of ATP in heart muscle; the possibility of cooperativity in skeletal muscle cannot be completely ruled out. In neither skeletal muscle (Spruce *et al.*, 1987; N. W. Davies and P. R. Stanfield, unpublished observations) nor in heart (Findlay, 1988b; Lederer and Nicholls, 1989) does the concentration of Mg^{2+} significantly alter the apparent affinity for ATP, though in heart Mg^{2+} may be crucial in one element of nucleotide regulation of the channel (see below; Findlay, 1988a, b; Lederer and Nicholls, 1989). The fact that channel closure by ATP occurs in the absence of Mg^{2+} is inconsistent with closure being associated with hydrolysis of the nucleotide.

It is also well known that nonhydrolyzable analogs of ATP have an action similar to that of ATP itself, again indicating that ATP is neither hydrolyzed in producing channel closure nor presumably in unbinding. Other nucleotides mimic the action of ATP, but higher concentrations are required. In general, the order of effectiveness is ATP $\geqslant$ AMP-PNP > ADP > AMP > CTP $\geqslant$ GTP $\geqslant$ XTP $\geqslant$ UTP $\geqslant$ ITP (Kakei *et al.*, 1985; Spruce *et al.*, 1987). Alterations of the ribose of ATP (for example, replacing it with 2′ deoxyribose) reduce the effectiveness of the nucleotide, though the effect of the change in structure is less than if the adenine base is replaced (Spruce *et al.*, 1987). Adenosine and adenine are reported to be ineffective in heart (Kakei *et al.*, 1985), but do produce channel closure in mammalian skeletal muscle (Weik and Neumcke, 1989) if the concentration is high enough.

The ADP/ATP Ratio

There is an additional element in the regulation by ADP that may be important. Several authors have reported that in the presence of ADP, ATP

is less effective at closing channels, and it is widely assumed that the ADP/ATP ratio is important physiologically in channel regulation. This idea was first put forward for the channel in pancreatic B-cells (Misler *et al.*, 1986), and is also true in heart muscle (Findlay, 1988a; Lederer and Nicholls, 1989). Spruce *et al.* (1987) could find no evidence for such an influence of ADP in skeletal muscle, a result recently confirmed by N. W. Davies and P. R. Stanfield (unpublished observations), in both the presence and absence of Mg^{2+} (see below).

It is possible that the difference between skeletal and heart muscle is associated with the difference in the Hill coefficient for the channel closing action of ATP. Recent work by Lederer and Nicholls (1989) has linked the effect of ADP to the nature of the cooperativity between nucleotide binding sites. Following work by Findlay (1988a, b), Lederer and Nicholls (1989) found that in heart muscle in the *absence* of internal Mg^{2+} ADP induced channel closure, though less effectively than did ATP. However, in the presence of Mg^{2+} ADP opposed the channel closing action of ATP. They propose two nucleotide binding sites on the channel (consistent with their value for the Hill coefficient of 2), which they call S1 and S2. Lederer and Nicholls (1989) propose that when ATP binds to S1, it increases the affinity of site S2 for ATP, and that binding there closes the channel. Binding of MgADP to site S1 reduces the binding of ATP through competitive inhibition and hence decreases ATP binding to the channel closing site S2. The physiological importance of the ADP/ATP ratio as a regulator of these channels remains uncertain, however, since relatively high concentrations of ADP appear to be required to produce the effect. MgGDP has a similar effect to that of MgADP.

Effect of Internal pH on ATP Binding

Rather more important may be a charge in intracellular pH, known to occur in exercising muscle (e.g., Pan *et al.*, 1988). In excised patches of frog skeletal muscle, decreasing the internal pH (pH_i) markedly reduces the inhibitory effect of ATP. Since this effect occurs both in the presence and absence of Mg^{2+}, it is not primarily associated with a change in the degree of protonation of ATP (Davies, 1990). The effect of changing pH_i on the activity of K_{ATP} channels both in the absence and presence of ATP is shown in Fig. 2. Similar experiments on cardiac muscle by Lederer and Nicholls (1989) show only a small change in the inhibitory effect of ATP, and lowering pH_i from 7.25 to 6.25 increased the Hill coefficient for ATP binding from 2 to 3. Recent experiments on frog skeletal muscle show the Hill coefficient is unchanged at 1 on lowering pH_i from 7.2 to 6.3 (N. W. Davies, N. B. Standen, and P. R. Stanfield, unpublished observations).

