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Effects of P1060 and aprikalim on whole-cell currents in rat portal vein; inhibition by glibenclamide and phentolamine

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1 Smooth muscle cells of the rat portal vein were dispersed by enzymatic treatment and recordings of whole-cell currents were made by the voltage-clamp technique. The effects of the potassium (K) channel openers, P1060 (0.3–10 μM) and aprikalim (3–30 μM) on these currents were investigated. Antagonism of these agents by glibenclamide and phentolamine was also studied.

2 When cells were clamped at − 10 mV, P1060 (1 μM) and aprikalim (3 μM) each induced a slowly-developing K-current (I_{KCO}), the noise of which gradually increased. The rate of onset of I_{KCO} was greater for P1060 than for aprikalim. Current-voltage plots showed that P1060 and aprikalim each caused an approximately 25 mV negative shift of the reversal potential at zero current.

3 P1060 (1 μM) and aprikalim (3 μM) each inhibited the slowly activating, slowly inactivating delayed rectifier current, I_{TO}.

4 Addition of MgATP (5 mM) to the recording pipette inhibited the generation of I_{KCO} by P1060 (1 μM) and reduced the accompanying inhibition of I_{TO}.

5 Stationary fluctuation analysis of the current noise associated with I_{KCO} induced by P1060 (1 μM) or aprikalim (3 μM) at a holding potential of − 10 mV indicated that the unitary conductance of the underlying K-channels was 10.5 pS at 0 mV under the quasi-physiological conditions of the experiment.

6 In the absence of K-channel openers, neither phentolamine (30–100 μM) nor glibenclamide (1 μM) affected the magnitude of control non-inactivating currents. However, phentolamine (30–100 μM), but not glibenclamide (1 μM) inhibited the control delayed rectifier current, I_{TO}.

7 After induction of I_{KCO} by P1060 (1 μM) or aprikalim (3 μM), subsequent exposure to glibenclamide (1 μM) or phentolamine (30 μM) inhibited this current. After aprikalim-induced reduction of I_{TO} had developed, subsequent exposure to glibenclamide was able partially to reverse the inhibition of I_{TO} whereas phentolamine was without effect. Pre-exposure to glibenclamide (1 μM) prevented both the generation of I_{KCO} by aprikalim (3 μM) and the inhibitory effect of this agent on I_{TO}.

8 It is concluded that P1060 and aprikalim each induce the current I_{KCO} by opening the same small conductance, ATP-sensitive K-channel (K_{ATP}), an effect which can be inhibited by glibenclamide or phentolamine. The opening of K_{ATP} by both P1060 and aprikalim probably involves competition between these agents and ATP for the ATP-control site associated with the channel. Inhibition of the delayed rectifier current, I_{TO}, by P1060 and aprikalim was glibenclamide-sensitive and may be caused by the induction of a state of run-down in the channel which underlies this current.

Keywords: P1060; aprikalim; phentolamine; glibenclamide; rat portal vein; voltage clamp; ATP-sensitive potassium channels

Introduction

The potassium (K) channel openers such as levcromakalim, pinacidil and aprikalim comprise a structurally-diverse group of compounds which cause membrane hyperpolarization and relaxation of smooth muscle (Edwards & Weston, 1990). The target channel of these agents in smooth muscle is the subject of some debate and several authors have suggested that a calcium-activated K-channel is involved (Hu *et al.*, 1990; Silberberg & van Breemen, 1990). However, indirect evidence such as the ability of the sulphonylureas like glibenclamide to inhibit the actions of these agents has always raised the possibility that they exert their effects in smooth muscle via the opening of an ATP-sensitive K-channel (K_{ATP}).

Until recently, direct evidence of this was lacking. However, Noack *et al.* (1992a,c) recently showed that exposure to levcromakalim or depletion of intracellular ATP induced K-currents in the rat portal vein. These currents (I_{KCO} and I_{met}, respectively) were inhibited by glibenclamide and were carried by a K-channel with a unitary conductance in the range 10–20 pS (stationary fluctuation analysis; quasi-physiological K-gradient). Noack *et al.* (1992a,c) concluded that I_{KCO} and I_{met} were carried by the same K-channel and that this channel possessed characteristics similar to those of a Type 1 K_{ATP} channel (Ashcroft & Ashcroft, 1990).

The ability of the sulphonylureas to inhibit the K-channel openers extends to a growing number of chemically-diverse agents (see review by Edwards & Weston, 1993). Perhaps the best known of these other substances is the α-adrenoceptor antagonist, phentolamine, which was demonstrated by McPherson & Angus (1989) to inhibit the effects of the K-channel openers in dog coronary artery. Since this report, it has been shown that inhibition of the openers by phentolamine extends not only to other smooth muscle preparations (Murray *et al.*, 1989) but also to the pancreatic β-cell (Plant & Henquin, 1990). In addition, the ability of other imidazolines such as efaroxan and antazoline to inhibit K_{ATP} has been reported in insulin-secreting cells (Dunne, 1991; Chan *et al.*, 1991; Jonas *et al.*, 1992).

The present study in the rat portal vein was designed to investigate whether the structurally-dissimilar K-channel openers P1060 (a potent, non-chiral pinacidil analogue) and aprikalim (a thioformamide) exert effects on K-currents similar to those previously reported for levcromakalim (Noack *et al.*, 1992a,c). In addition, the inhibitory actions of the sulphonylurea, glibenclamide, were compared with those of the 2-substituted imidazoline, phentolamine.

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Methods

All experiments were performed on single smooth muscle cells acutely isolated from male Sprague-Dawley rats (150–200 g), previously killed by stunning and cervical dislocation.

Isolation of cells

Portal veins were dissected into a nominally calcium-free physiological salt solution (Klöckner & Isenberg, 1985) and cleaned of extraneous connective tissue under a dissecting microscope. The vein was opened along its longitudinal axis and placed in a collagenase solution originally described for the separation of guinea-pig bladder smooth muscle cells (Klöckner & Isenberg, 1985). The tissue was agitated for 35 min in the enzyme solution at 37°C, washed in the same solution free of enzyme and cut into 4 segments. These segments were then triturated with a wide bore, smooth tipped pipette in Kraftbrühe (KB-medium; Klöckner & Isenberg, 1985). Cells were stored at 4°C in KB-medium and used within 9 h of separation. All experiments were performed at room temperature (22–26°C). Patch pipettes were made from Pyrex glass (H15/10, Jencons, UK) and had a resistance of 2–4 MOhm when filled with internal solution.

