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Characterization of K_{ATP} channels in intact mammalian skeletal muscle fibres

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1 The aim of this study was to characterize the K_{ATP} channel of intact rat skeletal muscle (rat flexor digitorum brevis muscle). Changes in membrane currents were recorded with two-electrode voltage-clamp of whole fibres.

2 The K_{ATP} channel openers, levcromakalim and pinacidil (10–400 μM), caused a concentration-dependent increase in whole-cell chord conductance (up to approximately 1.5 mScm^{-2}). The activated current had a weak inwardly rectifying current-voltage relation, a reversal potential near E_K and nanomolar sensitivity to glibenclamide – characteristic of a K_{ATP} channel current. Concentration-effect analysis revealed that levcromakalim and pinacidil were not particularly potent (EC_{50} ~186 μM, ~30 μM, respectively), but diazoxide was completely inactive.

3 The ability of both classical K_{ATP} channel inhibitors (glibenclamide, tolbutamide, glipizide and 5-hydroxydecanoic acid) and a number of structurally related glibenclamide analogues to antagonize the levcromakalim-induced current was determined. Glibenclamide was the most potent compound with an IC_{50} of approximately 5 nM. However, the non-sulphonylurea (but cardioactive) compound 5-hydroxydecanoic acid was inactive in this preparation.

4 Regression analysis showed that the glibenclamide analogues used have a similar rank order of potency to that observed previously in vascular smooth muscle and cerebral tissue. However, two compounds (glipizide and DK13) were found to have unexpectedly low potency in skeletal muscle.

5 These experiments revealed K_{ATP} channels of skeletal muscle to be at least 10× more sensitive to glibenclamide than previously found; this may be because of the requirement for an intact intracellular environment for the full effect of sulphonylureas to be realised. Pharmacologically, K_{ATP} channels of mammalian skeletal muscle appear to resemble most closely K_{ATP} channels of cardiac myocytes.

Keywords: Skeletal muscle; ATP-sensitive, K_{ATP} potassium channel; glibenclamide; 5-hydroxydecanoic acid; levcromakalim; cromakalim; tolbutamide; diazoxide

Introduction

K_{ATP} channels are a widely distributed family of potassium-selective ion channels which are closed by intracellular ATP and serve to couple cell metabolism to cell excitability. Their roles in mammalian tissues have been reviewed by several authors (in all tissues: Ashcroft & Ashcroft, 1990; in all muscle: Davies *et al*., 1991 and in smooth muscle: Quayle *et al*., 1997). There is now strong evidence that K_{ATP} channels exist as a complex of an inwardly rectifying potassium channel (Kir6.2) and a sulphonylurea binding receptor protein (SUR, Inagaki *et al*., 1995). Some activators of K_{ATP} channels (e.g., diazoxide and Mg^{2+}-ADP) and sulphonylurea inhibitors (e.g. glibenclamide) act through interaction with the SUR, whilst ATP itself seems to bind directly to Kir6.2 to affect channel closure (Tucker *et al*., 1997). It is also clear that several subtypes of SUR exist (Inagaki *et al*., 1996), and with the additional possibility of these receptors combining with differing isoforms of Kir6.2, there are many potential pharmacologically distinguishable subtypes of K_{ATP} channel complexes.

A few studies have investigated the pharmacology of functional skeletal muscle K_{ATP} channels in isolated membrane patches (Weik & Neumcke 1990; Allard & Lazdunski 1993; Barrett-Jolley & Davies 1997). However, in this paper, we have used two-electrode voltage clamp to investigate, for the first time, properties of K_{ATP} channels in whole and intact mammalian skeletal muscle and their modulation by a number of K_{ATP} channel selective agents.

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Methods

Preparation

Single skeletal muscle fibres were isolated from rat flexor digitorum brevis muscle by use of collagenase as described previously (McKillen *et al*., 1994).

Electrophysiology

Membrane currents were recorded with two-electrode voltage clamp of whole fibres by an NPI Turbo-TEC 10C amplifier, filtered at 1 kHz and connected directly to a PC at a sample rate of 3 kHz. The difficulties usually associated with the two-electrode voltage clamp of large cells such as skeletal muscle fibres (for example, 'space-clamp') were obviated by using fibres which were short and broad (Bekoff & Betz 1977; McKillen 1993; McKillen *et al*., 1994) and by monitoring only slowly developing currents. From both optical and electrical measurements (capacitance transients, not shown), the surface area of 'visible' membrane of these fibres is estimated to be in the order of $2e^{-3}$ cm^2. Experiments were performed at room temperature (23–26°C).

For all the experiments described in this paper, external potassium was 40 mM (see later) thus, assuming internal potassium to be 145 mM, E_K was −32 mV. We used a pulse protocol which consisted of holding the cell at 0 mV for 5 s, then stepping to +32 mV (for 100 ms), −32 mV (for 100 ms), −60 mV (for 100 ms) and then returning to 0 mV. This enabled us to monitor the resting potassium current (at 0 mV) whilst periodically monitoring the current-voltage relationship of the cells. The predominant resting conductance of these fibres was of chloride, but after replacement of extracellular Cl^- with gluconate, background (leak) conductance in these fibres was typically less than 300 nS.

Experimental protocol and data analysis

Isolated skeletal muscle fibres were allowed to settle on the bottom of a sylgard lined tissue chamber so that membrane currents could be recorded with two-electrode voltage clamp. The fibres were superfused with bathing solution (see below) at a flow rate of approximately 1 ml min^{-1}. Drugs were added directly to the perfusion media. This design allowed steady-state concentration-effect curves to be constructed when the electrophysiological effects of the various drugs were being assessed.

The first part of this study involved characterizing the actions, particularly the potency, of levcromakalim in the skeletal muscle cell. As will be shown, levcromakalim is not a potent K_{ATP} channel opener in this preparation and the highest concentration of levcromakalim utilized was 400 μM. In the second part of the study, designed to calculate the potency of glibenclamide and its analogues, we first activated K_{ATP} currents with levcromakalim (100 μM), then added various concentrations of the compounds (in the range of 1 nM to 300 μM) and reduced the current back toward the original baseline.

