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Relaxant Effects of the Potassium Channel Activators BRL 38227 and Pinacidil on Guinea-pig and Human Airway Smooth Muscle, and Blockade of Their Effects by Glibenclamide and BRL 31660

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SUMMARY: The airways relaxant effects and mechanism of action of the potassium channel activators BRL 38227 and pinacidil have been compared in guinea-pig and human airways. BRL 38227 was a potent relaxant in guinea-pig isolated trachealis ($IC_{50} = 4.9 \times 10^{-7}$ M against spontaneous tone) and human isolated bronchi ($IC_{50} = 4.75 \times 10^{-7}$ M against histamine-induced tone) and was eight- and six-fold more potent respectively than pinacidil. The relaxant effects of both compounds were shown to be markedly attenuated by glibenclamide (10^{-5} M) and BRL 31660 (10^{-5} M), with the nature of the blockade being species/tissue dependent. Glibenclamide (20 mg/kg iv) also inhibited the protective effects of BRL 38227 (50 μg/kg iv) and pinacidil (500 μg/kg iv) on histamine-induced changes in airways resistance and dynamic compliance in the anaesthetized guinea-pig, although the effects were short-lived. That both BRL 38227 and pinacidil owed their relaxant effects to potassium channel activation was supported by their ability to stimulate $^{42/43}K$ efflux from guinea-pig trachealis preloaded with the radiotracer at concentrations of 10^{-7}–10^{-5} M and 10^{-5} M respectively. Pretreatment with either glibenclamide (10^{-5} M) or BRL 31660 (10^{-5} M) ablated the response to both compounds. These studies show that two mechanistically distinct potassium channel blockers, glibenclamide and BRL 31660, do not substantially differentiate between the actions of BRL 38227 and pinacidil, although differences do occur, particularly at high concentrations in vitro.

INTRODUCTION

Potassium ions play an important role in the maintenance of membrane potential in smooth muscle cells. Under resting conditions the plasma membrane is more permeable to potassium than to sodium or calcium ions, and since potassium ions are present at higher concentration within the cytosol than outside the cell (whereas the reverse is true for sodium and calcium ions), the dominance of the potassium permeability creates a negative potential within the cell.[1] Stimulation of smooth muscle cells by various means raises sodium and calcium ion permeability, thereby depolarizing the membrane and increasing levels of intracellular calcium ions either as a result of influx through calcium channels or via release from intracellular stores, with subsequent contraction.[2]

Many compounds are known to directly inhibit calcium entry via high threshold L-type voltage-dependent channels,[3] but are unable to prevent the increase in intracellular calcium which arises from calcium entry through other channels or by its release from intracellular stores.[4] Moreover, whilst these calcium channel blockers are of value as vasodilators in the treatment of cardiovascular disorders,[5] they have not proved to be of benefit as bronchodilators in asthma.[6–8] A compound with a broader profile of pharmacological action to that of the calcium channel blockers should have a greater therapeutic potential, particularly in the treatment of respiratory disorders.

We have compared the effects of BRL 38227 (the active enantiomer of cromakalim)[9,10] and pinacidil (a structurally distinct potassium channel activator recently shown to share a similar mechanism of action)[11] (Fig. 1) as airways relaxants in vitro and as bronchodilators in vivo. In order to further understand the mechanism by which the potassium channel activators relax airway smooth muscle, we have also investigated the effects of two potassium channel blockers, the insulin secretagogue glibenclamide[12] and the anti-arrythmic agent BRL 31660 (Fig. 1)[13] on the relaxant activity of BRL 38227 and pinacidil. In addition, we have studied the ability of these blockers to attenuate potassium efflux in guinea-pig isolated trachealis in response to both BRL 38227 and pinacidil.

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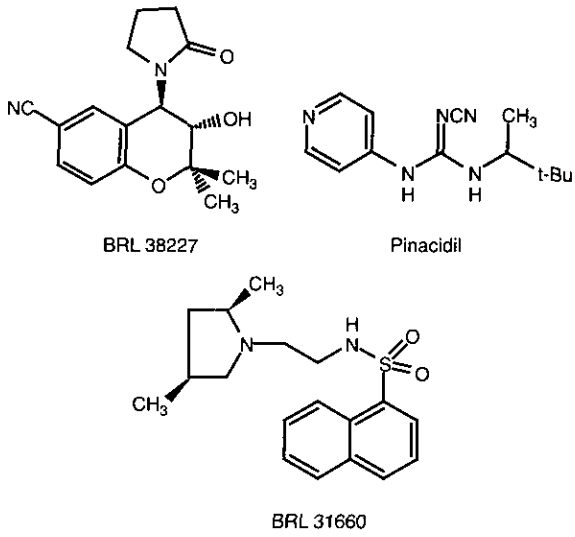

Fig. 1 Chemical structures of BRL 38227, pinacidil and BRL 31660.