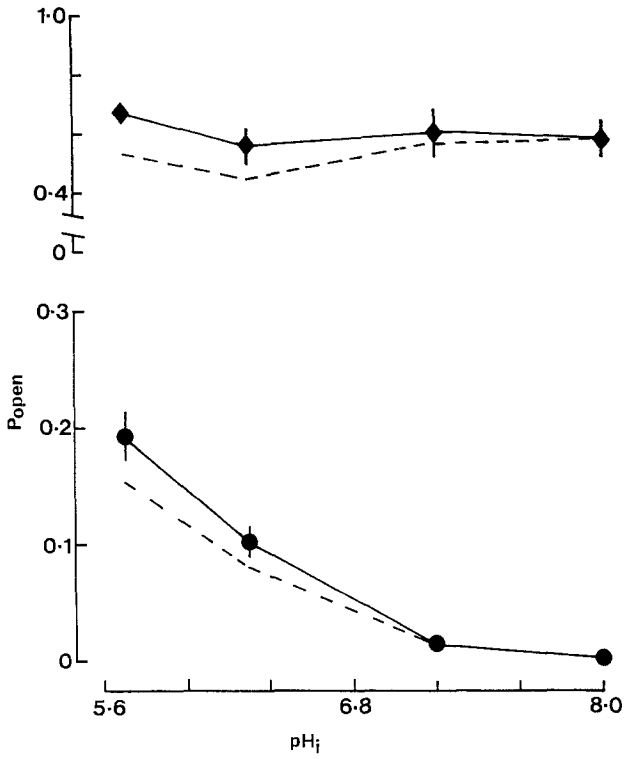


Fig. 2. The effect of pH_i on P_{open} of K_{ATP} channels recorded in an excised inside-out patch from adult frog skeletal muscle in the absence (◆, upper graph) and presence (●, bottom graph) of 0.5 mM ATP_i and 1 mM $MgCl_2$. The membrane potential was 0 mV and the K^+ gradient was $10_o/120_i$. Reproduced with permission from Davies (1990).

Rundown of Channel Activity and Effects of G-Proteins

In insulin-secreting cells, rundown of channel activity in the absence of ATP has been widely reported, and there is growing evidence that phosphorylation is necessary to restore or maintain K_{ATP} activity. There is also evidence for activation by GTP-binding proteins, which are probably part of the pathway by which peptide hormones activate K_{ATP} in these cells. All of this work has been reviewed recently (Ashcroft, 1988; Petersen and Dunne, 1989; De Weille *et al.*, 1989; Rorsman and Trube, 1990).

Rapid rundown has not been widely reported in muscle tissues, though Trube and Hescheler (1984) reported some decline in activity over several minutes in cardiac muscle. In general, though, K_{ATP} activity in muscle seems to persist for long periods in the absence of ATP. Some recent work does suggest the involvement of G-proteins in muscle, however. Parent and Coronado (1989) reincorporated K_{ATP} channels of rabbit T-tubules into

bilayers, and found that activity ran down rapidly under these conditions. Activity could be restored by internal GTP-γ-S and Mg^{2+}, or by the G-protein activator AlF_4. These authors suggest that a G-protein regulates the channels, and that in native membranes (where rundown does not occur) the K_{ATP} and G-protein are already intimately associated. Neumcke and Weik (1990) found that channels from the surface membrane of mouse skeletal muscle were insensitive to these G-protein activators, and suggest that the regulation of K_{ATP} channels in surface and T-tubule membranes may be different. In heart cells, Kirsch *et al.* (1990) found that GTP-γ-S or certain preactivated G-protein α subunits partially restored K_{ATP} channel activity in the presence of ATP, and therefore suggest that a G-protein may modulate ATP sensitivity in this tissue. Other workers have found that F^- stabilizes channel activity in patches from cardiac cells (Zilberter *et al.*, 1988). It is also likely that the actions of vasodilator peptides in arterial smooth muscle (Standen *et al.*, 1989; Nelson *et al.*, 1990a) occur by way of G-proteins.

