Effects of BRL 38227 on potassium currents in smooth muscle cells isolated from rabbit portal vein and human mesenteric artery

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1 Single smooth muscle cells were isolated from the rabbit portal vein and the human mesenteric artery and whole cell currents recorded at room temperature from either cell type by the whole cell voltage clamp technique.

2 In the rabbit portal vein cells addition of 10 μM BRL 38227 induced a quasi-instantaneous, voltage-insensitive and time-independent current which had a reversal potential of -75 mV under experimental conditions where the calculated E_K was -83 mV .

3 Cells were held at 0 mV and BRL 38227 was added cumulatively to construct a dose-response relationship. BRL 38227 ($0.03\text{--}10\text{ }\mu\text{M}$) caused a dose-dependent outward shift in the holding current with an EC_{50} of $1.3\text{ }\mu\text{M}$.

4 BRL 38227 ($10\text{ }\mu\text{M}$) had no effect on the delayed rectifier K^+ current measured in the presence of 5 mM tetraethylammonium and no effect on the Ca^{2+} -activated K^+ current measured in the presence of 5 mM 4-aminopyridine. Similarly BRL 38227 had no effect on the Ca^{2+} current.

5 The BRL 38227-induced current was blocked by glibenclamide ($10\text{ }\mu\text{M}$) and phentolamine ($100\text{ }\mu\text{M}$), specific blockers of the ATP-sensitive K^+ current in single cells.

6 In human isolated mesenteric artery cells, BRL 38227 ($10\text{ }\mu\text{M}$) induced a glibenclamide-sensitive current similar to, but smaller than, that observed in the rabbit portal vein.

7 We conclude that in these cells, BRL 38227 activates a potassium conductance which has the electrophysiological and pharmacological characteristics of ATP-sensitive K^+ channels.

Keywords: Smooth muscle; rabbit portal vein; human mesenteric artery; BRL 38227; potassium currents

Introduction

BRL 38227 [(–)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-1-benzopyran-3-ol]] is one of a novel class of compounds, termed potassium (K^+) channel openers, that have been shown to produce relaxation of various visceral and vascular smooth muscles (Hamilton & Weston, 1989). Their main mode of action is the activation of a potassium conductance resulting in hyperpolarization of the resting membrane potential. At present, several different potassium channels have been proposed as sites of action of these drugs. Beech & Bolton (1989c) suggested that the mode of action of cromakalim, the mixture of BRL 38227 and its (+)-enantiomer, was a pharmacological alteration of the time-dependent voltage-sensitive delayed rectifier current. Others have identified an ATP-sensitive K^+ conductance which is activated by cromakalim (Standen *et al.*, 1989) while both small (Okabe *et al.*, 1990) and large (Gelband *et al.*, 1989) conductance Ca^{2+} -activated K^+ channels have been proposed as the possible site of action for the potassium channel openers.

The whole cell K^+ currents present in the rabbit portal vein have been well characterized by Beech & Bolton (1989a,b). They identified a tetraethylammonium (TEA)-sensitive Ca^{2+} -activated K^+ current responsible for both spontaneous transient outward currents and a noisy sustained outward current elicited by depolarization positive of 0 mV . They also found a delayed rectifier K^+ current activated at potentials positive of -40 mV and a voltage-dependent K^+ current with fast kinetics. In the same cell Hume & Leblanc (1989), under different conditions, identified four currents including a background K^+ current, a delayed rectifier K^+ current and two

distinct Ca^{2+} -activated K^+ currents as suggested by earlier single channel studies (Inoue *et al.*, 1985; 1986).

The objective of the present study was to identify the BRL 38227-sensitive currents in cells isolated from both the rabbit portal vein and human mesenteric artery. BRL 38227 was found to elicit a time-independent voltage-insensitive K^+ conductance. The blockade of this conductance by glibenclamide and phentolamine suggested the site of action of BRL 38227 was the ATP-sensitive K^+ channel.

