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Two types of ATP-sensitive potassium channels in rat portal vein smooth muscle cells

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1 Single-channel recordings were made from single, enzymatically isolated smooth muscle cells of rat portal vein by the patch-clamp technique.

2 Unitary potassium currents were identified through two types of K-channels with conductances in 60:130 mM K-gradient of 50 and 22 pS; these are referred to as LK and MK channels respectively.

3 The LK channels became extremely active if isolated patches were created into nucleotide-free solution; activity was inhibited by ATP applied to the inner surface of the patch with a half maximal inhibition (K_i) of 11-23 μM. Channel activity declined and disappeared with time and could be regenerated by a brief application of Mg-ATP or a nucleoside diphosphate such as UDP (in the presence of Mg). LK channel activity was rarely stimulated by levcromakalim and not by pinacidil (K-channel openers, KCOs) but was blocked by glibenclamide.

4 Activity of MK channels declined if isolated patches were created into nucleotide free solution; activity reappeared if UDP or ATP alone (in the presence of Mg) was applied; pinacidil or levcromakalim in the presence of ATP or UDP further increased channel activity which was blocked by glibenclamide.

5 The LK channel inhibited by ATP_i is very similar in its conductance and other properties to the K_{ATP} channel described in tissues other than smooth muscle, in its conductance and properties the MK channel resembles the K_{NDP} channel we have previous described as present in other smooth muscles and opening in responses to KCOs.

Keywords: Smooth muscle; ATP-sensitive K channels; glibenclamide

Introduction

ATP-sensitive potassium (K_{ATP}) channels have been thoroughly investigated in cardiac and pancreatic β-cells (reviewed by Ashcroft, 1988; Nichols & Lederer, 1991). A most important property of K_{ATP} channels is dramatic channel openings in isolated patches in the absence of ATP (Noma, 1983; Trube & Hescheler, 1984; Ashcroft & Kakei, 1989; Tung & Kurachi, 1991) and potent inhibition of the K_{ATP} channel activity by ATP with the half-maximal inhibition (K_i) at about 17-25 μM in rat cardiac cells (Findlay, 1988; Lederer & Nichols, 1989) and 4 μM in rat pancreatic β-cells (Ashcroft & Kakei, 1988). In smooth muscle, however, there is still much controversy about the existence of K_{ATP} channels and their properties. Whole-cell currents have been observed in smooth muscle cells from rat portal vein (Noack *et al.*, 1992b). rabbit pulmonary artery (Clapp & Gurney, 1992) and canine coronary artery (Xu & Lee, 1994) which were induced by dialysing the cells with ATP-free solution. These whole-cell currents could be inhibited by adding ATP to the pipette solution and by extracellular glibenclamide, a suggested specific inhibitor of the K_{ATP} channels. However, the K_{ATP} channels in cardiac and pancreatic β-cells have always been defined at the single channel level. In smooth muscle cells, the data from single channel studies of K_{ATP} channels are limited and variable; channel conductance ranging from 7-280 pS have been reported and some channel types showed voltage or Ca-dependency (Standen *et al.*, 1989; Inoue *et al.*, 1989; Kajioka *et al.*, 1990; 1991; Kovacs & Nelson, 1991; Lorenz *et al.*, 1992; Bonev & Nelson, 1993; Furspan & Webb, 1993; Kamouchi & Kitamura, 1994). In more recent studies in freshly isolated vascular smooth muscle cells, in contrast to observations on cardiac and β-cells, no channel activity could be observed after isolated patches were created into the ATP-free solutions unless nucleoside diphosphates (NDPs) or potassium channel openers (KCOs) with NDPs or ATP were present (Kajioka 1991; Beech *et al.*, 1993a,b; Kamouchi & Kitamura 1994; Zhang & Bolton, 1995). We therefore designated these channels K_{NDP} (Beech *et al.*, 1993a,b; Zhang & Bolton, 1995). Others have recently confirmed our observations independently in other smooth muscles (Halliday *et al.*, 1994; 1995).

Based on the observations that no channel activity could be seen in the absence of ATP unless NDPs were present, we suggested that NDP, instead of ATP, was the more important regulator of these K_{NDP} channels in smooth muscle cells (Beech *et al.*, 1993a; Zhang & Bolton, 1995). Since NDPs are able to reactivate K_{ATP} channels after their inactivation in cardiac cells (Tung & Kurachi, 1991) and pancreatic β-cells (Dunne & Petersen, 1986), one explanation for the difficulty in observing K_{ATP} channel activity in smooth muscle might have been that the K_{ATP} channel in smooth muscle had already inactivated (dephosphorylated was suggested). We found, however, that channel dephosphorylation did not explain the observations (Zhang & Bolton, 1995).

In the present studies, we show that there are two kinds of ATP-sensitive potassium channels in smooth muscle cells isolated from rat portal vein, one of larger conductance (LK, 50 pS) which is inhibited by ATP_i, is not sensitive to KCOs, and which seems closely similar to K_{ATP} channels in heart and elsewhere, and another smaller conductance (MK, 22 pS) channel stimulated to open by NDP, ATP, or ATP and KCOs which resembles the K_{NDP} channel we have previously described in rabbit portal vein (Beech *et al.*, 1993a,b) and in rat small mesenteric artery smooth muscle cells (Zhang & Bolton, 1995).

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Methods

Single cell dispersion

Adult, male Wistar rats (150–250 g) were killed by cervical dislocation. Each portal vein was carefully cleaned of fat and connective tissue with fine scissors under a dissecting microscope. The vein was cut into small pieces and then incubated in a low-Ca (10 μM) and Mg-free physiological salt solution (PSS) at 37°C for 5 min. Tissue was then moved to the same solution which contained 1 mg ml^{-1} collagenase (type 1A, Sigma), 0.5 mg ml^{-1} pronase (Calbiochem) and 1 mg ml^{-1} bovine albumin (Sigma) at 37°C for 25 min. The tissues was washed with enzyme-free solution and triturated by sucking in and out of a wide bore, smooth-tipped pipette to obtain single smooth muscle cells. The cells were used for experiments within 6 h of separation during which time they were stored at 4°C in low-Ca (10 μM) solution. All experiments were performed at room temperature (22°C).

Electrophysiology

Cell-attached and isolated inside-out patch recording (Hamill *et al.*, 1981) were used in the experiments. The patch amplifier was an RK300 (Biologic). Patch pipettes were made from borosilicate glass (Plowden & Thompson); they had resistance of 2–4 MΩ after fire-polishing, and were coated with Sylgard (Dow Corning). Data were either stored on FM-tape (Racal) (3.75 in s^{-1}, low-band pass 1250 Hz) or captured on-line to a 486 PC for data analysis after filtering (100 Hz or 1 kHz, 4-pole Bessel filter, Barr and Stroud) and digitization (300 Hz or 4 kHz) using a 1401CED interface and software.

Data analysis

Unitary current amplitude was determined by fitting amplitude histograms with Gaussian distributions. The channel open-state probability (P_o) was calculated using the following equation

$$P_o = \sum_{i=1}^{N} \left(\frac{t_i i}{TN}\right)$$

where the t_i is the time spent with $i = 1,2,3.....$, N channels open, N is the number of channels, i is unitary current and T is the sample length. The calculation assumes the maximum number of unitary current levels observed in a patch to be equal to the number of active channels, N, in the patch. All data were expressed as mean ± s.e.mean.

Solutions

Low-Ca solution (no Mg added) for the isolation of single cells (mM): NaCl 130, KCl 5, $CaCl_2$ 0.01, HEPES 10, glucose 10, pH 7.4 with NaOH. Mg-free high K bath solution (mM): NaCl 9, KCl 120, HEPES 18, glucose 10 mM, EDTA 5, pH 7.4 with KOH. For Mg-containing bath solution, $MgCl_2$ 0.1, 1, 2 or