Single-cell electrophysiology

The whole-cell configuration of the patch clamp technique (Hamill *et al.*, 1981) was employed using an Axopatch-1C amplifier (Axon Instruments). The settling time of the system was less than 500 μs.

Voltage commands and data acquisition were performed on-line with an AT-compatible computer equipped with an Axon TL-1 interface. For cell stimulation and for recording and analysing data, the pClamp 5.5 programme was used (Axon Instruments). Data were stored on a digital audio tape recorder (Sony) and the evoked membrane currents were monitored continuously on a Gould Windograf recorder. The data described were not corrected by linear leak subtraction.

The bath containing the isolated cells was perfused with a Ca^{2+}-free physiological salt solution at approximately 0.7 ml min^{-1} using a peristaltic pump (LKB Microperpex); a second identical pump was used to remove the solution from the recording chamber. The effects of drugs were examined by adding appropriate quantities to the reservoir perfusing the bath so that responses could be examined under equilibrium conditions. Results are expressed as the mean ± s.e.mean with the number of observations in parentheses.

Drugs and solutions

The composition of the solutions used was as follows (mM): pipette (internal) NaCl 5, KCl 120, $MgCl_2$ 1.2, K_2HPO_4 1.2, glucose 11, HEPES 10, EGTA 1.2, oxalacetic acid 5, sodium pyruvate 2, sodium succinate 5, pH 7.3. In certain experiments 5 mM MgATP was also included in the pipette solution from which $MgCl_2$ was then omitted; bath (external) NaCl 125, KCl 4.8, $MgCl_2$ 3.7, KH_2PO_4 1.2, glucose 11, HEPES 10, EGTA 1, pH 7.3 aerated with O_2.

The enzyme solution for cell separation comprised (mM): KOH 130, $CaCl_2$ 0.05, taurine 20, pyruvate 5, creatine 5, HEPES 10, collagenase (1 mg ml^{-1}; Sigma Type VIII), pronase (0.2 mg ml^{-1}; Calbiochem), fatty acid-free albumin (1 mg ml^{-1}; Sigma). The pH was adjusted to 7.4 with methanesulphonic acid.

The following drugs were used: aprikalim (formerly RP52891, Rhône Poulenc Rorer), P1060 (N'-cyano-N-(3-pyridyl)-N''-(*t*-butyl)guanidine; Leo), glibenclamide (Sigma), phentolamine hydrochloride (Sigma). These agents were made up as 20 mM stock solutions in dimethyl sulphoxide (except phentolamine hydrochloride which was dissolved in twice distilled water).

Results

In the voltage clamp mode, the whole-cell currents recorded from freshly isolated rat portal vein cells were identical to those reported by Noack *et al.* (1992a,c). When cells were held at − 90 mV and stepped for 500 ms to test potentials ranging between − 80 mV and + 30 mV (10 mV increments), a current with fast activation and fast inactivation kinetics was observed. This current exhibited activation and inactivation time-courses very similar to those of an A-current. In addition, when cells were depolarized to test potentials positive to − 30 mV, a slowly activating, slowing inactivating current developed (Figure 1). This delayed rectifier-type current was designated I_{TO} (transient outward current) by Noack and co-workers (1992a). Following the complete inactivation of I_{TO}, a steady current level that exhibited no inactivation was observed. This current alone was available when cells were clamped at − 10 mV to inactivate the transient currents described above (Figure 1) and was designated I_{NI} (Noack *et al.*, 1992a). The magnitude of I_{TO} was determined by digital subtraction of I_{NI} (induced when stepping to test potentials from a holding potential of − 10 mV) from those currents induced when stepping to identical test potentials from − 90 mV. Measurement of I_{TO} at 200 ms after stepping from the command potential ensured that the A-like current had completely inactivated and therefore did not contaminate the values obtained for I_{TO}.

Generation of I_{KCO} by P1060 and aprikalim

P1060 and aprikalim each caused a concentration-dependent increase in the outward current when the cells were clamped at − 10 mV (Figure 2). This current was designated I_{KCO} and its magnitude was concentration-dependent over the range 300 nM–10 μM for P1060 and 3 μM–30 μM for aprikalim. When the effects of approximately EC_{80} concentrations of P1060 (1 μM) and aprikalim (3 μM) on I_{KCO} were compared, the maximum increase in I_{KCO} following exposure to P1060 was achieved after 7.0 ± 1.2 min compared with 11.4 ± 2.6 min in the presence of aprikalim (see Figure 2; $P<0.05$, Student's t test, $n = 5–6$).

Single-channel conductance underlying the effects of P1060 and aprikalim

The effects of P1060 and aprikalim were compared with those previously obtained for levcromakalim and for ATP-depletion (Noack *et al.*, 1992a,c). From the current noise signals (see Figure 2) generated by these K-channel openers at a constant holding potential (− 10 mV), recording segments of 2 s duration were sampled for further analysis. From these, amplitude histograms were constructed and Gaussian distributions fitted to them by computer. For each cell, the maximum number of possible observations was obtained and ranged from 5 to 54 (mean = 14, $n = 11$). This number was dependent on the rate of onset of the increase in holding current for each individual cell following exposure to P1060 or aprikalim. The mean current (μ) and current variance (σ^2) obtained at different stages of the experiment were analysed as previously described (Noack *et al.*, 1992a).

Using data derived from a total of 8 cells of comparable size, I_{KCO} induced by P1060 (1 μM) and by aprikalim (3 μM) was calculated to consist of single channel currents of 0.41 pA ± 0.08 pA ($n = 4$) and 0.41 ± 0.05 pA ($n = 4$), respectively. There was thus no difference between the target channels of these two agents. When the single channel conductance was calculated according to the Goldman-Hodgkin-Katz equation for both P1060 and aprikalim, a value of 10.5 pS (at 0 mV and under the quasi-physiological potassium gradient of the experiments) was obtained. For both agents, the best fitted curves gave values for the number of channels (N) which contributed to the generation of I_{KCO} in the range 200 to 2000 per cell.