Methods

Cell preparation

Rabbit portal vein Cells were isolated by the technique of Beech & Bolton (1989a). Adult rabbits of either sex weighing $2\text{--}3\text{ kg}$ were killed by i.v. injection of phenobarbitone. The main branch of the portal mesenteric vein was removed, placed in physiological saline solution (PSS) and dissected free of fat and connective tissue. It was then cut into four small equal sized pieces which were then placed into a low- Ca^{2+} PSS for $10\text{--}20\text{ min}$. The tissue was subsequently incubated for 30 min at 37°C in low- Ca^{2+} -PSS containing papain (1 mg ml^{-1}), dithiothreitol (1 mM) and bovine serum albumin ($1\text{--}2\text{ mg ml}^{-1}$). The tissue was placed in more enzyme-free low- Ca^{2+} PSS and triturated with a wide-bore Pasteur pipette to dislodge the cells. Calcium in the medium was then increased to about 0.7 mM , and the cells were stored at 4°C for use within 9 h .

Human mesenteric artery Fat attached to sections of stomach or small intestine removed during routine surgery at St

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Figure 1 Effects of BRL 38227 on whole cell K^+ currents in cells isolated from the rabbit portal vein. (a) Representative current traces from a single cell in normal PSS held at -60 mV and stepped from -90 mV to 10 mV with 20 mV increments in the absence and presence of $10 \mu\text{M}$ BRL 38227 (bath applied). (b) The I - V relationship of the K^+ current, measured at the peak of the current, in normal PSS for six cells (mean with s.e. mean shown by vertical bars) under control conditions ($\circ$) and in the presence of $10 \mu\text{M}$ BRL 38227 ($\bullet$). The current augmented by BRL 38227 had an apparent reversal potential of -75 mV.

Thomas's Hospital was immediately cut away, placed into cold physiological saline solution (PSS), and small arteries (0.5 – 3 mm, outer diameter) were dissected out. Arteries were then opened longitudinally and cut transversely into small equally sized sections. These sections were used immediately or were stored for up to two days in PSS at 4°C for further isolation. To obtain isolated cells, four sections of tissue were incubated in Ca^{2+} -free PSS for 30 min at 37°C . The tissue was then placed in 2 ml low- Ca^{2+} PSS containing papain (1.5 – 2.5 mg ml^{-1}), collagenase (1 – 1.7 mg ml^{-1} , Sigma Type XI), dithiothreitol (1 mM) and bovine serum albumin (2.5 – 3.5 mg ml^{-1}) for 50 – 60 min at 37°C . Following digestion the

tissue was then placed in enzyme-free Ca^{2+} -free PSS, gently triturated and stored in a similar fashion to the rabbit portal vein cells.

Electrophysiology

Voltage clamp and cell current clamp experiments were carried out at room temperature (23° – 27°C) by use of an Axopatch 1A patch clamp amplifier (Axon Instruments, Foster City, CA, U.S.A.). Patch pipettes, which were made from borosilicate glass, had resistances of 2 – 6 $\text{M}\Omega$ when filled with normal intracellular solution. Corrections for cell capacitance

Figure 2 Action of BRL 38227 on the whole cell K^+ currents in the presence of 5 mM tetraethylammonium (TEA) to block the Ca^{2+} -activated K^+ current. (a) Traces from a typical cell in the presence of 5 mM TEA held at -60 mV and stepped over a range of potentials from -100 mV to $+40$ mV with 20 mV increments in the absence (left) and presence of BRL 38227. In (b) the current-voltage relationship of the TEA (5 mM)-insensitive potassium current before ($\circ$) and after ($\bullet$) the addition of BRL 38227 ($10 \mu\text{M}$) is shown for 13 cells (mean with s.e. mean shown by vertical bars). The apparent reversal potential of the BRL 38227-stimulated current was again -75 mV. (c) Spontaneous transient outward currents (STOCs) were elicited from a cell held at 0 mV. TEA dose-dependently inhibited the STOCs; however, addition of BRL 38227 (BRL) still induced a large outward current in the presence of TEA. On removal of TEA, STOCs returned and there was a slight increase in the BRL 38227-induced current. Addition of glibenclamide (Glib) completely inhibited the BRL 38227-induced current with no effects on STOCs.