Kinetics of K_{ATP} Channels

The kinetics of K_{ATP} channels are complex, even in the absence of ATP, and show variability between muscle types. They have been investigated mainly in cardiac and skeletal muscle, and less is known about the quantitative behavior of these channels in smooth muscle. In all preparations the openings of K_{ATP} channels occur in bursts, indicating the presence of multiple closed states. The number of states inferred from distributions of open and closed times varies from one open and two closed states in cultured mouse skeletal muscle (Woll *et al.*, 1989) to two open and four closed states in frog skeletal muscle (Spruce *et al.*, 1987; N. W. Davies, N. B. Standen, and P. R. Stanfield, unpublished observations). Figure 3 shows single-channel records and the distributions of open and closed times from measurements on skeletal muscle, plotted with time on a logarithmic scale (see, for example, McManus *et al.*, 1987; Sigworth and Sine, 1987). The need to postulate at least four closed states seems clear. P_{open} is influenced slightly by membrane potential, and the kinetic properties appear to depend partly on $(V - E_K)$ in both heart and skeletal muscle (Zilberter *et al.*, 1988; Quayle, 1988). Since P_{open} is influenced only slightly by voltage, K_{ATP} channels show little time dependence following a voltage perturbation (Kakei and Noma, 1984; Kakei *et al.*, 1985; Spruce *et al.*, 1985), making a kinetic study of whole cell current difficult.

Kinetics in the Absence of Internal ATP

In the absence of internal ATP, K_{ATP} channel activity is high in most tissues, P_{open} being about 0.8 at 0 mV in cell-attached patches of guinea pig

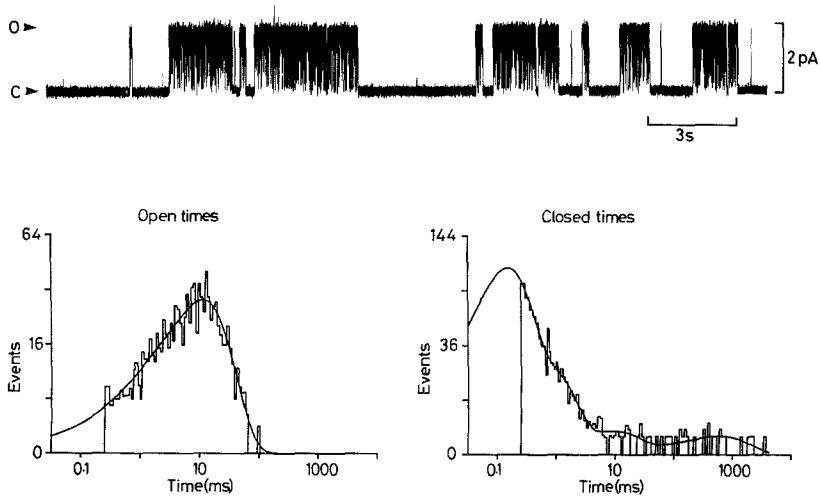


Fig. 3. Example of the activity of a single K_{ATP} channel recorded from an inside-out patch from frog skeletal muscle in the absence of ATP_i. Openings are upwards, membrane potential 0 mV, K^+ gradient $10_o/120_i$. The open and closed time distributions of this patch are shown below. Event durations have been log-binned and a minimum resolution of 200 μs was imposed on the data. Open times were fit by an exponential function with two components with time constants of 1.45 and 8.97 ms and closed times were fit with four components with time constants of 0.09, 0.53, 8.48, and 630.64 ms, respectively (N. W. Davies, N. B. Standen, and P. R. Stanfield, unpublished).

ventricular myocytes (open-cell configuration produced by treatment with saponin; Kakei *et al.*, 1985), 0.5 in inside-out patches of frog skeletal muscle (Spruce *et al.*, 1987; Davies, 1990), 0.23 in inside-out patches of cultured mouse skeletal muscle (Woll *et al.*, 1989), and 0.35 at -90 mV in rat arterial smooth muscle (Standen *et al.*, 1989). Most of the information on K_{ATP} channel kinetics has been obtained from excised patches of membrane under different conditions of ionic concentration, membrane potential, and time resolution of recording. Table II lists some of the values obtained for the time constants of the distributions of open and closed times.

