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Block of human aorta Kir6.1 by the vascular K_{ATP} channel inhibitor U37883A

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1 A human aorta cDNA library was screened at low stringency with a rat pancreatic Kir6.1 cDNA probe and a homologue of Kir6.1 (hKir6.1) was isolated and sequenced.

2 Metabolic poisoning of *Xenopus laevis* oocytes with sodium azide and application of the K^+ channel opener drug diazoxide induced K^+ channel currents in oocytes co-injected with cRNA for hKir6.1 and hamster sulphonylurea receptor (SUR1), but not in oocytes injected with water or cRNA for hKir6.1 or SUR1 alone.

3 K^+ channel currents due to hKir6.1 + SUR1 or mouse Kir6.2 + SUR1 were strongly inhibited by 1 μM glibenclamide. K^+-current carried by hKir6.1 + SUR1 was inhibited by the putative vascular-selective K_{ATP} channel inhibitor U37883A (IC_{50} 32 μM) whereas current carried by Kir6.2 + SUR1 or *Shaker* K^+ channels was unaffected.

4 The data support the hypothesis that hKir6.1 is a component of the vascular K_{ATP} channel, although the lower sensitivity of hKir6.1 + SUR1 to U37883A compared with native vascular tissues suggests the need for another factor or subunit. Furthermore, the data suggest that pharmacology of K_{ATP} channels can be determined by the pore-forming subunit as well as the sulphonylurea receptor and point to a molecular basis for the pharmacological distinction between vascular and pancreatic/cardiac K_{ATP} channels.

Keywords: ATP-sensitive K^+ channel; Kir6.1; Kir6.2; sulphonylurea receptor; *Shaker*; U37883A; artery

Abbreviations: DMSO, dimethylsulphoxide; hKir, human inward rectifier K^+ channel; K_{ATP}, ATP-sensitive K^+ channel; SUR, sulphonylurea receptor; U37883A, 4-morpholinecarboximidine-*N*-1-adamantyl-*N*′-cyclohexyl hydrochloride

Introduction

ATP-sensitive K^+ channels (K_{ATP} channels) are expressed in blood vessels of animals and humans where they act as a vasodilatory mechanism (reviewed by Quayle *et al*., 1997). The channels may be spontaneously active or inactive, they are stimulated by hypoxia or ischaemia, and may be stimulated or inhibited by agonists at G-protein-coupled receptors such as calcitonin-gene-related peptide receptors and endothelin receptors respectively (reviewed by Beech, 1997). Electrophysiological data suggest the presence of at least two subtypes of K_{ATP} channel in vascular smooth muscle cells (Beech *et al*., 1993; Zhang & Bolton, 1996; Beech, 1997). K_{ATP} channels are also expressed in endothelial cells where their opening may result in release of relaxant factors such as nitric oxide (Katnik & Adams, 1995; Langheinrich & Daut, 1997). The vascular K_{ATP} channels are a target for the antihypertensive and antianginal agents diazoxide and nicorandil (which activate K_{ATP} channels) and the antidiabetic sulphonylureas glibenclamide and tolbutamide (which potently inhibit some vascular K_{ATP} channels) (reviewed by Edwards & Weston, 1997). Although K_{ATP} channels are expressed widely throughout the body there are pharmacological differences between vascular and non-vascular K_{ATP} channels: for example, levcromakalim activates the vascular but not the pancreatic K_{ATP} channel, diazoxide activates the vascular but not the cardiac K_{ATP} channel, and the guanidine U37883A inhibits the vascular but not the pancreatic or the cardiac K_{ATP} channels (Meisheri *et al*., 1993; Ludens *et al*., 1995; reviewed by Edwards & Weston, 1993; 1997). Therefore, the possibility exists for selective pharmacological modulation of K_{ATP} channel subtypes. Further developments in subtype-selective K_{ATP} channel drugs may be aided by the study of cloned K_{ATP} channels subunits that can be investigated in isolation in expression systems and where the molecular sites of drug action can be elucidated.