Figure 3 The effects of BRL 38227 on the time-dependent delayed rectifier current (a) and on the quasi-instantaneous current (b, c) in the presence of tetraethylammonium 5 mM. Panel (a) shows the time-dependent current, calculated by subtracting the quasi-instantaneous current measured at the end of the downstroke of the capacitance artifact at the beginning of the voltage step from the peak current. This time-dependent current was plotted before (○) and after (●) the addition of BRL 38227. (b) The plot of the quasi-instantaneous current as measured previously in the presence (●) and absence (○) of BRL 38227. (c) The current induced by BRL 38227 was calculated by subtracting the currents shown in (b).

and series resistance were not deemed necessary for the experiments carried out. Data were stored on computer hard disc and DAT tape, digitized with a Labmaster DMA TL-1-125 interface, and analysed by the pClamp 5.0 programme (Axon Instruments). Since BRL 38227 affected a non-inactivated current, 'leak' currents were not normally calculated and subtracted except where stated.

Solutions

Physiological saline solution (PSS) contained (in mM): NaCl 130, KCl 5, MgCl₂ 1.2, CaCl₂ 1.5, glucose 10 and HEPES 10. This and all other solutions were buffered to pH 7.2 with NaOH. Low-Ca²⁺ PSS was identical, except that only 10 μM CaCl₂ was added to the solution.

The external solution for measuring K currents was PSS, while the pipette solution contained (in mM): KCl 140, HEPES 10, MgCl₂ 1, EGTA 1, and Na₂ATP 1. For measurement of the Ca²⁺ channel current, cells were incubated in a solution containing (in mM): NaCl 120, KCl 5, MgCl₂ 1.2, BaCl₂ 10, glucose 10, and HEPES 10. The internal solution for measuring the Ba²⁺ current contained (in mM): CsCl 130,

MgCl₂ 2.5, HEPES 10, EGTA 10 and Na₂ATP 1. Stock solutions of BRL 38227 (1 mM) were made up in a 10% dimethylsulphoxide (DMSO) solution whereas glibenclamide (10 mM) stock was made up daily in 100% DMSO.

Papain, dithiothreitol (DTT), bovine serum albumin (BSA), 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA), adenosine 5'-triphosphate (ATP), ethylene glycol bis-(β -aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) and glibenclamide were all obtained from Sigma. BRL 38227 was kindly provided by Dr T. Hamilton at SmithKline Beecham Pharmaceuticals Ltd, Harlow, Essex.

Results

Effects of BRL 38227 on the whole cell K⁺ currents of the rabbit portal vein

The currents elicited from cells dialysed with high potassium intracellular solution, and held at -60 mV, were recorded over a range of test potentials from -100 mV to +40 mV. Time-dependent K⁺ currents were detected at all test potentials positive of -40 mV (Figure 1); these increased in a voltage-dependent manner as described by Beech & Bolton (1989b) and Hume & Leblanc (1989). Under normal conditions in physiological saline the resting potential measured in current clamp was -32 mV $\pm$ 8 (mean $\pm$ s.d., n = 19). Application of BRL 38227 (10 μM) produced the onset of an outward shift in the holding current at -60 mV which reached a sustained maximum after 30 s-1 min, at which time the resting membrane potential was seen to have hyperpolarized to -53 mV $\pm$ 14 (n = 10). Concurrent with the outward shift in the holding current was an increase in the slope of the current voltage (I - V) relationship over the whole range of test potentials. The current voltage relationships in the presence and absence of BRL 38227 intersected at -75 mV, indicating that current elicited by the drug reversed at this potential. The calculated E_K under these conditions was -83 mV.

To study the specific K⁺ conductances being augmented by BRL 38227, 5 mM TEA, externally applied, was used to block the Ca²⁺ activated K⁺ current (Figure 2a). Addition of 5 mM TEA to the extracellular solution had no significant effect on the resting membrane potential measured in current clamp. As described by Beech & Bolton (1989b), the TEA-insensitive current appeared to consist predominantly of a delayed rectifier. By use of the same protocol as above, currents were elicited over a range of test potentials from -100 mV to +40 mV stepping from a holding potential of -60 mV (Figure 2a and b). Addition of BRL 38227 (10 μM) produced a similar response to that already described in Figure 1, with an increase in the slope of the I - V plot and reversal of the BRL 38227 stimulated current near -75 mV. Similarly the resting membrane potential was raised to -53 mV $\pm$ 11 (n = 25).

