British Journal of Pharmacology (1999) 128, 1636–1642
http://www.stockton-press.co.uk/bjp

Functional and electrophysiological effects of a novel imidazoline-based K_{ATP} channel blocker, IMID-4F

*,[1]Grant A. McPherson, [1]Karen L. Bell, [1]Joanne L. Favaloro, [2]Masahiro Kubo & [2]Nicholas B. Standen

[1]Department of Pharmacology, Monash University, Clayton, Victoria 3168, Australia and [2]Department of Cell Physiology and Pharmacology, University of Leicester, Leicester, LE1 9HN

1 The functional and electrophysiological effects of IMID-4F (2-[*N*-(2,6-dichlorophenyl)-*N*-(4-flurorobenzyl)amino]imidazoline), a fluoro-benzyl derivative of clonidine, on vascular K_{ATP} channels were investigated. In pig coronary artery, IMID-4F inhibited the vasorelaxation response to the K_{ATP} channel opener levcromakalim with a pK_B value of approximately 7.1. IMID-4F (30 μM) did not affect the vasorelaxation response to sodium nitroprusside (SNP).

2 In rat mesenteric artery smooth muscle cells IMID-4F (1–10 μM) caused a concentration-dependent depolarization of membrane potential. IMID-4F (10 μM) abolished the hyperpolarizing effects of levcromakalim (10 μM).

3 In patch clamp experiments using rat mesenteric artery smooth muscle cells, K_{ATP} channel currents induced by levcromakalim (10 μM) were inhibited by IMID-4F (0.3–3 μM) in a concentration-dependent manner. The calculated IC_{50} for IMID-4F inhibiting K_{ATP} channel current was approximately 0.8 μM.

4 Radioligand binding studies using bovine aortic smooth muscle cell membranes showed that IMID-4F (30 μM) did not displace binding to the K_{ATP} channel opener [^{3}H]-P1075. However, both levcromakalim (10 μM) and glibenclamide (10 μM) caused significant displacement of [^{3}H]-P1075.

5 These studies show that the imidazoline compound IMID-4F is one of the most potent antagonists of arterial K_{ATP} channels identified. Vasorelaxation, hyperpolarization and K^+ currents through K_{ATP} channels were all inhibited by IMID-4F at micromolar concentrations. Radioligand binding studies indicate that IMID-4F does not bind to the same site as levcromakalim or as glibenclamide. Considering other evidence, it is likely that IMID-4F acts by interacting directly with the pore of the K_{IR} channel, rather than through the sulphonylurea subunit of the K_{ATP} channel complex.

Keywords: Imidazolines; levcromakalim; smooth muscle relaxation; K_{ATP} channels; K_{ATP} channel antagonists; pig coronary artery; rat mesenteric artery

Abbreviations: SNP, sodium nitroprusside; IMID-4F, (2-[*N*-(2,6-dichlorophenyl)-*N*-(4-flurorobenzyl)amino]imidazoline)

Introduction

Although first identified in cardiac muscle (Noma, 1983), K_{ATP} channels have been best characterized in the pancreatic β-cell where they play an important role in the regulation of insulin secretion (Ashcroft & Ashcroft, 1990). Apart from cardiac tissue, K_{ATP} channels have also been identified in skeletal muscle as well as in both vascular and non-vascular smooth muscle (Quayle *et al*., 1997). The K_{ATP} channel has recently been shown to consist of two structural subunits, a sulphonylurea receptor (SUR) and an inward rectifying channel (K_{IR}) (Inagaki *et al*., 1995, Aguilar-Bryan *et al*., 1995). More recent work has indicated that the vascular K_{ATP} channel is likely to be a complex of SUR2B and K_{IR}6.1 (Yamada *et al*., 1997). The interest in K_{ATP} channels has been fuelled by the identification of synthetic agents (K_{ATP} channel openers) that exert their effect by opening K_{ATP} channels in a variety of tissues (Challinor-Rogers & McPherson, 1994). This mechanism of action of K_{ATP} channel openers has been established primarily through the finding that sulphonylureas such as glibenclamide (a potent and selective K_{ATP} channel antagonist in the pancreas (Ashford, 1990)), are able to antagonize the functional and electrophysiological actions of the K_{ATP} channel openers in vascular and non-vascular smooth muscle.

*Author for correspondence:
E-mail: grant.mcpherson@med.monash.edu.au

Apart from the sulphonylureas, a number of chemically unrelated groups of compounds have also been shown to antagonize the smooth muscle relaxant actions of K_{ATP} channel openers in vascular and non-vascular smooth muscle (Challinor-Rogers & McPherson, 1994). In 1989 we showed that imidazolines such as alinidine, and chemically related compounds including phentolamine, were able to antagonize the functional and electrophysiological actions of K_{ATP} channel openers, including levcromakalim, in vascular and non-vascular smooth muscle (McPherson & Angus 1989; 1990; Challinor-Rogers *et al*., 1994; see Challinor-Rogers & McPherson, 1994). Subsequent studies have shown that phentolamine and other imidazolines also inhibit K_{ATP} channels in the pancreatic β-cell (Dunne *et al*., 1995), although the pA_2 or pK_B values calculated for these imidazoline compounds have shown them to be some 30–100 times less potent than glibenclamide itself.