K_{ATP} channels are formed by the co-assembly of two different proteins (one pore-forming and one regulatory subunit) that are each repeated four times within one channel, giving an octamer (reviewed by Aguilar-Bryan *et al*., 1998; Ashcroft & Gribble, 1998). Two different pore-forming subunits (Kir6.1 and Kir6.2) have been cloned from pancreas and two regulatory subunits (SUR1 and SUR2) have been cloned from pancreas and brain respectively. The Kir6.1 gene, unlike the Kir6.2 gene, has introns, although no splice variants have been reported (Inagaki *et al*., 1995a; Erginel-Unaltuna *et al*., 1998). The SUR genes have multiple introns and at least two splice variants of each are known (Aguilar-Bryan *et al*., 1998). In this study we cloned a pore-forming K_{ATP} channel subunit from human aorta. The hKir6.1 subunit was functionally expressed and characterized in terms of its requirement for co-expression with sulphonylurea receptor, K^+-selectivity, voltage-dependence and sensitivity to block by the putatively vascular-selective agent U37883A. Comparisons have been made with Kir6.2.

Methods

A human aorta cDNA library prepared in λDR2 bacteriophage vector was purchased from Clontech. The coding sequence (1.2 kb in size) of uKATP-1 (rat K_{ir}6.1; accession number

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D42145) was used as a probe to screen the library. The probe was labelled with ^{32}P-α-deoxy cytidine triphosphate using oligolabelling kit according to manufacturer's (Pharmacia) instructions. The library was screened using the radiolabelled probe under low stringency hybridization conditions (25% formamide, 0.75 M NaCl, 75 mM sodium citrate, 1 × Denhardt's, 20 mM sodium phosphate, pH 6.5, 0.1% sodium dodecyl sulphate, 0.1 mg ml^{-1} sonicated salmon sperm DNA, 10% dextran sulphate, and 1×10^6 c.p.m. ml^{-1} of probe at 42°C) followed by washing with a buffer containing 300 mM NaCl, 30 mM sodium citrate and 0.1% sodium dodecyl sulphate (pH 7.0) at 42°C for 1 h. After screening 360,000 plaques, two positively hybridizing clones were isolated. The positive cDNA clones were subcloned into pKS-bluescript (Strategene) after *in vivo* excision from λDR2. Sanger's dideoxy sequencing revealed two identical clones, a partial clone of 1.8 kb and a full-length clone of 2.5 kb. The longer clone was used for subsequent studies and is referred to as hK_{ir}6.1.

Mouse K_{ir}6.2 (mKir6.2; accession number D50581) and hamster SUR1 (accession number L40623) were gifts kindly provided by S. Seino (Division of Molecular Medicine, Center for Biomedical Science, Chiba University School of Medicine, Japan) and L. Aguilar-Bryan (Baylor College of Medicine, Houston, U.S.A.) respectively. *Shaker* (N6-46 deletion) was a gift from R.W. Aldrich (Stanford University, U.S.A.).

The hKir6.1, mKir6.2 and hamster SUR1 cDNAs were subcloned into pKS-globin vector, such that the cDNA fragments were flanked by the 5′ and 3′ untranslated regions of *Xenopus* globin sequence. cRNA from all three linearized pKS-globin cDNA constructs was prepared using the T7 Megascript kit (Ambion) according to manufacturer's instructions.

Xenopus laevis were killed by an over-dose of tricaine anaesthetic followed by destruction of the brain and spinal cord. Oocytes were surgically removed from the abdomen of *Xenopus laevis* and transferred into Ca^{2+} free Ringer's solution. Follicular layer cells were dissociated from the oocytes by incubating the oocytes in collagenase (Sigma type 1A) solution at 22°C for 2 h. Forceps were used to peel off residual follicular cells and the oocytes were stored in 24 well plates (NUNC) containing Barth's solution (pH 7.4) overnight. Oocytes were micro-injected with 50 nl of water or cRNA (20–25 ng) encoding: K_{ir}6.2 alone; SUR1 alone; hK_{ir}6.1 alone; K_{ir}6.2 and SUR1; hK_{ir}6.1 and SUR1; *Shaker* (N6-46). Injected oocytes were incubated in Barth's solution at 19°C and the medium was changed every 24 h.

Electrophysiological measurements were made between 24 and 96 h after micro-injecting water or cRNA species and using a GeneClamp 500 amplifier (Axon Instruments, U.S.A.). Recording electrodes were pulled from borosilicate tubing (Clark Electromedical, U.K.) and had resistances of 0.5-5 MΩ when filled with 3 M KCl solution. Current and voltage signals were digitized by a 1401 analogue-to-digital converter (Cambridge Electronic Design, U.K.) and stored on a PC.