Further evidence that the K⁺ conductance stimulated by BRL 38227 was not the Ca²⁺-activated K⁺ current is illustrated in Figure 2c. Spontaneous transient outward currents (STOCs), which are the result of the opening of calcium-dependent K⁺ channels stimulated by the spontaneous cyclical release of calcium from an intracellular store (Benham & Bolton, 1986), were evoked by holding a cell at 0 mV (Figure 2c). Addition of 2.5 mM TEA completely inhibited the STOCs, confirming block of the Ca²⁺-dependent K⁺ current. Subsequent application of BRL 38227 (10 μM) resulted in a large outward current. The STOCs returned upon removal of TEA with very little apparent effect on the BRL 38227 response. The response to BRL 38227 was then completely inhibited by glibenclamide (1 μM), a selective inhibitor of the ATP-sensitive K⁺ current. Neither BRL 38227 nor glibenclamide appeared to affect STOCs.

Figure 4 Effects of BRL 38227 on the Ca^{2+} -activated K^+ current in cells held at 0 mV in the presence of 4-aminopyridine 5 mM. (a) Representative traces from a cell held at 0 mV and stepped to +10 mV and +70 mV in the presence and absence of BRL 38227. (b) The I-V relationship of the Ca^{2+} -activated K^+ current for 4 cells (mean with s.e.mean shown by vertical bars) before (○) and after (●) addition of BRL 38227.

On closer examination of the TEA-insensitive K^+ conductance (Figure 2a) it was possible to separate out two distinct components which were differentially affected by BRL 38227. The first component was a time-independent quasi-

Figure 5 The availability of the tetraethylammonium (TEA)-insensitive K^+ current in the absence (○) and presence (●) of BRL 38227 in five cells (mean with s.e.mean shown by vertical bars). Cells were stepped for 10 s to a conditioning potential between -80 mV to +20 mV with 20 mV increments and then stepped to a test potential of +40 mV. The plot shows the current elicited from the test potential from each conditioning step; the current magnitude is expressed as a fraction of the test potential current elicited for each cell after the -80 mV conditioning pulse under control conditions.

Figure 6 The effects of glibenclamide 10 μM (a, b) and phentolamine 100 μM (c, d) on the control instantaneous current (a, c) and the BRL 38227-induced instantaneous current (b, d). Glibenclamide (a, $n = 7$, mean ●, with s.e.mean shown by vertical bars) and phentolamine (c, $n = 3$, ■) had no effect on the control (○, □) instantaneous current. Plot (b) shows the current-voltage relationship of the quasi-instantaneous current ($n = 3$) under control conditions (○) after the addition of BRL 38227 (●) and finally in the presence of BRL 38227 and glibenclamide (▽). Plot (d) shows similar results for the quasi-instantaneous current in a cell under control conditions (□) in the presence of BRL 38227 (■) and after addition of BRL 38227 and phentolamine (△).

instantaneous current which could be estimated at the beginning of the current trace, at the end of the down-stroke of the capacitance artifact. Superimposed upon this was a time-dependent current which rose to a peak and then decayed slowly. Subtraction of the instantaneous current from the total peak current in the presence of 5 mM TEA (Figure 3a) resulted in a time-dependent voltage-sensitive current which activated at potentials positive of -40 mV. This current was equivalent to delayed rectifier as described by Hume & Leblanc (1989), and was unaffected by BRL 38227. The application of BRL 38227 in the presence of 5 mM TEA (Figure 2a) did, however, increase markedly the instantaneous component of the TEA-insensitive current.

Analysis of the quasi-instantaneous current (Figure 3b) showed that BRL 38227 increased the current at all potentials. The BRL 38227-induced current had a reversal potential of -75 mV. This is similar to the calculated K^+ conductance reversal potential, under the experimental conditions used, of -83 mV. Subtraction of the quasi-instantaneous current in the presence and absence of BRL 38227 gave a measure of the BRL 38227-induced current (Figure 3c). The current-voltage relationship of the induced current was linear between -100 mV to +10 mV and showed increasing inward rectification at potentials positive of this. The linear nature of the current-voltage relationship indicated the BRL 38227-induced current showed little voltage-dependence.