As already indicated, the kinetics show some dependence on $(V - E_K)$ and in cardiac muscle closed times generally decrease and open times increase as membrane potential approaches E_K from either a negative or a positive level (Zilberter *et al.*, 1988). In mammalian skeletal muscle, the effect is less marked, for while closed times behave similarly to those of cardiac muscle, open times increase monotonically with depolarization and do not appear to reach a maximum close to E_K (Woll *et al.*, 1989). Quayle (1988) investigated the effect of varying $[K^+]_o$ on the kinetics of K_{ATP} channels of frog skeletal muscle. Like Woll *et al.* (1989), he found that open times increased with depolarization, although lowering $[K^+]_o$ at a given voltage did result in an

Table II. Open and Closed Time Constants of K_{ATP} under Different Conditions in the Absence of ATP

Tissue	K_o^+/K_i^+ (mM)	$V - E_K$ (mV)	Open times (ms)	Closed times ms)	References
Guinea pig ventricular cells	145/133	−80	1.88	0.38	Trube and Hescheler (1984)
	150/150	−40	0.87	0.09	Qin *et al.* (1989)
Rat ventricular cells	300/300	−40	2.70	0.50	Zilberter *et al.* (1988)
	300/300	+40	12.10	0.2	Zilberter *et al.* (1988)
Frog skeletal muscle	60/120	−40	1.85	0.41	Spruce *et al.* (1985)
			5.65	4.56	
				14.23	
	60/120	−20	0.59	0.26	Spruce *et al.* (1985)
			11.56	1.80	
				12.41	
	10/120	+60	0.30	0.07	Davies *et al.* (unpublished)
			5.04	0.23	
				6.59	
				245.09	
	60/120	−40	2.94	0.27	Quayle (1988)
				3.18	
				11.34	
	60/120	+40	0.98	0.08	Quayle (1988)
			10.96	0.90	
				5.56	
	60/120	+40	1.13	0.13	Spruce (1986)
			16.90	1.91	
				11.10	
				625.0	
Mammalian skeletal muscle	160/160	+50	8.79	0.30	Woll *et al.* (1989)
				70.0	

increase in mean open time as expected from a dependence on $(V - E_K)$. The time constant of the fastest component of the closed time distribution went through a minimum between −40 and +20 mV with 60 mM $[K^+]_o$ ($E_K = -17$ mV).

Burst Kinetics of K_{ATP} Channels

Long bursts of activity are a common property of K_{ATP} channels (see, for example, Figs. 1 and 3). In work on frog skeletal muscle, Spruce (1986), Spruce *et al.* (1987), and Quayle (1988) classified closures belonging to the two shortest elements of the total population as occurring within bursts and found either no effect of voltage on burst duration (Spruce, 1986; mean duration 400 ms at both −60 and +20 mV) or only a weak effect (Quayle, 1988; 170 ms at −60 mV, 220 ms at +20 mV). Woll *et al.* (1989) report that the gaps between bursts decrease with depolarization.

The distribution of bursts shows several components: the distribution of burst durations is best fitted by three exponentials and there are also multiple components if the bursts are characterized in terms of the number of openings they contain (Quayle, 1988, N. W. Davies, N. B. Standen, and P. R. Stanfield, unpublished observations). Correlations exist between the durations of openings within a burst and between one burst duration and the next, an observation that has implications for the way the different states of the channel are linked together (see below; Colquhoun and Hawkes, 1987). Burst kinetics of cardiac K_{ATP} channels have not yet been investigated in detail.

Modulation of K_{ATP} Channel Kinetics by Internal pH

The kinetics of K_{ATP} channels of frog skeletal muscle are affected by internal pH (pH_i; Davies *et al.*, 1989a; Davies, 1990) in the absence of ATP. The main observation is that there is an increase in mean open time as pH_i is increased. This increase is accompanied by a rise in the number of brief closures and an increase in the burst duration. These effects of pH_i appear more pronounced at positive potentials (Davies *et al.*, 1989).

The Effect of Intracellular ATP on Channel Kinetics

As previously mentioned, an increase in internal [ATP] decreases channel P_{open} with a K_i in excised patches of between 20 and 140 μM (Table I). In summary, the effects of ATP on channel kinetics are first to reduce the duration of bursts and the number of openings per burst (see Fig. 1) and second to increase the proportion of the population of closures that are very long. There are other changes in open and closed times, with open times being reduced and closures lengthened. The reduction in open time implies that ATP can bind to the channel when it is in an open state. Saturating concentrations of ATP result in complete channel closure.

A major effect of intracellular ATP is to reduce burst length and the number of openings per burst. In experiments on skeletal muscle, Spruce *et al.* (1987) showed that 1 mM ATP reduced the mean burst duration to 1.4% of that in the absence of the nucleotide (from approximately 240 to 3.5 ms). The mean number of openings per burst was reduced to 2.4% of control (from 86 to 2). The general result is evident from examination of Fig. 1, especially if the records in the presence of 2 mM ATP are compared with those in the control.