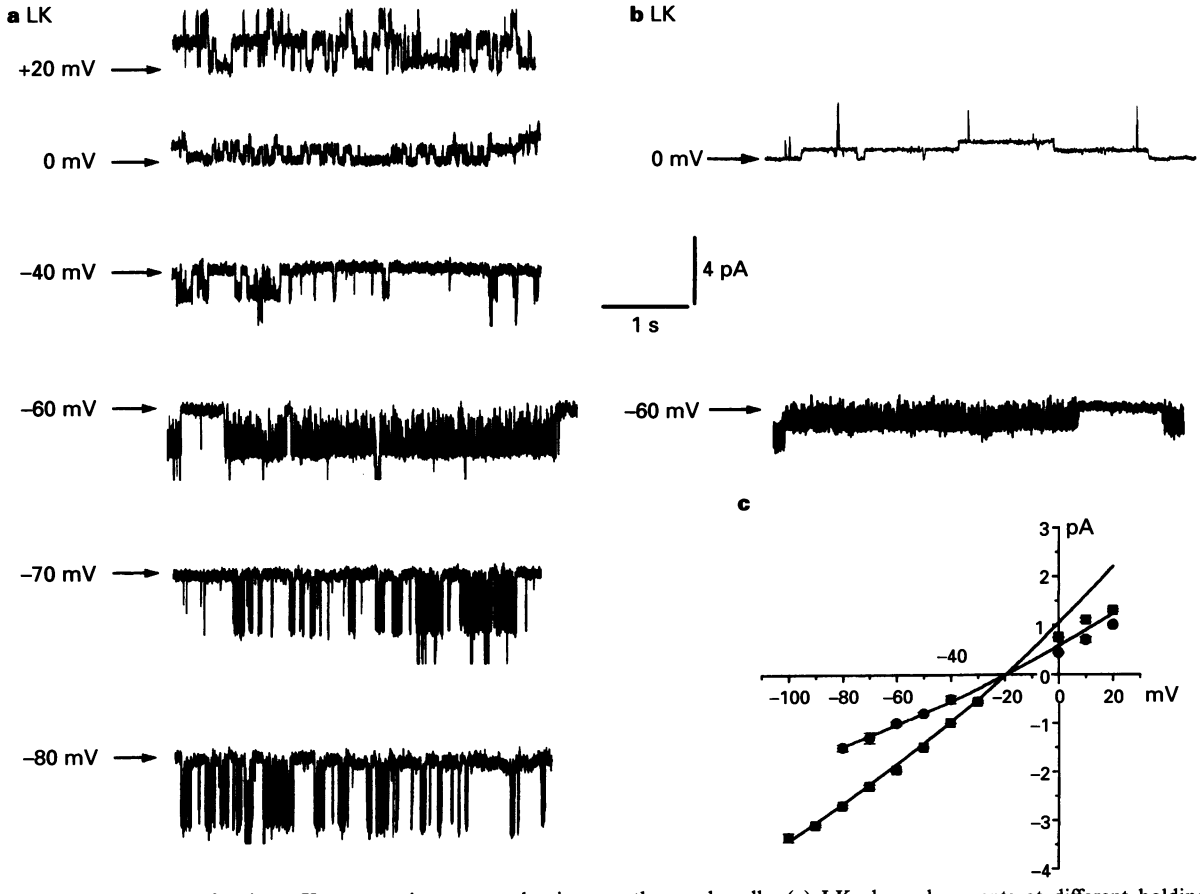

Figure 1 Two sizes of unitary K-currents in rat portal vein smooth muscle cells. (a) LK channel currents at different holding potentials in an inside-out patch; 60 mM K was in the pipette solution and Mg-free high K (130 mM) solution was in the bath. Note that the second channel opening level was cut off due to the saturation of the amplifier when holding potentials were more negative than −60 mV. (b) MK channel currents at 0 and −60 mV in a different patch from that in (a); 3 mM Mg high K solution was in the bath. (c) Current-voltage relationship for the LK channel (■ $n = 29$ and 39 at −60 and 0 mV, respectively; $n = 3–5$ for other holding potentials) and MK channel (●, $n = 2–12$). The lines are derived from the Goldman-Hodgkin-Katz current equation fitted to the points. Conductances of the LK and the MK channel were 49 pS and 22 pS.

3 mM was added and EDTA was replaced by EGTA. EGTA or EDTA has no effect on channel activity in the present experiments. ATP was added as the Na-salt and was prepared freshly on the day of the experiments. Pinacidil, levcromakalim and glibenclamide were prepared as 100 mM stock solution in dimethylsulphoxide (DMSO). The maximal concentration of DMSO applied was 0.1% which had no effect on LK and MK channel activities. Pipette solution (mM): NaCl 80, KCl 60, $MgCl_2$ 1.2, HEPES 10, glucose 10, pH 7.4 with NaOH.

Drugs

ATP (adenosine triphosphate), ATP-γ-s (adenosine 5'-O-3(thiotriphosphate)), EGTA (ethlyglycol-*bis*-(β-aminoethyl)-*N,N,N',N'* tetraacetic acid), EDTA (ethylenediaminetetraacetic acid), HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid), uridine diphosphate (UDP) and glibenclamide were from Sigma. Pinacidil monohydrate was from Leo Pharmaceutical Company (Denmark). Levcromakalim ((-)-Ckm) was a gift from Dr T. Hamilton (SKB).

Results

Channel conductance and rectification

Two sizes of unitary K-channel current were observed in cell-attached and isolated inside-out patches of rat portal vein smooth muscle cells. Most of the inside-out patches were held either at −60 mV or 0 mV; the current direction was outward (upward) at holding potentials more positive than 0 mV and inward (downward) at holding potentials more negative than −20 mV (K reversal potential was −19.6 mV; Figure 1). The amplitude of the larger unitary current was -1.93 ± 0.008 pA ($n = 29$ patches) at −60 mV and 0.67 ± 0.018 (Figure 1a, $n = 38$ patches) at 0 mV, respectively; the amplitude of the smaller unitary current was -0.98 ± 0.032 ($n = 12$ patches) at −60 mV and 0.42 ± 0.008 pA ($n = 6$ patches) at 0 mV (Figure 1b), respectively. The current-voltage relationships for channels in inside-out patches were nearly linear when current was inward with slope conductances (for current between −60 and −20 mV) of 49 pS (squares) and 22 pS (circles) (Figure 1c); very similar results were obtained for the two channel types in cell-attached patches. The reversal potentials for both unitary currents was close to the K equilibrium potential (−19.6 mV, Figure 1c). These two channels with larger and smaller current amplitude will be termed the LK channel and the MK channel, respectively.

When inside-out or cell-attached patches were held at negative holding potentials both MK and LK channels showed fluctuations in the open state (probably incompletely resolved brief channel closings) which decreased when the patches were held at more positive potentials (Figure 1a,b). The probability of the open state (P_o) of both channel types was neither appreciably voltage-modulated nor calcium-sensitive (for inside-out patches Ca-free solution was at both sides of the cell membrane and EGTA or EDTA 5 mM was in the bathing solution). In four inside-out patches, when holding potentials were changed from −100 mV to +30 mV by steps of 20 mV, P_o of the LK channel fluctuated by less than 10% of its original value and the changes were not obviously related to voltage.

When the cell membrane was held at positive potentials, the Ca-activated large conductance K-channel (BK channel) was also activated (Figure 1b). Apart from these three channels, a channel with much smaller current amplitude could also be

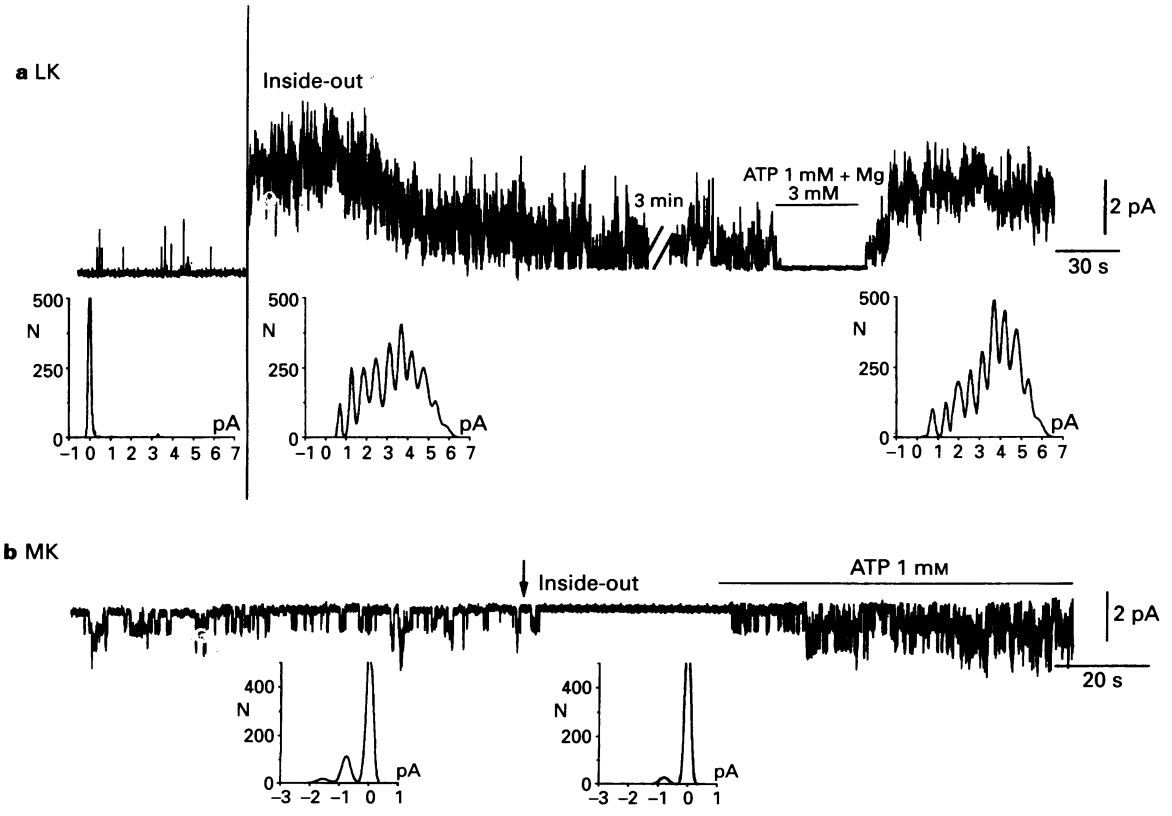

Figure 2 Channel activity in inside-out patches. (a) Activation of LK channel upon creating an inside-out patch into nucleotide-free solution (vertical line). Record initially from a cell-attached patch held at 0 mV; 60 mM K solution was in the pipette and Mg-free solution was in the bath. The first two amplitude histograms were constructed from periods of 40 s before and immediately after the inside-out patch was created. The third amplitude histogram was constructed from a period of 40 s after ATP and Mg was washed out. (b) Inactivation of the MK channel when isolated inside-out patch was created into nucleotide-free solution. The patch was initially cell-attached and was held −60 mV; an inside-out patch was created at the time indicated by arrow; 60 mM K solution was in the pipette and 3 mM Mg solution was in the bath.

observed in a few patches ($n=3$) when they were held continuously at −60 mV. This channel, with a conductance of 2.3 pS in the range of −100 to −60 mV, did not open when the patches were held at 0 mV. No effort were made to investigate the properties of this channel because it could be observed only occasionally.