The action of BRL 38227 (10 μM) on the Ca^{2+} -activated K^+ current was investigated, under conditions designed to minimize other currents. Cells were held at a potential of 0 mV to

Figure 7 The concentration-response curve to BRL 38227 ($10\ \mu\text{M}$). Cells were held at $0\ \text{mV}$ and the outward current elicited by increasing doses of BRL 38227 was measured and expressed as a fraction of the current stimulated by a $1\ \mu\text{M}$ dose (mean with s.e.mean shown by vertical bars, $n = 5$).

inactivate maximally the delayed rectifier K^+ current (Beech & Bolton, 1989b, see also Figure 5). 4-Aminopyridine (4-AP, $5\ \text{mM}$) was also present in order to block any residual delayed rectifier. When cells were then stepped over a range of test potentials from $0\ \text{mV}$ to $+70\ \text{mV}$, a noisy, non-inactivating current, which appeared to be comprised predominantly of a Ca^{2+} -activated K^+ current, was elicited. Application of BRL 38227 ($10\ \mu\text{M}$), as shown in Figure 4, had no effect on this outward current under these conditions.

The voltage-dependence of the BRL 38227-induced conductance was further investigated by studying the availability of TEA-insensitive current (Figure 5). Cells were held for $10\ \text{s}$ at

Figure 8 Effects of BRL 38227 on the whole cell calcium current: (a) shows representative traces from a cell held at $-60\ \text{mV}$ and stepped to $-20\ \text{mV}$ and $0\ \text{mV}$ before and after the addition of BRL 38227. Plot (b) shows the current-voltage relationship of the peak calcium current (mean with s.e.mean shown by vertical bars, $n = 4$) in the absence ($\circ$) and presence ($\bullet$) of BRL 38227.

potentials between $-80\ \text{mV}$ and $+20\ \text{mV}$, and then stepped to a test potential of $+40\ \text{mV}$ for $100\ \text{ms}$. The peak of the current at the test voltage for each prepulse potential was measured and normalized to the current elicited by the $-80\ \text{mV}$ prepulse step for each cell. This procedure was carried out in 5 cells in the presence and absence of BRL 38227 ($10\ \mu\text{M}$). BRL 38227 caused an upward parallel shift in the availability of the current, indicating that the induced current did not share the sensitivity of the delayed rectifier current to depolarization of the conditional potential. It is noteworthy that the quasi-instantaneous current in control cells also did not inactivate as the holding potential was depolarized.

To identify the current induced by BRL 38227, the effects of glibenclamide and phentolamine, both blockers of the ATP-sensitive K^+ conductance (Standen *et al.*, 1989; Plant & Henquin, 1990), were investigated. In the absence of BRL 38227, neither glibenclamide ($10\ \mu\text{M}$) nor phentolamine ($100\ \mu\text{M}$) had an effect on the instantaneous current evoked from cells held at $-60\ \text{mV}$ and stepped over the range of potentials from $-100\ \text{mV}$ to $+40\ \text{mV}$ (Figure 6a,c). However, after pretreatment with BRL 38227, application of glibenclamide ($10\ \mu\text{M}$) completely blocked any previously induced current (Figure 6b) leaving only the control instantaneous conductance. Under identical conditions, phentolamine ($100\ \mu\text{M}$) had similar effects, completely reversing any stimulation of the current by BRL 38227 (Figure 6d).

The dose-response relationship to BRL 38227 was constructed over a range of concentrations from $30\ \text{nM}$ to $10\ \mu\text{M}$. Cells were held at a potential of $0\ \text{mV}$, and the outward shift in the holding potential induced by several sequentially added concentrations of drug measured. Data in each cell were normalised to the effect observed in $1\ \mu\text{M}$ BRL 38227, as illustrated in Figure 7. The dose-response relationship was fitted to a sigmoid curve using a Hill Coefficient of 1 (i.e. assuming no cooperativity) and an EC_{50} of $1.3\ \mu\text{M}$ was calculated.