There is currently a great deal of interest in compounds that antagonize K_{ATP} channels because they may have important therapeutic uses in the treatment of a number of pathological conditions including cardiac arrhythmia and diabetes mellitus (type II). In addition, novel K_{ATP} channel blockers may prove to be useful tools for studying vascular smooth muscle physiology. This paper presents evidence that the imidazoline-based compound IMID-4F, potently blocks vascular K_{ATP} channels.

Methods

Porcine coronary artery

Porcine hearts were obtained from freshly killed pigs at an abattoir. The right circumflex artery was rapidly removed and placed in ice-cold physiological Kreb's solution composition (mM): NaCl 119, KCl 4.7, $MgSO_4.7H_2O$ 1.17, KH_2PO_4 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11. The artery was cut into 4 mm long segments and each segment was suspended on stainless steel hooks, connected to a force transducer and bathed in Kreb's solution at 37°C in 25 ml jacketed glass organ baths. The tissue was continuously gassed with 5% CO_2 in O_2. Changes in isometric force were recorded *via* a Grass FT03C force transducer. Vessels were left to equilibrate under zero force for 30 min then an initial force of 5 *g* was applied. After another 30 min, the force was re-adjusted to 5 *g* and the tissues were left for a further 30 min. Subsequently, a potassium depolarizing solution, composition (mM): KCl 123, $MgSO_4.7H_2O$ 1.17, KH_2PO_4. 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11, was added. This response was used to determine the maximum constrictor response of the tissue. The vessels were then washed and left until the response returned to the initial baseline, before commencing the experiment.

After this period each coronary ring was submaximally (approximately 60%) constricted with the thromboxane-mimetic U46619 (10–30 nM), then a cumulative concentration-response curve to levcromakalim (10 nM–30 μM) (or in some cases sodium nitroprusside; 1 nM–30 μM), was then constructed (0.5 log increments). Only one concentration-response curve was obtained on any one coronary artery ring. Each concentration of IMID-4F to be tested was added 20 min prior to submaximally constricting the tissue with U46619. Preliminary experiments indicated that this incubation period was sufficient for equilibrium antagonism to be produced.

Electrophysiological studies on the rat mesenteric artery

Wistar Kyoto rats were killed by CO_2 asphyxia. The mesentery was rapidly removed and placed in ice cold Kreb's solution. A segment of rat small mesenteric artery, 2 mm in length, corresponding to a third or fourth order branch from the superior mesenteric artery, was mounted in a small vessel myograph as previously described (McPherson, 1992). Briefly, two 40 μm wires were threaded through the lumen of the vessel segment. One wire was attached to a stationary support driven by a micrometer, while the other was attached to an isometric force transducer. Vessels were allowed to equilibrate under zero force for 30 min. Using the diameter of the vessel, calculated from the distance between the two mounting wires, a passive diameter-tension curve was constructed as previously described (McPherson, 1992). From this curve the effective transmural pressure was calculated. The vessel was set at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg. Vessel diameters at an equivalent transmural pressure of 100 mmHg (D_{100}) were approximately 300 μm in diameter.

The intracellular membrane potential of a single smooth muscle cell was monitored in the same vessel described above. A conventional glass electrode (1.5 mm blanks, World Precision Instruments Inc., U.S.A.) filled with 0.5 M KCl (tip resistance approximately 80–100 MΩ) was used to impale a single smooth muscle cell. The microelectrode was positioned by a Burleigh Inchworm motor driven by a 6000 series controller (Burleigh, U.S.A.). The microelectrode was advanced using 0.5 μm steps until a stable impalement was achieved. The bath (15 ml volume) containing the vessel was part of a 25 ml recirculating system that contained a jacketed organ bath, where the Kreb's solution was warmed to 37°C and oxygenated, and where drugs could be added. This design allowed cumulative steady-state concentration-effect curves to levcromakalim in the presence and absence of IMID-4F to be constructed. Whole cell membrane potential data were captured by the use of the data acquisition system (CVMS Version 2.0, World Precision Instruments, U.S.A.).

Study of ATP-sensitive K^+ currents in the rat mesenteric artery

K_{ATP} currents in vascular smooth muscle were studied in cells from the rat small mesenteric artery as previously described (Kubo *et al*., 1997). Male Wistar Kyoto rats (200–400 g) were killed by stunning followed by cervical dislocation. Mesenteric arteries were removed and placed in an ice-cold buffer, composition (mM): NaCl 136, KCl 5.6, Na_2HPO_4 0.42, NaH_2PO_4 0.44, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, HEPES 10, pH 7.4 with NaOH. Isolated rat mesenteric artery myocytes were obtained using a method described previously (see Kubo *et al*., 1997). Cells were stored at 4°C and used within 8 h.

The effects of externally applied IMID-4F on ATP-dependent K^+ (K_{ATP}) currents were examined on the rat mesenteric artery myocyte using the conventional whole cell configuration of the patch clamp technique (Kubo *et al*., 1997). Two bath (external) solutions were used during the course of the experiment. The first, containing 6 mM K^+, contained (mM): NaCl 134, KCl 6, $MgCl_2$ 1, CaCl2 0.1 and HEPES 10, pH 7.2. A high K^+ solution (140 mM K^+) was also used and was identical to this except all NaCl was replaced with KCl. The pipette (intracellular) solution contained (mM): KCl 110, KOH 30, HEPES 10, EGTA 10, $MgCl_2$ 1, $CaCl_2$ 0.1, Na_2ATP 0.1, NaADP 0.1, GTP 0.2, pH 7.2.