Again in skeletal muscle, Spruce *et al.* (1985) found that 0.5 mM ATP may have shortened the faster time constant of the open time probability density function but left the longer one unaltered. The same concentration of ATP had no effect on the time constants of the probability density function for closed times. Spruce *et al.* (1985) argued that ATP placed channels in a

closed state too long-lived to contribute to their measured histograms. The hypothesis that ATP induces very long-lived closures appears correct since computing the mean open time ($\bar{t}_o$) and the mean closed time ($\bar{t}_c$) from the probability density functions of Spruce *et al.* (1985) shows that there is little change with ATP when long closures are excluded. $\bar{t}_o$ was 5.29 ms in the absence of ATP and 5.37 ms in 0.5 mM ATP, while $\bar{t}_c$ was 1.03 ms in the absence of ATP and 0.94 ms in its presence. Since the relation $P_{\text{open}} = \bar{t}_o/(\bar{t}_o + \bar{t}_c)$ must hold, the very large reduction in P_{open} in ATP can only have occurred because of the increase in the number of long closures which Spruce *et al.* (1985) excluded from their probability density function. Spruce *et al.* (1987; Fig. 3 of that paper) also show that if the binomial theorem is used to test whether channels in a multi-channel patch open and close independently of each other, the predicted probabilities of 0, 1, 2, 3, etc. channels being open are a good fit to the observed probabilities in the absence of ATP. However, in the presence of ATP the predictions fit well only if the number of channels assumed to be active is reduced. The result is also consistent with ATP producing long closures, effectively silencing individual channels for a significant part of the recording period.

Other authors, working on heart muscle, have emphasized changes in open and closed times as important in the action of ATP. In particular, Kakei *et al.* (1985) showed that in ventricular cells ATP increased fast and slow time constants of the closed time distribution and reduced both fast and slow time constants of the open time distribution. However, more recently Qin *et al.* (1989) have shown that ATP does not alter the durations of openings and closures occurring within bursts, a result more consistent with that of Spruce *et al.* (1985, 1987).

Step Changes in ATP Concentration

One method of examining the rates with which ATP acts on channel P_{open} is to apply stepwise changes in ATP concentration. To achieve this, Qin and Noma (1988) developed a method where an excised patch can be moved from one solution to another through a separating "oil gate", the complete change of solution being accomplished within about 6 ms. Using this method with excised, inside-out patches of guinea-pig ventricular cells and ensemble averaging the responses, Qin *et al.* (1989) showed that upon stepping from 1 or 5 mM ATP to an ATP-free solution there was a latent period of about 1 s before K_{ATP} channels started to open. This latency was unexplained, but once channels began to open, P_{open} increased along an exponential time course, consistent with a rate constant for the unbinding of ATP of $3.2\,s^{-1}$. On switching back to an ATP-containing solution, the activity decreased exponentially without obvious delay. The time constant was dependent on

[ATP], its reciprocal being proportional to [ATP], consistent with a Hill coefficient of 1 for ATP binding. A complication in the result was that the fall in P_{open} followed one of two rates, corresponding to closing, or binding rate constants of either 51.7 or 5.6 mM^{-1} s^{-1}, and possibly indicating two states of sensitivity to ATP. The result that binding of a single ATP appears to close the channel is in contrast to results obtained from measurements of steady-state P_{open} as a function of [ATP] (see Table I).

Rapid changes in ATP concentration are also possible by flash photolysis of "caged ATP," a method that has been used to investigate ATP binding to K_{ATP} channels of rat heart cells (Nicholls *et al.*, 1990). The result is complicated by the fact that the caged-ATP compound itself closed K_{ATP} channels although its inhibitory effect was less than that of ATP. The time constant (τ = 300 ms) of channel closure following photolytic degradation of 2 mM caged ATP was slower than those (9.7 and 89 ms) measured with 2 mM ATP by Qin *et al.* (1989). An additional complication, however, is that flash photolysis of 2 mM caged ATP will almost certainly yield an ATP concentration less than 2 mM, perhaps partly explaining the slower rate of channel closure.