Previous work has suggested that cromakalim, the racemic mixture of BRL 38227 and its enantiomer, caused an inhibition of the inward current carried by Ca^{2+} channels in vascular cells (Okabe *et al.*, 1990; Post *et al.*, 1991). We therefore examined the effects of BRL 38227 on the whole cell current through Ca^{2+} channels, using a caesium based pipette solution and an external solution containing $10\ \text{mM}\ \text{Ba}^{2+}$. The Ba^{2+} current evoked from a holding potential of $-60\ \text{mV}$ was apparent positive of $-30\ \text{mV}$ and peaked at around $+10\ \text{mV}$. As shown in Figure 8, BRL 38227 ($10\ \mu\text{M}$) had no significant effect on the $I-V$ plot or kinetics of the Ba^{2+} current. BRL 38227 $50\ \mu\text{M}$ also had no effect on the Ba^{2+} current in the one cell in which it was tested.

Effect of BRL 38227 on the potassium current in human mesenteric artery

The action of BRL 38227 on the K^+ current in cells isolated from the human mesenteric artery was studied (Figure 9) under conditions similar to those previously used in the experiments with the rabbit portal vein. Cells were held at a potential of $-60\ \text{mV}$ and stepped over a range of test potentials from $-100\ \text{mV}$ to $+20\ \text{mV}$. K^+ currents were elicited at test potentials positive of $-30\ \text{mV}$ and appeared to have very little time-dependence. Application of BRL 38227 ($10\ \mu\text{M}$) induced a time-independent current, particularly apparent at more negative test potentials, with a reversal potential of $-75\ \text{mV}$. A further increase in the BRL 38227 concentration to $50\ \mu\text{M}$ did not appear to increase the current augmented by $10\ \mu\text{M}$ BRL 38227 suggesting full activation of the current in these cells was achieved by a maximum dose of $10\ \mu\text{M}$. The current activated by BRL 38227 was then inhibited by the blocker of the ATP-sensitive K^+ channel, glibenclamide ($10\ \mu\text{M}$).

It is noteworthy that the dominant current at positive potentials is a noisy, TEA- and Ca-sensitive K^+ current which

Figure 9 Effects of BRL 38227 (10 and 50 μM) on whole cell K^+ currents in a cell isolated from the human mesenteric artery. The cell was held at -60 mV and stepped to test potentials of -100 mV , -60 mV , -20 mV and $+20\text{ mV}$ under control conditions, in the presence of 10 μM and 50 μM BRL 38227 and after the addition of 50 μM BRL 38227 and 10 μM glibenclamide.

is insensitive to both BRL 38227 and glibenclamide (Figure 9, see Aaronson & Smirnov, 1991, for description of outward current in these cells). Figure 10 illustrates that the blockade of this current with 5 mM TEA unmasks the effect of 10 μM BRL 38227 at positive potentials. As in the rabbit portal vein, the BRL 38227-stimulated current shows no evidence for voltage- or time-dependence, and demonstrates a reversal potential between -70 and -80 mV .

It is of interest that even though these cells demonstrate a substantial outward current at positive potentials, an inward tail current is often present when cells are stepped back to -60 mV (e.g. Figure 9). This tail current must therefore represent a current which is either partially or entirely carried by an ion other than K^+ . The tail current is especially prominent in cells buffered with a low (0.2 mM) EGTA concentration, and is also most prominent at potentials at which the voltage-

gated Ca^{2+} current is maximal, suggesting that it may be Ca^{2+} -activated (Smirnov & Aaronson, unpublished). It is also markedly stimulated by BRL 38227, especially at 50 μM , and this stimulation is reversed by glibenclamide (Figure 9).

Discussion

The results of these experiments suggest that in the rabbit portal vein and human mesenteric artery, BRL 38227 activates a K^+ conductance which is voltage-insensitive and time-independent. The activation of this conductance results in a hyperpolarization of the resting cell membrane potential. The similarity of apparent reversal potential of this current (-75 mV) to the calculated reversal potential for a pure K^+ current of -83 mV suggests the conductance elicited is carried by K^+ . This is in agreement with other investigators using K^+ channel openers (Weir & Weston, 1986; Cook, 1988; Quast & Cook, 1989).