In general, the voltage-clamped skeletal muscle fibres were viable for only a limited time (less than 30 min on average), and whilst there were some examples where full concentration-effect curves could be constructed to both the levcromakalim and the glibenclamide analogues this was not generally the case. Thus in the majority of studies, 2 to 3 different concentrations of either levcromakalim, glibenclamide or the glibenclamide analogues were assessed for activity. Mean concentration-effect curves were constructed by averaging responses at a number of different concentrations. To calculate the potency of levcromakalim itself, and for glibenclamide and its analogues at reversing levcromakalim (100 μM)-induced currents, mean concentration-effect data were analysed by use of a non-linear curve fitting to obtain estimates of potency ($pD_2 = -\log EC_{50}$ for levcromakalim and $pIC_{50} = -\log IC_{50}$ for glibenclamide and analogues) by use of

$$\text{current} = \text{max. current} \times C^{n_H}/(C^{n_H} + K_{50}{}^{n_H}) \quad \text{equation 1.}$$

where C is the concentration of drug, n_H is the slope (+ve for activation −ve for inhibition). K_{50} is either the EC_{50} or the IC_{50} and these values represent the midpoint parameters; the EC_{50} is the concentration of levcromakalim causing 50% of the maximum current or, in the case of the glibenclamide analogues, IC_{50} is the concentration which inhibits the K_{ATP} current to 50% (maximum current = 100%). The midpoint parameters were actually estimated as $-\log(EC_{50}$ or $IC_{50})$ by making the appropriate substitution (see Black & Shankley, 1985). Errors for pD_2 or pIC_{50} quoted in the text were generated by the non-linear curve fitting programme (Microcal Origin) and give an estimate of the confidence limit for each parameter. Other values are quoted as means ± s.e.mean where *n* is the number of measurements. Correlation and regression statistics were calculated with Microsoft Excel's analysis toolpack.

Solutions and materials

The solution used whilst dissecting and treating with collagenase (3 mg ml^{-1}) was (in mM): NaCl 154, KCl 5, Na_2HPO_4 1, HEPES 10, $MgCl_2$ 1.2 and $CaCl_2$ 1.2; pH 7.4 with NaOH. After collagenase treatment, the muscle was triturated and then stored for up to six hours in (mM): NaCl 154, KCl 5, Na_2HPO_4 1, HEPES 10 and $MgCl_2$ 1.2; pH 7.4 with NaOH. The bathing solution used during experiments was (mM): Na_2SO_4 77, Na_2HPO_4 1, K gluconate 40, $MgSO_4$ 1.2, $CaSO_4$ 8, HEPES 10 and glucose 10. pH was raised to 7.4 with NaOH (1 M stock – approximately 2.5 ml l^{-1}).

Voltage electrodes (fabricated from 1.2 mm filamented, thick walled borosilicate glass, Clarke Electromedical) had a resistance of ~20 MΩ when filled with 1 M KCl. Current electrodes (1.2 mm filamented, thin walled borosilicate glass, Clarke Electromedical) were filled with: K_3 citrate 1 M, KCl 0.2 M and EGTA 2 mM. When filled, these current electrodes had resistances of between 4 and 8 MΩ.

Drugs

Drugs used and their sources were: levcromakalim (a gift from SmithKline Beecham, U.K.); glibenclamide (Sigma, St. Louis, U.S.A.), pinacidil, diazoxide and 5-hydroxydecanoic acid (RBI, Natick, MA, U.S.A.). A number of novel analogues structurally related to glibenclamide were also used (see Challinor-Rogers *et al*., 1995 for additional details). Their structures and laboratory code numbers are listed in Figure 1. The compounds were glibenclamide: and DK31 (amidoethyl-benzene-sulphonylureas); DK1 and DK2 (sulphonamides), DK37 (benzenesulphonylurea) and DK13 (benzamide analogue). All other chemicals were purchased from Sigma.

5-Hydroxydecanoate was prepared as a 100 mM stock in water, all other drugs were made up at 10 to 300 mM stocks in dimethylsulphoxide (DMSO) and added to the bathing solution immediately before use. Final concentrations are given where appropriate and maximum DMSO exposure was 0.2%. DMSO alone had no noticeable effect on membrane currents at up to 0.5% (data not shown).

Results

Characteristics of levcromakalim-induced currents

By use of the voltage protocol described in the Methods (see also Figure 2), whole-cell currents were recorded in the absence and in the presence of the K_{ATP} channel opener, levcromakalim (100 μM). The application of levcromakalim (100 μM) evoked an increase in current measured at +32 mV, 0 mV and −60 mV, but not at the potassium equilibrium potential, E_k (−32 mV). Glibenclamide (1 μM) reduced this levcromakalim-induced current back to near control levels (Figure 2). The addition of glibenclamide (up to 100 μM) alone had no effect on whole-cell currents (data not shown).

The onset of action of levcromakalim was rapid. Figure 3a shows mean current (at +32 mV, 0 mV and −32 mV) recorded over a period of approximately 10 min, during the application of levcromakalim and glibenclamide. Application of levcromakalim (100 μM) to the perfusing bath solution resulted in a substantial increase in whole-cell conductance.