In these experiments K_{ATP} currents were induced by first changing the external bathing solution from 6–140 mM KCl followed by the addition of levcromakalim (10 μM). Under these circumstances a glibenclamide sensitive current develops (Kubo *et al*., 1997). The effect of IMID-4F was then assessed by adding increasing concentrations of this compound and assessing the level of reversal of levcromakalim induced current produced.

Currents were recorded with an Axopatch 200A amplifier at a bandwidth of 10 kHz, and stored on DAT tape, or onto the hard disk of a microcomputer after digitization with an Axon Instruments A–D interface. Records were filtered at 2 kHz for display. Patch clamp recordings were made at room temperature, 18–22°C.

Radioligand binding studies

Bovine aorta smooth muscle cell membranes were prepared by adapting the method of Carman-Krzan *et al*. (1997) and binding experiments were carried out. Bovine aortae were obtained from the local abattoir and stored in ice-cold storage buffer composition (mM): NaCl 139, KCl 5, $MgCl_2$ 1.2, HEPES (4-(2-hydroxyethyl)-1-piperazine-ethane-sulphonic acid) 5, EGTA (ethylene glycol-bis-(2-aminoethylether)-N.N.N′,N′-tetra-acetic acid) 1; pH = 7.4). The aortae were then cleared of fat and connective tissue and the smooth muscle dissected from the inner elastic layer. The smooth muscle was then minced in 3 vol g^{-1} (wet weight) of the mincing buffer, composition (mM): HEPES 10, EGTA 1, phenylmethylsulphonyl fluoride (PMSF) 0.2, pepstatin A 0.2, leupeptin 10, soybean trypsin inhibitor (10 $\mu g\ ml^{-1}$) and

homogenized. The homogenate was then sieved through a fine nylon mesh, then centrifuged at $100 \times g$ for 10 min at 4°C. The supernatant was aspirated and further centrifuged at $4.8 \times 10^4 \times g$ for 15 min at 4°C. The resulting pellet was then suspended in approximately 20 volumes of HEPES buffer composition (mM): NaCl 139, KCl 5, $MgCl_2$ 2, HEPES 20, (pH = 7.4), and stored at −80°C.

Drug displacement experiments were carried out using the method of Löffler-Walz & Quast (1998). The membrane preparation (0.2–0.5 mg ml^{-1} protein) was incubated with [^{3}H]-P1075 (1 nM), and the cold displacing drug, levcromakalim (10 μM), glibenclamide (10 μM) or IMID-4F (30 μM) in a total volume of 1 ml in incubation buffer, composition (mM): NaCl 139, KCl 5, $MgCl_2$ 25, $CaCl_2$ 1.25, HEPES 20, pH = 7.4, which included an ATP-regenerating system (creatine phosphate 20 mM, Na_2ATP 3 mM, and creatine phosphokinase 50 U ml^{-1}) at 37°C for 30 min. The ATP-regenerating system was included as it has previously been shown that both MgATP and an ATP-regenerating system are required to obtain stable binding to [^{3}H]-P1075 (Löffler-Walz & Quast, 1998). The incubation was stopped by adding 4 ml of ice-cold quench solution, composition (mM): NaCl 154, Tris 50; pH = 7.4. The bound and free ligand were then separated by filtration under vacuum over glass microfibre filters (Whatman GF/B). The filters were washed twice with 5 ml of quench solution and counted for [^{3}H] in 2.5 ml scintillant (EcoLite, ICN Biomedicals, U.S.A.).

Analysis of results

Porcine coronary artery The contraction to U46619 (10–30 nM) was taken as 100% response and relaxation produced by different concentrations of levcromakalim expressed as a percentage of this response. The percentages obtained were then represented graphically and the pD_2 ($-\log EC_{50}$) value calculated as the concentration of relaxant required to cause 50% of the maximal relaxant response.

It was observed that at high concentrations, IMID-4F (10–30 μM) shifted the levcromakalim concentration-response curve in a non-parallel fashion. Also, the slope of the curve was reduced and the maximum response to levcromakalim appeared to be depressed. This suggested that the type of antagonism displayed by IMID-4F was non-competitive, preventing us from the use of Schild analysis (Jenkinson, 1991) as a means of determining the potency of the active ions. Instead, an 'apparent pK_B' was estimated, based on a single concentration of antagonist.

The following equation was used:

$$\text{Apparent } pK_B = -\text{Log}([\text{Antagonist Conc., M}]/[\text{Concentration ratio} - 1]) \quad (1)$$

The single concentration of IMID-4F used to calculate the apparent pK_B was 1 μM and selected on the basis that it produced a parallel-like shift in the levcromakalim concentration-response curve without overtly affecting the maximal levcromakalim response.

Statistics

Results are given as mean ± s.e.mean. Statistical analysis was carried out as stated in the text, but in general groups of two were analysed using Student's *t*-test and groups of more than two by Analysis of Variance (ANOVA), with *post hoc* comparisons by the Student-Newman-Keuls test. All statistical calculations were performed using SigmaStat (Jandel Scientific, U.S.A.).

