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Inhibition of vascular K_{ATP} channels by U-37883A: a comparison with cardiac and skeletal muscle

[2]G.C. Wellman, [2]R. Barrett-Jolley, [2]H. Köppel, [2]D. Everitt & *,[1]J.M. Quayle

[1]Department of Medicine, Cardiovascular Research Institute, Leicester University, Robert Kilpatrick Clinical Sciences Building, Leicester Royal Infirmary, P.O. Box 65, Leicester, LE2 7LX and [2]Department of Cell Physiology and Pharmacology, Leicester University, University Road, Leicester LE1 9HN

1 The aim of this study was to investigate the selectivity of the ATP-sensitive potassium (K_{ATP}) channel inhibitor U-37883A (4-morpholinecarboximidine-*N*-1-adamantyl-*N*′-1-cyclohexyl). Membrane currents through K_{ATP} channels were recorded in single muscle cells enzymatically isolated from rat mesenteric artery, cardiac ventricle and skeletal muscle (flexor digitorum brevis). K_{ATP} currents were induced either by cell dialysis with 0.1 mM ATP and 0.1 mM ADP, or by application of synthetic potassium channel openers (levcromakalim or pinacidil).

2 U-37883A inhibited K_{ATP} currents in smooth muscle cells from rat mesenteric artery. Half inhibition of 10 μM levcromakalim-induced currents occurred at a concentration of 3.5 μM.

3 Relaxations of rat mesenteric vessels caused by levcromakalim were reversed by U-37883A. 1 μM levcromakalim-induced relaxations were inhibited at a similar concentration of U-37883A (half inhibition, 1.1 μM) to levcromakalim-induced K_{ATP} currents.

4 K_{ATP} currents activated by 100 μM pinacidil were also studied in single myocytes from rat mesenteric artery, skeletal muscle and cardiac ventricle. 10 μM U-37883A substantially inhibited K_{ATP} currents in vascular cells, but had little effect in skeletal or cardiac myocytes. Higher concentrations of U-37883A (100 μM) caused a modest decrease in K_{ATP} currents in skeletal and cardiac muscle. The sulphonylurea K_{ATP} channel antagonist glibenclamide (10 μM) abolished currents in all muscle types.

5 The effect of U-37883A on vascular inward rectifier (K_{IR}) and voltage-dependent potassium (K_V) currents was also examined. While 10 μM U-37883A had little effect on these currents, some inhibition was apparent at higher concentrations (100 μM) of the compound.

6 We conclude that U-37883A inhibits K_{ATP} channels in arterial smooth muscle more effectively than in cardiac and skeletal muscle. Furthermore, this compound is selective for K_{ATP} channels over K_V and K_{IR} channels in smooth muscle cells.

Keywords: K_{ATP} channel; potassium channel; U-37883A; U37883A; PNU-37883A; smooth muscle; potassium channel opener

Abbreviation: U-37883A, 4-morpholinecarboximidine-*N*-1-adamantyl-*N*′-1-cyclohexyl

Introduction

ATP-sensitive potassium (K_{ATP}) channels serve various important functions in pancreatic β-cells, muscle cells, neurones, and other cell types (Aguilar-Bryan *et al*., 1998; Ashcroft & Gribble, 1998). K_{ATP} channels are inhibited by intracellular ATP and activated by intracellular nucleoside diphosphates, enabling them to link cellular metabolism to excitability. This underlies the role of K_{ATP} channels in processes such as insulin secretion, hypoxic vasodilation of arterial smooth muscle, and protection of cardiac muscle and neurones during hypoxia and ischaemia. K_{ATP} channels are also the target of synthetic inhibitors and activators in clinical use. Anti-diabetic sulphonylureas stimulate insulin secretion by inhibiting K_{ATP} channels in pancreatic β-cells, while K_{ATP} channel activators which relax smooth muscle are used in hypertensive crises and angina (Quayle *et al*., 1997).

K_{ATP} channels are important regulators of the cell membrane potential of arterial smooth muscle cells. Channel activity is set by several factors, including the cellular metabolic state as well as vasoconstrictor and vasodilator receptor-linked second messenger pathways (Quayle *et al*., 1997). As in other cells, K_{ATP} channels in smooth muscle are inhibited by glibenclamide and other sulphonylureas (Noack *et al*., 1992; Russell *et al*., 1992; Beech *et al*., 1993; Quayle *et al*., 1995). Glibenclamide has been widely used to study the physiological role of these channels in arteries (Quayle *et al*., 1997). Antagonism of vasorelaxation by glibenclamide has been used to implicate K_{ATP} channel activation as an underlying cause of the response. However: (1) the selectivity of sulphonylureas for K_{ATP} channels is not absolute, as they inhibit other cellular processes (e.g. Cocks *et al*., 1990; Ribalet *et al*., 1996); (2) the sulphonylurea receptor may couple to other ion channels (e.g. Beech *et al*., 1993; Ämmälä *et al*., 1996; Quayle *et al*., 1997); (3) the presence of K_{ATP} channels in the vascular endothelium, cardiac muscle, skeletal muscle and other cells complicates interpretation of studies on intact tissue. There is therefore a need for other, tissue-selective, K_{ATP} channel inhibitors.