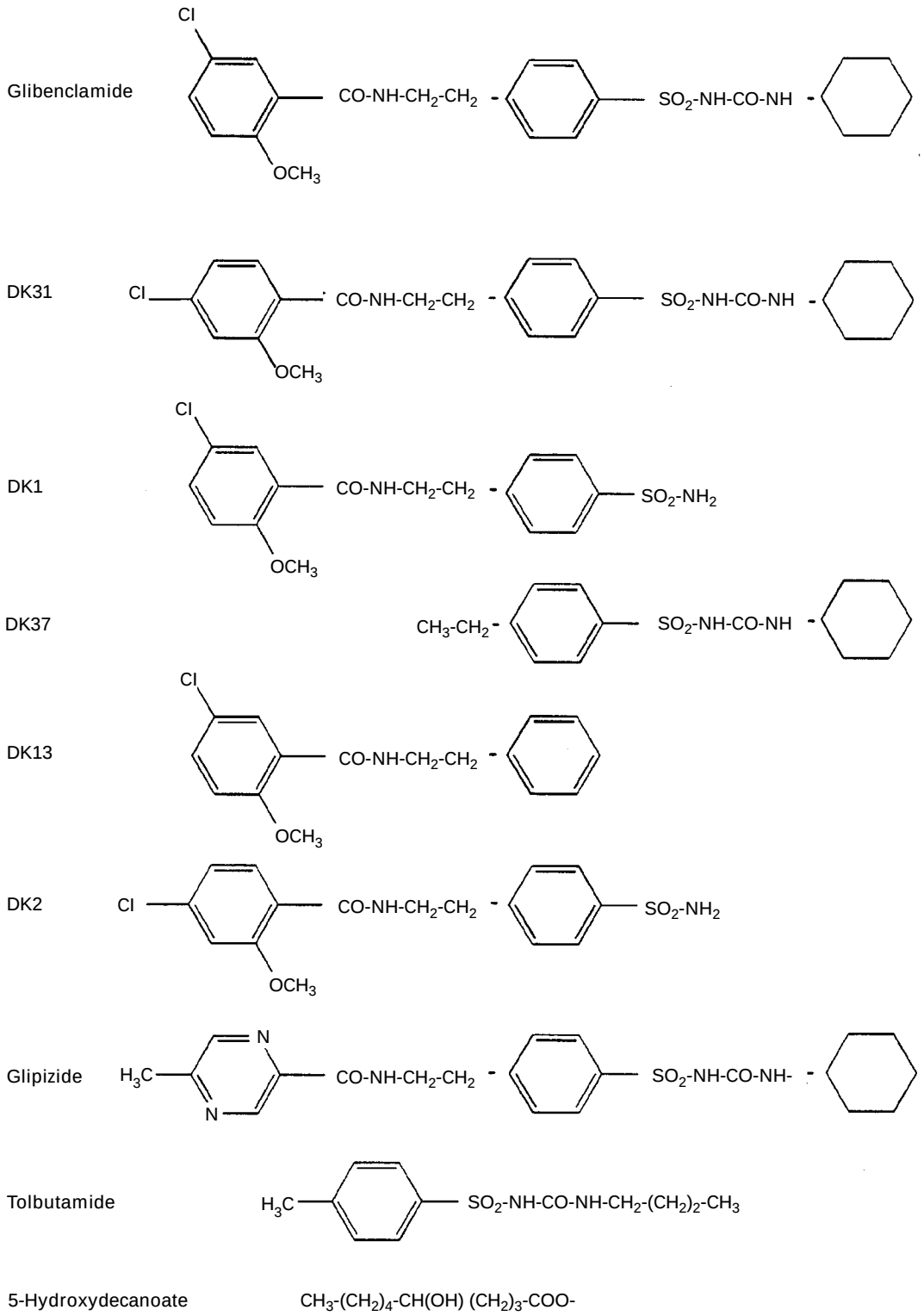

Figure 1 Structures of all the K_{ATP} channel inhibitors used in this study.

The response generally reached a maximum within 10 to 15 min following the addition of levcromakalim to the bath solution, and was largely reversed by the addition of a low concentration of glibenclamide (10 nM). Figure 3c shows the current voltage-relationship of the levcromakalim (100 μM)-activated current. This was obtained by subtracting the current in the absence of levcromakalim from the current in its presence (Figure 3b). This difference current reversed very close to E_K as would be expected from a K_{ATP}-mediated current.

The potency of levcromakalim was determined in a separate series of experiments. We applied increasing concentrations of levcromakalim to muscle fibres and recorded the K_{ATP} currents evoked (Figure 4a). The concentration-effect curve obtained from these experiments is shown in Figure 5. We could not obtain a maximum response to the highest concentration of levcromakalim that we utilized (400 μM). Consequently, the data were normalized such that the response to levcromakalim 200 μM (a concentration used in all experiments) was assigned the value of 100%. With this normalized data, and by use of

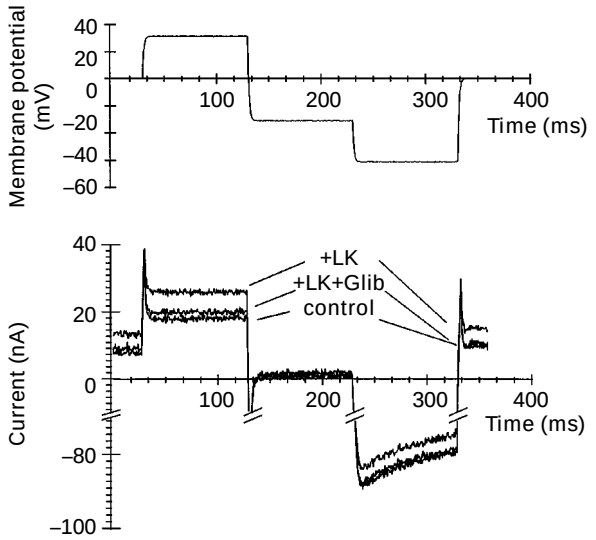

Figure 2 Activation of glibenclamide-sensitive current by levcromakalim. At five second intervals, the membrane potential was stepped from 0 mV, to +32 mV (for 100 ms) then to −32 mV (100 ms) then −60 mV (100 ms) and finally back to 0 mV. In the example shown, (a) shows the voltage recorded before and during the application of drugs. (b) Shows the membrane currents recorded in control solution, 100 μM levcromakalim (LK) and 100 μM levcromakalim with 1 μM glibenclamide (LK + Glib), as indicated. Membrane conductance, and outward currents were increased by levcromakalim, and this effect was reversed by glibenclamide. The current at −32 mV ($\sim E_K$) was little affected by these K_{ATP} active drugs. Note that the dominant inward current under these conditions was inwardly rectifying. However, the y-axis has been broken to emphasize the activation of the glibenclamide-sensitive outward current.

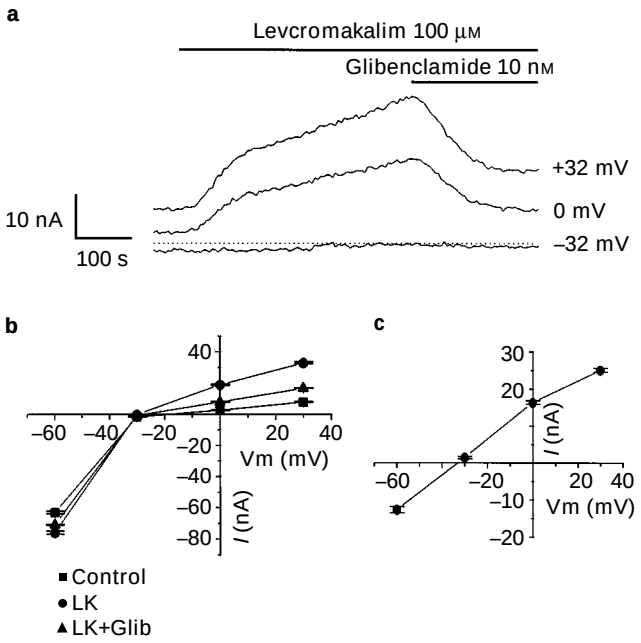

Figure 3 Levcromakalim activated a K_{ATP} current. (a) Typical trace showing current measured at −32 mV, 0 mV and +32 mV. Levcromakalim (100 μM) and glibenclamide (10 nM) were added as indicated by the bars. (b) Current-voltage curves from (c), plotted as the mean of 10 points recorded in control, 100 μM levcromakalim (LK) and levcromakalim with 10 nM glibenclamide (Glib). (c) The levcromakalim-activated difference current (levcromakalim-control) which reversed near to E_K.

the curve-fit, we calculated a maximum current of 176.6% (of the 200 μM levcromakalim response) with a pD_2 value of 3.73 ± 0.17, $n = 19$. Levcromakalim (200 μM) evoked a current of 65 ± 15 nA ($n = 7$). Pinacidil, another widely active K_{ATP} channel opener was also active in this preparation (Figure 4b) and whilst again, we did not obtain a true maximum current, by our curve-fitting programme we estimated a pD_2 of 4.5 ± 0.3 ($n = 11$) (Figure 5). Diazoxide (up to 300 μM) failed to activate any K_{ATP} current in any of the 5 fibres tested (Figure 4b and 5).