Drugs

Drugs used and their sources were: levcromakalim (Beecham, U.K.); sodium nitroprusside (Sigma Chemical Co., U.S.A.); U46619-methyl acetate solution (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$, Cayman Chemicals, U.S.A.); IMID-4F base (2-[*N*-(2,6-dichlorophenyl)-*N*-(4-flurorobenzyl) amino]imidazoline) was synthesized by Eva Campi at the Department of Chemistry, Monash University. Figure 1 shows the structure of IMID-4F. Levcromakalim and IMID-4F were made up in 100% methanol, SNP was dissolved in H_2O. All drugs were then diluted in Kreb's solution immediately prior to use.

For the radioligand binding studies: [^{3}H]-P1075 (specific activity 119 Ci $mmol^{-1}$) was obtained from Amersham (U.S.A.). Pepstatin A, leupeptin, soybean trypsin inhibitor, phenylmethysulphonylfluoride, creatine phosphokinase, creatine phosphate and glibenclamide were obtained from the Sigma Chemical Co. (U.S.A.) and Na_2ATP was from ICN Biomedicals (U.S.A.).

Results

Antagonism of levcromakalim responses by IMID-4F

In pig coronary artery, constricted with U46619, IMID-4F antagonized the vasorelaxant response to levcromakalim in a concentration-dependent manner. Figure 2a shows mean concentration-effect curves for levcromakalim in the absence and presence of increasing concentrations of IMID-4F. As noted earlier, the type of antagonism displayed by this compound is non-competitive in nature in that the concentration-effect curves to levcromakalim are shifted to the right in a non-parallel fashion with suppression of the maximal response. Based on the right-ward shift in the levcromakalim concentration-effect curve the calculated pK_B

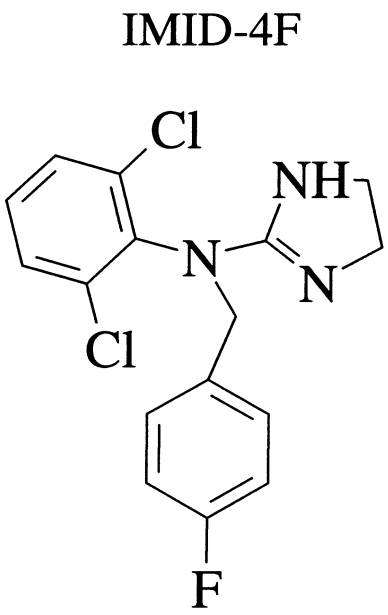

Figure 1 The chemical structure of IMID-4F used in the present study.

for IMID-4F (calculated at 1 μM) was 7.10 ± 0.10 ($n=4$). IMID-4F did not affect the vasoconstrictor response to U46619 (as expressed as a percentage of the maximum tissue response to the potassium depolarizing solution; Figure 2b), at concentrations up to 30 μM. Figure 2c shows that IMID-4F (30 mM) had no effect on the pD_2 value for vasorelaxation elicited by SNP (10–30 μM). Relaxation to SNP (pD_2): control: 5.93 ± 0.3, plus IMID-4F (30 μM): 5.63 ± 0.20, $n=4$, $P>0.05$, Student's t-test).

Electrophysiological actions of IMID-4F on the rat small mesenteric artery

Smooth muscle cells of the rat small mesenteric artery ($D_{100} = 320 \pm 22$ μm, $n=3$) had a resting membrane potential of -60 ± 1 mV ($n=3$ cells, 3 different vessels). Figure 3a,b show that IMID-4F (0.1–10 μM) caused a significant concentration-dependent membrane depolarization of approximately 9 mV (membrane potential: control -61.0 ± 1.8;