One candidate for a smooth muscle selective K_{ATP} channel inhibitor is U-37883A (also known as PNU-37883A). This compound antagonizes the relaxations of rabbit mesenteric arteries caused by synthetic K_{ATP} channel openers such as levcromakalim (Meisheri *et al*., 1993). Indeed, U-37883A, like glibenclamide, has been used to implicate K_{ATP} channel involvement in a number of vascular responses *in vivo* and *in vitro* (e.g. Meisheri *et al*., 1993; Ohrnberger *et al*., 1993; Smith *et*

* Author for correspondence; E-mail: JMQ2@le.ac.uk

al., 1994; Minkes *et al*., 1995; de Witt *et al*., 1996). There is some evidence that U-37883A may show tissue selectivity, as it inhibits K_{ATP} channels in follicular cells of *Xenopus* oocytes but not in pancreatic β-cells (Guillemare *et al*., 1994a). However, there is no direct evidence that U-37883A is a selective inhibitor of vascular K_{ATP} channels. We describe here the inhibitory actions of U-37883A on K_{ATP} currents in single smooth muscle cells isolated from rat mesenteric artery. This is compared with the antagonistic actions of the compound on relaxations caused by a K_{ATP} channel opener (levcromakalim) in intact vessels. Tissue selectivity was investigated by testing the effects of U-37883A on K_{ATP} currents in cardiac and skeletal muscle cells from rat. Furthermore, selectivity of U-37883A for K_{ATP} channels was studied by recording currents through other potassium channels present in arterial smooth muscle cells. We conclude that U-37883A shows selectivity for K_{ATP} channels in vascular smooth muscle.

Methods

The experiments in this paper used tissue from male adult Wistar rats. Rats were stunned prior to being killed by cervical dislocation.

Tension recordings

Rat mesenteric arteries were dissected in a physiological saline solution containing (mM): NaCl 137, KCl 5.4, $MgCl_2$ 1, NaH_2PO_4 0.44, Na_2HPO_4 0.46, $NaHCO_3$ 4.17, $CaCl_2$ 2, HEPES 10, glucose 10, pH adjusted to 7.4 with NaOH. Isometric tension was recorded in a small vessel myograph at 37°C, as described previously (Langton *et al*., 1996). The bath solution consisted of (mM): NaCl 137, KCl 5.4, $MgCl_2$ 1, NaH_2PO_4 0.44, Na_2HPO_4 0.46, $NaHCO_3$ 25, $CaCl_2$ 2, glucose 10, and was equilibrated with gas containing 5% CO_2 and 95% O_2 to maintain the pH at 7.4. Arteries were contracted by increasing the extracellular potassium concentration to 20 mM and by adding 2 μM phenylephrine. U-37883A had no effect on this induced tone in the absence of levcromakalim.

Isolation of smooth muscle cells

Single cells were isolated from rat mesenteric or, in the case of inward rectifier potassium currents, coronary arteries using an enzymatic digestion procedure described previously (e.g. Kubo *et al*., 1997). After dissection, the arteries were transferred to an isolation solution of identical composition to the dissection solution detailed above except that the calcium concentration was reduced to 0.1 mM. For dissociation, a two stage enzymatic treatment was used. First, the arteries were incubated in 0.1 mM Ca^{2+} isolation solution containing papain (Sigma, 1–1.5 mg ml^{-1}) and dithioerithritol (Sigma, 1 mg ml^{-1}) for 25–35 mins at 35°C. This was followed by an incubation in fresh 0.1 mM Ca^{2+} isolation solution containing collagenase (Sigma, Type F, 1–1.5 mg ml^{-1}) and hyaluronidase (Sigma, Type I-S, 1 mg ml^{-1}) for 10–20 mins at 35°C. Arteries were then transferred to enzyme-free isolation solution, washed several times, and single cells obtained by trituration through a fire polished pasteur pipette. Cells were stored in isolation solution on ice and used on the day of preparation.

Isolation of cardiac myocytes

Male adult Wistar rats were heparinized (1000 u kg^{-1}), stunned, and killed by cervical dislocation. The chest was then opened and the aorta incised and cannulated *in situ* with an 18 gauge needle. The heart was excised, mounted on a Langendorff column, and perfused for 4 min at a rate of 14 mls min^{-1} at 37°C with a solution containing (mM): NaCl 134, NaH_2PO_4 1.2, $MgSO_4$ 1.2, KCl 4, HEPES 10, glucose 10, pH 7.4. After this time the perfusion solution was switched to one containing 0.8 mg ml^{-1} collagenase (Worthington, Type II) and 0.1 mg ml^{-1} protease (Sigma). The calcium level at this point was raised to 80 μM to enhance collagenase activity. The heart was perfused with enzyme containing solution for 8–10 min, and then for a further 10 min with a solution containing (mM): NaCl 108, NaH_2PO_4 1.2, $MgSO_4$ 1.2, KCl 4, HEPES 10, glucose 10, taurine 50, pH 7.4. Thereafter, the heart was removed from the cannula, the atria trimmed away, and the ventricles chopped with scissors. Cells were released by trituration through a pasteur pipette and stored at room temperature.

Isolation of skeletal muscle cells

Skeletal muscle cells were isolated from the flexor digitorum brevis muscle of rat, as previously described (McKillen *et al*., 1994). Muscles were dissected and then incubated in 3 mg ml^{-1} collagenase (Sigma, Type 1) dissolved in saline containing (mM): NaCl 146.3, KCl 4.75, $CaCl_2$ 1, Ca_2HPO_4 0.95, $MgCl_2$ 0.5, HEPES 9.5, pH adjusted to 7.4 with NaOH. Muscles were incubated for 30 min at 4°C, followed by 90 min at 37°C. Single cells were isolated in fresh Ca^{2+} free saline by trituration through a fire polished pasteur pipette.