Previous publications have suggested the presence of at least three K^+ currents in single cells isolated from the rabbit portal vein, as discussed in the introduction, which could be considered as the site of action for BRL 38227. Work by Beech & Bolton (1989c) has shown that cromakalim does not act upon the voltage-sensitive K^+ current described as I_{Kr} (Beech & Bolton, 1989a) a transient, rapidly inactivating current present at only hyperpolarized holding potentials.

In the same paper (Beech & Bolton, 1989c) postulated that cromakalim (the racemic mixture of BRL 38227 and its enantiomer) acted upon the delayed rectifier K^+ current to short-circuit the voltage gate of the channels and render the current voltage-insensitive and time-independent. Their conclusions were based on the similarity in potency of the channel blockers phencyclidine, quinidine and 4-AP for the delayed rectifier and the cromakalim-induced currents. Our experiments in the presence of TEA (5 mM) to block the Ca^{2+} -activated K^+ current present in both visceral (Benham & Bolton, 1986; Bolton & Lim, 1989) and vascular (Beech & Bolton, 1989b) smooth muscle cells do not suggest any action of BRL 38227 on the delayed rectifier K^+ current. Rather it appears from our work that the current induced by BRL 38227 is superimposed upon the delayed rectifier K^+ current. Evidence for this is two fold. Firstly, subtraction of the quasi-instantaneous current from the total current elicited in the presence of 5 mM TEA gave a measure of the time-dependent current referred to previously as the delayed rectifier (Beech & Bolton, 1989; Hume & Leblanc, 1989). The magnitude of the time-dependent current was unaffected by 10 μM BRL 38227 (Figure 3). Secondly, BRL 38227 caused a parallel upward shift in the availability of the TEA-insensitive current, indicating the activation of a holding potential-insensitive conduc-

Figure 10 Effects of BRL 38227 on the whole cell K^+ current in human mesenteric artery cells in the presence of 5 mM tetraethylammonium (TEA). (a) The effect of BRL 38227 in a cell held at -60 mV and stepped to -100 mV and $+20\text{ mV}$. (b) Currents elicited from 1 cell in the presence of 5 mM TEA, and the presence (●) and absence (○) of BRL 38227 (10 μM).

tance that was independent of the voltage-sensitive delayed rectifier current, which retained its characteristic potential sensitivity.

Several researchers have suggested the site of action of the K^+ channel openers to be one of the Ca^{2+} -activated K^+ channels. Gelband *et al.* (1989) suggested cromakalim increased the P_{open} of a large conductance Ca^{2+} -activated K^+ channel with similar properties to channels found in a variety of visceral and vascular smooth muscle cells. Others have described nicorandil activating a Ca^{2+} -activated K^+ channel with a conductance of 30 pS (Inoue *et al.*, 1989). More recently, Kajioka *et al.* (1990) presented evidence of nicorandil acting upon a low conductance Ca^{2+} -dependent and ATP-sensitive K^+ in the rat portal vein. Our observations in both rabbit portal vein and human mesenteric artery cells, however, do not indicate a significant action of BRL 38227 on the TEA- and Ca^{2+} -sensitive current. In the rabbit portal vein, currents elicited by stepping positive from a holding potential of 0 mV in the presence of 4-AP (5 mM) should reflect primarily the activation of Ca^{2+} -activated K^+ current. BRL 38227 had no significant effect on the current-voltage relationship under these conditions. We observed spontaneous transient outward currents (STOCs), which have been shown to be caused by Ca^{2+} activated K^+ channels opened by Ca^{2+} released from intracellular stores (Benham & Bolton, 1986). These were blocked almost completely by 2.5 mM TEA, a concentration that had only a minor effect on the response to BRL 38227.

Human mesenteric cells also exhibit prominent STOCs and a noisy K^+ current which is markedly diminished by high concentrations of intracellular EGTA or BAPTA and which is blocked by TEA with an IC_{50} of 0.8 mM (Aaronson & Smirnov, 1991). This current, which is very pronounced at positive potentials (Figure 9, step to +20 mV), is also unaffected by BRL 38227, even at high concentrations.