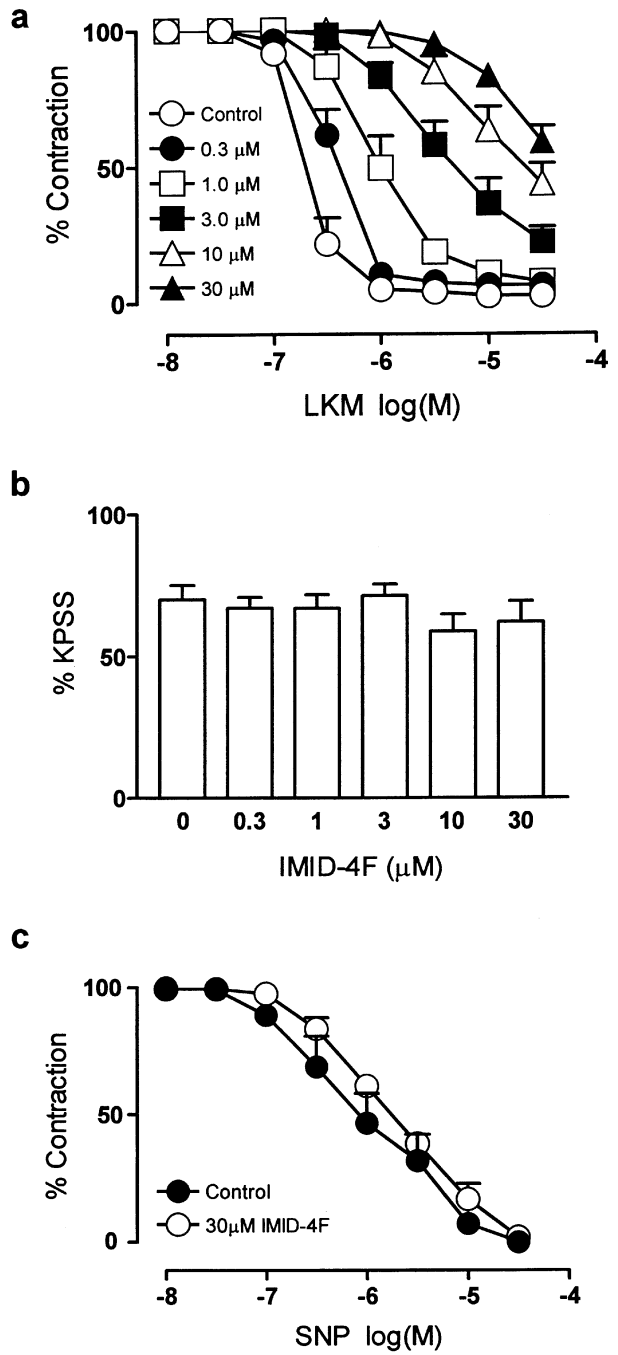

Figure 2 (a) Mean levcromakalim concentration-response curves obtained in the isolated pig coronary artery in the absence and in the presence of IMID-4F (0.3–30 μM, $n=12$). (b) Effect of IMID-4F (0.3–30 μM) on the vasoconstrictor response to U46619 expressed as a percentage of the response to KPSS ($n=12$). (c) Mean sodium nitroprusside (SNP) concentration-response curves obtained in the isolated pig coronary artery in the absence and presence of IMID-4F 30 μM ($n=4$). Data are presented as mean $\pm$ s.e.mean.

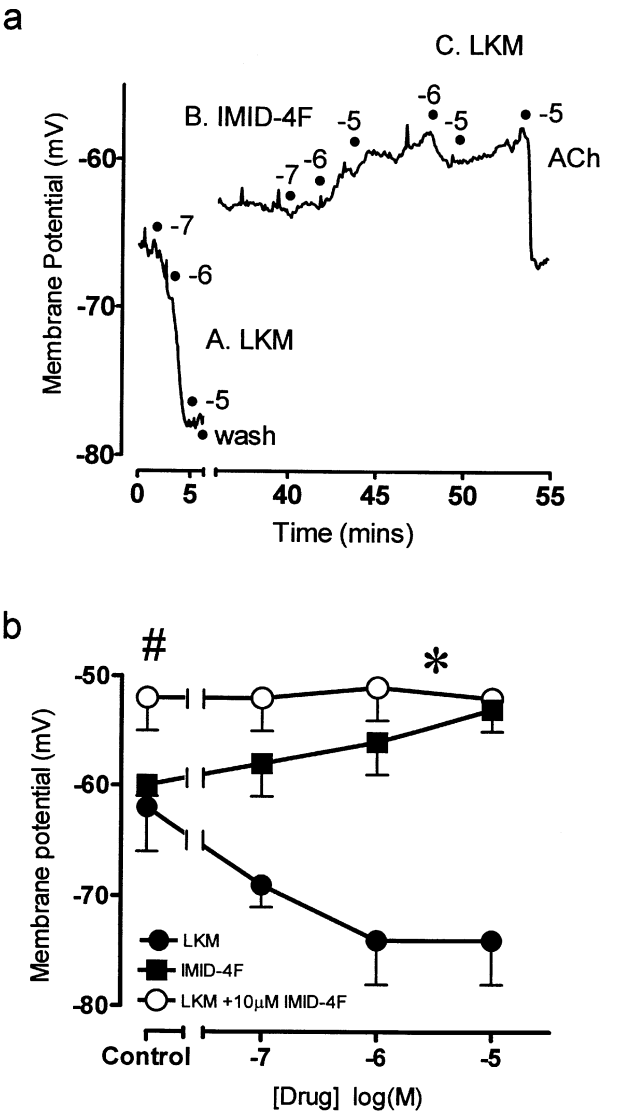

Figure 3 Electrophysiological effects of IMID-4F on membrane potential in the rat small mesenteric artery. (a) Representative trace obtained in a single vessel showing: (A) The effect of levcromakalim (0.1–10 μM) alone. (B) The effect of IMID-4F (0.1–10 μM) alone. (C) The effect of levcromakalim (1 and 10 μM) in the presence of the highest concentration of IMID-4F (10 μM). Acetylcholine (ACh, 10 μM), an agent that causes hyperpolarization through mechanisms other than K_{ATP} channel opening, still hyperpolarized membrane potential. Concentrations are given as log (M). (b) Mean concentration-effect curves obtained to levcromakalim alone (0.1–10 μM), IMID-4F alone (0.1–10 μM) and levcromakalim (0.1–10 μM) obtained in the presence of IMID-4F (10 μM). Data is presented as mean $\pm$ s.e.mean. (#Resting membrane potential is significantly more positive in the presence of IMID-4F (10 μM), $P<0.05$, Student's t-test; *levcromakalim response is significantly inhibited in the presence of IMID-4F 10 μM, $P<0.05$, two-way repeated measures ANOVA, $n=3$).

plus IMID-4F (10 μM) -52.7 ± 1.9, $n=6$, $P<0.05$, Student's unpaired t-test.).