The extracellular bathing solution during recordings was usually either Ringer's solution (mM): NaCl 115, KCl 2, $CaCl_2$ 1.8, HEPES 10, pH 7.4, or 90-K^+ solution (mM): KCl 90, $CaCl_2$ 1.8, HEPES 5, $MgCl_2$ 1, pH 7.4. When bath solutions with different K^+ concentrations are described the solution was based on the 90-K^+ solution and NaCl replaced KCl. Drug solutions were prepared as 1000 × stock solutions and diluted to 1 × prior to use. Glibenclamide (Sigma) and diazoxide (Sigma) stock solutions were prepared in dimethylsulphoxide (DMSO) while U37883A (Pharmacia & Upjohn) was prepared in deionized water. U37883A is 4-morpholine-carboximidine-*N*-1-adamantyl-*N*′-cyclohexyl hydrochloride (Figure 2D). Sodium azide (Sigma) was added directly to bath solution. When recording K_{ATP} currents, oocytes were perfused with 90 mM K^+ solution. During recordings, oocytes were placed in a 0.2-ml recording chamber through which solution flowed at about 2 ml min^{-1}.

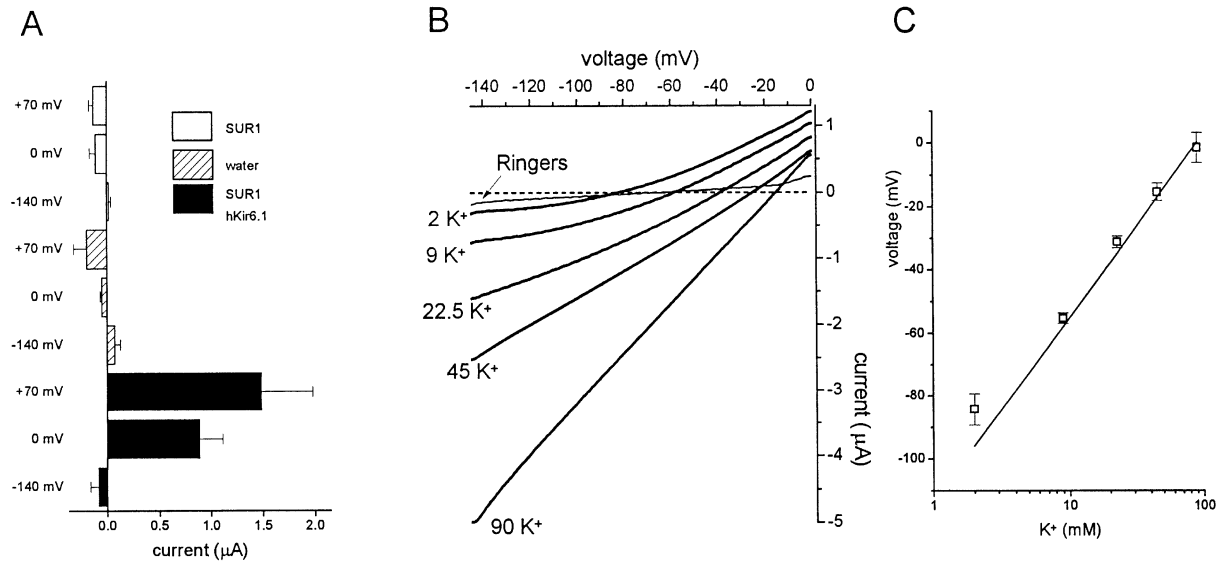

Figure 1 Co-expression of hKir6.1 and SUR1 produces functional K^+-selective channels in *Xenopus* oocytes. (A) Mean ± s.e.mean current amplitudes in response to the combined bath-application of 3 mM sodium azide and 0.2 mM diazoxide in Ringer's solution. All recordings were made from one batch of oocytes which had been injected 2–4 days previously with SUR1 cRNA (n = 5), water (n = 6), or SUR1 and hKir6.1 cRNAs (n = 27). Current amplitudes are shown for three different voltages (−140 mV, 0 mV and +70 mV) sampled during a ramp protocol. (B) Six current traces from one oocyte expressing hKir6.1 and SUR1, each during a ramp change in voltage from −150 mV to 0 mV. The five thicker traces were recorded after the response to 3 mM azide and 0.2 mM diazoxide and with 2, 9, 22.5, 45 and 90 mM K^+ in the bath solution. The record marked 'Ringers' was recorded in Ringer's solution and before azide and diazoxide were applied. (C) A plot of reversal potential against extracellular K^+ concentration. The open squares with error bars are the mean ± s.e.mean reversal potentials for azide- and diazoxide-induced currents in three oocytes expressing hKir6.1 and SUR1. The thick straight line is the Nernst K^+-equilibrium potential assuming an intracellular K^+ concentration of 90 mM.