Hume & Leblanc (1989) described a time-independent K^+ current in rabbit portal vein cells that was inhibited by the removal of extracellular Ca^{2+} ; this current may be equivalent to the quasi-instantaneous current we observed in the absence of BRL 38227. It is important to note, however, that this current was not affected by concentrations of glibenclamide and phentolamine that completely blocked the effect of BRL 38227 on the quasi-instantaneous current. Therefore, we do not feel that BRL 38227 increased the existing quasi-instantaneous current; rather it induced a new time-independent conductance.

It has been postulated that part of the relaxant action of cromakalim is an inhibition of the voltage-operated Ca^{2+} current (Okabe *et al.*, 1990). Post *et al.* (1991) showed, however, that BRL 38227 did not inhibit I_{Ca} in colonic smooth muscle. Our observation that BRL 38227 did not reduce I_{Ca} in the rabbit portal vein is therefore consistent with this latter report.

The final class of potassium channels to be considered as a possible site of action for ' K^+ channel openers' are the ATP-sensitive K^+ channels. Since their initial discovery in cardiac cells (Noma, 1983), ATP-sensitive channels have been identi-

fied in a variety of tissues including pancreatic β -cells (Cook & Hales, 1984), skeletal muscle (Spruce *et al.*, 1985) and more recently in vascular (Standen *et al.*, 1989) smooth muscle. In other tissues this channel has been shown to have a high selectivity for K^+ ions, to exhibit little voltage sensitivity and to inwardly rectify at potentials positive of +20 mV (for review see Ashcroft & Ashcroft, 1990). Our observation that BRL 38227 induced a current with a reversal potential near the K^+ equilibrium potential and a linear current-voltage relationship which begins to inwardly rectify at positive potentials are in complete agreement with these observations. Further evidence of the action of BRL 38227 being on ATP-sensitive K^+ channels is the blocking of the BRL 38227-induced current by phentolamine and by the sulphonylurea compound, glibenclamide. Both drugs have previously been shown to inhibit selectively the relaxant actions of K^+ channel openers in a variety of smooth muscles (McPherson & Angus, 1989; Murray *et al.*, 1989; Nielsen-Kudsk *et al.*, 1990). In other tissues they have been shown to be selective inhibitors of the ATP-sensitive K^+ current (glibenclamide, Standen *et al.*, 1989; Belles *et al.*, 1987; phentolamine, Plant & Henquin, 1990). Thus under our experimental conditions the current induced by BRL 38227 appears to have the characteristics of the ATP-sensitive K^+ current, as described in other tissues. Final confirmation of the nature of this current must, however, await single channel recordings, which we have not yet carried out.

In the cells isolated from the human mesenteric artery, BRL 38227 produced similar results to those obtained in the rabbit portal vein cells. BRL 38227 caused the appearance of a time-independent current even at very negative test potentials, which was specifically blocked by glibenclamide, and which had a reversal potential between -70 and -80 mV. It appears therefore BRL 38227 induced a K^+ conductance in the human mesenteric artery cells which has similar characteristics to that present in the rabbit portal vein. It is interesting, however, that in human mesenteric artery cells, BRL 38227 also increased the magnitude of an inward tail current which could not have been caused by this time-dependent K^+ current. Although we have not studied this tail current in detail, its enhancement by low EGTA_i and by I_{Ca} suggests that it is Ca^{2+} -activated. We speculate that, as in other types of smooth muscle cell (Chopra *et al.*, 1990), BRL 38227 may inhibit Ca^{2+} uptake into the sarcoplasmic reticulum. Thus, Ca^{2+} entering through voltage gated Ca^{2+} channels during depolarizing steps might be sequestered to a smaller extent in the presence of BRL 38227, leading to a rise in subplasmalemmal $[Ca^{2+}]_i$ and the stimulation of a Ca^{2+} -dependent tail current. Confirmation of this possibility must, however, await additional study.

In conclusion, it appears that BRL 38227 induces a current through channels which have the pharmacological and physiological characteristics of an ATP-sensitive K^+ conductance. The existence of this current in both cell models studied, taken in conjunction with the work of other investigators, suggests this conductance may be ubiquitous in vascular smooth muscle cells.

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