Responses to levcromakalim (0.1 – 10 μM) were obtained in the absence and presence of IMID-4F (10 μM). In the absence of IMID-4F, levcromakalim caused a concentration-dependent hyperpolarization of the resting membrane potential from -62 ± 4 mV to -74 ± 4 mV ($n=3$ cells, three different vessels); a hyperpolarization of approximately 12 mV. This hyperpolarization to levcromakalim (up to 10 μM) was completely inhibited by IMID-4F (10 μM) ($P<0.05$, 2-way repeated measures ANOVA, Figure 3b).

Effects of IMID-4F on K_{ATP} channel currents

The ability of IMID-4F (0.3 and 3 μM) to reverse levcromakalim (10 μM) induced current was assessed in rat mesenteric myocytes. When held at a potential of -60 mV and in the presence of high K^+ (140 mM) the basal inward current was -25 ± 10 pA ($n=3$). After the addition of levcromakalim (10 μM) the inward current increased to -124 ± 50 pA ($n=3$). This levcromakalim-induced inward current was rapidly inhibited by IMID-4F in a concentration-dependent manner: Figure 4a shows a typical recording. In three cells, the currents observed in the presence of 0.3 μM and 3 μM IMID-4F respectively were -104 ± 42 and -33 ± 10 pA. The concentration-response curve for inhibition is shown in Figure 4b, and the pIC_{50} for IMID-4F against K_{ATP} current was found to be 6.22 ± 0.01 ($n=3$), corresponding to an IC_{50} of approximately 0.8 μM.

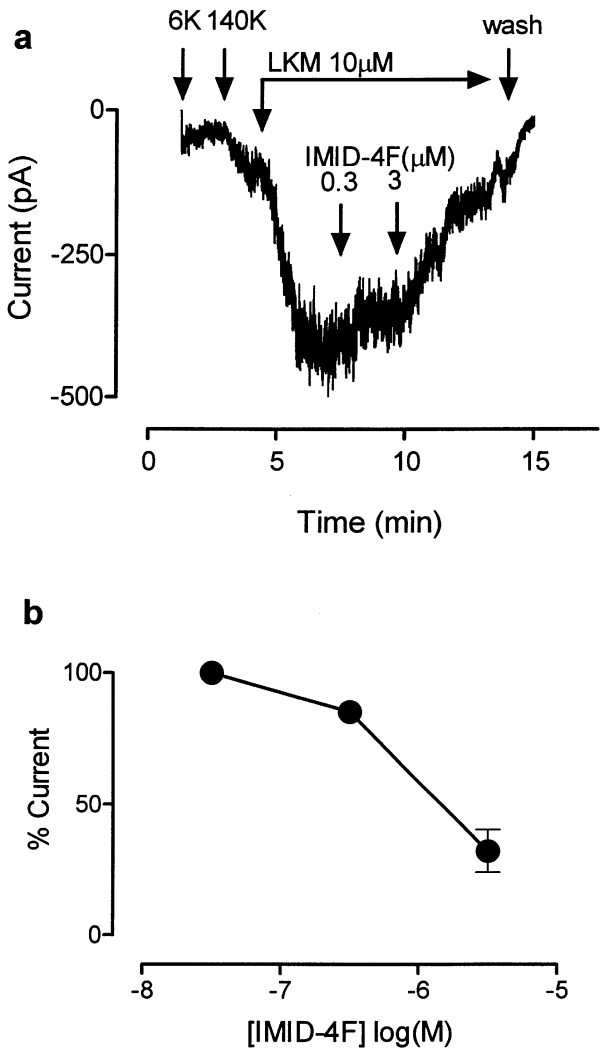

Figure 4 Representative trace obtained in a single cell showing the production of K_{ATP} channel K^+ current with levcromakalim (10 μM), then reversal of this current with increasing concentrations of IMID-4F (0.3 – 3 μM).

Radioligand binding studies

In drug displacement experiments in bovine aortic smooth muscle membranes [^{3}H]-P1075 binding was significantly reduced by the presence of levcromakalim and glibenclamide, but not IMID-4F (% control binding: levcromakalim 55 ± 7; glibenclamide 51 ± 4; IMID-4F 101 ± 4, $P<0.05$, ANOVA, $n=5$, Figure 5).

Discussion

The main finding from this study is that the fluoro-phenyl derivative of alinidine, IMID-4F, is a potent antagonist of the K_{ATP} channel opening effects of levcromakalim on vascular smooth muscle. In 1989 we were the first to show that imidazolines (e.g. alinidine and phentolamine) based compounds were antagonists of the vascular smooth muscle relaxant actions of cromakalim (McPherson & Angus, 1989; 1990). Using identical techniques as those used in the present experiments, the apparent pK_B for alinidine in rat thoracic aorta was calculated at approximately 5.5 (Challinor-Rogers *et al*., 1994). The calculated pK_B for IMID-4F was calculated to be 7.10 which represents an overall increase in potency of 40 fold over that displayed by alinidine. The present study also showed that the antagonism of levcromakalim vasorelaxant responses was specific for this vasodilator since IMID-4F (30 μM) failed to affect the vasorelaxant responses to sodium nitroprusside, a nitro-vasorelaxant compound that causes its effect by activating cyclic GMP. This is consistent with previous findings which showed that imidazoline based compounds specifically inhibited vasorelaxant responses to K_{ATP} channels openers (McPherson & Angus, 1989).