Determination of the potency of glibenclamide analogues

In order to calculate the potency of glibenclamide and its analogues, we first activated K_{ATP} currents with levcromakalim (100 μM), then added various concentrations of the compounds (in the range of 1 nM to 100 μM) and reduced the current back toward the original baseline. Figure 6 shows examples of this protocol and compares the action of glibenclamide (Figure 6a), DK13 (Figure 6b) and DK2 (Figure 6c). By use of the results obtained by curve-fitting the mean concentration-response curves, the rank order of potency (based on pIC_{50} data) of the compounds was: glibenclamide (8.31) > DK31 (7.91) > DK1 (6.15) > glipizide (5.60) > DK2 (5.48) > DK37 (5.28) > tolbutamide (4.98) > DK13 (3.89) (5-hydroxydecanoic acid was completely inactive at 100 and 300 μM). Table 1 summarizes this data. Figure 7a shows the mean concentration-effect curves for the classical K_{ATP} channel inhibitors (glibenclamide, glipizide, tolbutamide and 5-hydroxydecanoic acid) and Figure 7b shows the mean concentration-effect curves for the five 'DK' glibenclamide analogues. All the compounds tested, at a sufficient concentration, completely abolished the K_{ATP} current, except DK13 (and 5-hydroxydecanoate). Figure 6b shows that the maximum concentration of DK13 (100 μM) was unable to reverse fully activation of K_{ATP} currents by levcromakalim.

Discussion

In this paper we have used two-electrode voltage-clamp to investigate K_{ATP} channels of rat skeletal muscle and their inhibition by a series of glibenclamide analogues. On the basis of the sensitivity of this preparation to levcromakalim and pinacidil (but not diazoxide) and the high potency with which glibenclamide analogues (but not 5-hydroxydecanoic acid) antagonize the action of levcromakalim, it would appear that skeletal muscle K_{ATP} channels have properties most similar to, but distinct from cardiac muscle.

K_{ATP} channel openers

Previously, single channel studies on isolated membrane patches have shown mammalian skeletal muscle K_{ATP} channels to be weakly activated by potassium channel openers (Allard & Lazdunski, 1993; Weik & Neumcke, 1990; Hussain *et al.*, 1994). Allard & Lazdunski (1993) calculated EC_{50}s for cromakalim (the racemic equivalent to levcromakalim) and pinacidil to be 220 and 125 μM, respectively, with a requirement for the presence of low concentrations (~ 500 μM) of cytoplasmic ATP. However, although finding similar activation with levcromakalim and pinacidil, Weik & Neumcke (1990) and Hussain *et al.* (1994) showed that high concentrations of cytoplasmic ATP (for example, 1 mM) completely prevented the action of these openers. It was therefore an open question as to whether K_{ATP} channels would

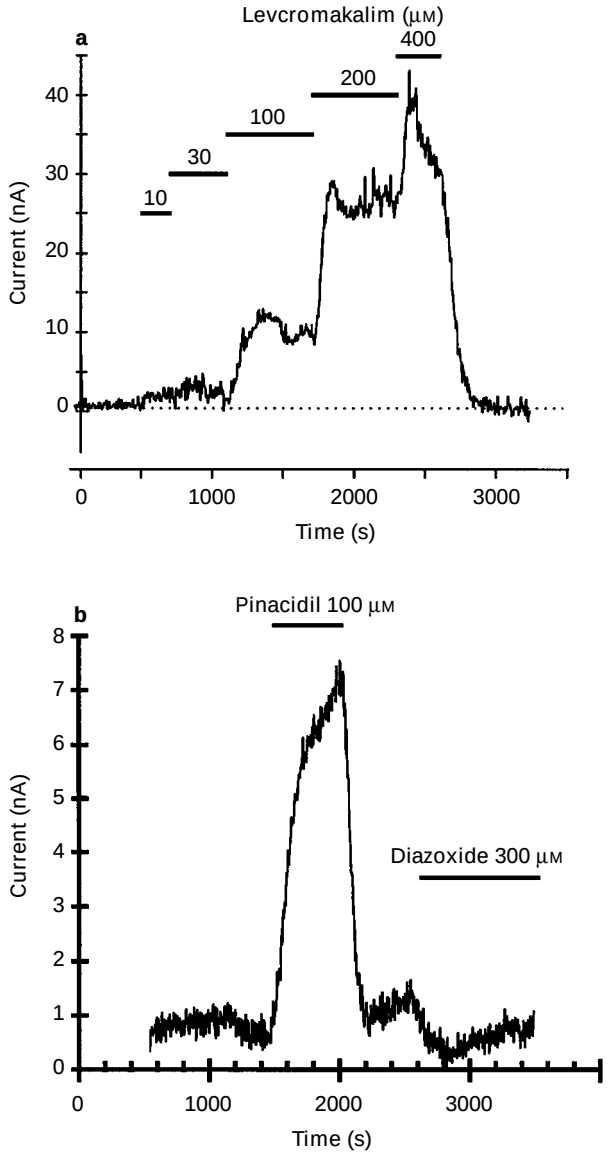

Figure 4 Effect of potassium channel openers on whole-cell K_{ATP} currents. Membrane currents were recorded at 0 mV whilst potassium channel openers were applied to the muscle fibres. (a) Effect of increasing concentrations of levcromakalim (as indicated by the bar). (b) Application of 100 μM pinacidil activated a current whereas application of 300 μM diazoxide did not.

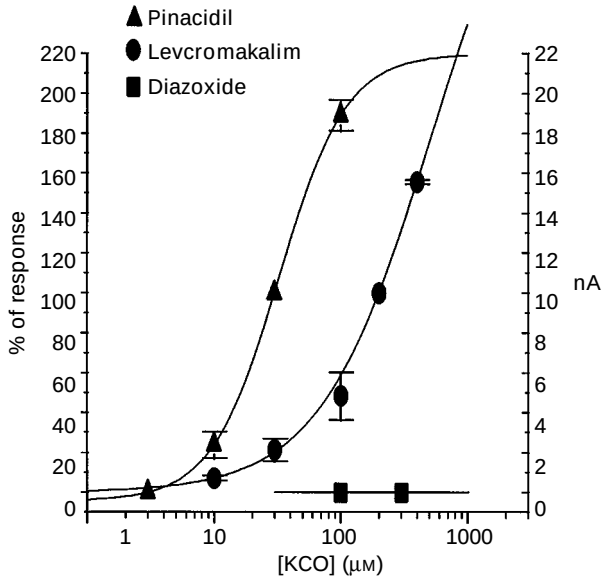

Figure 5 Potassium channel opener concentration-effect curves. The concentration-effect curves were constructed from experiments such as that shown in Figure 4. We could not achieve a true maximum and so we normalized current amplitude to that produced by levcromakalim 200 μM (=100%). The line was fitted to a sigmoidal curve (equation 1) with an estimated maximum of 176.7±18% of the 200 μM levcromakalim current (this corresponds to ~115 nA) and a pD_2 of 3.73±0.17 (slope 1.23±0.2) (n=19). Pinacidil data were fit (equation 1) with an estimated maximum of 215%, a pD_2 of 4.5±0.3 and slope 1.6±0.5 (n=11). Diazoxide had no effect up to 300 μM. Note, the y-axis on the left is current expressed as a percentage; the y-axis on the right is in nA and refers only to the diazoxide data.