Results

The open reading frame of hKir6.1 cDNA sequence is identical to that of KCNJ8 (accession number D50312) which was isolated from a human lung cDNA library (Inagaki *et al* 1995a), and 97.7% identical to the rat homologue uKATP-1 (Inagaki *et al* 1995b).

Micro-injection of hKir6.1 cRNA into oocytes did not induce basal current and current could not be induced by levcromakalim, diazoxide or metabolic poisoning with sodium azide, and there were no effects of glibenclamide or U37883A ($\geqslant 5$ recordings from each of 20 batches of oocytes; data not shown). In contrast, co-injection of cRNA for hKir6.1 and SUR1 led to the expression of a small basally-active voltage-independent K^+-current, and additional larger K^+-currents could be induced by bath-application of 3 mM sodium azide and further current was induced by the addition of 200 μM diazoxide ($n=5$) but not 10 μM levcromakalim ($n=3$) (data not shown). The induced K^+-currents were always abolished by the addition of 1 μM glibenclamide ($n=49$) (e.g. Figure 2). Figure 1A shows mean current amplitudes in response to the combined addition of 3 mM sodium azide and 200 μM diazoxide for a single batch of 38 oocytes from one animal. In water and SUR1-alone injected oocytes small inward deflections in the current records occurred at positive voltages. However, when hKir6.1 was included with SUR1 there were marked outward currents at 0 and +70 mV and slight inward currents at −140 mV. Therefore, K^+-current could be induced in oocytes co-injected with hKir6.1 and SUR1.

The K^+-selectivity of hKir6.1+SUR1 channels was confirmed by varying the extracellular K^+ concentration during continuous recordings from oocytes co-expressing hKir6.1 and SUR1 cRNAs. The reversal potential for azide- and diazoxide-induced current changed progressively from about −85 mV to about −10 mV upon raising the K^+ concentration from 2–90 mM (Figure 1B). Mean values for the reversal potential deviated from those predicted for a K^+-selective electrode only at low extracellular K^+ concentrations, consistent with there being high K^+-selectivity combined with a low permeability for Na^+ (Figure 1C).

Current carried by hKir6.1 and SUR1 channels was inhibited by U37883A or glibenclamide (Figure 2). Bath-application of U37883A (100 μM) led to at least 50% inhibition of azide and diazoxide-induced current at −30 mV whether there was a high concentration of K^+ in the bath solution and thus inward K^+-current (Figure 2A) or a low concentration of K^+ and thus outward K^+-current (Figure 2B). The block was removed upon wash-out of the U37883A from the bath solution. Glibenclamide was more potent and effective, abolishing the induced current (Figure 2A,B). Current-voltage relationships for U37883A- and glibenclamide-sensitive currents were essentially identical (Figure 2C). This suggests that both compounds blocked the same channel and that the action of U37883A was not voltage-dependent. Percentage block of current by 100 μM U37883A was not significantly different at −140 mV (block to 29.85±8.37% of the control current amplitude, $n=6$) compared with block at +70 mV (41.35±13.21% of control, $n=6$).