A number of chemically diverse compounds are known to antagonise the K_{ATP} channel opening effects of levcromakalim. Most notable of these are the sulphonylureas typified by glibenclamide. Previous studies (see McPherson *et al*., 1997) have shown that glibenclamide behaves as a classical competitive antagonist of levcromakalim mediated vasorelaxant responses in a number of vascular preparations, with a

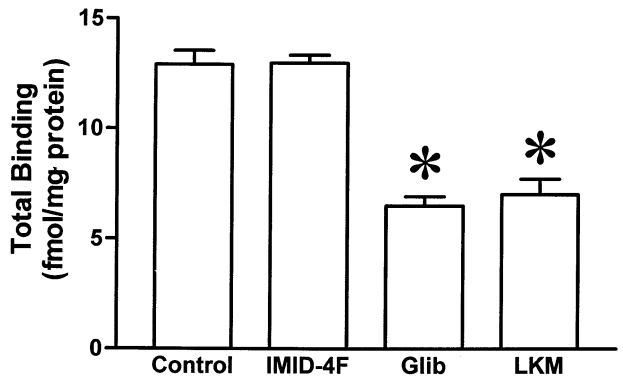

Figure 5 [^{3}H]-P1075 binding to membranes prepared from bovine aorta. [^{3}H]-P1075 binding was significantly reduced in the presence of levcromakalim (10 μM) and glibenclamide (10 μM), but not reduced in the presence of IMID-4F (30 μM). (* = significantly reduced, $P<0.05$, ANOVA, $n=5$).

pA_2/pK_B of approximately seven and slope value near unity. Thus when using sulphonylurea based compounds the levcromakalim concentration-effect curve is shifted to the right in a parallel manner with no obvious effect on the maximum response. Another potent group of levcromakalim antagonists that we have identified are the lipophilic quaternary ions such as tetraphenylphosphonium ($pK_B = 7.2$; McPherson & Piekarska, 1994; Piekarska & McPherson, 1997). While displaying the same potency as glibenclamide, tetraphenylphosphonium (and related compounds) show a markedly different type of antagonism as that displayed by the sulphonylureas. In the case of the lipophilic quaternary ion, these compounds cause a non-parallel shift to the right of the levcromakalim concentration-effect curve and, at higher concentrations, suppress the maximum vasorelaxant response that levcromakalim can elicit. At this time it is uncertain where lipophilic quaternary ions interact with the K_{ATP} channel. However previous studies with other quaternary ions have suggested that they interact with the pore of the K^+ channel to inhibit K^+ efflux from the cell (McPherson & Piekarska, 1994).

The potency and nature of the antagonism displayed by IMID-4F in this study is similar to that displayed by the lipophilic quaternary ions. IMID-4F caused a non-competitive antagonism of the K_{ATP} channel opening effects of levcromakalim, in pig coronary artery. This antagonism was specific for the K_{ATP} channel as sodium nitroprusside induced vasorelaxation was unaffected by IMID-4F. IMID-4F also antagonized the levcromakalim-induced hyperpolarization response in rat mesenteric artery, and inhibited K^+ currents which had been induced by opening of K_{ATP} channels by levcromakalim in isolated rat mesenteric artery smooth muscle cells. IMID-4F did not inhibit binding of [^{3}H]-P1075 to K_{ATP} channels, although levcromakalim and glibenclamide both significantly inhibited [^{3}H]-P1075 binding. This indicated that IMID-4F acts at a different site to levcromakalim or glibenclamide. Since imidazolines can be positively charged at physiological pH (McPherson & Piekarska, 1994) it is possible that IMID-4F may affect K_{ATP} channel opening through an interaction with the channel pore. This idea is supported by the recent finding that phentolamine, an imidazoline, inhibited pancreatic K_{ATP} channels by directly interacting with the K_{IR} component of this structure (Proks & Ashcroft, 1997).

Previous studies by us (McPherson & Angus, 1991) using the rat small mesenteric artery have shown that K_{ATP} channel antagonists (alinidine, glibenclamide) and the quaternary ion, tetraphenylphosphonium (Zhang *et al*., 1998) cause a concentration-dependent depolarization of smooth muscle cells in this preparation. We have suggested that, in this tissue, K_{ATP} channels are spontaneously opened and hold the membrane potential approximately 10 mV more negative than would otherwise be expected (McPherson & Angus, 1991). Consistent with this idea, IMID-4F, at concentrations expected to antagonize K_{ATP} channels (1–10 μM), also caused a small concentration-dependent depolarization of this magnitude. In addition to antagonizing the spontaneously opened K_{ATP} channels present in this tissue we also confirmed that IMID-4F (10 μM) completely antagonized the hyperpolarizing responses to levcromakalim observed in the rat small mesenteric artery. Since IMID-4F caused depolarization of the rat mesenteric artery, it may be expected that this compound would also potentiate vasoconstrictor responses. However, IMID-4F did not affect resting tension in pig coronary artery, nor did it increase the contraction response to U46619 (McPherson & Bell, unpublished observations).

In conclusion the results from this study have identified an imidazoline based compound, IMID-4F, which is a potent antagonist of K_{ATP} channels. The nature of the antagonism and binding studies indicate that this compound acts *via* a different mechanism to the sulphoylurea compounds, probably by interacting with the K_{ATP} channel pore directly.