Channel activity on creation of isolated inside-out from cell-attached patches

A total number of 329 cell-attached patches were used to investigate the activity of LK and MK channels in cell-attached patches. Among these LK channel activity was observed alone in 18 patches while MK channel activity was observed alone in 65 patches; LK and MK channel activity was observed in the same patch on 9 occasions. The remainder of the patches ($n=237$) showed neither LK nor MK channel activity at −60 mV or 0 mV in the cell-attached patch mode.

The two types of K-channel showed a conspicuous difference in behaviour when isolated patches were formed by pulling cell-attached patches into ATP-free solution. LK channels, inactive in the cell-attached mode, showed a dramatic increase in activity when an isolated inside-out patch was created into ATP-free solution (Figure 2a) in 74 patches (out of 123 patches from which an inside-out patch was created successfully from the cell-attached patch mode). However, if LK channels were active in the cell-attached mode then activity declined upon creating an inside-out patch; this decline could not be correlated with any obvious factor such as time since cell dispersion. If MK channels showed no activity in the cell-attached mode, no activity appeared when an isolated inside-out patch was created into nucleotide-free solution (see later, Figure 5). If MK channels were active in the cell-attached mode, activity generally declined on creating an inside-out patch into nucleotide-free solution (11 out of 13 patches, Figure 2b); in two patches activity increased slightly (P_o increased 0.13±0 to 0.19±0.03). These effects could be seen unequivocally when only one channel type was active in a patch; they were also apparent when both channels were present (Figure 4). There was no obvious reason why LK and MK channels were active in some cells in the cell-attached mode and quiescent in others.

Effects of ATP

After initially dramatic channel activity appeared in the isolated inside-out patch mode and in the absence of nucleotide, LK channel activity gradually decreased over 40 s to a few minutes (Figure 2a). Figure 2a shows an inside-out patch in which up to 6 channels opened simultaneously immediately after the patch was isolated; after about 9 min, only 4 channels could be seen still active. ATP (1 mM) in the presence of 3 mM Mg abolished the remaining channel activity. After washing out ATP, activity of the LK channels recovered fully. It was necessary for Mg^{2+} to be present in order for ATP to reactivate the LK channels. Thus ATP, as on K_{ATP} channels of cardiac and pancreatic β-cells, had dual effects on LK channel activity, i.e. inhibiting LK channel openings and reactivating rundown LK channel activity after washing out MgATP. In patches in which LK channel activity had been produced by isolating patches into nucleotide-free solution, ATP (100 μM or 1 mM) in the absence or presence of Mg (1–3 mM) could

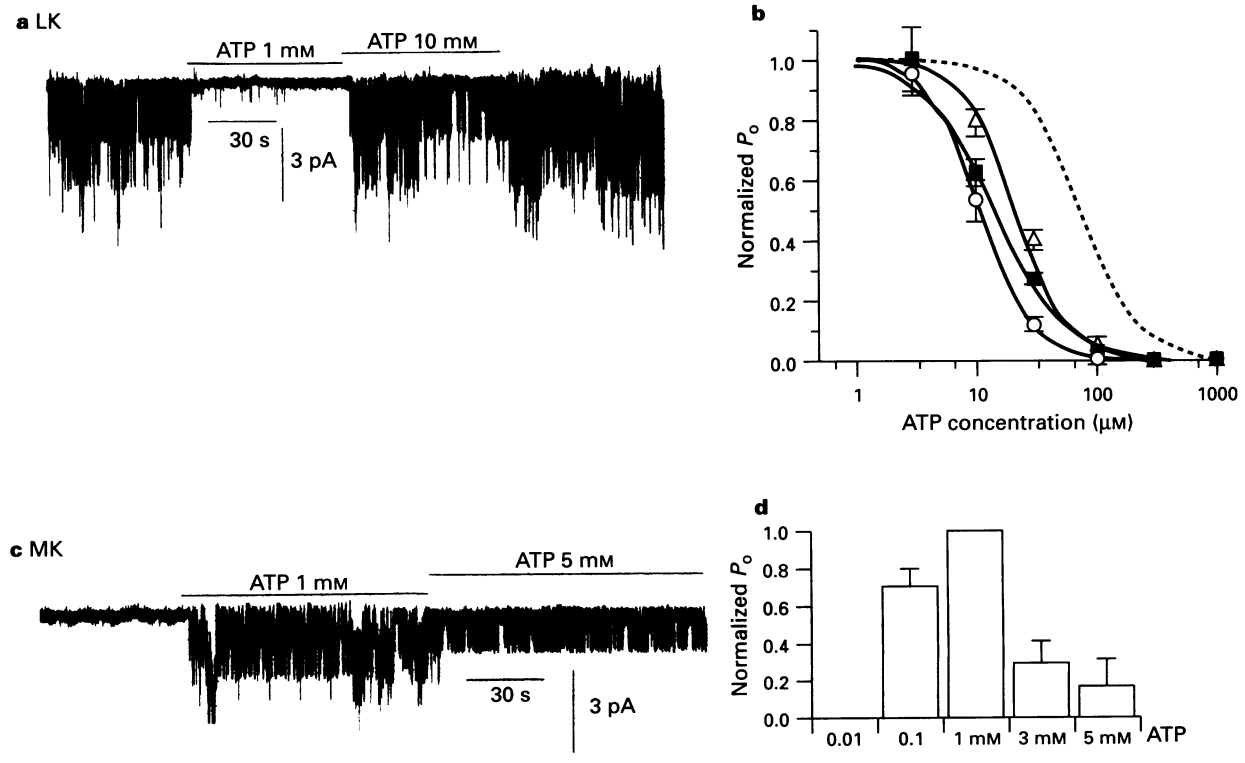

Figure 3 Opposite effects of ATP on the LK and the MK channel activities in isolated inside-out patches. (a) Inhibition of LK channel by ATP (1 mM) and 10 μM) in an inside-out patch. The patch was held at −60 mV and Mg-free high K solution was the bathing solution. ATP 1 mM (in the presence of 3 mM Mg) and 10 μM (in the absence of Mg) was applied to the patch via the bathing solution. (b) Concentration-response relationship for ATP inhibiting the LK channel activity in the presence and absence of Mg. P_o of the LK channel in the absence of ATP was normalized at 1. The solid and dotted lines were drawn according to the equation described in the text. K_i for ATP in the absence of Mg (○, $n=12$), in the presence of 0.1 mM Mg (■, $n=5$) and in the presence of 1 mM Mg (△, $n=5$) were 11, 14 and 23 μM, respectively. The dotted line was the expected shift of the concentration-response relationship of ATP in the presence of 1 mM Mg assuming Mg bound ATP was inactive. (c) Activation of MK channel by ATP in an inside-out patch. The patch was held at −60 mV. ATP 1 and 5 mM (in the presence of 2 mM Mg) was applied to the patch via the bathing solution. (d) Concentration-response relationship for ATP activation of MK channel activity; $n=3-5$.