Electrophysiology of smooth and cardiac muscle cells

Whole cell K_{ATP} currents were recorded from single vascular and cardiac myocytes using the patch clamp technique (Hammill *et al*., 1981). The pipette (intracellular) solution contained (mM): KCl 107, KOH 33, EGTA 10, $MgCl_2$ 1, HEPES 10, pH 7.2. Na_2ATP (0.1 mM) and NaADP (0.1 mM) were added to this pipette solution on the day of the experiment, and the pH readjusted to 7.2 with KOH. Seals were formed, and whole cell recordings established, in an extracellular solution containing (mM): NaCl 134, KCl 6, $MgCl_2$ 1, $CaCl_2$ 0.1, HEPES 10, glucose 10, pH adjusted to 7.4 with NaOH. In some experiments after obtaining the whole cell configuration the extracellular solution was changed to one containing (mM): KCl 140, $MgCl_2$ 1, $CaCl_2$ 0.1, HEPES 10, glucose 10, pH adjusted to 7.4 with NaOH. Current recordings were filtered at 2 or 5 kHz.

Electrophysiology of skeletal muscle cells

Membrane currents were recorded by two microelectrode voltage clamp of single skeletal muscle fibres using an NPI Turbo-TEC 10C amplifier, filtered at 1 kHz, and recorded directly onto a computer at 3 kHz, as previously described (Barrett-Jolley & McPherson, 1998). The extracellular solution used to record currents contained (mM): Na_2SO_4 77, Na_2HPO_4 1, K gluconate 40, $MgSO_4$ 1.2, $CaSO_4$ 8, HEPES 10, glucose 10, pH adjusted to 7.4 with NaOH. A low chloride solution was used in order to minimise the chloride conductance, which is the dominant resting conductance in these cells.

Data analysis

Currents were analysed with pCLAMP6 (Axon Instruments, Foster City, CA, U.S.A.) or custom written software. Due to the slow time course of action of U-37883A (Figures 2 and 3), only one or two concentrations of the compound were generally tested on any single cell. Steady state inhibition was

measured at the end of each period of U-37883A application. Glibenclamide was added at the end of the experiment and the extent of inhibition was calculated as the fraction of the 10 μM glibenclamide-sensitive current blocked by U-37883A. The 10 μM glibenclamide-sensitive current is equivalent to the K_{ATP} current in these cells (Quayle *et al*., 1995; Kubo *et al*., 1997; Barrett-Jolley & McPherson, 1998). Concentration-effect data were analysed and fitted using a non-linear curve fitting algorithm in Jandel SigmaPlot to estimate the half inhibition constant (K_i) using Equation 1:

$$\frac{I_{U37883A}}{I_{CON}} = \frac{1}{1 + \left(\frac{[U37883A]}{K_i}\right)^n} \quad (1)$$

where I_{CON} is the 10 μM glibenclamide-sensitive current in the absence of U-37883A, $I_{U37883A}$ is the 10 μM glibenclamide-sensitive current in the presence of U-37883A, [U37883A] is the U-37883A concentration, and n is the slope factor.

Solutions, chemicals, drugs

Experiments were conducted at room temperature, and compounds were applied to the cell by perfusing the experimental chamber. Chemicals and drugs were obtained from Sigma, unless otherwise stated. Pinacidil was obtained from RBI. Levcromakalim was provided by Smith Kline Beecham, and U-37883A by Pharmacia and Upjohn Company. Levcromakalim and pinacidil were prepared as 10 or 100 mM stock solutions in DMSO, and U-37883A was prepared as a 10 mM stock solution in water.

Results

U-37883A inhibits levcromakalim-induced relaxations of rat mesenteric artery

Levcromakalim relaxes mesenteric arteries by activating K_{ATP} channels (e.g. Ohrnberger *et al*., 1993; Quayle *et al*., 1997). U-37883A is a putative K_{ATP} channel blocker, and, consistent with this, it reversed 1 μM levcromakalim-induced relaxations of rat mesenteric arteries mounted in a myograph (Figure 1a). U-37883A caused 50% reversal (the IC_{50}) at a concentration around 1 μM (Figure 1b). These experiments suggest that U-37883A inhibits K_{ATP} channels activated by levcromakalim, so antagonizing relaxations.

K_{ATP} currents in mesenteric artery myocytes

K_{ATP} currents were recorded from single smooth muscle cells isolated from rat mesenteric artery (Kubo *et al*., 1997). A holding potential of -60 mV was used, and the cell was dialyzed with a pipette solution including 0.1 mM ATP and 0.1 mM ADP and 10 mM EGTA. These conditions facilitate recording of K_{ATP} currents and minimize currents through voltage-dependent potassium channels and calcium-activated potassium channels. As illustrated in Figure 2, increasing the extracellular $[K^+]$ from 6–140 mM, and therefore the driving force on K^+ from an outward to an inward direction, led to development of an inward current. In a sample of 17 cells, membrane current increased from -12.5 ± 2.5 pA in 6 mM $[K^+]_o$, to -82.6 ± 17.4 pA in 140 mM $[K^+]_o$. Exposure of the cell to 10 μM levcromakalim further enhanced current to -216.3 ± 23.5 pA. Both resting and levcromakalim-induced currents are primarily through K_{ATP} channels in these cells (e.g. Russell *et al*., 1992; Beech *et al*., 1993; Quayle *et al*., 1995; Kubo *et al*., 1997). For instance, membrane current fell to -29.5 ± 4.9 pA in the presence of 10 μM glibenclamide. Furthermore, previous studies have shown that these currents show little voltage-dependence, and are blocked by increasing the intracellular ATP concentration (Russell *et al*., 1992; Beech *et al*., 1993; Quayle *et al*., 1995; Kubo *et al*., 1997).