This project was supported by a Project Grant from the NH&MRC of Australia and a Wellcome–Ramaciotti Travel Grant (WRA168, GAM). We also acknowledge the MRC and the British Heart Foundation, who contributed to the support in the U.K. We are grateful to Vivien Khalil and Magdy Iskander who initially synthesised IMID-4F. We thank Eva Campi from the Department of Organic Chemistry, Monash University, for synthesising IMID-4F.

References

AGUILAR-BRYAN, L., NICHOLS, C.G., WECHSLER, S.W., CLEMENT, J.P., BOYD, A.E., GONZALEZ, G., HERRERA-SOSO, H., NGUY, K., BRYAN, J. & NELSON, D.A. (1995). Cloning of the β cell high affinity sulphonylurea receptor: a regulator of insulin secretion. *Science,* **268,** 423.

ASHCROFT, S.H. & ASHCROFT, F.M. (1990). Properties and functions of ATP-sensitive K-channels. *Cell Signal,* **2,** 197–215.

ASHFORD, M.L.J. (1990). Potassium channels and modulation of insulin secretion. In: Cook N.S. (ed). *Potassium channels: structure, classification, function and therapeutic potential.* Ellis Horwood: Chichester. pp. 306–313.

CARMEN-KRZAN, M., KRZAN, M. & SCHUNACK, W. (1997). Pharmacological properties of cardiovascular histamine H_1 receptor binding sites: characterisation with 2 phenylhistamines. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **355,** 431–437.

CHALLINOR-ROGERS, J.L., HAY, T.K.C. & MCPHERSON, G.A. (1994). Comparison of the cromakalim antagonism and bradycardic actions of a series of novel alinidine analogues in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **350,** 158–166.

CHALLINOR-ROGERS, J.L. & MCPHERSON, G.A. (1994). Potassium channel openers and other regulators of K_{ATP} channels. *Clin. Exp. Pharm. Physiol.,* **21,** 583–597.

DUNNE, M.J., HARDING, E.A., JAGGAR, J.H., SQUIRES, P.E., LIANG, R., KANE, C., JAMES, R.F.L. & LONDON, N.J.M. (1995). Potassium channels, imidazolines and insulin-secreting cells. *Ann. N.Y. Acad. Sci.,* **763,** 242–261.

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

JENKINSON, D.H. (1991). How we describe competitive antagonists: three questions of usage. *Trends Pharmacol. Sci.,* **12,** 53–54.

KUBO, M., QUAYLE, J.M. & STANDEN, N.B. (1997). Angiotensin II inhibition of ATP-sensitive K^+ currents in rat arterial smooth muscle cells through protein kinase C. *J. Physiol.,* **503,** 489–496.

LÖFFLER-WALZ, C. & QUAST, U. (1998). Binding of K_{ATP} channel modulators in rat cardiac membranes. *Br. J. Pharmacol.,* **123,** 1395–1402.

MCPHERSON, G.A. & ANGUS, J.A. (1989). Phentolamine and structurally related compounds selectively antagonise the vascular actions of the K^+ channel opener, cromakalim. *Br. J. Pharmacol.,* **97,** 941–949.

MCPHERSON, G.A. & ANGUS, J.A. (1990). Characterisation of responses to cromakalim and pinacidil in smooth and cardiac muscle by use of selective antagonists. *Br. J. Pharmacol.,* **100,** 201–206.

MCPHERSON, G.A. & ANGUS, J.A. (1991). Evidence that acetylcholine-mediated hyperpolarization of the rat small mesenteric artery does not involve the K^+ channel opened by cromakalim. *Br. J. Pharmacol.,* **103,** 1184–1190.

MCPHERSON, G.A. (1992). Optimal conditions for assessing vascular reactivity in small resistance arteries in the small vessel myograph. *Clin. Exp. Pharm. Physiol.,* **19,** 815–825.

MCPHERSON, G.A. & PIEKARSKA, A.E. (1994). Antagonism by lipophilic quaternary ions of the K^+ channel opener, levcromakalim, in vascular smooth muscle. *Br. J. Pharmacol.,* **112,** 1223–1229.

MCPHERSON, G.A., CHOI, R.T., KONG, D.C. & ISKANDER, M.N. (1997). The thromboxane-A_2 and K_{ATP} channel antagonist actions of a series of sulphonylurea derivatives in pig coronary artery. *Eur. J. Pharmacol.,* **324,** 193–200.

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

PIEKARSKA, A.E. & MCPHERSON, G.A. (1997). Structure-activity relationship of quaternary ions of levcromakalim induced relaxation in pig coronary artery. *Eur. J. Pharmacol.,* **322,** 37–44.

PROKS, P. & ASHCROFT, F.M. (1997). Phentolamine block of K_{ATP} channels is mediated by Kir6.2. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 11716–11720.

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

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

ZHANG, W., BOLTON, T.B., PIEKARSKA, A.E. & MCPHERSON, G.A. (1998). The electrophysiological effects of tetraphenylphosphonium on vascular smooth muscle. *Eur. J. Pharmacol.,* **347,** 119–123.

(Received April 28, 1999
Revised July 2, 1999
Accepted September 6, 1999)