almost totally abolish channel activity of the LK channel (P_o was reduced by 95%–100%, $n=25$; Figures 2a, 3a), suggesting both free ATP and Mg-ATP were efficient in inhibiting LK channel activity. However, in order to establish the concentration-effect relationship for the inhibition of the LK channel by ATP in the presence and absence of Mg, involvement of the following factors must be considered: Mg inhibits the LK channel from the internal surface of the patch (unpublished observations). Also LK channel activity runs down, and it was not easy to get stable recordings for long periods when patches were held more negative than −40 mV (reversal potential for K in the present experiments was about −19.6 mV) To overcome the above difficulties the following strategies were used when constructing the Mg-ATP concentration-effect relationship: (i) The activity of the LK channel in the presence of Mg without ATP was given a relative value of 1.0; 0.1 and 1 mM Mg were used in the experiments because, at a Mg concentration higher than 2 mM, channel activity was low. Over the range of ATP concentrations used, in the presence of 1 mM Mg, about 85% of total ATP is Mg-ATP (free-ATP and Mg-ATP concentrations were calculated by using the EQCAL programme). (ii) ATP (1 mM) in the presence of 2 or 3 mM Mg was used to prevent channel run down, i.e. ATP 1 mM with 2 or 3 mM Mg was always applied first for 90–120 s to the internal surface of the patches to inhibit fully and to reactivate the LK channel before a single test concentration of ATP with or without Mg, or Mg alone, was applied. Test concentrations of ATP with or without Mg were followed by washout with ATP-free solution to get full activity of the LK channel (Figure 3a). (iii) The patches were held either at −60 mV for short periods or held at 0 mV. Figure 3b shows the concentration-effect relationship for the inhibition of LK channel activity by ATP in the absence (circles) and in the presence of Mg 0.1 (filled squares) and 1 mM (triangles). The solid lines were drawn according to the equation $1/[1+K/(ATP)^n]$ (where ATP is the ATP concentration (μM), K_i = the concentration of ATP (μM) producing half-maximal inhibition n = Hill coefficient). K_i for ATP in the absence, in the presence of 0.1 mM, or 1 mM Mg was 11, 14 and 23 μM, respectively. In the absence, in the presence of 0.1 mM, or 1 mM Mg n for ATP was 2, 1.5 and 1.8, respectively. In the presence of Mg, the concentration-response relationship was shifted to the right, indicating that Mg interacted with the effect of ATP on LK. In the presence of 1 mM Mg, about 85% of ATP was bound to Mg. The dotted line in Figure 3b shows the expected shift of the concentration-response relationship of ATP in the presence of 1 mM Mg ($K_i=73$ μM), if LK channel activity was inhibited only by free-ATP. The actual shift (K_i 23 μM) was smaller than the expected.

As mentioned above, it was rare to see MK channel activity in isolated inside-out patches in the absence of nucleotide. However, when ATP (1 mM) was applied in the presence of Mg (2–3 mM) to the internal surface of 41 patches, the MK channel was activated in 9 of these. The MK channel could be activated by ATP either in patches in which the MK channel had been active in the cell-attached mode (Figure 2b), or in patches in which no MK channel activity had been observed (Figure 4a). Lower concentration of ATP (10 μM) had no effect on MK channel activity; when ATP (1 mM) had stimulated MK channel activity it was inhibited by up to 80% by increasing the ATP concentration to 3–5 mM (Figure 3c), i.e.

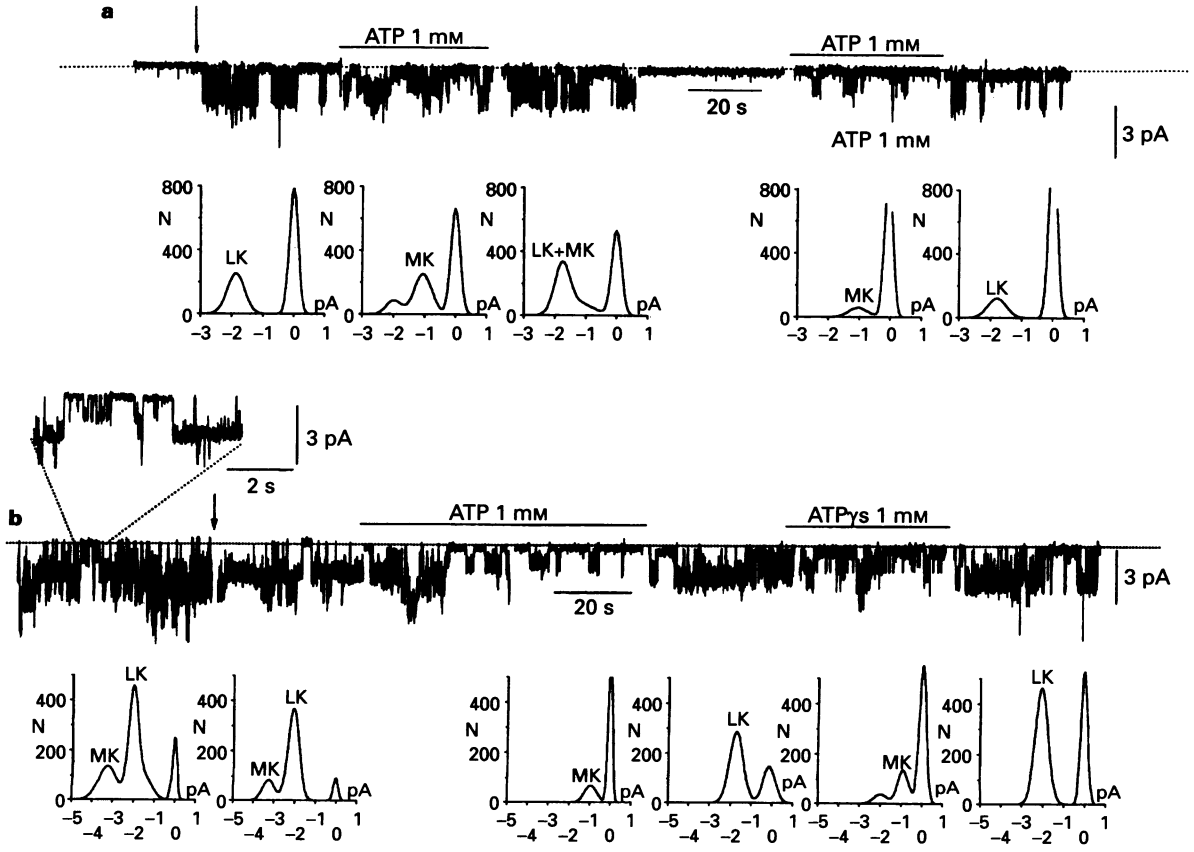

Figure 4 Reciprocal effects of ATP on LK channel and the MK channel activities in patches in which both channel types were present. The patches were held at −60 mV; 60 mM K solution was in the pipette and 3 mM Mg high K solution was in the bath. Inside-out patches were formed from cell-attached patches at times indicated by arrows. ATP or ATP-γ-s (1 mM) in the presence of 3 mM Mg were applied in the bathing solution during periods indicated by bars. Amplitude histograms were constructed from a period of 40 s. In (a), the first, third and fifth amplitude histograms were in the absence of ATP. In (b) the first amplitude histogram was from the cell-attached patch; the second, fourth and sixth from the isolated inside-out patch in nucleotide-free solution. A portion of expanded trace is shown to reveal two unitary current sizes.

the concentration-effect relationship for ATP was bell-shaped (Figure 3d). Washout of ATP did not cause potentiation of MK channel activity.

The inhibitory action of ATP on the LK channel was accompanied by a stimulating action of ATP on MK channel activity in some patches, which is demonstrated in Figure 4. Both cells in Figure 4 were bathed in 3 mM Mg solution. In Figure 4a, the cell-attached patch was without channel openings but when an inside-put patch was formed into nucleotide-free solution, the LK channel was activated. ATP (1 mM) totally abolished LK channel activity and activated the MK channel at the same time (the second peak in the histogram is also MK channel activity). After washing out ATP, the LK channel was reactivated and MK channel activity was reduced in the ATP-free solution. After LK and MK channel activity had totally disappeared, ATP (1 mM) reactivated the MK channel and, after washout, some LK channel activity. Similar results were observed in 3 other patches where simultaneously the LK channel was inhibited and the MK channel activated in the presence of Mg-ATP and then LK channel was activated and MK channel activity disappeared when ATP was washed out. In Figure 4b, both LK channel and MK channel activity were present in the cell-attached patch mode which could be seen more clearly on a fast time-base. After an inside-out patch was created into nucleotide-free solution, activity of both LK and MK channels decreased. When ATP (1 mM) was applied to the patch, activity of the LK channel decreased and eventually disappeared while MK channel activity was little altered. After washing out ATP, MK channel activity disappeared and the LK channel was reactivated. ATP-γ-S, an analogue of ATP, had a similar but possible weaker effect than ATP on LK and MK channel activity (Figure 4b).