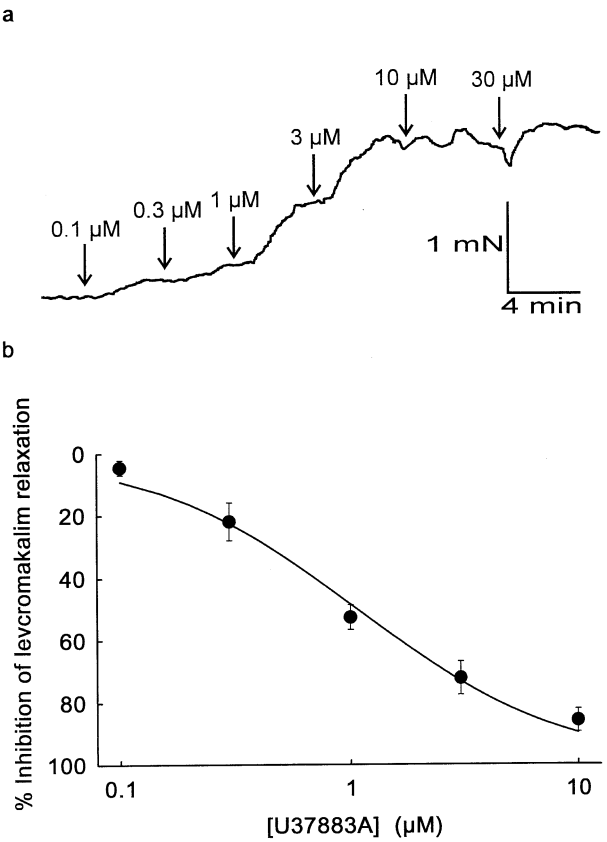

Figure 1 U-37883A antagonizes levcromakalim-induced relaxations of rat mesenteric artery. (a) Reversal of a dilation to 1 μM levcromakalim by U-37883A at the concentrations indicated. (b) Concentration-inhibition curve for U-37883A, obtained from experiments similar to that in (a). Data are the mean $\pm$ s.e.mean ($n = 6$ arteries). The line was fitted to the data according to equation 1 in the text and with a half inhibition constant of 1.1 μM and a coefficient of 0.97.

U-37883A inhibits levcromakalim-induced K_{ATP} currents in rat mesenteric artery

Initial patch clamp experiments with U-37883A were designed to investigate whether the observed inhibition of levcromakalim-induced relaxations by this compound could be explained by inhibition of K_{ATP} currents. As illustrated in Figure 2a, U-37883A inhibited K_{ATP} currents activated by levcromaklim in rat mesenteric artery myocytes. In six cells exposed to 10 μM levcromakalim, 10 μM U-37883A reduced glibenclamide-sensitive currents from -233.0 ± 43.1 to -59.2 ± 17.9 pA.

We next sought to examine the concentration-dependence of action of U-37883A. Due to the relatively slow time course of action of the compound (Figure 2a), only one or two concentrations were tested on any individual cell. The fractional inhibition of 10 μM levcromakalim-induced K_{ATP} current by U-37883A (0.1–10 μM) is summarized in Figure 2b. The half inhibition constant for U-37883A (3.5 μM) is comparable to that determined from reversal of levcromakalim relaxations (1.1 μM, Figure 1b).

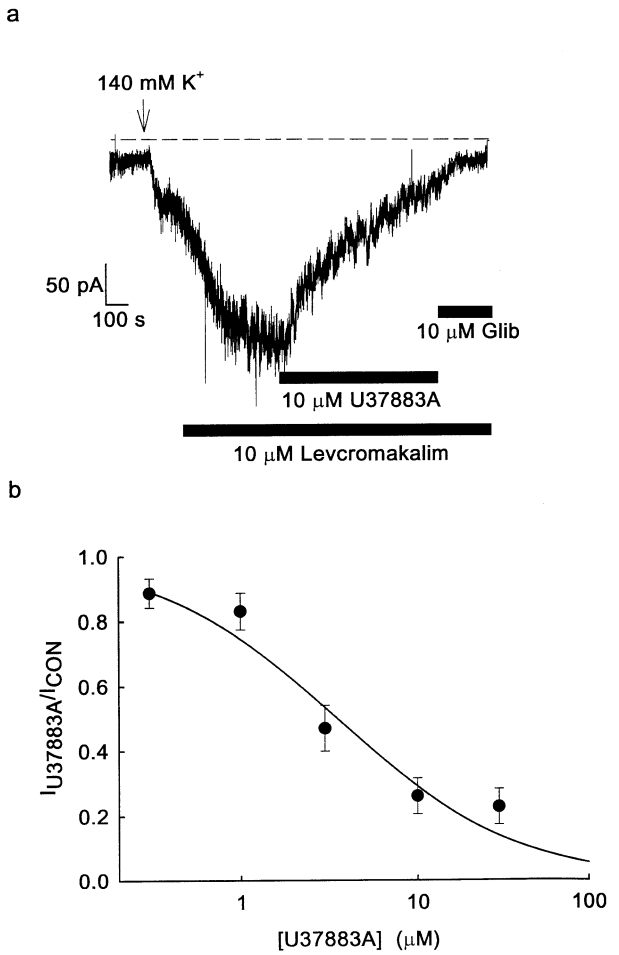

Figure 2 U-37883A inhibits K_{ATP} currents in smooth muscle cells. (a) Recording of K_{ATP} current in a myocyte isolated from rat mesenteric artery. The membrane potential was −60 mV. The extracellular solution was changed from one containing 6 mM K^+ to a 140 mM K^+ solution at the arrow. The pipette solution contained 140 mM K^+ and 0.1 mM ATP and ADP. Levcromakalim, U-37883A and glibenclamide were present in the extracellular solution where indicated. The dashed line indicates the zero current level. (b) Concentration-effect curve for U-37883A inhibition of 10 μM levcromakalim-induced K_{ATP} currents. The line is fitted to equation 1 of the text with a K_i of 3.52 μM and a coefficient of 0.85. Data are the mean of 3–7 observations and are plotted ± s.e.mean.