Kinetic Model of the K_{ATP} Channel

The kinetic behavior of K_{ATP} channels is not fully understood, though models or state diagrams must account for the presence of at least two open and four closed states. Spruce (1986) proposed a scheme (scheme 1, below; see also Stanfield, 1987) which includes a fourth, long-duration closed state, occupancy of which is influenced by internal [ATP]:

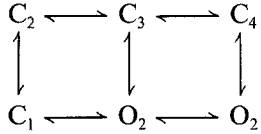
$$C_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_2 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} C_3 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} O_1 \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} O_2 \qquad (1)$$

$$O_1 \underset{k_5}{\overset{k_{-5}}{\rightleftarrows}} C_4$$

The proposal of scheme 1 (Spruce, 1986) was generated using the matrix methods of Colquhoun and Hawkes (1982) to optimize the fit to experimental probability density functions for open and closed states as well as to the measured channel P_{open} using appropriate transition rates between states. The main effect of increasing [ATP] was to increase k_5. Interestingly, given the recent results of Qin *et al.* (1989), in scheme 1 k_{-5} was given a value between 2 and 4 s^{-1} (the rate for unbinding being 3.2 s^{-1} in Qin *et al.*, 1989) while k_5 was 250 s^{-1} in 1 mM ATP (the rate for binding being 52 mM^{-1} s^{-1} in Qin *et al.*, 1989). Small changes, of uncertain significance, occurred in other transition rates.

The placing of the ATP-regulated step as occurring from only one of the open states is partly arbitrary, and it is as likely that the channel can bind

ATP in any open or short-lived closed state. It is already clear that Spruce's (1986) model must be modified. The observation of multiple components of bursts, of correlations within bursts, and of correlations in length between consecutive bursts (Quayle, 1988; N. W. Davies, N. B. Standen, and P. R. Stanfield, unpublished observations) has implications for the way states are connected. While it is premature to give an alternative quantitative scheme here, a change in the connectivity of the states can be suggested that will account for some of the observations on bursts. Scheme (2) shows one such form of the necessary modifications (see Colquhoun and Hawkes, 1987):



(2)

Here states C_1 and C_2 are long-lived closures such as occur between bursts, while C_3 and C_4 are short-lived closures within bursts. Clearly, further work is needed to produce a kinetic scheme that explains more fully how K_{ATP} channels open and close in the absence of ATP and how ATP alters that behavior.

Possible Roles of K_{ATP} in Muscle

Considerations of possible functions for K_{ATP} channels in muscle have to take into account two important factors. The first is the high ATP sensitivity of the channel, with half block occurring at 20–140 μM, while internal ATP is at millimolar concentrations. In muscle this may merely result in the observed low resting P_{open}, and it has been calculated that opening of very few K_{ATP} channels could have substantial effects, both in cardiac and smooth muscle (Carmeliet *et al.*, 1990; Nelson *et al.*, 1990b). A more serious problem is that internal ATP is well buffered, changing only slowly in ischemia or under metabolic exhaustion, while shortening of the cardiac action potential, for example, occurs more rapidly. Two possible resolutions have been proposed; either the submembrane [ATP] changes more than total intracellular ATP, or alternative mechanisms of channel regulation exist. Present measurements of ATP do not have sufficient resolution to decide the first point, but there is increasing evidence, discussed above, that other factors may influence channel P_{open}. It seems clear that a complete understanding of the functions of K_{ATP} channels will require more detailed knowledge of the ways in which they are regulated. Here we shall consider some of the roles that have been proposed for these channels in muscle; these are summarized in Fig. 4.