Effect of UDP and KCOs

In our previous work (Beech *et al.*, 1993a,b; Zhang & Bolton, 1995) we found that in rabbit portal vein and rat mesenteric artery smooth muscle cells, nucleoside diphosphate (NDP) was able to activate a K-channel which was termed the K_{NDP} channel and that this was the target channel of KCOs. In the present studies, we found that NDP stimulated activity of both LK and MK channels. Figure 5 shows that the K_{NDP} channel and the MK channel have similar characteristics. In Figure 5a MK channel activity was observed neither in a cell-attached patch nor immediately after an inside-out patch was formed. Pinacidil (10 μM) alone had no effect in this or in other isolated patches (but did in cell-attached patches). ATP (1 mM), in the presence of pinacidil activated the channel. Channel activity ceased soon after ATP was washed out. UDP (1 mM) reactivated the channel in the absence of pinacidil. The trace on a fast time base shows that the channel activated by ATP and pinacidil, and the channel activated by UDP, have the same characteristics. Figure 5b shows a patch where ATP alone (in the presence of Mg) could not activate the MK channel unless the KCO, pinacidil, was present. Similar results were also obtained in 7 other cells where the MK channel was activated by pinacidil or (-)-Ckm only in the presence of ATP. Pinacidil or (-)-Ckm also increased MK channel activity already activated by ATP. Thus, the MK channel was more active in the

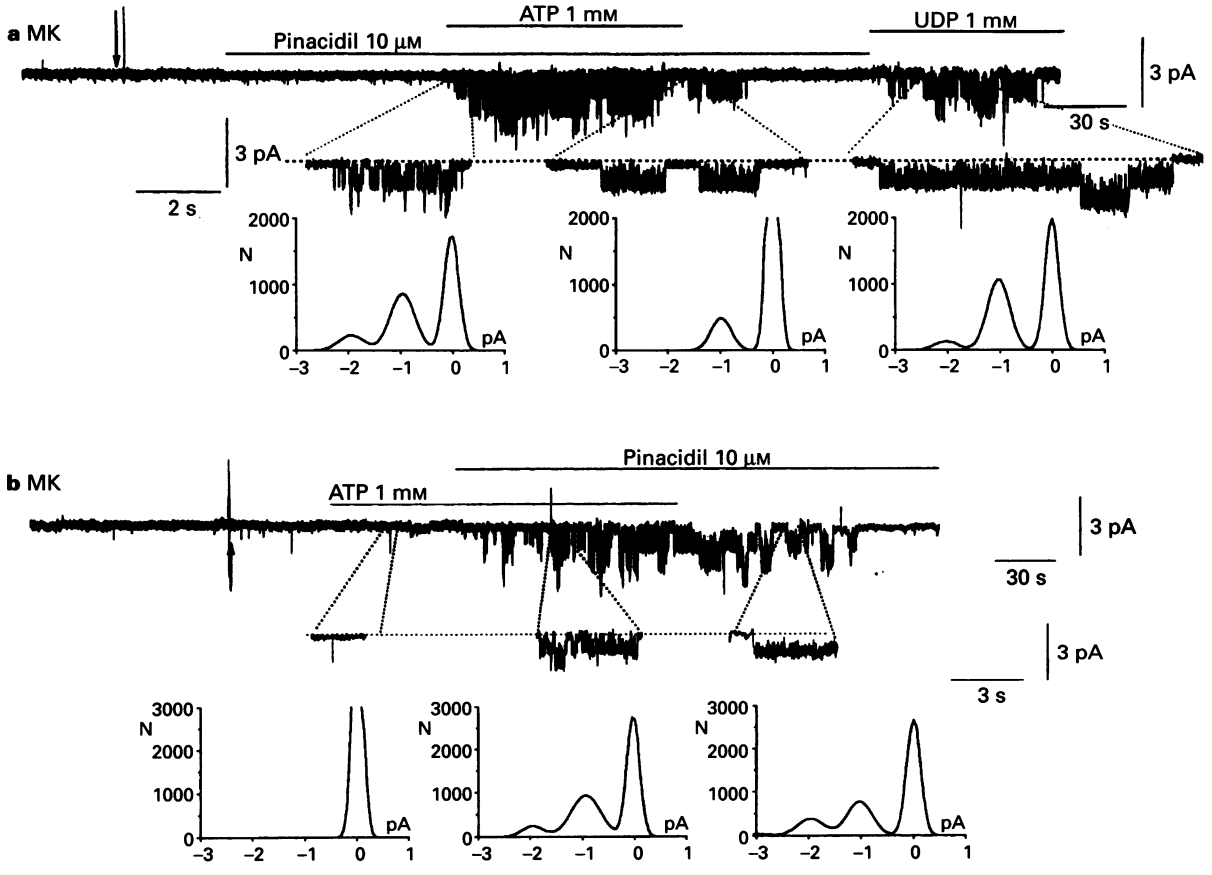

Figure 5 Effects of ATP, UDP and pinacidil on MK channel activity. The patches were held at −60 mV; 60 mM K solution was in the pipette and 3 mM Mg high K solution was in the bath. Inside-out patches were created at times indicated by arrows. ATP (1 mM), pinacidil (10 μM), and UDP (1 mM) in the presence of 3 mM Mg were applied in the bathing solution during periods indicated by bars. Amplitude histograms were constructed from periods of 40 s in (a) in pinacidil and ATP, in pinacidil after washing out ATP, and in UDP respectively and in (b) in ATP alone, in ATP and pinacidil, and in pinacidil alone respectively.

presence of KCOs and ATP than in the presence of ATP alone, although sometimes, as in Figures 2b and 4, ATP alone was sufficient to activate the MK channel.

In contrast, KCOs did not generally activate LK channel activity. With time in ATP-free solution, LK channel activity gradually ran down (Figure 2a). UDP (1 mM) in the presence of Mg (1–2 mM) reactivated LK channel activity in 15 out of 20 patches in which LK channel activity had run down (Figure 6a). Figure 6a shows an inside-out patch in which LK channel was present but channel activity had became very low (P_o was 0.0071). UDP in the presence of Mg (2 mM), but not in its absence, increased P_o (to 0.17). Similar results were observed in another three patches. Thus, UDP requires the presence of Mg in order to reactivate LK channel activity. UDP had no effect on the LK channel when it was already active (not shown).

LK channel activity in either cell-attached ($n=7$) or inside-out patch ($n=18$) recordings was generally unaffected by application of (-)-Ckm (up to 100 μM) or pinacidil (up to 100 μM). In only two patches from 18 inside-out patches was there evidence of a transient stimulation of LK channel activity upon a first application of (-)-Ckm (10 μM); however, this stimulation could not be repeated in the same patch although the channels were still active (no rundown). If LK channel activity was reduced by application of ATP at a low concentration (10–100 μM), (-)-Ckm (10 μM) and pinacidil (10 μM) were also found to be without effect ($n=10$). Figure 6b shows that (-)-Ckm (10 μM) did not affect LK channel activity in the presence or absence of UDP (1 mM).

Inhibition of the LK channel and the MK channel activity by glibenclamide

Figure 7 shows the effect of glibenclamide (10 μM) on LK and MK channel activity. In inside-out patches, glibenclamide concentration-dependently inhibited LK channel activity (Figure 7a). As in isolated inside-out patches of rat cardiac cells (Ripoll *et al.*, 1993) glibenclamide up to 100 μM did not totally block LK channel activity which could be abolished by 100 μM ATP. The amplitude histograms, which were constructed from a period of 40 s in each case (control, glibenclamide 10, 30 and 100 μM), show that glibenclamide inhibited channel activity but did not affect the unitary current amplitude of the LK channel. Figure 7b shows the concentration-response relationship for glibenclamide in inhibiting LK channel activity; K_i of glibenclamide was about 3 μM. These experiments were done in the absence of Mg^{2+}; in four patches, the presence of glibenclamide at its IC_{50} (3 μM) reduced P_o to an average of 62±4%, compared to 44±9% in the same patches if Mg^{2+} was absent. This is very different from the 1000 fold greater sensitivity of K_{ATP} to glibenclamide in whole-cell recording from β-cells when Mg^{2+} was present (Lee *et al.*, 1994) but similar to the result in isolated inside out patches of rat cardiac cell (Venkatesh *et al.*, 1991; Findlay, 1993).

Figure 7c shows the effect of glibenclamide on MK channel activity induced by ATP and (-)-Ckm in an inside-out patch. ATP 1 mM activated the Mk channel and (-)-Ckm 10 μM increased the channel activity in the continuous presence of ATP. Glibenclamide (10 μM) inhibited about 85% of the MK