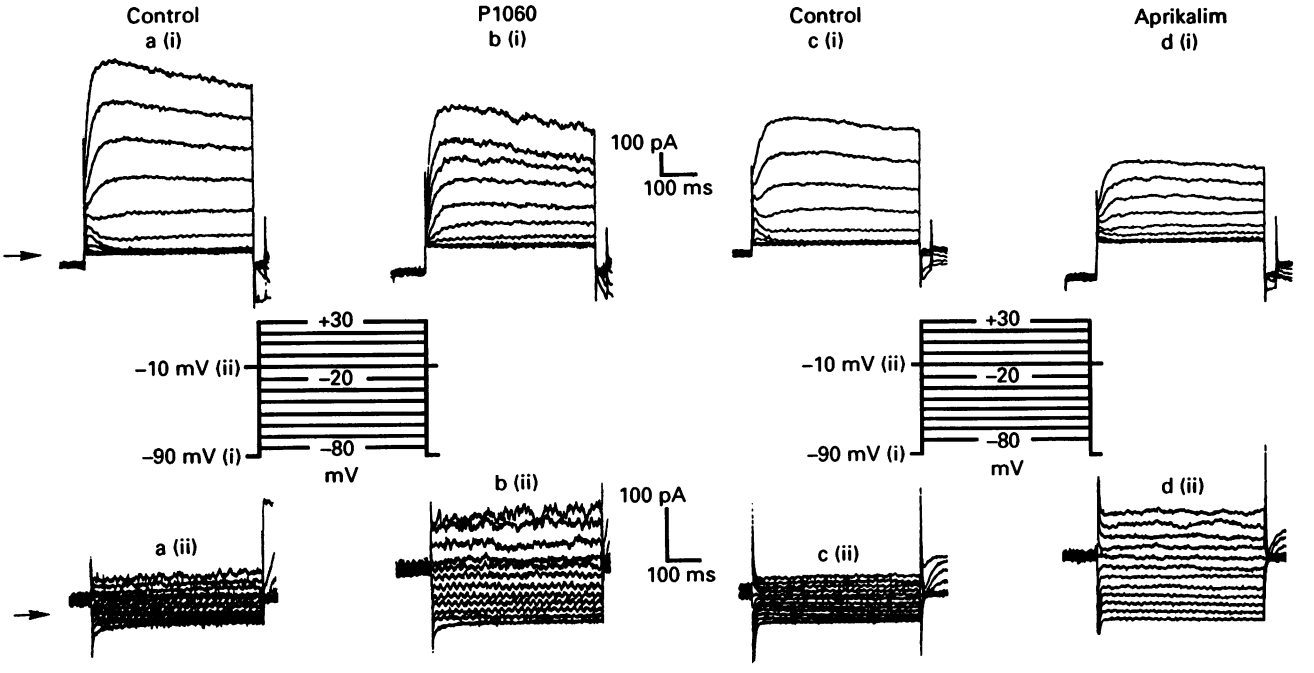

Figure 1 Typical traces demonstrating the inhibition of the delayed rectifier current (I_{TO}) and the induction of the non-inactivating current (I_{KCO}) by (a,b) P1060 (1 μM) and (c,d) aprikalim (3 μM). Control currents (a,c) and currents in the presence of P1060 (b) or aprikalim (d) are displayed for comparison. The upper traces show the delayed rectifier current (I_{TO}) derived by digital subtraction of the non-inactivating currents available when stepping to a series of test potentials from a holding potential of − 10 mV (ii), from the total currents available when stepping to the same series of test potentials from a holding potential of − 90 mV (i). The lower traces are the currents obtained when stepping to the test potentials from a holding potential of − 10 mV. Note the inhibition of I_{TO} in b(i) and d(i) and the generation of I_{KCO} in b(ii) and d(ii). Traces a,b and c,d were derived from two different cells. The arrows indicate the zero current level.

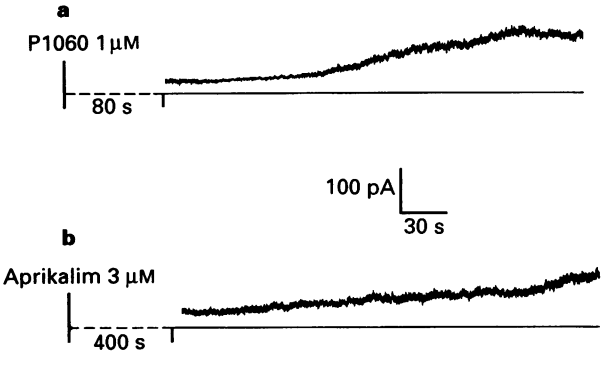

Figure 2 Typical development of I_{KCO} in two different cells from rat portal vein following exposure to (a) P1060 (1 μM) and (b) aprikalim (3 μM). Holding potential − 10 mV. Note the characteristic increase in current noise during the development of I_{KCO} and that the rate of onset of I_{KCO} was faster for P1060 than for aprikalim.

Noise analysis theory predicts a decrease in current noise when the mean value for the open probability is larger than 0.5. Values obtained for μ and σ^2 indicated that both aprikalim and P1060 could each increase the open probability to a value in the range 0.5–0.6 (6 out of 11 cells).

Effects of P1060 and aprikalim on I-*V relationships*

To determine the effects of P1060 and aprikalim in more detail, current-voltage (*I*-V) experiments were carried out in the presence of P1060 (1 μM) or aprikalim (3 μM), concentrations which represented the respective EC_{80} values for the generation of I_{KCO}. Stepping to a range of test potentials from alternate holding potentials of − 90 mV and − 10 mV allowed the generation of total currents (inactivating plus non-inactivating currents) or non-inactivating currents alone, respectively. Subsequent digital subtraction of the non-activating current from the total current at each test potential permitted the evaluation of the effects of P1060 and aprikalim on the delayed rectifier current component, I_{TO}.