METHODS

Guinea-pig isolated tracheal spirals

Tension experiments

Tracheal spiral strips (two per animal) from male Dunkin-Hartley guinea-pigs (300–600 g) were prepared and suspended under isometric conditions in oxygenated Krebs solution. Tension was allowed to develop spontaneously and was maintained at 2 g by frequent adjustment. For the blocking experiments, glibenclamide or BRL 31660 was added 20 min prior to the potassium channel activators. BRL 38227 and pinacidil were added in a cumulative fashion and the inhibitory effects were calculated as a percentage of the relaxation induced by a supramaximal concentration of isoprenaline (10^{-3} M) added at the end of the experiment. Only one concentration-effect experiment was performed in a tissue and for the blocking experiments a single concentration of glibenclamide or BRL 31660 was tested prior to examining the effects of either BRL 38227 or pinacidil.

Potassium efflux experiments

Trachea excised from the above animals were cleaned of adhering fat and connective tissue and opened by longitudinal section. The trachealis muscle was dissected free from the organ, divided into three segments and each segment was randomly assigned to the various treatment groups. No attempt was made to remove epithelium. Following a preincubation at 37°C in 2.5 ml of Krebs solution bubbled with 95% O_2: 5% CO_2, tissues were loaded with $^{42/43}K$ (37–74 MBq/l) at 37°C in 2.5 ml of Krebs solution for 60 min. Efflux was followed by transferring tissues through a sequence of 17 washing samples at 37°C, with a resident time in each wash of 3 min. When present, BRL 38227 or pinacidil was added to tubes 11–14. In the majority of experiments in which the effects of the potassium channel blockers glibenclamide (10^{-5} M) and BRL 31660 (10^{-5} M) were tested, these were added only to tubes 11–14, preliminary experiments having shown them to be equi-effective whether added in this manner or present throughout the efflux experiment. Neither concentration of glibenclamide nor BRL 31660 elicited any effect on basal efflux. At the end of the efflux period, the tissues were blotted and the radioactivity in both tissues and washing samples was measured by γ-counting. Efflux was calculated as a rate coefficient (fractional loss of radioactivity from the tissue per minute, expressed as a percentage).

Human isolated bronchi

Macroscopically normal lung tissue was obtained from patients of both sexes undergoing surgery for carcinoma of the lung and was maintained at 4°C overnight in Krebs solution prior to use. Spiral strips were mounted under isometric conditions at 2 g tension in 10 ml tissue baths containing Krebs solution bubbled with 95% O_2/5% CO_2 at 37°C. Tension was maintained at 2 g by frequent adjustment over a 90 min equilibration period prior to drug studies. For spasmolytic experiments, compounds were added using a cumulative protocol against tension induced by an approximately EC_{70} concentration of histamine (5×10^{-6} M) in addition to any spontaneous tone present. For the blocking experiments, tension was induced by the same concentration of histamine and the tissue was equilibrated for 20 min in the presence of either glibenclamide or BRL 31660 prior to treatment with the test compound. Only one concentration-effect experiment was performed in a tissue and in the blocking experiments a single concentration of glibenclamide or BRL 31660 was tested prior to examining the effects of either BRL 38227 or pinacidil.

Bronchoconstriction in conscious guinea-pigs

Male guinea-pigs (400–460 g) were dosed orally with BRL 38227, pinacidil or the vehicle and placed in a Perspex chamber of approximately 8 l capacity. At various times subsequent to dosing, the animals were challenged for 4 min with a histamine aerosol generated over 20 s from a 5 mM solution of histamine diphosphate using a Monaghan 675 ultrasonic nebulizer (power setting 7). Individual groups of guinea-pigs were taken for each time point and the time from the introduction of the aerosol to collapse was recorded. Those animals not collapsing within the

4 min observation time were considered to be fully protected.