be opened by levcromakalim and pinacidil in intact skeletal muscle fibres where one would expect ATP concentrations to be in the millimolar range (Dawson *et al*., 1978, suggest ~4 mM and never falling below 2.5 mM, even under conditions of extreme exhaustion or fatigue). In fact we find that we can activate tens of nA of K_{ATP} channel current with either pinacidil or levcromakalim (10 nA at 0 mV ≅313 nS or ~150 μScm^{-2}, see Methods). Metabolic exhaustion of these fibres (not shown) can activate hundreds of nA of K_{ATP} channel current and so a very approximate estimation would be that 200 μM levcromakalim activates about 10% of all available K_{ATP} channels in these intact fibres. It may be argued that we do not know for sure the intracellular concentration of ATP, which is true, but, firstly there is absolutely no basal K_{ATP} current under control conditions, and secondly, the isolated muscle fibres can still be quite readily 'twitched' (not shown) by an appropriate electrical stimulus. It is then surprising that intact skeletal muscle fibres are sensitive to potassium channel openers in this intact preparation in a similar range (30–185 μM) to that seen in single channel experiments (Weik & Neumcke, 1990; Allard & Lazdunski, 1993; Hussain *et al*., 1994). Like Weik & Neumcke (1990), we found skeletal muscle K_{ATP} channels to be completely insensitive to diazoxide, one of the strongest pharmacological tools for classifying K_{ATP} channels. For whilst pinacidil and levcromakalim show greater activity in smooth muscle than cardiac muscle or pancreatic β-cells (e.g., cardiac: EC_{50}>30 μM, Escande *et al*., 1988; pancreas: EC_{50} ~50 μM, Dunne *et al*., 1990; vascular smooth muscle: EC_{50} ~1.5 μM, Quayle *et al*., 1990; Russell *et al*., 1992; EC_{50} ~20 nM, Quast & Cook 1988), diazoxide is active in smooth muscle and pancreatic β-cells, but appears to be completely inactive on cardiac muscle (pancreas and vascular smooth muscle: Quast & Cook, 1988; cardiac: Faivre & Findlay, 1989). The pharmacological profile of potassium channel openers in skeletal muscle is then most similar to cardiac muscle and least like that of vascular smooth muscle.

Classical sulphonylurea compounds

We find glibenclamide (and tolbutamide) to be much more effective at inhibiting mammalian skeletal muscle K_{ATP} channels than previously demonstrated. For example, Allard & Lazdunski (1993), showed mammalian skeletal muscle K_{ATP} channels in cell free patches to be inhibited by glibenclamide with an apparent K_i of 190 nM and our group found previously (in patches excised from muscle fibres isolated under identical conditions to the present study, Barrett-Jolley & Davies, 1997), an apparent glibenclamide K_i of 60 nM. However, in the experiment described in the present study, we calculated a glibenclamide K_i of ~6 nM (estimated to be equal to $IC_{50}=10\hat{}(-pIC_{50})$). We also found tolbutamide potency to

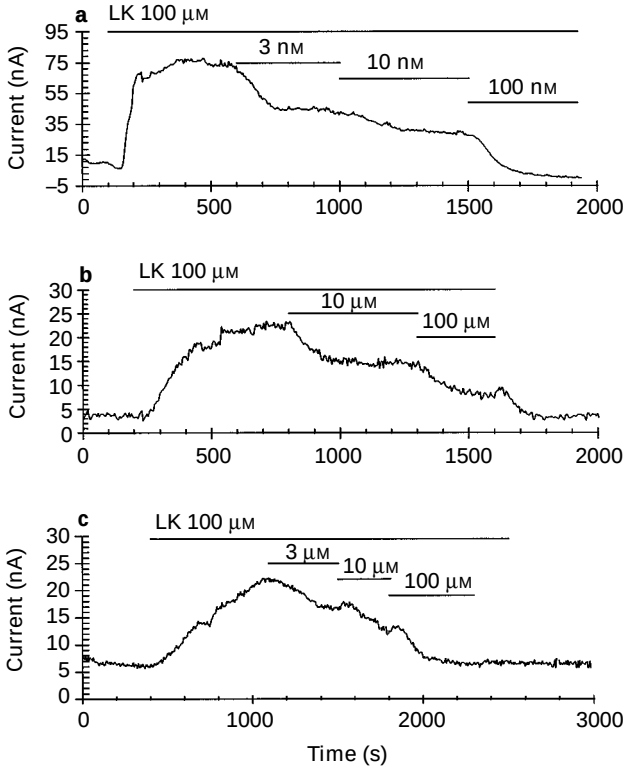

Figure 6 Inhibition of K_{ATP} currents by glibenclamide and its analogues. (a, b and c) Currents recorded at 0 mV. K_{ATP} currents were first activated by levcromakalin (LK; 100 μM) and then inhibited by either glibenclamide or an analogue. Inhibition by (a) glibenclamide, (b) DK13 and (c) DK2 is shown.

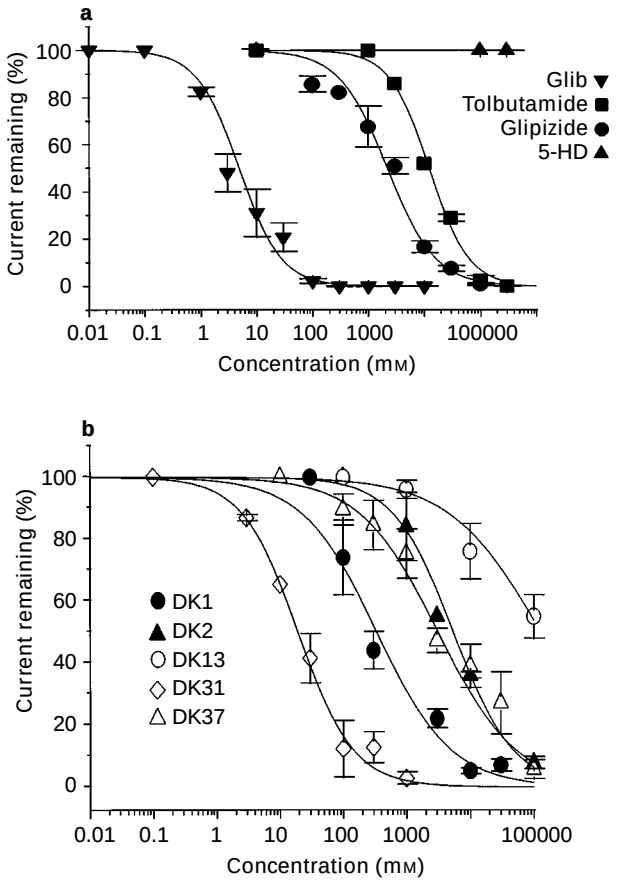

Figure 7 Mean concentration-effect curves for the series of K_{ATP} channel inhibitors. (a) Mean current-effect curves for the classical K_{ATP} channel antagonists; (b) data for the 'DK' series of glibenclamide (Glib) analogues. In each case the data were fitted with equation 1. The best estimate for the curve parameters are given in Table 1.