On stepping to a series of test potentials from a holding potential of − 10 mV, the generation of I_{KCO} by P1060 (1 μM) and aprikalim (3 μM) was characterized by an increase in the magnitude of the evoked non-inactivating currents over the voltage range employed (− 80 to + 30 mV; Figure 3). At 0 mV, for example, the magnitude of currents in the presence of P1060 and aprikalim was 142.4 ± 26.9 pA and 88.9 ± 14.42 pA, respectively ($n = 6$) compared with control values of 22.9 ± 3.5 pA and 15.3 ± 4.3 pA, respectively ($n = 6$). It can be seen from the *I*-V plots (Figure 3) that the reversal potential of the non-inactivating current complex was moved in a hyperpolarizing direction in the presence of P1060 and aprikalim. Prior to exposure to either agent, control currents reversed near − 30 mV but in the presence of these openers the reversal potential was shifted to approximately − 55 mV (Figure 3). For both P1060 and aprikalim, respective control and test curves intersected at approximately − 78 mV, a value close to the theoretical value for E_K (− 83 mV) in these experiments and indicating that I_{KCO} was a K-current.

P1060 (1 μM) and aprikalim (3 μM) each inhibited the delayed rectifier current, I_{TO}. In the case of aprikalim, this inhibition was evident at all test potentials over the activation range of I_{TO}, whereas P1060 only inhibited I_{TO} at potentials positive to 0 mV (control 280.4 ± 28.0 pA, P1060 240.5 ± 21.9 pA, $n = 7$; control 252.7 ± 61.0 pA, aprikalim 121.5 ± 25.3 pA, $n = 7$ at 0 mV, Figure 3). Treatment of cells with vehicle alone (dimethyl sulphoxide, 0.06%) had no effect on the rat portal vein K-currents ($n = 3$, data not shown).

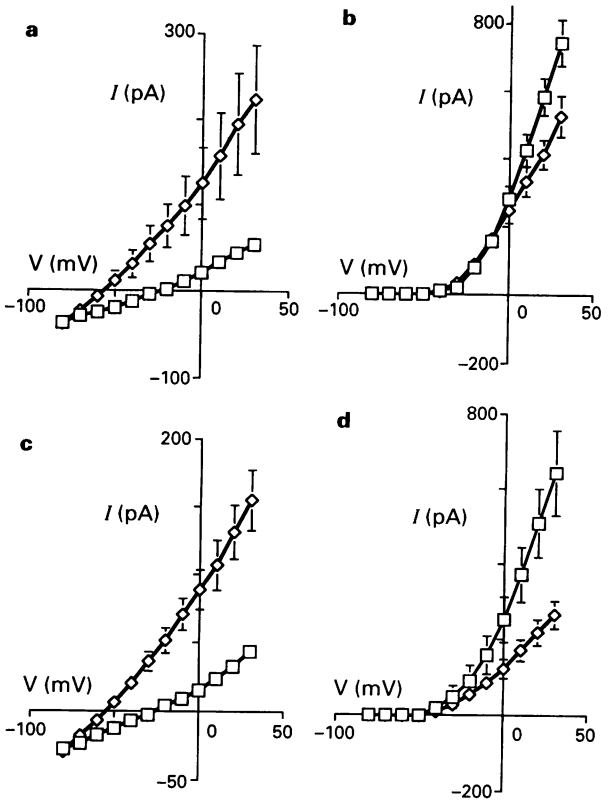

Figure 3 The effects of P1060 (1 μM, ◇; a,b) and aprikalim (3 μM, ◇; c,d) on the whole-cell currents in isolated single cells from rat portal vein. (a) and (c) illustrate the generation by these K-channel openers of I_{KCO} on stepping to test potentials from a holding potential of − 10 mV. (b) and (d) show the effects of the K-channel openers on the transient outward current (I_{TO}) calculated by digitally subtracting the current available when stepping from a holding potential of − 10 mV from that available when stepping from a holding potential of − 90 mV. (□) Control currents. Each point represents the mean value derived from 6 observations ± s.e.mean.

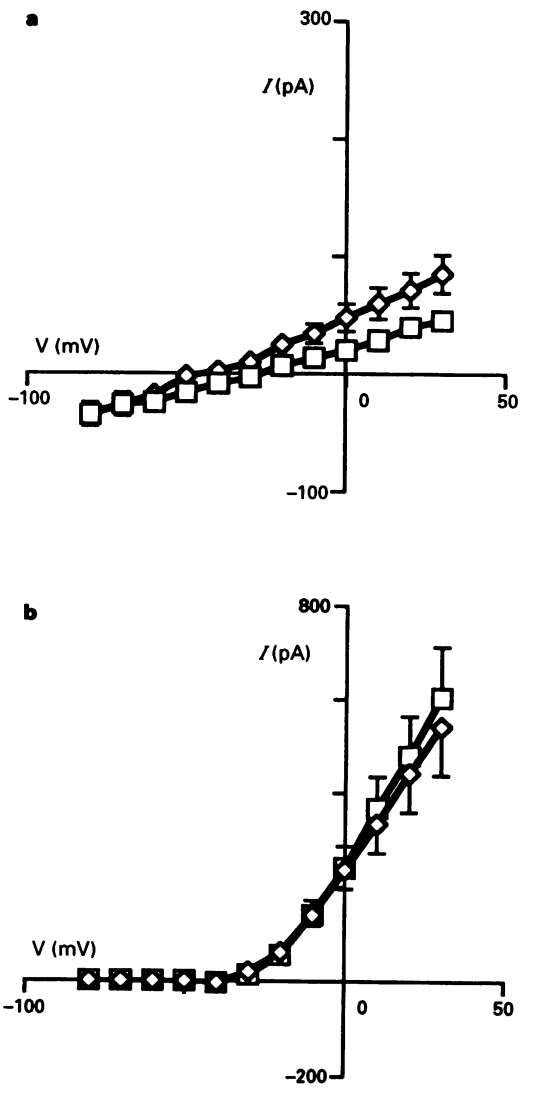

Figure 4 The effect of P1060 (1 μM, ◇) on currents induced in single cells from rat portal vein when the pipette (intracellular) solution contained 5 mM MgATP. (a) Generation of I_{KCO} by P1060 on stepping to test potentials from a holding potential of − 10 mV. (b) Effects of P1060 on the transient outward current (I_{TO}) calculated by digitally subtracting the current available when stepping from a holding potential of − 10 mV from that available when stepping from a holding potential of − 90 mV. (□) Control currents obtained in the presence of 5 mM intracellular ATP. Each point represents the mean value derived from 5 observations ± s.e.mean.