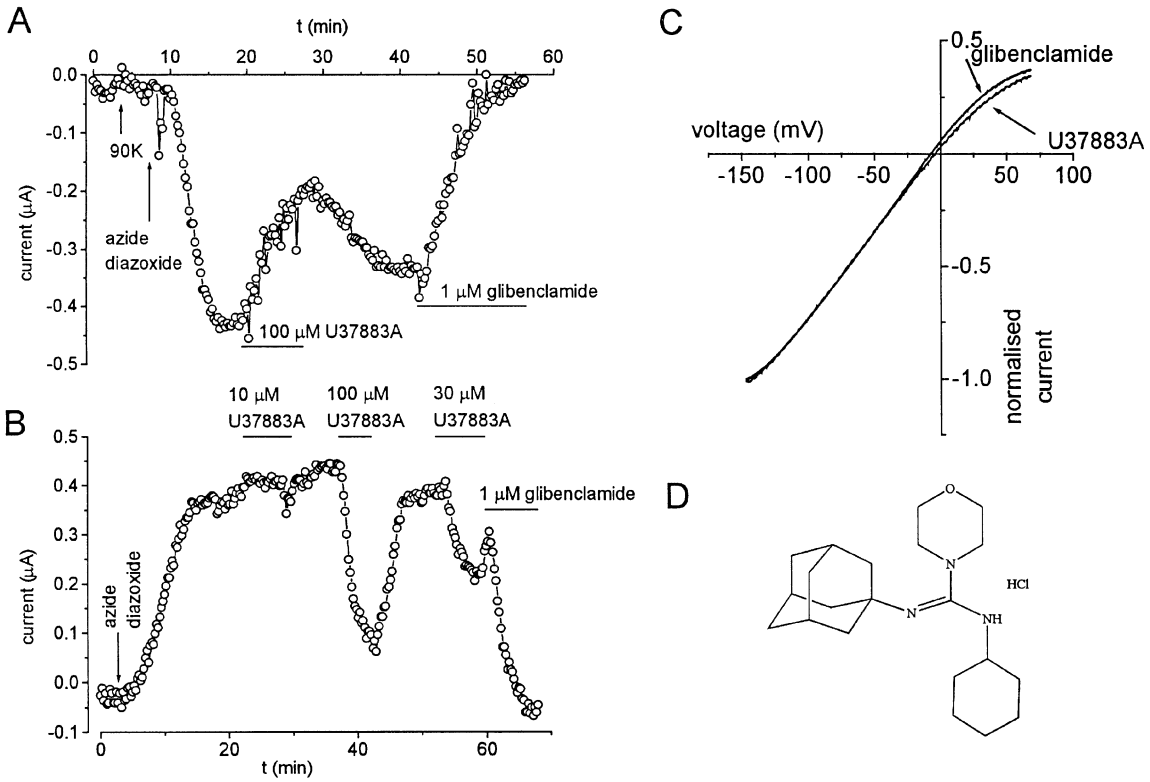

Figure 2 Inhibition of currents through hKir6.1/SUR1 channels by U37883A and glibenclamide. All recordings were made from oocytes expressing hKir6.1 and SUR1. (A) Plot of current amplitude at −30 mV. The bath solution was changed from Ringer's to 90 mM K^+ solution, and 3 mM sodium azide and 0.2 mM diazoxide were added to the bath as indicated by the vertical arrows. The horizontal solid bars indicate the addition of 100 μM U37883A and 1 μM glibenclamide to the bath solution. (B) Plot of current amplitude at −30 mV. The bath solution was the Ringer's solution throughout the experiment, and 3 mM sodium azide and 0.2 mM diazoxide were added to the bath as indicated by the vertical arrow. The horizontal solid bars indicate the addition of 10, 100, 30 μM U37883A and 1 μM glibenclamide to the bath solution. (C) Superimposed current-voltage relationships for currents taken from the experiment described in (A) and which were blocked by 100 μM U37883A or 1 μM glibenclamide. Currents were measured during a ramp change in voltage from −150 mV to +70 mV and the amplitudes have been normalized to the current size at −150 mV. (D) Structure of U37883A (4-morpholinecarboximidine-*N*-1-adamantyl-*N*′-cyclohexyl hydrochloride).

Co-expression of Kir6.2 with SUR1 also enabled the induction of K^+-current by azide and diazoxide. The currents were similar to those carried by hKir6.1+SUR1 channels although there was no evidence of basal K^+-current prior to the application of azide and diazoxide. Most strikingly, current carried by Kir6.2+SUR1 channels was essentially resistant to 100 μM U37883A (Figure 3A). In six out of ten recordings in Ringer's solution, 100 μM U37883A induced a small block of Kir6.2+SUR1 channels but on average the inhibition was less than 10% (Figure 3B). By contrast, currents carried by