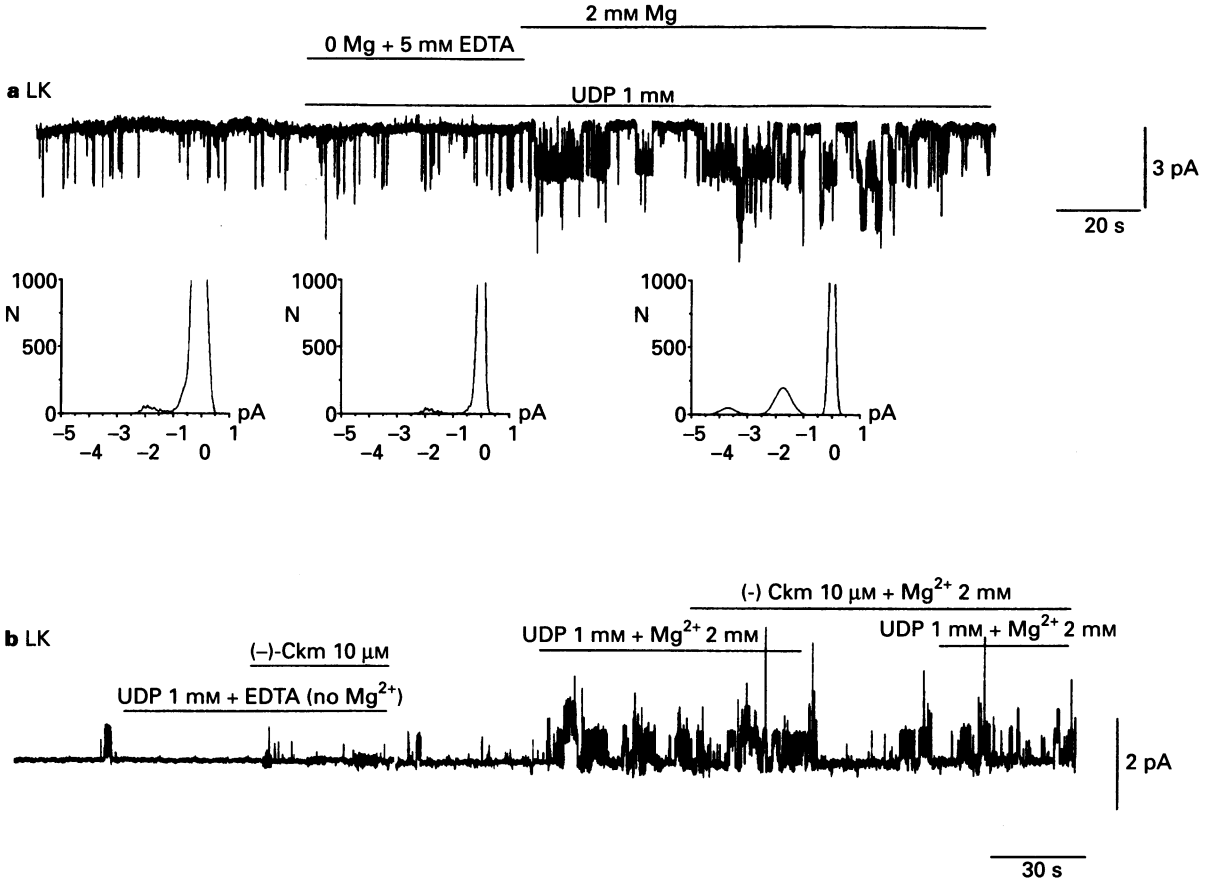

Figure 6 Effects of UDP and lack of effect of (-)-Ckm on LK channel activity. (a) An inside-out patch was held at −60 mV and the recording started about 3 min after an inside-out patch was created; 60 mM K solution was in the pipette and Mg-free high K solution was in the bath. Amplitude histograms were constructed from periods of 40 s. First, second and third histograms correspond to the trace above in the absence of UDP, in the presence of UDP without Mg, and in the presence of UDP and Mg, respectively. (b) An inside-out patch was held at 0 mV. The recording started about 5 min after the inside-out patch was created and when LK channel activity had become low; 60 mM K solution was in the pipette and Mg-free high K solution was in the bath. UDP stimulated LK channel activity if Mg was present; (-)-Ckm had no further effect.

channel activity induced by ATP (1 mM) and (-)-Ckm (10 μM) in this patch and inhibited by an average of 92±8% in three patches (P_o was reduced from 0.17±0.08 to 0.02±0.007). In three inside-out patches, glibenclamide 1 or 10 μM strongly inhibited the channel activity induced by UDP (1 mM) (Figure 7d, P_o was reduced from 0.38±0.14 to 0.0014±0.0011). It seems that the channel activated by UDP was more sensitive to glibenclamide.

Discussion

The LK channel we describe here for the first time in smooth muscle is exceedingly similar in properties to the K_{ATP} channel in cardiac and pancreatic β-cells. One characteristic property of K_{ATP} channels is the dual effects of ATP, i.e. inhibition of channel activity by ATP when channels are active and re-activation of channel activity by Mg-ATP after it has run down. These effects of ATP on the LK channel were well demonstrated in the present experiments on rat portal vein smooth muscles. Another characteristic property of K_{ATP} channels is the dramatic increase in activity seen when isolated inside-out patches are created into ATP-free solution.

In rat cardiac cells, the inhibition of the K_{ATP} channels activity by ATP could be described as a sigmoid function of ATP with a Hill coefficient of 2 and K_i or 17–30 μM. Free ATP and Mg-ATP were both effective with Mg-ATP slightly more efficient (Findlay, 1988; Lederer & Nichols, 1989). In rat pancreatic β-cells, however, only free ATP was a potent inhibitor of the K_{ATP} channels with a K_i of 4 μM and Mg-ATP had little inhibitory effect. In the present experiments, The concentration-response relationship for the inhibition of the LK channel activity by ATP (when Mg was absent) could be fitted with coefficient of 2, suggesting there were two binding sites for ATP. In the presence of 0.1 and 1 mM Mg, the concentration-response relationship for the inhibition of LK channel activity by ATP was shifted to the right and K_i was increased from 11 to 14 and to 23 μM, respectively. If free ATP were the only effective form of ATP to act on LK channels in the present experiments, in the presence of 1 mM Mg the K_i of 11 μM would be expected to shift to 73 μM. The actual shift (to 23 μM) was less than this, perhaps because Mg itself depresses activity and prevents the full extent of the rightward shift in sensitivity to ATP being manifested. However, as shown in Figures 2a and 3a and described in the results, 0.1 and 1 mM ATP inhibited 95% – 100% of the LK channel activity but 2 or 3 mM Mg in the presence of these concentrations of ATP would reduce free ATP concentration to less than 10% (i.e. free ATP less than 10 or 100 μM). Thus it is also possible that, as in cardiac cells, Mg-ATP and free ATP are both effective inhibitors but not equally so.

Apart from the response to ATP, other characteristics of the

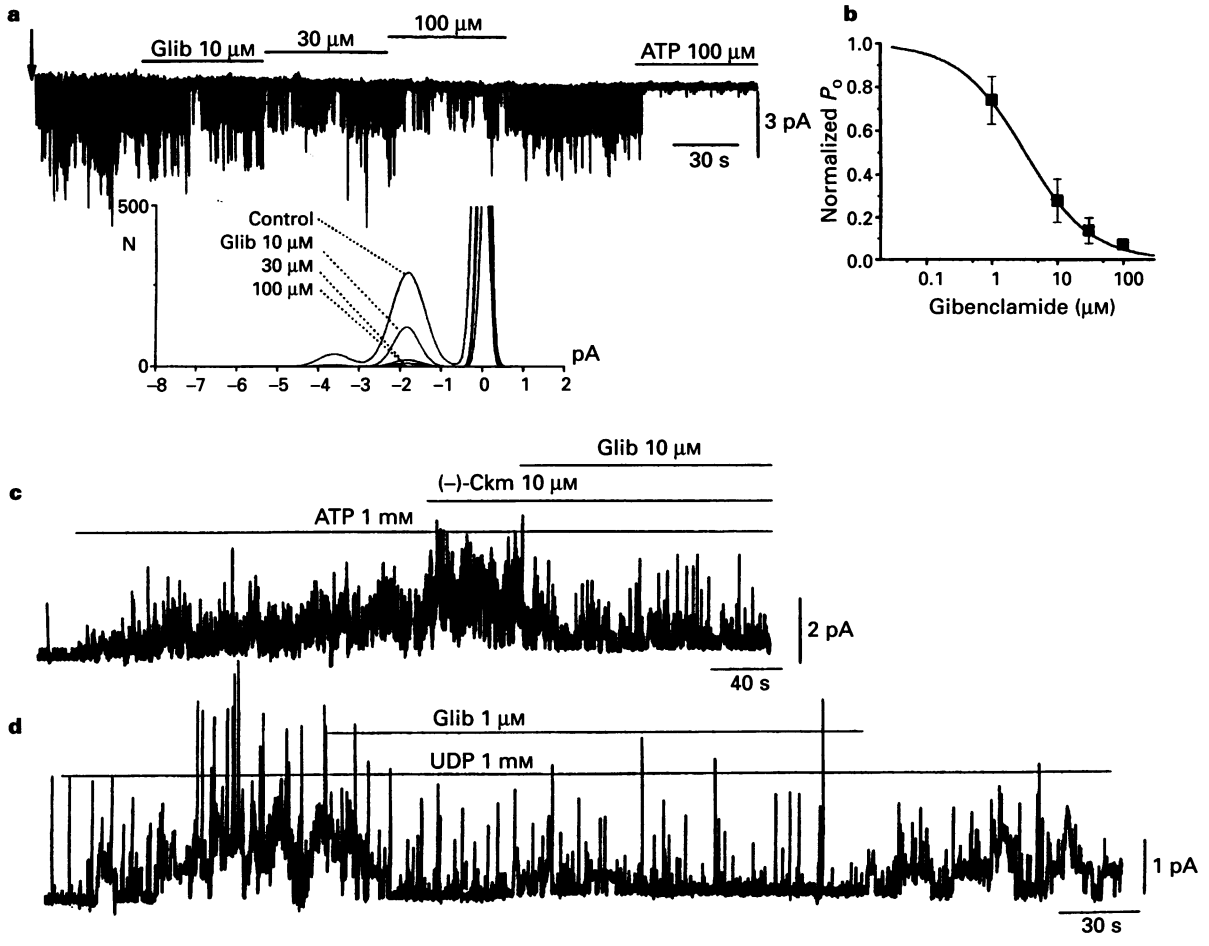

Figure 7 Inhibition of LK and MK channel activities by glibenclamide. (a) Concentration-dependent inhibition of LK channel activity by glibenclamide in an inside-out patch. The patch was held at −60 mV and Mg-free high K solution was in the bath. The superimposed amplitude histograms were constructed from a period of 40 s before and during application of glibenclamide 10, 30 and 100 μM. (b) Concentration-response relationship of glibenclamide on LK channel activity in inside-out patches (each point 2–5 cells). K_i was about 3 μM. (c) Inhibition of MK channel activity by glibenclamide in an outside-out patch. The patch was held at 0 mV and MK channel was activated by ATP and levcromakalim (in the presence of 2 mM Mg). (d) Glibenclamide inhibited the channel activity induced by UDP (in the presence of 2 mM Mg) in an inside-out patch. There is Ca-activated K-channel activity in this patch.