Table 1 The potency of glibenclamide and analogues at inhibiting levcromakalim activated K_{ATP} currents in rat skeletal smooth muscle cells

Compound	*pIC_{50}*	*Slope (n_H)*	n
Glibenclamide	8.31±0.09	1.16±0.08	31
DK31	7.91±0.06	1.00±0.07	21
DK1	6.15±0.19	0.74±0.08	19
Glipizide	5.60±0.18	1.03±0.05	20
DK2	5.48±0.07	0.93±0.11	13
DK37	5.28±0.14	0.71±0.08	24
Tolbutamide	4.98±0.04	1.24±0.06	12
DK13	3.89±0.21	0.61±0.14	15

be approximately 10× that obtained previously (Woll *et al.*, 1989). Binding experiments have also shown K_{ATP} channels of skeletal muscle to be very sensitive to sulphonylureas (Gopalakrishnan *et al.*, 1991; Dickinson *et al.*, 1997) but the differences between single channel glibenclamide K_is (60–190 nM) and the K_i calculated here (~6 nM) are the most interesting, because the experimental conditions are relatively similar. One of the reasons that glibenclamide may be more active in the intact preparation than in the cell free patch experiments is that sulphonylureas are probably more potent in the presence of intracellular Mg^{2+}-nucleotides, than in their absence. This has been suggested previously for other preparations (e.g. Dickinson *et al.*, 1997; Löffler & Quast, 1997; Gribble *et al.*, 1997), but our present experiments may provide an illustration of the importance of the intracellular milieu. Gribble *et al.*, (1997) suggested that sulphonylureas, when added to oocytes expressing SUR1-K_{ATP} channels, interacted with a very high affinity SUR site to abolish the activating effect of Mg^{2+}-ADP, and thus unmasked inhibition by free ADP. This effect then enhances the inhibition caused by sulphonylureas in their own right. Thus, the presence of intracellular nucleotides may account for, in part, the much greater sensitivity in intact voltage-clamped skeletal muscle fibres than that seen in cell free patches. A comparison of the ability of glibenclamide to block K_{ATP} channels of skeletal muscle with that in other tissues indicates that skeletal muscle sensitivity falls between that displayed in the pancreatic β-cell and that displayed in vascular smooth muscle. It is difficult to compare individual IC_{50}s from functional studies with those of binding experiments. However, in pancreatic β-cells, glibenclamide seems to have a potency in the low namomolar range (Schmid-Antomarchi *et al.*, 1987; Panten *et al.*, 1989), whereas in vascular smooth muscle the potency of glibenclamide is in the order of 100× lower (Buckingham *et al.*, 1989; Wilson, 1989; Challinor & McPherson, 1993; Quayle *et al.*, 1995). Our calculated value of ~6 nM is in fact rather similar to that seen in cardiac muscle (cardiac muscle: IC_{50} 7 nM, (Findlay, 1992; Krause *et al.*, 1995).

Other glibenclamide analogues

Recently, Challinor-Rogers *et al.* (1995) showed, with many of the same compounds used in the present study, that there was a strong correlation between the potency of the glibenclamide analogues at high affinity sulphonylurea receptors in the brain (assessed by calculating their pK_i values against [^{3}H]-glibenclamide binding) and the potency of the same compounds

as levcromakalim antagonists in smooth muscle from the rat aorta. The ranked potency order of the glibenclamide analogues in rat skeletal muscle: glibenclamide > DK31 > DK1 > glipizide > DK2 > DK37 > DK13 is similar to that in the cortex and in vascular smooth muscle (Challinor-Rogers *et al*., 1995). Regression analysis (not shown) of pIC_{50} values obtained in the rat skeletal muscle (present study) against the values (pK_i calculated in the radioligand binding studies in the cerebral cortex ($pK_i = 1.13 \times pIC_{50} + 0.70$, adjusted $r^2 = 0.66$, $P < 0.05$), and pK_b calculated for inhibition of levcromakalim relaxation in vascular smooth muscle ($pK_b = 0.82 \times pIC_{50} + 0.26$, adjusted $r^2 = 0.93$, $P < 0.0005$) shows two interesting features. Firstly, as one might expect, the correlation between rat aorta and skeletal muscle was stronger than that between cerebral cortex and skeletal muscle. Secondly, only two compounds, glipizide and DK13, were not near the brain pK_i v pIC_{50} regression line. Without these, skeletal muscle and cerebral cortex data do correlate reasonably well ($pK_i = 1.37 \times pIC_{50} - 1.22$; adjusted $r^2 = 0.95$, $P < 0.005$, the fit now excluding DK13 and glipizide themselves). Using this equation relating potency at skeletal muscle and cerebral cortex K_{ATP} channels (excluding DK13 and glipizide themselves), we anticipated that the potency of glipizide would have been approximately 40 nM (pIC_{50} 7.4) and DK13 $\sim 5\ \mu$M (pIC_{50} 5.3), but glipizide and DK13 were surprisingly weak in skeletal muscle (IC_{50} $\sim 2\ \mu$M and 125 μM, respectively). It is possible that this low sensitivity of skeletal muscle K_{ATP} channels to glipizide and DK13 reflects the presence of a subtly different subtype of SUR in skeletal muscle, and this may prove useful information in construction of tissue selective K_{ATP} ligands.

5-Hydroxydecanoic acid

Single channel and whole-cell patch clamp studies have shown the antiarrhythmic agent, 5-hydroxydecanoic acid, to be an effective blocker of cardiac myocytes K_{ATP} channels both before and after (simulated) ischaemia (Notsu *et al*., 1992a,b). However, it was completely inactive in our skeletal muscle voltage-clamp experiments and to our knowledge it has not yet been shown to be active in any other tissue. Some studies suggest that 5-hydroxydecanoate activity in the heart is ischaemia selective (e.g. Auchampach *et al*., 1992; Shultz *et al*., 1997), if this were the case, we would not have expected to see block of levcromakalim-activated currents in our experiments. Future experiments will determine if skeletal muscle K_{ATP} channels are indeed sensitive to 5-hydroxydecanoic acid under conditions of ischaemia or metabolic stress.