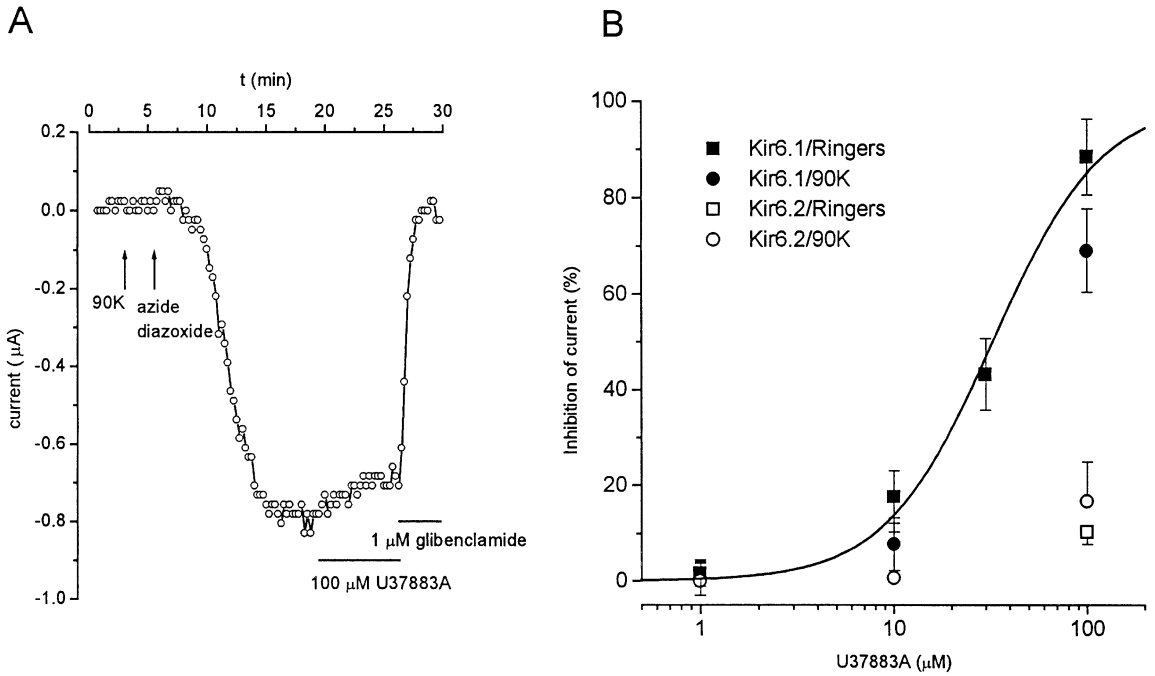

Figure 3 Lack of effect of U37883A on current carried by mKir6.2. Currents were recorded from oocytes expressing mKir6.2 and SUR1, or hKir6.1 and SUR1. (A) Plot of current amplitude at -30 mV in an oocyte expressing Kir6.2 and SUR1. The bath solution was changed from Ringer's to 90 mM K^+ solution, and 3 mM sodium azide and 0.2 mM diazoxide were added to the bath as indicated by the vertical arrows. The horizontal solid bars indicate the addition of 100 μM U37883A and 1 μM glibenclamide to the bath solution. (B) Mean$\pm$s.e.mean ($n=5-10$) percentage inhibition of azide- and diazoxide-induced current by various concentrations of bath-applied U37883A. All measurements were made at -30 mV. Oocytes expressing hKir6.1 and SUR1 were recorded from in Ringer's bath solution (filled squares) or 90 mM K^+ bath solution (filled circles). Oocytes expressing Kir6.2 and SUR1 were also recorded from in Ringer's (open squares) or 90 mM K^+ solution (open circles). If s.e.mean bars are not visible they are smaller than the symbol size. The smooth curve is the Hill equation fitted to the hKir6.1/Ringer's data points. It has a slope of 1.6 and 50% inhibition occurs at 32 μM U37883A.