Effect of addition of MgATP to the recording pipette

Since I_{KCO} generated by levcromakalim is carried by ATP-sensitive K-channels (Noack *et al.*, 1992c), we investigated whether the addition of a relatively high concentration (5 mM) of MgATP to the pipette solution could modify the effects of P1060 on K-currents in the rat portal vein. With this concentration of MgATP in the pipette, neither the time course of development (data not shown) nor the magnitude of I_{NI} or I_{TO} was affected (compare control values in Figures 3 and 4). In comparison, inclusion of 5 mM MgATP in the pipette inhibited the stimulation of I_{KCO} by 1 μM P1060 and reduced the inhibitory effect of P1060 on I_{TO} (compare Figures 3 and 4).

Effects of glibenclamide and phentolamine

Addition of glibenclamide (1 μM) to the recording chamber had no significant effect on either I_{NI} or I_{TO} (Figure 5). Phentolamine (30 μM–100 μM) had no effect on I_{NI} but caused concentration-dependent inhibition of I_{TO} (Figure 5). The degree of inhibition of the effects of the K-channel openers by glibenclamide or phentolamine was dependent on the timing of exposure to these agents. Thus, if tissues were first exposed to P1060 (1 μM) or aprikalim (3 μM), the I_{KCO} which developed was fully reversed by subsequent exposure to glibenclamide (1 μM; Figure 6) but only partially reversed by phentolamine (30 μM; Figure 6). Aprikalim (3 μM)-induced inhibition of I_{TO} was partially reversed by the subsequent addition of glibenclamide (1 μM; Figure 7) to the recording chamber whereas phentolamine was without effect. However, prior exposure to glibenclamide (1 μM) completely prevented both the development of I_{KCO} by aprikalim (3 μM) and the associated inhibition of I_{TO} (Figure 7). Pretreatment with phentolamine also prevented the development of I_{KCO} but failed to prevent the inhibition of I_{TO} by aprikalim (Figure 8).

Discussion

Similarities between structurally-different K-channel openers

The present study in rat portal vein has demonstrated that the K-channel openers P1060 (a cyanoguanidine, pinacidil

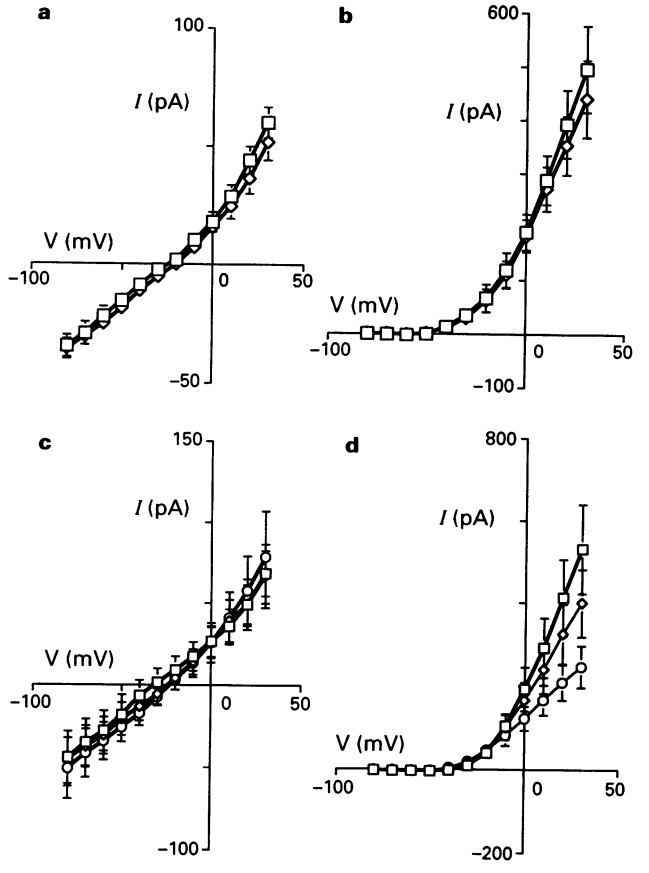

Figure 5 Effects of glibenclamide (1 μM, ◇; a,b) and phentolamine (30 μM, ◇; 100 μM, ○; c,d) on currents in rat portal vein cells. (a) and (c) show effects on non-inactivating currents obtained on stepping to test potentials from − 10 mV. (b) and (d) illustrate the effects on the transient outward current (I_{TO}) calculated by digitally subtracting the current available when stepping from − 10 mV from that available when stepping from − 90 mV. (□) Indicates control currents. Each point represents the mean value derived from 3 to 7 observations ± s.e.mean.