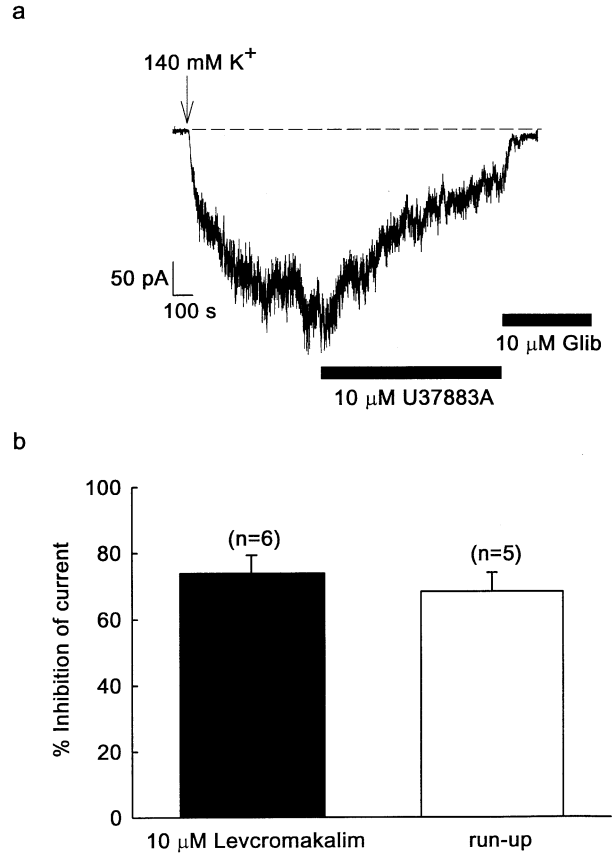

Figure 3 Inhibition of K_{ATP} currents by U-37883A in the absence of levcromakalim. (a) Effect of 10 μM U-37883A on the K_{ATP} current in a mesenteric artery cell induced by dialysis with a pipette solution containing 0.1 mM ATP and 0.1 mM ADP. The holding potential was −60 mV. The dashed line indicates the zero current level. (b) Mean inhibition caused by 10 μM U-37883A in the absence and presence of 10 μM levcromakalim.

U-37883A inhibits K_{ATP} currents induced by cell dialysis with solutions containing 0.1 mM ATP and ADP

The functional and electrophysiological data described above are consistent with inhibition of levcromakalim-induced K_{ATP} currents by U-37883A. Previous studies have provided evidence that U-37883A acts at a separate site from levcromakalim, for instance it is not a competitive antagonist of levcromakalim binding (Meisheri *et al.*, 1995). However, it is not known whether U-37883A inhibits K_{ATP} currents only after they have been activated by synthetic K^+ channel openers. To address this issue, we have examined the effects of U-37883A on basal K_{ATP} currents induced by cell dialysis with a pipette solution containing low (0.1 mM) levels of ATP and ADP (Figure 3a). U-37883A (10 μM) inhibited these basal glibenclamide-sensitive K_{ATP} currents from −99.0 ± 38.6 pA to −28.4 ± 11.3 pA ($n=5$ cells). The mean percentage inhibition of basal K_{ATP} current was not significantly different to that seen in the presence of 10 μM levcromakalim (68.4 ± 5.7% vs 73.9 ± 5.5%, respectively, $P=0.51$, Figure 3b).

Effect of U-37883A on K_{ATP} currents in cardiac and skeletal muscle

It has been suggested that U-37883A may inhibit K_{ATP} channels in vascular smooth muscle cells more effectively than in pancreatic β-cells (Guillemare *et al.*, 1994a). We have investigated the tissue selectivity of U-37883A directly by comparing the actions of this compound on K_{ATP} currents recorded from rat vascular, skeletal and cardiac myocytes. In this series of experiments, currents were activated by the K_{ATP} channel opener pinacidil (Quayle *et al.*, 1995). A concentration of 100 μM was used as K_{ATP} channels are less sensitive to openers in cardiac and skeletal muscle cells than in the vasculature (Quayle *et al.*, 1997; Barrett-Jolley & McPherson, 1998). The extracellular potassium concentration was 6 mM for experiments on cardiac and smooth muscle, and 40 mM for those on skeletal muscle. The holding potential was 0 mV in all cases, and currents are therefore in the outward direction. K_{ATP} currents in cardiac and skeletal muscle cells were less sensitive to inhibition by U-37883A than in arterial smooth muscle (Figure 4). For example, 10 μM inhibited the glibenclamide-sensitive current activated by 100 μM pinacidil by 89.8 ± 1.8% ($n=6$) in vascular myocytes. In contrast, this concentration of U-37883A caused little change in cardiac muscle or skeletal muscle myocytes. The percentage inhibition of K_{ATP} current by 10 μM U-37883A in cardiac, skeletal and vascular smooth muscle cells is shown in Figure 5. A higher