LK channel in the present experiments were also similar to those of the K_{ATP} channel in cardiac and pancreatic β-cells. The unitary conductance of the K_{ATP} channels in cardiac cells was 55–62 pS (Trube & Hescheler, 1984; Kakei *et al.*, 1985) when external K concentration was about 50–70 mM. In the present experiments, the unitary conductance of the LK channel was 49 pS when external K concentration was 60 mM. Thus the unitary conductance of the LK channels in rat portal vein cells was very similar to that of the K_{ATP} channels in cardiac cells. Even in the absence of internal Mg, the channels showed inward rectification, conductance being smaller when the current was outward (Figure 1c) which is also found in cardiac and pancreatic β-cells (Findlay, 1987; Ashcroft & Kakei, 1989).

However, unlike K_{ATP} channels in cardiac cells (Shen *et al.*, 1991), the LK channel in rat portal vein smooth muscle cells was rarely stimulated by KCOs ((-)-Ckm and pinacidil) in cell-attached patches or in isolated patches even in the presence of NDP (Figure 6b). This surprising result, however, would support further the theory that the target channel of KCOs in smooth muscle was the MK or K_{NDP} channel (see below).

The MK channel described in the present experiments had properties similar to the K_{NDP} channels described in rabbit portal vein and rat mesenteric artery smooth muscle cells in our previous publications (Beech *et al.*, 1993a; Zhang & Bolton, 1995) and similar to the channels activated by NDP and KCOs (pinacidil and levcromakalim) reported in rabbit portal vein smooth muscles by others (Kajioka *et al.*, 1991; Kamouchi & Kitamura, 1994). These channels have the following common characteristics: (i) they were activated by NDPs, or by KCOs in the presence of ATP or NDP; (ii) they were not active in the absence of nucleotide; (iii) they have rather smaller channel conductance of 20–24 pS when external K was 60 mM (15 pS when external K was 6 mM, Kajioka *et al.*, 1991) (Figure 1a; Beech *et al.*, 1993a; Zhang & Bolton, 1995). However, the K_{NDP} channel was not activated by ATP (Beech *et al.*, 1993a; Zhang & Bolton, 1995). In our previous experiments (Beech *et al.*, 1993a; Zhang & Bolton, 1995), we did not test the effect of ATP on the K_{NDP} channels after they were activated by NDPs at the single channel level. However, ATP (1 mM) included in the pipette solution in the presence of 2 mM Mg inhibited about 63% of the whole-cell currents induced by 1 mM GDP (Beech *et al.*, 1993a). Kamouchi & Kitamura (1994) reported that K_i for ATP inhibiting the channel activity induced by 1 mM UDP and 100 μM pinacidil in rabbit portal vein was between 200 μM and 1 mM in the absence or presence of Mg. In the present studies, channel activity of the MK channel induced by 1 mM ATP in the presence of 3 mM Mg could be inhibited by a higher concentration of ATP. Thus, the MK channel and similar channels were not very sensitive to the inhibitory action of ATP, particularly in the presence of Mg.

In the cardiac cells, the dominant channel activity in the cell-attached patch was the inward rectifier (K_{IR}) (Sakmann & Trube, 1984; Trube & Hescheler, 1984) which gradually inactivated when a patch was isolated into ATP-free solution (Trube & Hescheler, 1984). K_{IR} could be re-activated by intracellular ATP after its inactivation (Takano *et al.*, 1990), a similar process to that shown in Figure 2b in the present experiments. The conductance of K_{IR} was about 25–30 pS in 140:140 K gradient and channel openings showed bursts (Sakmann & Trube, 1984; Trube & Hescheler, 1984); these channel characteristics were similar to the MK channel in the present experiments. K_{IR} might also exist in smooth muscle (Quayle *et al.*, 1993). We tried to identify an inward rectifier current in rat portal vein smooth muscle cells. In whole-cell mode, there was no apparent inward rectification of the current induced by ramp voltage changes from −120 mV to +20 mV. However, when barium 0.5–1 mM (concentrations suggested to block K_{IR} specifically) was applied to the cell, current in the inward direction was reduced. Thus there was a barium-sensitive current in rat portal vein smooth muscles (unpublished observations). In inside-out patches, the MK channel showed only slight inward rectification even in the presence of Mg which does not support the idea that the MK channel and the K_{IR} channels are the same channels, although rectification of K_{IR} inward current in cardiac cells was lost at least partially in isolated patches (Matsuda, 1988). Clearly, more work needs to be done to clarify the relationship between the MK channel and inward rectifier K channels.

The discovery of the inhibitory effect of antidiabetic sulphonylurea drugs upon the K_{ATP} channel in pancreatic β-cells (Sturgess *et al.*,1985) has been followed by their adoption as specific blockers of this type of K channel (Quast & Cook, 1989). Nevertheless, quantitative electrophysiological studies of the effects of the most potent sulphonylurea, glibenclamide, have been few in both pancreatic β-cells (Zunkler *et al.*, 1988; Sturgess *et al.*, 1988) and cardiac cells (Venkatesh *et al.*, 1991; Findlay, 1993; Ripoll *et al.* 1993). In cardiac cells, K_i values of glibenclamide for inhibiting the K_{ATP} channel in inside-out patches were about 0.5–6 μM (Venkatech *et al.*, 1991; Findlay, 1993; Ripoll *et al.*, 1993); complete inhibition of K_{ATP} channel activity could not be observed even in the presence of a high concentration (300 μM) (Ripoll *et al.*, 1993). Such data are in close agreement with our present results. Sulphonylureas were generally 2–3 orders of magnitude more potent in blocking K_{ATP} channels from pancreatic β-cells than from cardiac cells (Zunkler *et al.*, 1988; Venkatesh *et al.*, 1991; Ripoll *et al.*, 1993). However, the efficiency of sulphonylureas as blockers of K_{ATP} channels might depend on other factors which are involved in different experiments. It has been reported that intracellular ADP could relieve the K_{ATP} channel inhibition by glibenclamide (Venkatesh *et al.*, 1991; Ripoll *et al.*, 1993). More recently, it has been reported that inhibition of the K_{ATP} channel from insulin-secreting cells by glibenclamide was Mg-dependent; when intracellular Mg was omitted the K_i of glibenclamide for inhibiting whole-cell currents through K_{ATP} channels was increased from 2.1 nM to 3.6 μM (Lee *et al.*, 1994). However, Mg has no significant effect on the sensitivity of rat cardiac K_{ATP} channels to glibenclamide in isolated inside-out patches (Venkatesh *et al.*, 1991; Findlay, 1993). The sensitivity of K_{ATP} channels to glibenclamide in whole-cell and in isolated patch mode seems different (Findlay, 1993). In the present experiments, the sensitivity of the LK channel to glibenclamide was not significantly affected by Mg, and thus was similar to that of the K_{ATP} channel in cardiac muscle.

In our previous experiments we found that glibenclamide inhibited whole-cell currents through K_{NDP} channels with a K_i of 25 nM and that the K_i of glibenclamide was 8 times higher when inhibiting whole-cell currents induced by (-)-Ckm. In the present experiments, glibenclamide 1 or 10 μM totally abolish the channel activity induced by UDP but it was less efficient in inhibiting the channel activity induced by (-)-Ckm in the presence of ATP (Figure 7c, 7d). This could be a result of complex interactions between glibenclamide and KCO.

Kajioka *et al.* (1990) reported a small conductance K channel (10 pS with external K concentration of 6 mM) in rat portal vein smooth muscle cells which was activated by intracellular Ca and the KCO nicorandil and which was inhibited by 5 mM ATP. This small-conductance ATP-sensitive potassium channel has not been reported in other tissues and is apparently different from either the LK channel or the MK channel of the present experiments. Noack *et al.* (1992a,b) reported that a significant whole-cell current (I_{met}) could be induced in rat portal vein smooth cells during metabolic depletion which prevented any K-current in response to (-)-Ckm. We could not observe such a current, I_{met}, in rabbit portal vein (Beech *et al.*, 1993a) or in rat mesenteric artery (Zhang & Bolton, 1995) smooth muscle cells. We have also observed a significant whole-cell current induced by ATP-free pipette solution in rat portal vein smooth muscle cells (unpublished observations). Thus, as far as whole-cell potassium channels are concerned, rat portal vein smooth muscle cells have different properties from those of other smooth muscle cells and further work will be needed to elucidate the single channel

activity which underlies the whole cell currents described by others in rat portal vein smooth muscle cells (Kajioka *et al.*, 1990; Noack *et al.*, 1992a,b).

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References

ASHCROFT, F.M. (1988). Adenosine 5'-triphosphate-sensitive potassium channels. *Annu Rev. Neurosci.*, **11**, 97–118.