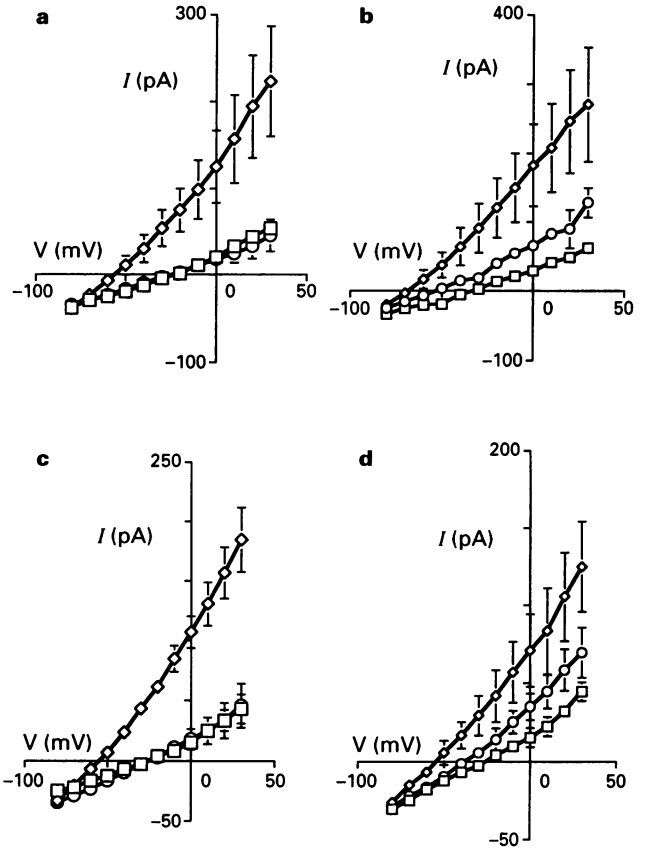

Figure 6 Effect of glibenclamide (1 μM; a,c) and phentolamine (30 μM; b,d) on the induction of I_{KCO} by P1060 (1 μM; a,b) and aprikalim (3 μM; c,d). (□) Control currents; (◇) currents in the presence of the respective K-channel opener; (○) currents in the presence of the K-channel opener and respective antagonist. Each point represents the mean value derived from 3 observations ± s.e.mean.

analogue) and aprikalim (a thioformamide) generate a non-activating K-current, I_{KCO}. In addition, P1060 and aprikalim each inhibited I_{TO}, a slowly activating, time- and voltage-dependent transient outward current, the properties of which were similar to those of a delayed rectifier current (see Bolton & Beech, 1992). The generation of I_{KCO}, together with the simultaneous reduction in I_{TO} produced by these agents are properties identical to those exhibited in rat portal vein by the benzopyran, levcromakalim (Noack *et al.*, 1992a). Thus, three structurally-dissimilar prototype K-channel openers produce qualitatively similar effects on macro K-currents which strongly suggests that all share common sites and mechanisms of action.

Such a conclusion is supported by analysis of the current noise associated with I_{KCO} in the present study. Using this procedure, a value of 0.4 pA was obtained for the single channel current from which the unitary conductance underlying I_{KCO} was calculated to be 10.5 pS at 0 mV under the quasiphysiological conditions of the present experiments. These values correlate well with the data obtained by Noack *et al.* (1992a,c) for levcromakalim and indicate that the same K-channel underlies the current I_{KCO} generated by the three types of K-channel opener. However, the results of the present study, together with those of previous investigations (Noack *et al.*, 1992a,c) give no support to the view (Standen *et al.*, 1989) that a K-channel of unitary conductance 146 pS represents the target of agents like P1060, aprikalim and levcromakalim.

Modulation of I_{KCO} by ATP: site of action of the K-channel openers

In the present study, inclusion of MgATP in the recording pipette inhibited the induction of I_{KCO} by P1060 suggesting that the K-channel underlying this current was ATP-sensitive. Such a view is consistent with that expressed in an earlier study (Noack *et al.*, 1992c) which also concluded that an ATP-sensitive K-channel (K_{ATP}; broadly similar to the Type 1 K_{ATP} described by Ashcroft & Ashcroft, 1990) carried I_{KCO}. In this investigation, Noack and coworkers (1992c) found that removal of substrates for the carboxylic acid cycle from the pipette solution (thus reducing the intracellular ATP concentration, $[ATP]_i$) induced a current (I_{met}) which declined with time and which had an underlying unitary conductance similar to that of I_{KCO} induced by levcromakalim. Furthermore, after 'run-down' of I_{met}, effects of levcromakalim could not be observed.

ATP is thought to regulate K_{ATP} by an interaction with two independent sites. At one of these, ATP binds and inhibits channel opening, whereas phosphorylation of the other site is a prerequisite for the flow of K-current (see Ashcroft, 1988; Edwards & Weston, 1993). The ability of K-channel openers to induce I_{KCO} (present study; Noack *et al.*, 1992a,c), the inhibition of this effect by inclusion of ATP in the recording pipette (present study) and the production of a current, I_{met}, with characteristics identical to those of I_{KCO}, by reduction of $[ATP]_i$ (Noack *et al.*, 1992c) combine to suggest that the K-channel openers compete with ATP for access to the ATP binding sites on K_{ATP} and that this is the basic mechanism