Bronchoconstriction in anaesthetized guinea-pigs

Male Dunkin-Hartley guinea-pigs (600–800 g) were anaesthetized with urethane (1.25/g kg iv). The trachea was cannulated to measure air flow (low pressure Ether UP1 differential pressure transducer connected to a heated Fleisch pneumotachograph), the carotid artery to measure mean blood pressure and the jugular vein for iv dosing. The oesophagus was ligated just above the thoracic region to give a maximum negative pressure reading (low pressure transducer, Ether UP1) in phase with the airflow signal. The reference port of the oesophageal pressure transducer was connected to the airway port of the pneumotachograph, so that airway pressure was subtracted from intraoesophageal pressure and the pressure differential due to the resistance screen of the pneumotachograph was removed. Airway resistance and dynamic lung compliance were calculated by the method of Dennis et al[14] using a specially designed analogue computer (G. R. Francis, SmithKline Beecham, Harlow).

Histamine challenges were given iv at 10-min intervals at a dose which produced at least a 100% increase in airways resistance. The mean increases in resistance and decreases in compliance produced by histamine in control animals were $162 \pm 9.34\%$ and $24.6 \pm 1.38\%$ ($n = 16$) from baseline values of 0.055 ± 0.004 cmH_2O ml/sec^{-1} and 1.49 ± 0.07 ml/cm H_2O. Once reproducible responses were obtained, glibenclamide or vehicle were given 15 or 30 min prior to BRL 38227 or pinacidil, which were themselves given 2 min before further challenge. Mean arterial blood pressure was measured immediately prior to challenge, at which point any effects of the compounds were maximal. The baseline blood pressure was 51 ± 8 mm Hg (mean $\pm$ SD, $n = 50$). The effects of the compounds on histamine-induced changes in resistance and compliance are expressed relative to the effect of the last histamine challenge prior to their administration.

Drugs and solutions

BRL 38227 and BRL 31660 were synthesized in the SmithKline Beecham Laboratories, the latter compound being used as its hemifumarate salt. Pinacidil was supplied by Leo Pharmaceuticals. BRL 38227 was dissolved in 10% dimethylsulphoxide at 10^{-3} M for the in vitro work. Pinacidil was dissolved in 70% ethanol at 10^{-2} M. Further dilutions of BRL 38227 and pinacidil were into water. For oral administration the compounds were suspended in 1% methylcellulose, the dose volume being 1 ml/kg body weight.

The Krebs solution used had the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.18 and glucose 11. The mixed potassium isotope ($^{42/43}K$) was supplied by the MRC Cyclotron Unit (Hammersmith Hospital, London) and contained ^{42}K and ^{43}K in an approximately 1:2 ratio.

Statistical analysis

Results are expressed as arithmetic means $\pm$ SEM or geometric means with 95% confidence limits. For the experiments in conscious animals, times of 240 s were allocated to animals that were totally protected for the purposes of calculating arithmetic mean collapse or cough times. Statistical analysis in these experiments was by one-tailed Mann-Whitney *U*-test.

RESULTS

Relaxant effects of BRL 38227 and pinacidil in vitro

Guinea-pig trachealis

BRL 38227 and pinacidil evoked a characteristic inhibition of spontaneous tone in guinea-pig isolated trachealis in a concentration-dependent manner (Fig. 2). Both compounds were capable of producing almost complete inhibition, and the concentrations found to achieve 50% of the maximum relaxation attained by isoprenaline (10^{-3} M) were 4.94 (3.60–6.77) $\times 10^{-7}$ M ($n = 10$) and 1.39 (0.83–2.33) $\times 10^{-6}$ M ($n = 10$) for BRL 38227 and pinacidil respectively. Prior equilibration of the tissues with the potassium channel blockers glibenclamide (3×10^{-7} to 10^{-5} M) or BRL 31660 (10^{-6}–10^{-4} M) for 30 min at each concentration reduced the effects of both BRL 38227 and pinacidil (Fig. 2). For BRL 38227, increasing concentrations of BRL 31660 resulted in a non-competitive antagonism of the relaxation achieved (Fig. 2A). With pinacidil, however, BRL 31660 produced a rightward shift of the concentration–response curve with no change in the maximum relaxation (Fig. 2B). Glibenclamide behaved differently in that low concentrations (3×10^{-7} to 10^{-6} M) evoked a parallel rightward shift of the BRL 38227 concentration–response curve, whereas at higher concentrations the maximum response was depressed (Fig. 2C). With pinacidil, glibenclamide evoked a rightward shift of the concentration–response curve over the range 3×10^{-7} to 10^{-6} M, had no further effect at higher concentrations (Fig. 2D).