ASHCROFT, F.M. & KAKEI, M. (1989). ATP-sensitive K channels in rat pancreatic β-cells: modulation by ATP and Mg ions. *J. Physiol.*, **416**, 349–367.

BEECH, D.J., ZHANG, H., NAKAO, K. & BOLTON, T.B. (1993a). K channel activation by nucleotide diphosphates and its inhibition by glibenclamide in vascular smooth muscle cells. *Br. J. Pharmacol.*, **110**, 573–582.

BEECH, D.J., ZHANG, H., NAKAO, K. & BOLTON, T.B. (1993b). Single channel and whole-cell K-currents evoked by levcromakalim in smooth muscle cells from the rabbit portal vein. *Br. J. Pharmacol.*, **110**, 583–590.

BONEV, A.D. & NELSON, M.T. (1993). ATP-sensitive potassium channels in smooth muscle cells from guinea pig urinary bladder. *Am. J. Physiol.*, **264**, C1190–C1200.

CLAPP, L.H. & GURNEY, A.M. (1992). ATP-sensitive K^+ channels regulate resting potential of pulmonary arterial smooth muscle cells. *Am. J. Physiol.*, **262**, H916–H920.

DUNNE, M.J. & PETERSEN, O.H. (1986). Intracellular ADP activates K^+ channels that are inhibited by ATP in an insulin-secreting cell line. *FEBS Lett*, **208**, 59–62.

FINDLAY, I. (1987). ATP-sensitive K channel in rat ventricular myocytes are blocked by internal divalent cations. *Pflügers Arch.*, **410**, 313–320.

FINDLAY, I. (1988). ATP^{4+} and ATP-Mg inhibit the ATP-sensitive K channel of rat ventricular myocyte. *Pflügers Arch.*, **412**, 37–41.

FINDLAY, I. (1993). Sulphonylurea drugs no longer inhibited ATP-sensitive K^+ channels during metabolic stress in cardiac muscle. *J. Pharmacol. Exp. Ther.*, **266**, 456–467.

FURSPAN, P.B. & WEBB, R.C. (1993). Decreased ATP sensitivity of a K^+ channel and enhanced vascular smooth muscle relaxation in genetically hypertensive rats. *J. Hypertens.*, **11**, 1067–1072.

HALLIDAY, F.C., AARONSON, P.I. & GURNEY, A.M. (1994). K^+ currents in isolated rabbit aortic smooth muscle cells. *J. Physiol.*, **477**, 44P.

HALLIDAY, F.C., GURNEY, A.M. & AARONSON, P.I. (1995). The glibenclamide-sensitive K^+ current in rabbit aortic myocytes is activated by intracellular GDP but not the absence of ATP. *Br. J. Pharmacol.*, **114**, 31P.

HAMILL, O.P., MARTY, E., NEHER, B., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for the high-resolution current recording from the cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.

INOUE, I., NAKAYA, Y., MORI, H. (1989). Extracellular Ca^{2+}-activated K-channel in coronary artery smooth muscle cells and its role in vasodilatation. *FEBS Lett.*, **255**, 282–284.

KAJIOKA, S., KITAMURA, K. & KURIYAMA, H. (1991). Guanosine diphosphate activates an adenosine 5'-triphosphate-sensitive K^+ channel in the rabbit portal vein. *J. Physiol.*, **444**, 397–418.

KAJIOKA, S., OIKE, M. & KITAMURA, K. (1990). Nicorandil opens a calcium-dependent potassium channel in smooth muscle cells of the rat portal vein. *J. Pharmacol. Exp. Ther.*, **254**, 905–913.

KAKEI, M., NOMA, A. & SHIBASAKI, T. (1985). Properties of adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. *J. Physiol.*, **363**, 441–462.

KAMOUCHI, M. & KITAMURA, K. (1994). Regulation of ATP-sensitive K^+ channels by ATP and nucleotide diphosphate in rabbit portal vein. *Am. J. Physiol.*, **266**, H1687–1698.

KOVACS, R.J. & NELSON, M.T. (1991). ATP-sensitive K^+ channels from aortic smooth muscle incorporated into planar lipid bilayers. *Am. J. Physiol.*, **261**, H604–H609.

LEDERER, W.J. & NICHOLS, C.G. (1989). Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. *J. Physiol.*, **419**, 193–211.

LEE, K., OZANNE, S.E., HALES, C.N. & ASHFORD, M.L. (1994). Mg^{2+}-dependent inhibition of K_{ATP} by Sulphonylureas in CR1-G1 insulin-secreting cells. *Br. J. Pharmacol.*, **111**, 632–640.

LORENZ, J.N., SCHNERMANN, J., BROSIUS, F.C., BRIGGS, J.P. & FURSPAN, P.B. (1992). Intracellular ATP can regulate afferent arteriolar tone via ATP-sensitive K^+ channels in the rabbit. *J. Clin. Invest.*, **90**, 733–740.

MATSUDA, H. (1988). Open-state substructure of inwardly rectifying potassium channels revealed by magnesium block in guinea-pig hearts cells. *J. Physiol.*, **397**, 237–258.

NICHOLS, C.G. & LEDERER, W.J. (1991). Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am. J. Physiol.*, **261**, H1675–1686.

NOACK, T., DEITMER, P., EDWARDS, G. & WESTON, A.H. (1992a). Characterization of potassium currents modulated by BRL 38227 in rat portal vein. *Br. J. Pharmacol.*, **106**, 717–726.

NOACK, T., EDWARDS, G., DEITMER, P. & WESTON, A.H. (1992b). Potassium channel modulation in rat portal vein by ATP depletion: a comparison with the effects of levcromakalim (BRL 38227). *Br. J. Pharmacol.*, **107**, 945–955.

NOMA, A. (1983). ATP-regulated single channels in cardiac muscle. *Nature*, **305**, 147–148.

QUAST, U. & COOK, N.S. (1989). Moving together: K^+ channel openers and ATP-sensitive K^+ channels. *Trends Pharmacol. Sci.*, **10**, 431–434.

QUAYLE, J.M., McCARRON, J.G., BRAYDEN, J.E. & NELSON, M.T. (1993). Inward rectifier K^+ currents in smooth muscle cells from rat resistance-sized cerebral arteries. *Am. J. Physiol.*, **265**, C1363–1370.

RIPOLL, C., LEDERER, W.J. & NICHOLS, C.G. (1993). On the mechanism of inhibition of K_{ATP} channels by glibenclamide in rat ventricular myocytes. *J. Cardiovasc. Electrophysiol.*, **4**, 38–47.

SAKMANN, B. & TRUBE, G. (1984). Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea pig heart. *J. Physiol.*, **347**, 641–657.

SHEN, W.K., TUNG, R.T., MACHULDA, M.M. & KURACHI, Y. (1991). Essential role of nucleotide diphosphates in nicorandil-mediated activation of cardiac ATP-sensitive K^+ channel. A comparison with pinacidil and lemakalim. *Circ. Res.*, **69**, 1152–1158.

STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activated ATP-sensitive channels in arterial smooth muscle. *Science*, **245**, 177–180.

STURGESS, N.C., ASHFORD, M.L.J., COOK, D.L. & HALES, C.N. (1985). The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet*, **ii**, 474–475.

STURGESS, N.C., KOZLOWSKI, R.Z., CARRINGTON, C.A., HALES, C.N. & ASHFORD, M.L.J. (1988). Effects of Sulphonylureas and diazoxide on insulin secretion and nucleotide-sensitive channels in an insulin-secreting cell lines. *Br. J. Pharmacol.*, **95**, 83–94.

TAKANO, M., QIN, D. & NOMA, A. (1990). ATP-dependent decay and recovery of K^+ channels in guinea-pig cardiac myocytes. *Am. J. Physiol.*, **258**, H45–H50.

TRUBE, G. & HESCHELER, J. (1984). Inward-rectifying channel in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pflügers Arch.*, **407**, 178–184.

TUNG, R.T. & KURACHI, Y. (1991). On the mechanism of nucleotide diphosphate activation of the ATP-sensitive K^+ channel in ventricular cells of guinea-pig. *J. Physiol.*, **437**, 239–256.

VENKATESH, N., LAMP, S.T. & WEISS, J.N. (1991). Sulphonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ. Res.*, **69**, 623–637.

XU, X-P. & LEE, K.S. (1994). Characterization of the ATP-inhibited K^+ current in canine coronary smooth muscle cells. *Pflügers Arch.*, **427**, 110–120.

ZHANG, H-L. & BOLTON, T.B. (1995). Activation by intracellular GDP, metabolic inhibition and pinacidil of a glibenclamide-sensitive K-channel in smooth muscle cells of rat mesenteric artery. *Br. J. Pharmacol.*, **114**, 662–672.

ZUNKLER, B.J., LENZEN, S., MANNER, K., PANTEN, U. & TRUBE, G. (1988). Concentration-dependent effects of tolbutamide, meglitinide, glipizide, glibenclamide and diazoxide on ATP-regulated K^+ channels in pancreatic β-cells. *Naunyn-Schmied. Arch. Pharmacol.*, **337**, 225–230.

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