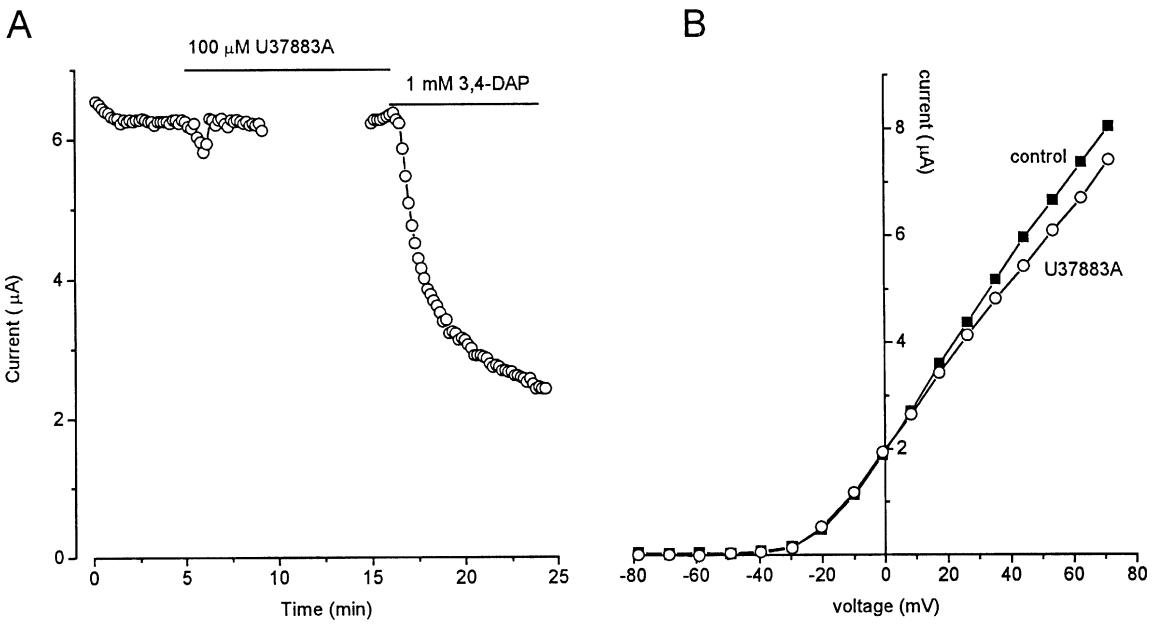

Figure 4 Lack of effect of U37883A on a voltage-gated K^+ channel. Current was recorded in Ringer's solution from an oocyte 2 days after injection of *Shaker* (N6-46) cRNA. (A) Plot of current amplitude elicited by a 500-ms square voltage step to +60 mV applied every 10 s from a holding potential of 80 mV. 100 μM U37883A and then 1 mM 3,4-diaminopyridine (3,4-DAP) were bath-applied as indicated. The break in the experiment is when a current-voltage relationship was determined for the *Shaker* channels (B). The control current-voltage relationship was sampled immediately prior to the beginning of the trace shown in (A). Leakage current was not subtracted.

hKir6.1 + SUR1 were always inhibited and the IC_{50} derived from the mean data was 32 μM (Figure 3B).

Selectivity of U37883A for Kir6.1 + SUR1 channels was further established by testing the effect of U37883A on a voltage-gated delayed rectifier K^+ channel, *Shaker* (N6-46), which had part of the N-terminus deleted to prevent fast inactivation. 3,4-Diaminopyridine (1 mM) inhibited but U37883A (100 μM) had no effect on current through *Shaker* (N6-46) channels ($n = 3$) (e.g. Figure 4).

Discussion

A K_{ATP} channel subunit has been cloned from a human aorta cDNA library. The clone belongs to the Kir6.1 family of inward rectifiers and is referred to as hKir6.1. The study is the first description of functional expression of the human homologue of Kir6.1. hKir6.1 does not form functional plasma membrane K^+ channels when expressed in isolation in *Xenopus* oocytes. However, when expressed together with a sulphonylurea receptor (SUR1) hKir6.1 forms metabolically-sensitive, mildly inwardly-rectifying, K^+-selective channels that are diazoxide and glibenclamide-sensitive. The putatively vascular-selective K_{ATP} channel inhibitor U37883A blocked currents carried by hKir6.1 + SUR1 channels but not Kir6.2 + SUR1 or *Shaker* (N6-46) channels. This suggests that U37883A is selective for some isoforms of K_{ATP} channel and that the binding site may be on the Kir6.1 protein or that the Kir6.1 protein acts allosterically to promote drug binding to SUR1.

Some investigators have observed that rat or mouse Kir6.1 can form functional plasma membrane K^+ channels when expressed in the absence of exogenous sulphonlyurea receptor in various expression systems including HEK293 cells (Inagaki *et al*., 1995b; Ämmälä *et al*., 1996), NIH3T3 cells (Ishida-Takahashi *et al*., 1998) and *Xenopus laevis* oocytes (Inagaki *et al*., 1995b). However, Yamada *et al*. (1997) did not observe any channel activity of mouse Kir6.1 expressed alone in HEK293T cells, and in >20 batches of oocytes we have not observed any function of human Kir6.1 expressed alone. Kir6.1 is not activated by K^+ channel opener drugs (e.g. pinacidil) nor is it sulphonylurea-sensitive in the absence of sulphonylurea receptor (Ämmälä *et al*., 1996; Ishida-Takahashi *et al*., 1998). Therefore, it is difficult to distinguish expressed Kir6.1 from endogenous background K^+ channels in the expression system and it seems plausible that results of previous studies are not proof that rat and mouse Kir6.1 can form independent channels, and thus are fundamentally different from human Kir6.1. Furthermore, it cannot yet be excluded that under some culture conditions and in some expression systems auxiliary proteins may be present that enable Kir6.1 to traffic to the plasma membrane or to form an active channel once in the plasma membrane. Whatever the reason for the lack of independent expression of hKir6.1 the fact has prevented us from testing of the effect of U37883A in the absence of sulphonylurea receptor.