SUR subtype in skeletal muscle

K_{ATP} channels exist as a complex between an inwardly rectifying potassium channel and a large accessory protein (SUR) which appears to confer most of the channels' pharmacological properties (see Introduction). So far, three forms of SUR have been identified; SUR1, SUR2A and SUR2B. Pancreatic β-cells are rich in SUR1 (Inagaki *et al*., 1995) and muscle (smooth, cardiac and skeletal) seems to express predominantly SUR2 (Inagaki *et al*., 1996). Inagaki *et al*. (1996), found that an isoform of SUR2, now termed SUR2A was present in cardiac and skeletal muscle, whereas Chutkow *et al*. (1996), using *in situ* hybridization, found SUR2A to be expressed exclusively in cardiac tissue. Isomoto *et al*. (1996) and Yamada *et al*. (1997) showed vascular smooth muscle to express SUR2B and the observation by Chutkow *et al*. (1996) that SUR2B was ubiquitous may reflect the presence of at least some vascular smooth muscle in most other whole tissue preparations. All of the SUR subtypes cloned to date have been identified in cerebral/brain tissue (Inagaki *et al*., 1995; 1996; Isomoto *et al*., 1996), this may be because of the heterogeneous nature of most brain preparations.

In terms of the potassium channel opener pharmacology of SUR subtypes, Inagaki *et al*. (1995) showed SUR1-K_{ATP} channels to be activated by diazoxide and Inagaki *et al*. (1996) found SUR2A-K_{ATP} channels to be activated by pinacidil, but not diazoxide. Isomoto *et al*. (1996) and Yamada *et al*. (1997) showed SUR2B-K_{ATP} channels to be activated by both agents (pinacidil and diazoxide), and so the pharmacological profile of potassium channel openers in intact skeletal muscle seems to be most like that of SUR2A-K_{ATP}.

Sulphonylurea sensitivities determined for cloned SUR2A-K_{ATP} channels were somewhat lower than may have been expected for cardiac tissue (Inagaki *et al*., 1996). Differences between the sulphonylurea sensitivities of cloned SUR2A-K_{ATP} channels and native K_{ATP} channels in cardiac myocytes (Inagaki *et al*., 1995 vs Krause *et al*., 1995 and Findlay, 1992) may reflect either differences in the recording conditions (e.g., presence of nucleotides), or the presence of subtypes of SUR2A which have yet to be identified. Our skeletal muscle K_{ATP} channel sulphonylurea K_is (glibenclamide: 6 nM, tolbutamide: 12 μM) are actually more like those determined for cloned SUR1-K_{ATP} channels (glibenclamide: ~ 1 nM, tolbutamide: 2 μM, Inagaki *et al*., 1995 and Gribble *et al*., 1997, respectively).

In conclusion, our two-electrode voltage-clamp system has proven to be a powerful preparation for analysis of K_{ATP} channel properties in whole, intact mammalian skeletal muscle. We have shown that the K_{ATP} channel sulphonylurea sensitivity of intact skeletal muscle fibres is 10 to 30 $\times$ greater than that in isolated patches and furthermore, skeletal muscle K_{ATP} channel pharmacology is (with the possible exception of 5-hydroxydecanoate) most similar to that in cardiac muscle. It remains to be seen whether 5-hydroxydecanoate becomes active in skeletal muscle under ischaemic conditions and whether, when cloned, the skeletal muscle SUR is identical to the SUR2A of cardiac muscle.

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References

ALLARD, B. & LAZDUNSKI, M. (1993). Pharmacological properties of ATP-sensitive K^+ channels in mammalian skeletal muscle. *Eur. J. Pharmacol.*, **236,** 419–426.

ASHCROFT, S.J.H. & ASHCROFT, F.M. (1990). Properties and functions of ATP-sensitive K^+ channels. *Cell. Signalling*, **2,** 197–294.

AUCHAMPACH, J.A., GROVER, G.J. & GROSS, J.A. (1992). Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc. Res.,* **26,** 1054–1062.

BARRETT-JOLLEY, R. & DAVIES, N.W. (1997). Kinetic analysis of the inhibitory effect of glibenclamide on K(ATP) channels of mammalian skeletal muscle. *J. Memb. Biol.,* **155,** 257–262.

BEKOFF, A. & BETZ, W.J. (1977). Physiological properties of dissociated muscle fibres obtained from innervated and denervated adult rat muscle. *J. Physiol.,* **271,** 25–40.

BLACK, J.W. & SHANKLEY, N.P. (1985). The isolated stomach preparation of the mouse: a physiological unit for pharmacological analysis. *Br. J. Pharmacol.,* **86,** 571–579.

BUCKINGHAM, R.E., HAMILTON, T.C., HOWLETT, D.R., MOOTOO, S. & WILSON, C. (1989). Inhibition by glibenclamide of the vasorelaxant action of cromakalim in the rat. *Br. J. Pharmacol.,* **97,** 57–64.

CHALLINOR, J.L. & MCPHERSON, G.A. (1993). Evidence that imidazol(id)ine- and sulphonylurea based antagonists of cromakalim act at different sites in the rat thoracic aorta. *Clin. Exp. Pharmacol. Physiol.,* **20,** 467–475.

CHALLINOR-ROGERS, J.L., KONG, D.C.M., ISKANDER, M.N. & MCPHERSON, G.A. (1995). Structure-activity relationships of glibenclamide analogues: A comparison of potency as levcromakalim antagonists in rat aorta vs affinity for [^{3}H]-glibenclamide binding to membranes from rat cerebral cortex. *J. Pharmacol. Exp. Ther.,* **273,** 778–786.

CHUTKOW, W.A., SIMON, C., BEAU, M. & BURABANT, C.F. (1996). Cloning, tissue expression and chromosomal localisation of SUR2, the putative drug-binding subunit of cardiac skeletal muscle, and vascular K_{ATP} channels. *Diabetes,* **15,** 1439–1445.

DAVIES, N.W., STANDEN, N.B. & STANFIELD, P.R. (1991). ATP-dependent potassium channels of muscle cells: their properties, regulation, and possible functions. *J. Bioenerg. Biomembr.,* **23,** 509–535.

DAWSON, M.J., GADIAN, D.G. & WILKIE, D.R. (1978). Muscular fatigue investigated by phosphorous nuclear magnetic resonance. *Nature,* **274,** 861–866.

DICKINSON, K.E.J., BRYSON, C.C., COHEN, R.B., ROGERS, L., GREEN, D.W. & ATWAL, K.S. (1997). Nucleotide regulation and characteristics of potassium channel opener binding to skeletal muscle membranes. *Mol. Pharmacol.,* **52,** 473–481.

DUNNE, M.J., ASPINALL, R.J. & PETERSEN, O.H. (1990). The effects of cromakalim on ATP-sensitive potassium channels in insulin-secreting cells. *Br. J. Pharmacol.,* **99,** 169–175.

ESCANDE, D., THURINGER, D., LEGUERIN, S. & CAVERO, I. (1988). The potassium channel opener cromakalim (BRL 34915) activates ATP-dependent K+ channels in isolated cardiac myocytes. *Biochem. Biophys. Res. Commun.,* **154,** 620–625.