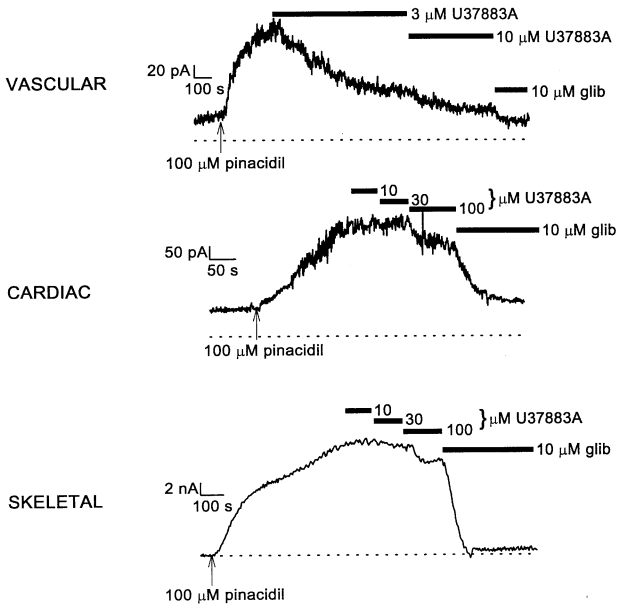

Figure 4 Inhibition of K_{ATP} channels in vascular, cardiac and skeletal muscle cells by U-37883A. The holding potential was 0 mV and the $[K^+]_o$ was 6 mM for vascular and cardiac cells, and 40 mM for skeletal muscle cells. Vascular and cardiac K_{ATP} currents were recorded by whole cell patch clamp, and cells were dialyzed with a pipette solution containing 100 μM ATP and 100 μM ADP. Currents in skeletal muscle cells were recorded by two microelectrode voltage clamp, and cells were therefore undialyzed. In all cases currents were induced by 100 μM pinacidil, and U-37883A added at the concentrations indicated in the figure. The dashed lines indicates the zero current levels.

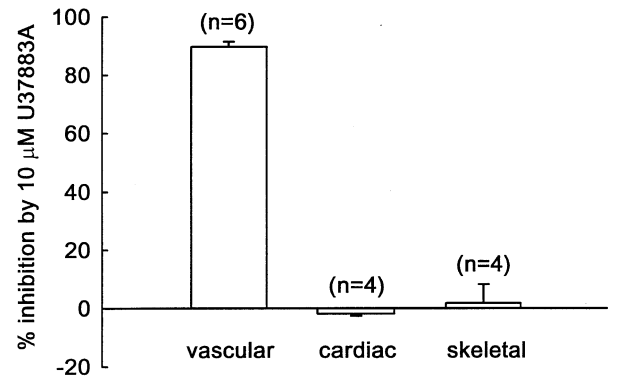

Figure 5 Selectivity of inhibition of vascular K_{ATP} channels by U-37883A. The figure shows the mean inhibition of 100 μM pinacidil-induced current by 10 μM U-37883A in experiments such as those illustrated in Figure 4. Data are the mean of six cells for vascular, and four cells for cardiac and skeletal muscle.

concentration of U-37883A (100 μM) produced modest inhibition of K_{ATP} current in cardiac muscle (38.4%, $n=2$) and skeletal muscle ($25.8\pm2.8\%$, $n=4$).

Effect of U-37883A on voltage-activated and inward rectifier potassium channels in arterial smooth muscle cells.

We also tested whether U-37883A inhibited voltage-activated potassium channels or inward rectifier potassium channels in smooth muscle. Whole cell K_V currents were recorded in myocytes from rat mesenteric artery in a physiological potassium gradient (6 mM $[K^+]_o$, 140 mM $[K^+]_i$) and with 10 μM glibenclamide in the extracellular solution to inhibit K_{ATP} channels. The holding potential was -70 mV and depolarizing test steps were applied between -65 and $+60$ mV in 5 mV increments. Figure 6 illustrates that 10 μM U-37883A had little effect on K_V currents, although some inhibition was apparent at higher concentrations of the compound. The peak whole cell currents for this cell in the presence or absence of U-37883A are plotted against voltage in Figure 6b. The mean percentage inhibition of K_V current by 10, 30 and 100 μM U-37883A at a test potential of $+40$ mV is shown in Figure 6c. The peak current induced by a depolarizing voltage step from -70 mV to $+40$ mV was 316.0 ± 51.1 pA in control conditions, and 292.1 ± 46.1 pA in the presence of 10 μM U-37883A ($n=4$ cells).

Inward rectifier potassium channels are present at low density in large mesenteric arteries in the rat (see Figures 2 and 3 and Kubo *et al.*, 1997; Quayle *et al.*, 1997). Recordings of K_{IR} current were therefore made in cells from the septal coronary artery (Quayle *et al.*, 1996; Robertson *et al.*, 1996). Currents were recorded at -60 mV in symmetrical 140 mM K^+ containing solutions, and in the presence of 10 μM glibenclamide to inhibit K_{ATP} currents (Quayle *et al.*, 1996). As with vascular K_V currents, a modest decrease in K_{IR} currents occurred at higher concentrations of U-37883A. In four cells the 50 μM barium-sensitive current at -60 mV, which is equivalent to the K_{IR} current (Quayle *et al.*, 1996; Robertson *et al.*, 1996), changed from -146.7 ± 36.9 pA in control, to -143.0 ± 42.5 pA, -121.0 ± 48.7 pA, and -106.7 ± 45.9 pA in the presence of 10, 30 and 100 μM U-37883A, respectively. The fraction of K_{IR} current remaining in the presence of compound was 1.006 ± 0.008 ($n=8$), 0.77 ± 0.008 ($n=6$), and 0.63 ± 0.013 ($n=4$), with 10, 30 and 100 μM U-37883A.