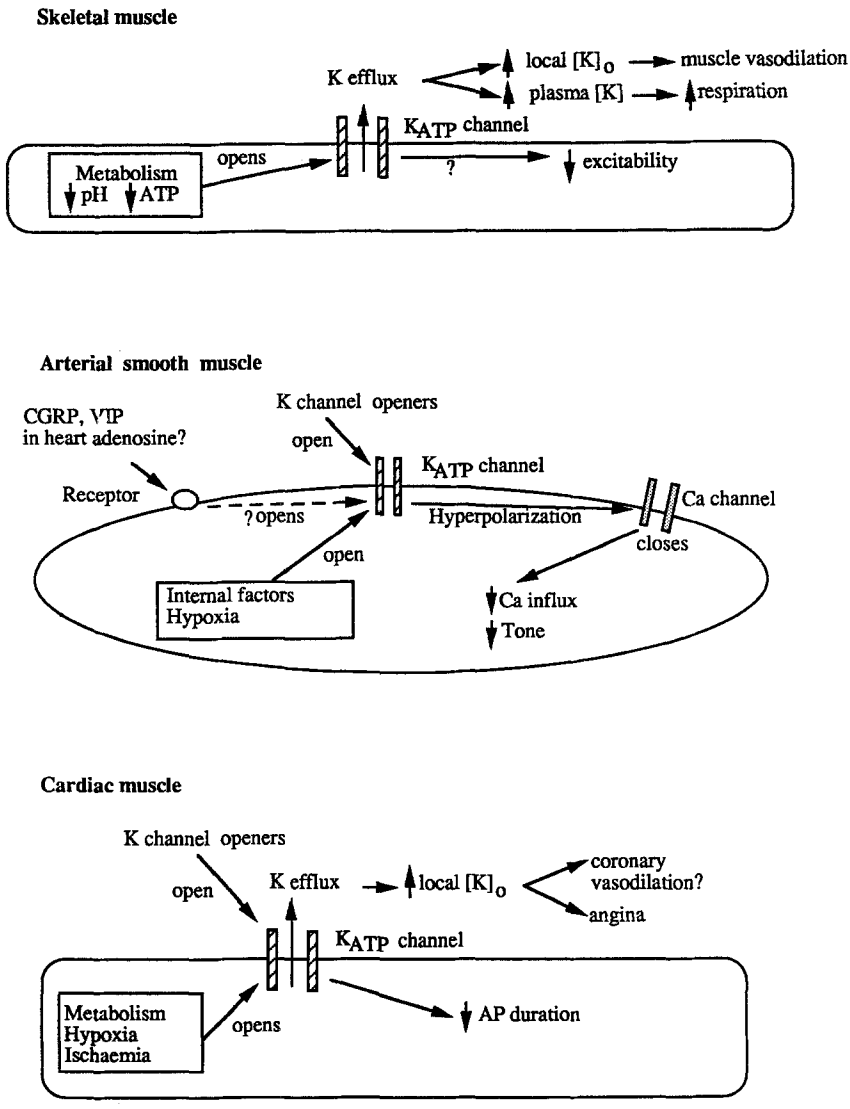


Fig. 4. Possible roles of K_{ATP} channels in muscle.

Effects of Hypoxia and Ischemia in Cardiac Muscle

In his original description of K_{ATP} channels, Noma (1983) suggested that their activation underlies the shortening of the cardiac action potential seen in hypoxia (Trautwein *et al.*, 1954), and that, by reducing contractile force, this might prevent further decline in intracellular ATP and so cell death.

Noma and Shibasaki (1985) also concluded that K_{ATP} channels underlie the hypoxia-induced outward current, and it has now been shown that the effects of hypoxia on action potential duration can be reversed by glibenclamide (Sanguinetti *et al.*, 1988; Wilde *et al.*, 1990; Carmeliet *et al.*, 1990). Hypoxia alone leads to a decrease in action potential duration, hyperpolarization, and decreased excitability (Vleugels *et al.*, 1980). Under conditions of ischemia, where hypoxia is associated with accumulation of metabolites, an increase in extracellular K^+ concentration occurs and causes depolarization (Hill and Gettes, 1980; Weiss and Shine, 1981; Kléber, 1983). This, together with the decrease in refractory period associated with the shortened action potential, may lead to arrhythmias (Harris *et al.*, 1954; Weiss and Shine, 1981). At least some of the K^+ accumulation seems to arise from efflux through K_{ATP} channels, since it is reduced by glibenclamide (Kantor *et al.*, 1987; Wilde *et al.*, 1989), which may also prevent arrhythmias in hearts made experimentally ischemic (Kantor *et al.*, 1989). Under these conditions K_{ATP} activation seems to be harmful; nevertheless its initial effect in reducing energy usage might be beneficial if reperfusion occurs before arrhythmic activity becomes pronounced. Their role in causing K^+ efflux might be important in other ways. K^+ accumulation leads to cardiac pain (Procacci and Zoppi, 1984), and may thereby cause cessation of physical activity, while it is also possible that a rise in $[K^+]_o$ contributes to vasodilation in the heart as it does in skeletal muscle (Scott *et al.*, 1970). It has been reported that adult-onset diabetic patients receiving tolbutamide showed a higher mortality from cardiovascular disease than did those receiving insulin or controls (University Group Diabetes Program, 1970).