FAIVRE, J.F. & FINDLAY, I. (1989). Effects of tolbutamide, glibenclamide and diazoxide upon action potentials recorded from rat ventricular muscle. *Biochem. Biophys. Acta,* **984,** 1–5.

FINDLAY, I. (1992). Inhibition of ATP-sensitive K^+ channels in cardiac muscle by the sulphonylurea drug glibenclamide. *J. Pharmacol. Exp. Ther.,* **261,** 540–545.

GOPALAKRISHNAN, M., JOHNSON, D.E., JANIS, R.A. & TRIGGLE, D.J. (1991). Characterization of binding of the ATP-sensitive potassium channel ligand, [^{3}H] glyburide, to neuronal and muscle preparations. *J. Pharmacol. Exp. Ther.,* **257,** 1162–1171.

GRIBBLE, F.M., TUCKER, S.J. & ASHCROFT, F.M. (1997). The interaction of nucleotides with the tolbutamide block of cloned ATP-sensitive K+ channel currents expressed in *Xenopus* oocytes: a reinterpretation. *J. Physiol.,* **504,** 35–45.

HUSSAIN, M., WAREHAM, A.C. & HEAD, S.I. (1994). Mechanism of action of a K+ channel activator BRL-38227 on ATP-sensitive K+ channels in mouse skeletal-muscle fibers. *J. Physiol.,* **478,** 523–532.

INAGAKI, N., GONOI, T., CLEMENT IV, J.P., NAMBA, N., INAZAWA, J., GONZALEZ, G., AGUILAR-BRYAN, L., SEINO, S. & BRYAN, J. (1995). Reconstruction of IK_{ATP}: An inward rectifier subunit plus the sulphonylurea receptor. *Science,* **270,** 1166–1170.

INAGAKI, N., GONOI, T., CLEMENT IV, J.P., WANG, C.-Z., AGUILAR-BRYAN, L., BRYAN, J. & SEINO, S. (1996). A family of sulphonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron,* **16,** 1011–1017.

ISOMOTO, S., KONDO, C., YAMADA, M., MATSUMOTO, S., HIGASHIGUCHI, O., HORIO, Y., MATSUZAWA, Y. & KURACHI, Y. (1996). A novel sulphonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J. Biol. Chem.,* **271,** 24321–24324.

KRAUSE, E., ENGLERT, H. & GÖGELEIN, H. (1995). Adenosine triphosphate dependent K currents activated by metabolic inhibition differ from those elicited by the channel opener rimakalim. *Pflügers Archiv.,* **429,** 625.

LÖFFLER, C. & QUAST, U. (1997). Pharmacological characterization of the sulphonylurea receptor in rat isolated aorta. *Br. J. Pharmacol.,* **120,** 476–480.

MCKILLEN, H.-C. (1993). A sarcolemmal vesicle preparation from rat isolated skeletal muscle suitable for patch-clamp experiments. *J. Physiol.,* **467,** 242P.

MCKILLEN, H.-C., DAVIES, N.W., STANFIELD, P.R. & STANDEN, N.B. (1994). The effect of intracellular anions on ATP-dependent potassium channels of rat skeletal muscle. *J. Physiol.,* **479,** 341–351.

NOTSU, T., OHHASHI, K., TANAKA, I., ISHIKAWA, H., NIHO, T., FUKUTAKE, K. & MIZOTA, M. (1992a). 5-Hydroxydecanoate inhibits ATP-sensitive K+ channel currents in guinea-pig single ventricular myocytes. *Eur. J. Pharmacol.,* **220,** 35–41.

NOTSU, T., TANAKA, I., TAKANO, M. & NOMA, A. (1992b). Blockade of the ATP-sensitive K+ channel by 5-hydroxydecanoate in guinea pig ventricular myocytes. *J. Pharmacol. Exp. Ther.,* **260,** 702–708.

PANTEN, U., BURGFIELD, J., GOERKE, F., RENNICKE, M., SCHWANSTECHER, M., WALLASCH, A., ZÜNKLER, B.J. & LENZEN, S. (1989). Control of insulin secretion by sulphonylureas, meglitnide and diazoxide in relation to their binding to the sulphonylurea receptor in pancreatic islets. *Biochem. Pharmacol.,* **38,** 1217–1229.

QUAST, U. & COOK, N.S. (1988). In vitro and in vivo comparison of two K+ channel openers, diazoxide and cromakalim, and their inhibition by glibenclamide. *J. Pharmacol. Exp. Ther.,* **250,** 261–271.

QUAYLE, J.M., BONEV, A.D., BRAYDEN, J.E. & NELSON, M.T. (1995). Pharmacology of ATP-sensitive K^+ currents in smooth muscle cells from rabbit mesenteric arteries. *Am. J. Physiol.,* **269,** C1112–C1118.

QUAYLE, J.M., NELSON, M.T. & STANDEN, N.B. (1997). ATP sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol. Rev.,* **77,** 1165–1232.

RUSSELL, S.N., SMIRNOV, S.V. & AARONSON, P.I. (1992). Effects of BRL38277 on potassium currents in smooth muscle cells isolated from rabbit portal vein and human mesenteric artery. *Br. J. Pharmacol.,* **105,** 549–556.

SCHMID-ANTOMARCHI, H., DE WEILLE, J., FOSSET, M. & LAZDUNSKI, M. (1987). The antidiabetic sulfonylurea glibenclamide is a potent blocker of the ATP-modulated K^+ channel in insulin secreting cells. *Biochem. Biophys. Res. Commun.,* **146,** 21–25.

SCHULTZ, J.E., QIAN, Y.Z., GROSS, G.J. & KUKREJA, R.C. (1997). The ischaemia-selective K_{ATP} channel antagonist, 5HD, blocks ischaemic preconditioning in the rat heart. *J. Mol. Cell. Cardio.,* **29,** 1055–1060.

TUCKER, S.J., GRIBBLE, F.M., ZHAO, C., TRAPP, S. & ASHCROFT, F.M. (1997). Truncation of Kir6.2 produces ATP-sensitive K+ channels in the absence of the sulphonylurea receptor. *Nature,* **387,** 179–183.

WEIK, R. & NEUMCKE, B. (1990). Effects of potassium channel openers on single potassium channels in mouse skeletal-muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **342,** 258–263.

WILSON, C. (1989). Inhibition by sulphonylureas of vasorelaxation induced by K^+ channel activators *in vitro*. *J. Auton. Pharmacol.,* **9,** 71–78.

WOLL, K.H., LONNENDONKER, U. & NEUMCKE, B. (1989). ATP-sensitive potassium channels in adult-mouse skeletal-muscle – different modes of blockage by internal cations, ATP and tolbutamide. *Pflügers Archiv.,* **414,** 622–628.

YAMADA, M., ISOMOTO, S., MATSUMOTO, S., KONDO, C., SHINDO, T., HORIO, Y. & KURACHI, Y. (1997). Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea sensitive but ATP-insensitive K^+ channel. *J. Physiol.* **499**(3), 715–720.

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