Discussion

This paper shows that the compound U-37883A is a selective inhibitor of vascular K_{ATP} channels. Whole cell K_{ATP} currents in smooth muscle cells from rat mesenteric artery were inhibited by U-37883A, with a K_i of 3.5 μM. In contrast, U-37883A caused little inhibition of K_{ATP} currents in skeletal and cardiac myocytes at a concentration of 10 μM. U-37883A did inhibit K_V and K_{IR} currents in smooth muscle cells to some extent, although this was only apparent at higher concentrations of the compound (100 μM). Finally, U-37883A appears well suited to study the functional effects of K_{ATP} channels in the vasculature, as it reversed levcromakalim-induced relaxations at concentrations that selectively inhibit vascular K_{ATP} channels.

Selectivity of U-37883A for vascular K_{ATP} channels

U-37883A is a more potent inhibitor of K_{ATP} channels in the vasculature than in skeletal and cardiac muscle (Figures 4 and 5). U-37883A also has little effect on K_{ATP} currents in the insulinoma-derived RINm5F cell line (Guillemare *et al.*, 1994a). However, the compound does block some non-vascular K_{ATP} channels. Follicular cells surrounding *Xenopus* oocytes possess K_{ATP} channels which are inhibited by U-37883A with a K_i of 0.26 μM (Guillemare *et al.*, 1994a). K_{ATP} channels in these follicular cells have other similarities to vascular channels (Guillemare *et al.*, 1994b). For instance, K_{ATP} channels in both cell types share similar sensitivities to synthetic potassium channel openers, and both are activated by receptors positively coupled to cyclic AMP-dependent

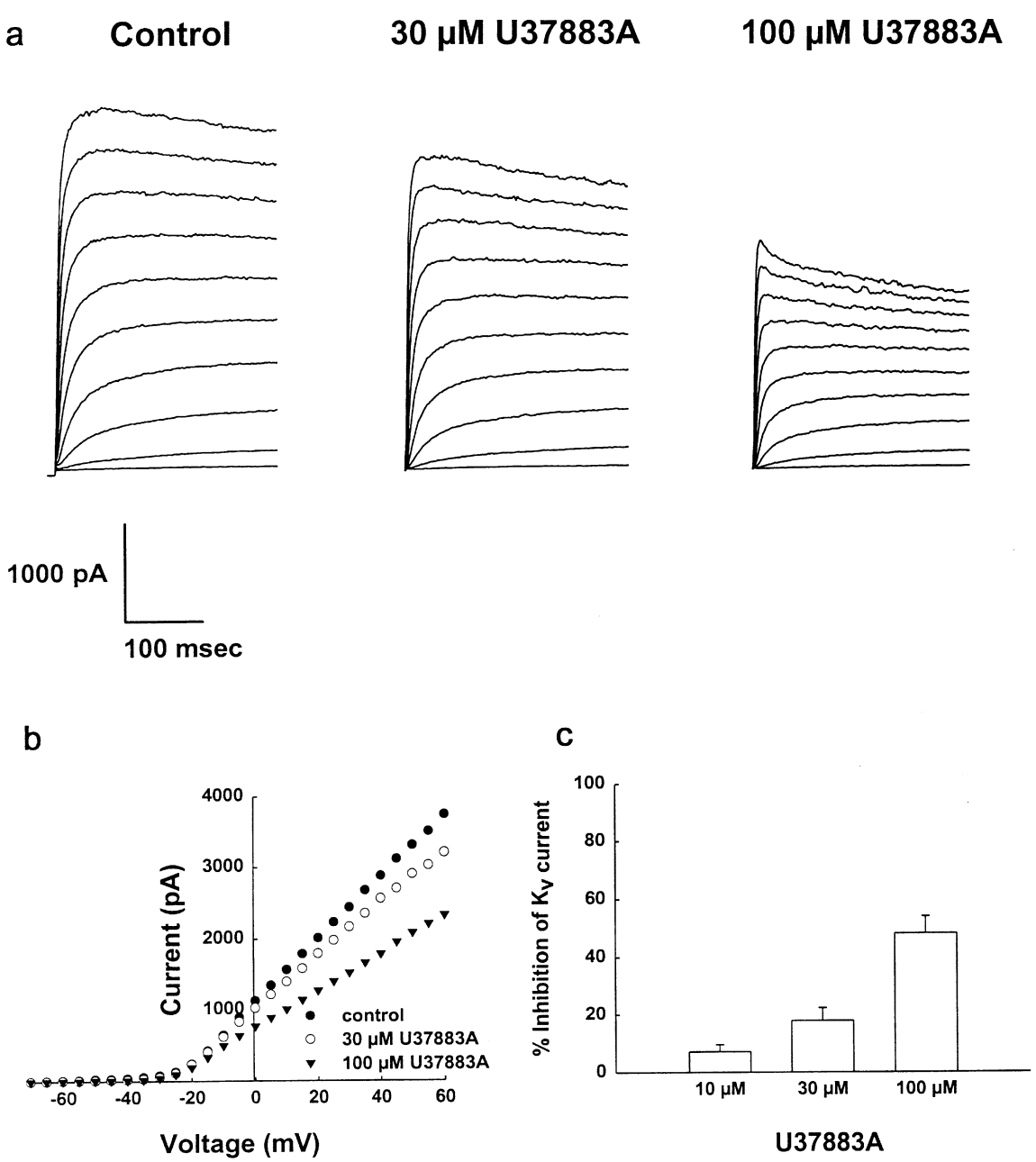

Figure 6 Inhibition of K_V currents in rat mesenteric artery by U-37883A. (a) A family of whole cell currents recorded in response to voltage steps from a holding potential of −70 mV to a test potential of between −30 and +60 mV, in 10 mV increments. Records are shown in the absence (control) and in the presence of 30 and 100 μM U-37883A. (b) Mean peak current-voltage relationships for the cell illustrated. (c) Percentage inhibition of peak K_V current recorded in response to a step from −70 mV to +40 mV in the presence of 10, 30 and 100 μM U-37883A for four cells.

protein kinase (Guillemare *et al*., 1994b; Wellman *et al*., 1998). K_{ATP} channels in kidney tubules are also inhibited by U-37883A, although half inhibition occurs at the higher concentration of around 50 μM (Wang *et al*., 1995).