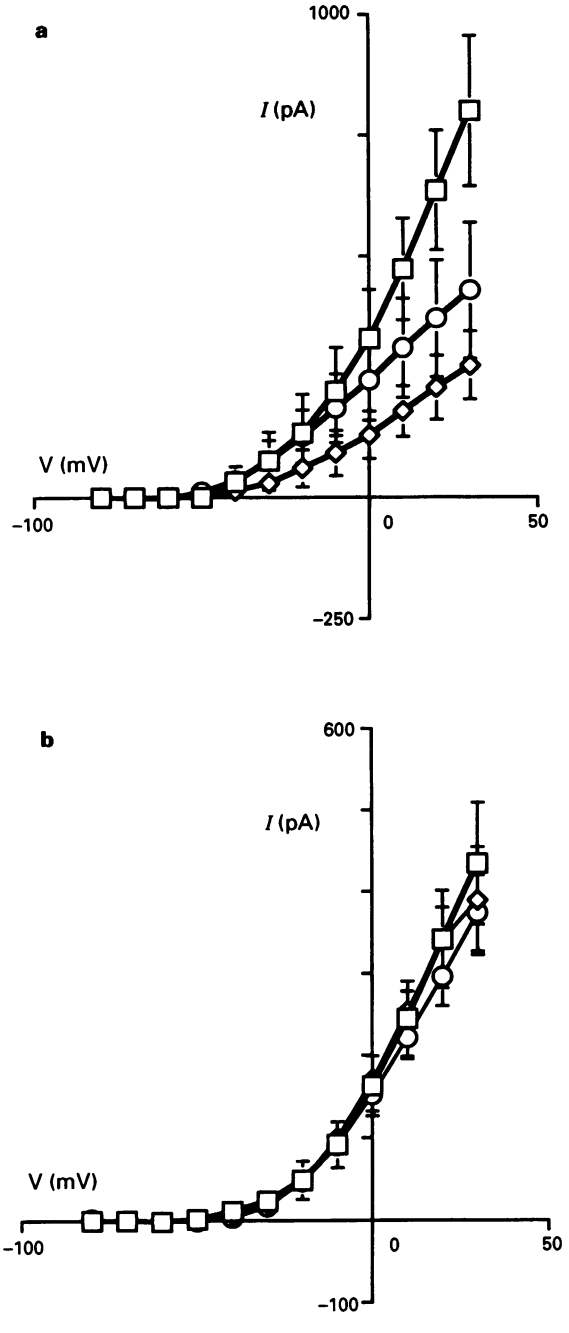

Figure 7 Effect of glibenclamide (1 μM) on the aprikalim (3 μM)-induced inhibition of I_{TO}. (a) Partial reversal of the aprikalim-induced inhibition of I_{TO} by glibenclamide. (b) Inhibition of the effect of aprikalim on I_{TO} by pre-exposure to glibenclamide. (□) Control current; (◇) current in the presence of aprikalim (a) or glibenclamide (b) alone; (○) current in the presence of both glibenclamide and aprikalim. Each point represents the mean value derived from 3 observations ± s.e.mean.

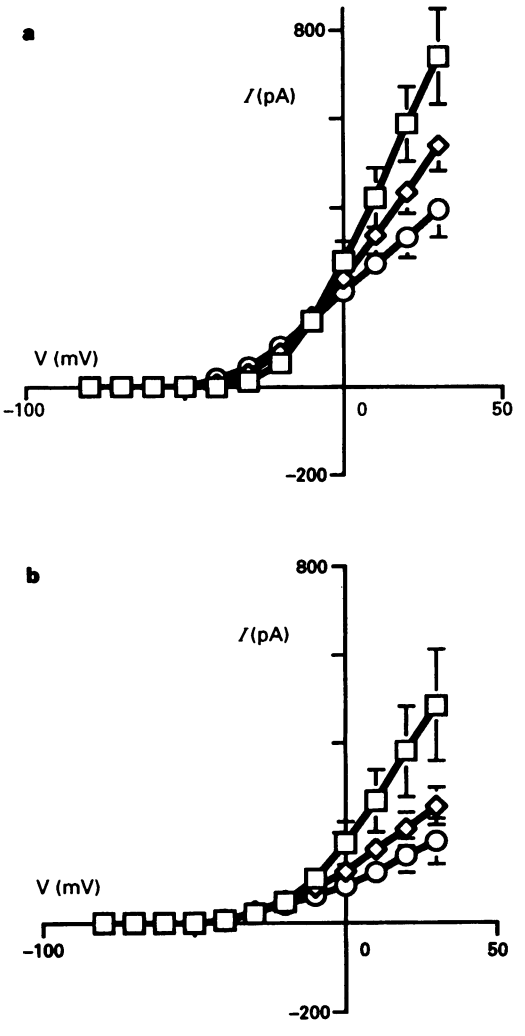

Figure 8 Effect of phentolamine (30 μM) on the inhibition of I_{TO} by P1060 (1 μM; a) and aprikalim (3 μM; b). (□) Control current; (◇) current in the presence of the respective K-channel opener; (○) current in the presence of the respective opener and phentolamine (30 μM). Each point represents the mean value derived from 3 observations ± s.e.mean.

which underlies the ability of these agents to open K_{ATP}. This suggestion, originally proposed by Thuringer & Escande (1989) following studies on the action of RP49356 in cardiac muscle, has received further support from an investigation of the effects of K-channel openers on ATP-sensitive chloride (Cl) currents (Sheppard & Welsh, 1992). These workers showed that levcromakalim, diazoxide and minoxidil sulphate closed the Cl-channels which underlie these currents. Since these Cl_{ATP} channels, in direct contrast to K_{ATP} in smooth muscle, close as ATP binding decreases, these data strongly support the view that the K-channel openers compete with ATP for the ATP-binding sites which modulate channel opening. The results further suggest that the (so-called) K-channel openers could exert effects wherever ATP binding sites exist with characteristics similar to those on K_{ATP} and Cl_{ATP}.

Inhibition of the delayed rectifier current, I_{TO}: possible link with I_{KCO}

Like K_{ATP}, the K-channels (K_V) responsible for carrying delayed rectifier currents must also be phosphorylated before they can be opened (see Peroso & Bezanilla, 1990). Thus, the ability of both P1060 and aprikalim (present study) and of levcromakalim (Noack *et al.*, 1990a) to inhibit the delayed rectifier current, I_{TO}, could be explained if these agents were able to prevent phosphorylation of K_V. This would induce a state of channel 'run-down' which would manifest itself as a reduction in the whole-cell current, I_{TO}. If the K-channel openers can indeed reduce the degree of phosphorylation of the delayed rectifier channel (K_V) with consequent reduction of I_{TO}, such an action could also be exerted at other ion channels which require phosphorylation prior to opening. One such channel is K_{ATP} itself (see Ashcroft, 1988) and it is possible that reduced phosphorylation and run-down could

account for the previously-reported anomalous closure of K_{ATP} by the K-channel openers (insulinoma cell, Dunne, 1990; cardiac ventricle, Faivre & Findlay, 1989) under certain conditions. In both the present study and in an earlier investigation (Noack *et al.*, 1992a), P1060, aprikalim and levcromakalim were each unable to increase the open probability of K_{ATP} to values greater than the range of 0.5–0.6. This suggests that the ability of all three agents to open K_{ATP} by decreasing ATP binding to this channel could be limited by the simultaneous induction of channel run-down.