There are ten amino acid differences between human and rat Kir6.1. These occur at positions 108–110 and 118, which are predicted to be extracellular and between the first membrane-spanning domain and the K^+-selectivity filter, and 246, 286, 348, 396, 421 and 422, which are on the C-terminal domain, which is predicted to be intracellular. In three of these positions (110, 286 and 396) the amino acid residues in the human sequence are the same as those in mouse Kir6.1. The most obvious differences between human and rat/mouse sequences are: serine in place of glycine (position 108); threonine in place of alanine (118); leucine in place of glutamine (246); and asparagine and threonine in place of cysteine and proline (421 and 422). If there is any functional significance to these differences it is not currently known.

Kir6.1 initially appeared to be fairly ubiquitously distributed in the rat, and this led to the naming of the rat cDNA clone as uKATP-1. However, Kir6.1 has been found to be absent from endothelial cell-lines, many endocrine clonal cell lines and some neurones (Inagaki *et al*., 1995b; Karschin *et al*., 1998). Therefore, although we have cloned hKir6.1 from a human aorta cDNA library and have not investigated the cell type from which the mRNA originated, it seems probable that the origin was vascular smooth muscle cells which are the major cell type in the aorta.

Guillemare *et al*. (1994) observed potent inhibition of P1060-induced K^+-current in folliculated *Xenopus* oocytes. IC_{50} values for inhibition of the current by U37883A and glibenclamide were 0.26 μM and 0.3 μM respectively. Similarly, the IC_{50} for U37883A-induced inhibition of pinacidil-induced relaxation of rabbit mesenteric artery was about 0.8 μM (Khan *et al*., 1997). Therefore, although Kir6.1 + SUR1 channels were blocked by U37883A, the concentrations required were comparatively high (IC_{50} 32 μM). Interestingly, Ca^{2+} antagonists are less potent blockers of cloned Ca^{2+} channels expressed in *Xenopus* oocytes compared with mammalian cell-lines (e.g. Grabner *et al*., 1996 *c.f*. Hockerman *et al*., 1997) and thus it is conceivable that the low potency of U37883A is simply a feature of the expression system. Alternatively, a factor or subunit that is present in arteries and follicular cells of oocytes but not defolliculated oocytes may be required for potent block by U37883A.

The data suggest that a difference between the sequences of Kir6.1 and Kir6.2 affects sensitivity to U37883A. The major difference between the two proteins exists at the distal section of the C-terminal domain, which is predicted to be intracellular and may be involved in the interaction between Kir6 and SUR (Aguilar-Bryan *et al*., 1998; Takano *et al*., 1998). Alternatively, there is a section of almost 20 amino acids (starting at position 101 in Kir6.1) that is present in human, rat and mouse Kir6.1 sequences but absent in human, rat and mouse Kir6.2. Although, it is not known that U37883A binds directly to Kir6.1, the latter sequence is an attractive possibility for a U37883A binding site because of its location at the extracellular lip of the channel's K^+ pore and considering the fact that U37883A is water soluble and perhaps membrane impermeant. Two of the species differences between Kir6.1 sequences also exist in this section of amino acids.

There has been debate over whether Kir6.1 normally functions as a plasma membrane channel or a mitochondrial membrane channel (Aguilar-Bryan *et al*., 1998; Takano *et al*., 1998). Biochemical data suggest the presence of the Kir6.1 protein in mitochondrial and plasma membranes, raising the possibility of a dual function (Suzuki *et al*., 1997). Electrophysiological data clearly show that Kir6.1 can be a functional plasma membrane K^+ channel when sulphonylurea receptor is expressed in a foreign expression cell system. Although it is not established whether Kir6.1 forms an essential part of a native K^+ channel there are strong indications that Kir6.1 forms part of a plasma membrane K_{ATP} channel subtype in vascular smooth muscle cells. There are similarities between the properties of expressed Kir6.1 + SUR2B/SUR1 channels (Yamada *et al*., 1997; Satoh *et al*., 1998; Takano *et al*., 1998) and the native K_{NDP} channels of rabbit portal vein smooth muscle cells (Beech *et al*., 1993). Characteristically, the channels both have a small unitary conductance, fail to open simply in the absence of ATP, are stimulated to open by Mg^{2+}/

nucleoside diphosphate complexes, and are relatively resistant to inhibition by ATP. A notable difference is that Kir6.1 + SUR2B channels are not as sensitive to inhibition by glibenclamide. Because SUR1s are more sensitive to glibenclamide than SUR2s it may be that an SUR1 variant is part of the rabbit portal vein K_{NDP} channel. The observation in this study that Kir6.1 is in some way involved with the sensitivity of K_{ATP} channels to U37883A supports the argument that the K_{NDP} subtype of vascular K_{ATP} channels contains Kir6.1.

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