Use of U-37883A in studies on intact tissue

U-37883A causes half inhibition of levcromakalim-induced relaxations of rat mesenteric arteries at around 1 μM (Figure 1). Meisheri and colleagues (1995) have previously shown that U-37883A inhibits the relaxations to several K_{ATP} channel openers in rabbit mesenteric artery at concentrations between 0.78 and 1.4 μM. Our data showing half inhibition of K_{ATP} currents in isolated vascular myocytes at around 3 μM suggests that U-37883A is acting as a K_{ATP} channel inhibitor in these intact tissue experiments, and supports the previous use of this compound as a K_{ATP} channel inhibitor in functional studies (Meisheri *et al*., 1993; Ohrnberger *et al*., 1993; Smith *et al*., 1994; Minkes *et al*., 1995; de Witt *et al*., 1996). For instance, in the cat pulmonary circulation, U-37883A blocked the vasodilation to the K_{ATP} channel openers levcromakalim and pinacidil (Minkes *et al*., 1995). U-37883A also reduced reactive hyperaemia, which is thought to result partially from K_{ATP} channel activation, in the cat hindquarters vascular bed (Minkes *et al*., 1995; de Witt *et al*., 1996). Higher concentrations of U-37883A (50 μM) do have nonvascular effects, reducing cardiac contractile force and conduction time (Humphrey *et al*., 1996). This may reflect inhibition of channels other than vascular K_{ATP} channels by U-37883A, as seen in this study (e.g. Figure 6).

Glibenclamide has been more widely used to study the functional role of K_{ATP} channels in smooth muscle (Quayle *et al*., 1997). Reversal of a particular effect (e.g. relaxation, membrane potential hyperpolarization) by glibenclamide has been used to implicate K_{ATP} channel involvement in many responses. This appears well founded, and molecular studies have shown that the sulphonylurea receptor is an integral component of the K_{ATP} channel, along with an inward rectifier potassium channel (Inagaki *et al*., 1995). However, sulphonylureas influence several other cellular processes, including the Na/K ATPase and chloride channels, and the sulphonylurea receptor may be able to couple to potassium channels other than the K_{ATP} channel (Ämmälä *et al*., 1996; Quayle *et al*., 1997). It is therefore advantageous to have other K_{ATP} channel blockers to support the use of glibenclamide. U-37883A appears well suited to this. Indeed, the vascular selectivity of U-37883A demonstrated in this study means it may have significant advantages in studies of the circulation of cardiac and skeletal muscle, where vascular and non-vascular effects of K_{ATP} channel inhibitors may need to be distinguished. In contrast, glibenclamide shows little selectivity between K_{ATP} channels in these different muscle types (Quayle *et al*., 1997; Barrett-Jolley & McPherson, 1998).

Site of action of U-37883A

The pharmacological properties of K_{ATP} channels depend on the cell type. For instance, the channel in β-cells is more sensitive to inhibition by sulphonylureas, while vascular K_{ATP} channels are more sensitive to activation by potassium channels openers. Studies on cloned K_{ATP} channels have provided evidence that the pharmacological profile of K_{ATP} channels in a particular tissue reflects the expression pattern of different channel isoforms (Inagaki *et al*., 1995; 1996; Yamada *et al*., 1997; Aguilar Bryan *et al*., 1998). The K_{ATP} channel is a hetero-oligomer composed of the sulphonylurea receptor (SUR) protein and an inward rectifier potassium channel subunit (Kir 6), with four of each subunit forming each channel (Clement *et al*., 1997). There are at least two isoforms of both sulphonylurea receptor (SUR1, SUR2) and Kir 6 (Kir 6.1, Kir 6.2) (Aguilar-Bryan *et al*., 1998; Babenko *et al*., 1998). There are also at least two important forms of SUR2 (SUR2A, SUR2B), formed by alternate splicing of mRNA (Aguilar-Bryan *et al*., 1998; Babenko *et al*., 1998). The distribution and the electrophysiological properties of the different clones suggests that the cardiac and skeletal muscle K_{ATP} channel is composed of Kir 6.2 and SUR2A (Inagaki *et al*., 1995; 1996; Aguilar Bryan *et al*., 1998; Babenko *et al*., 1998). The identity of the smooth muscle K_{ATP} channel is less certain, although the pharmacology of smooth muscle K_{ATP} channels corresponds closely to that of cloned channels which contain SUR2B (Yamada *et al*., 1997; Aguilar Bryan *et al*., 1998). It has been proposed by Babenko *et al*. (1998) that Kir 6.1/SUR2B and Kir6.2/SUR2B may underlie two different forms of K_{ATP} channel previously identified in smooth muscle (Beech *et al*., 1993; Zhang & Bolton, 1996). U-37883A may be useful in further defining the molecular basis of different K_{ATP} channels found in the vasculature.

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