Skeletal Muscle

In skeletal muscle it seems clear that K_{ATP} channels account for the increased K^+ permeability seen in metabolic exhaustion as discussed previously. The high density of K_{ATP} channels in skeletal muscle make it likely that these channels also play a role under more physiological conditions, for example in exercising muscle. In this context, the involvement of regulators in addition to ATP may be important. In particular, intracellular pH changes both more rapidly and substantially than ATP on exercise (e.g., Challis *et al.*, 1989). pH has a large effect on the sensitivity of K_{ATP} channels to ATP over the physiological pH range, such that a fall in pH_i increases P_{open} of K_{ATP} channels (Davies, 1990). One possibility, discussed by Spruce *et al.* (1987), is that the channels open to reduce excitability of muscle fibres and so cause them to rest. The primary causes of fatigue, however, appear to involve central rather than peripheral processes (Bigland-Ritchie and Woods, 1984),

so that if such a mechanism does occur it must be at the level of individual fibers, perhaps resting certain fibres within a motor unit.

It may be that the K^+ efflux through K_{ATP} channels, acting through a rise in extracellular K^+ concentration, is more important than their effects on excitability. K^+ is released from muscle on exercise (Fenn, 1938), leading to a substantial rise in arterial [K^+] (Band *et al.*, 1982; Medbo and Sejersted, 1990). This rise in [K^+] may have effects both locally in muscle and on arterial chemoreceptors. A local rise in [K^+] has been shown to cause vasodilation within skeletal muscle itself (Skinner and Powell, 1967; Scott *et al.*, 1970). A rise in plasma potassium in the appropriate range (up to about 6 mM) excites carotid body chemoreceptors, acting synergistically with hypoxia and leading to increased ventilation (Band *et al.*, 1985; Band and Linton, 1986; Burger *et al.*, 1988). In this way, increased arterial K^+ may contribute to the increase in respiration on exercise (Band and Linton, 1986; Burger *et al.*, 1988). It is not clear at present to what, if any, extent K_{ATP} channels are involved in the K^+ efflux from muscle in exercise. Clearly, they could provide a means to link both oxygen uptake (by ventilation rate) and its delivery to muscle (by vasodilation) to the metabolic state of muscle fibers. Future investigations of the possible role of K_{ATP} channels in K^+ efflux from exercising muscle and in the rise in arterial [K^+] on exercise will be of great interest in this respect.

Smooth Muscle

As discussed above, several workers have suggested that activation of K_{ATP} channels of smooth muscle underlies the response to K^+ channel opening drugs in certain tissues. It has recently been suggested that K_{ATP} channels of arterial smooth muscle may also be important in the vasodilatory response to hypoxia or ischemia. In isolated perfused hearts, Daut *et al.* (1990) found that coronary vasodilation induced by hypoxia was blocked by glibenclamide and mimicked by cromakalim. They propose that activation of K_{ATP} hyperpolarizes and so relaxes coronary arteries and arterioles. In the pulmonary circulation, hypoxia causes *vasoconstriction*, and this is also blocked by tolbutamide and mimicked by diazoxide (Robertson *et al.*, 1989). These workers suggest that in the lungs hypoxia may open K_{ATP} channels of cells other than smooth muscle, which then release a constricting factor (or reduce release of a dilating factor).

Several endogenous vasodilator substances also act at least partly through hyperpolarization. These include the peptides vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP), and acetylcholine, which exerts part of its action through a hyperpolarizing factor

released from the vascular endothelium (Taylor and Weston, 1988). Hyperpolarizations and relaxations to these agents in some cerebral and mesenteric arteries can be inhibited by glibenclamide, tolbutamide, or Ba^{2+} (Brayden *et al.*, 1989; Standen *et al.*, 1989). The most potent of the vasodilators, CGRP, activates single K^+ channels in cell-attached patches on arterial smooth muscle cells (Nelson *et al.*, 1990a). The mechanism of this activation is unknown; however, the peptide hormones galanin and somatostatin have been shown to activate K_{ATP} channels in insulin-secreting cells by way of a GTP-binding protein (Petersen and Dunne, 1989; De Weille *et al.*, 1989), and a similar mechanism might be involved in smooth muscle. Daut *et al.* (1990) found that the coronary vasodilator response to adenosine was inhibited by glibenclamide, and have therefore suggested that adenosine may activate K_{ATP} by such a route.

Thus it is possible that K_{ATP} channels in some smooth muscle tissues may form targets for neurotransmitters, probably acting by way of G-proteins. In resistance vessels activation of these channels, leading to hyperpolarization, may form a physiological mechanism for vasodilation. Since K_{ATP} channels are clearly targets for extracellular messengers in insulin-secreting cells, it is possible that such a role may be found also in cardiac and skeletal muscle.

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