Inhibitory effects of glibenclamide on I_{KCO}

As previously reported for levcromakalim (Noack *et al.*, 1992a), the induction of I_{KCO} by P1060 or aprikalim was inhibited by glibenclamide (1–10 μM). This effect could be observed either by application of glibenclamide after I_{KCO} had become established or by inclusion of glibenclamide in the bath solution prior to exposure to the K-channel opener. However, in the same concentration-range, glibenclamide itself had no effect on either I_{TO} or on the complex non-inactivating currents designated I_{NI}. This is the first clear demonstration in smooth muscle that even in micromolar concentrations, glibenclamide is a selective inhibitor of I_{KCO}, with essentially no such effect on the other calcium-independent outward currents, I_{TO} and I_{NI}. In whole portal veins bathed in normal, calcium-containing physiological salt solutions, glibenclamide (up to 10 μM) did not significantly modify spontaneous mechanical activity (Noack *et al.*, 1992b). Such an observation is consistent with the lack of effect of glibenclamide on I_{TO} and I_{NI} observed in the present study and further suggests that any effect of this sulphonylurea on calcium-dependent K-currrents is negligible.

An important objective of the present investigation was to determine the effect of glibenclamide on the inhibition of I_{TO} produced by P1060 and aprikalim since in an earlier study, Noack *et al.* (1992a) had reported that this phenomenon was glibenclamide-insensitive. Initial experiments were performed by first exposing the cells to either P1060 or aprikalim and allowing the inhibition of I_{TO} to develop followed by superfusion with glibenclamide in the continuing presence of the K-channel opener. Using this approach, identical to the one adopted by Noack *et al.* (1992a), reversal of the opener-induced inhibition of I_{TO} was seen in some cells, but not in others. Thus, in an attempt to clarify the position, further experiments were carried out by first exposing cells to glibenclamide and then to aprikalim, the opener which produced a greater inhibition of I_{TO} compared with P1060. Under these conditions in the continuing presence of glibenclamide, the ability of aprikalim to reduce the size of I_{TO} was abolished.

From these studies it is not possible to explain definitively why the relative timing of exposure to glibenclamide and aprikalim was so critical before consistent reversal of the aprikalim-induced inhibition of I_{TO} could be observed. It was suggested by Noack *et al.* (1992a) that the inhibition of I_{TO} caused by levcromakalim could be due to a decrease in the number of K_V channels in the phosphorylated state (i.e. an increase in the run-down of K_V). Antagonism of the K-channel openers by glibenclamide could involve inhibition of an A-kinase involved at a critical point in the glycolytic cascade (see Caro, 1990). This would shift this pathway in the direction of glycolysis and result in a net synthesis of ATP. If a reduction in the size of I_{TO} by the K-channel openers involves prevention of the phosphorylation of the delayed rectifier channel as already discussed, then a glibenclamide-driven increase in $[ATP]_i$ (under conditions in which there was a critical balance between ATP concentration and phosphorylation of the channel) could serve to reverse or prevent the inhibition of I_{TO} seen in the absence of glibenclamide. Thus, if such functional antagonism underlies glibenclamide-induced reversal of the inhibition of I_{TO} by aprikalim (present study), the relative contact times of glibenclamide and opener could be critical in determining the degree of observed antagonism.

Inhibitory effects of phentolamine on I_{KCO} *and* I_{TO}

In the present investigation, micromolar concentrations of phentolamine, like glibenclamide, had no effect on I_{NI} but inhibited the generation of I_{KCO} by both P1060 and aprikalim. Although phentolamine has previously been shown to inhibit K_{ATP} in insulin-secreting cells (Dunne, 1991; Jonas *et al.*, 1992), this is the first demonstration of its ability to inhibit a K-current in isolated smooth muscle cells and it explains the basis of the phentolamine-induced reversal of the mechano-inhibitory actions of K-channel openers in a variety of tissues (McPherson & Angus, 1989; Murray *et al.*, 1989).

In addition to its ability to inhibit I_{KCO}, phentolamine also produced a decrease in the magnitude of I_{TO}, especially at the upper end of the tested concentration-range. These effects of phentolamine were thus similar to those of the imidazoisoindole derivative, ciclazindol, which also inhibited both I_{KCO} and the delayed rectifier current, I_{TO} (Noack *et al.*, 1992b). Both ciclazindol (Noack *et al.*, 1992b) and phentolamine (Schwietert *et al.*, 1992; Weston, unpublished) stimulate the spontaneous mechanical activity of the rat portal vein and phentolamine induces marked rhythmic contractions in guinea-pig bladder detrusor muscle (Satake *et al.*, 1984). We thus suggest that it is the inhibition of the smooth muscle delayed rectifier which underlies the ability of ciclazindol, phentolamine and of other agents like antazoline (see Schwietert *et al.*, 1992) to increase spontaneous activity in these tissues. Such a view is supported by the finding that this mechanical effect is shared by the sotalol derivative E-4031 (Schwietert *et al.*, 1992), a compound known to be a potent inhibitor of the delayed rectifier channel in cardiac tissue (Wettwer *et al.*, 1991).

In contrast to its ability to inhibit I_{KCO}, phentolamine did not reverse the opener-induced inhibition of I_{TO}. Indeed, as already detailed, phentolamine itself inhibited I_{TO}, and there was a trend for this inhibition to increase in the joint presence of both phentolamine and the openers. Further experiments are clearly required to determine not only the mechanism which underlies antagonism of the K-channel openers by phentolamine but also that by which phentolamine inhibits I_{TO}.

Conclusions

The results of the present study in rat portal vein cells have shown that both P1060 and aprikalim induced I_{KCO}, a non-inactivating K-current and simultaneously inhibited I_{TO}, a current with the characteristics of a delayed rectifier. This pattern of K-current modulation was identical to that described in an earlier investigation with levcromakalim (Noack *et al.*, 1992a). It suggests that all three structurally-diverse K-channel openers share common mechanistic features and induce the current, I_{KCO}, by an action on a small conductance, glibenclamide- and ATP-sensitive K-channel. In the absence of the K-channel openers, glibenclamide itself had no effect on the control inactivating and non-inactivating currents. In addition, P1060 and aprikalim also inhibited the delayed rectifier current, I_{TO}, an effect which in the case of aprikalim was reversed by glibenclamide but not by phentolamine. It was found that phentolamine was itself able to inhibit I_{TO} in these cells, but that glibenclamide alone exerted no significant effect on this delayed rectifier current. Further studies are in progress to determine in more detail the mechanisms which underlie the actions of these K-channel modulators.

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