Human bronchi

In a similar manner, both BRL 38227 and pinacidil caused a near maximal and concentration-dependent inhibition of histamine (5×10^{-6} M)-induced contractions in human isolated bronchi (Fig. 3). The IC_{50} concentrations relative to the relaxant effects of iso-

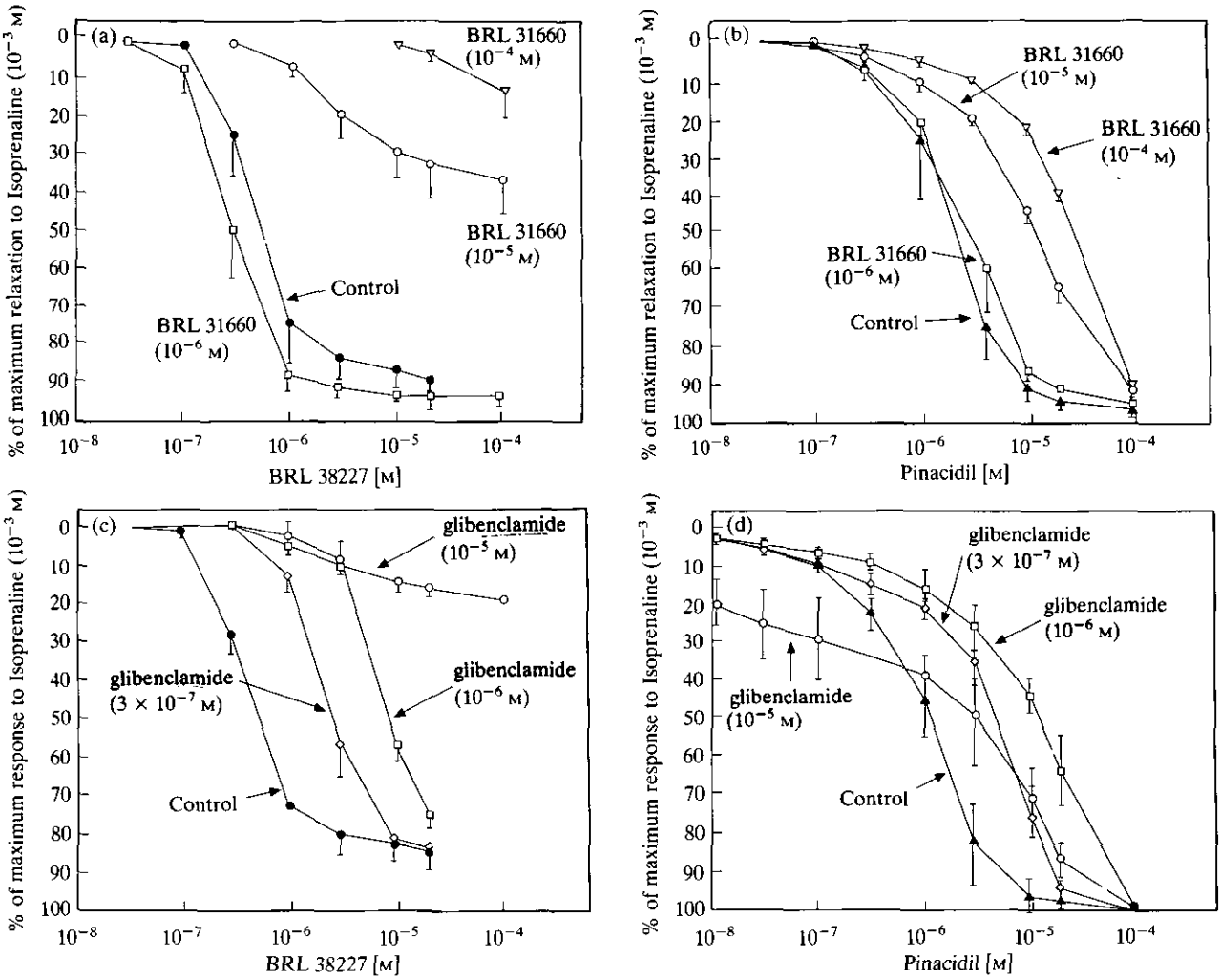

Fig. 2 Inhibition of spontaneous tone in guinea-pig trachealis by BRL 38227 (●, a and c) and pinacidil (▲, b and d) and blockade of these effects by BRL 31660 (a and b) at concentrations of 10^{-6} (□), 10^{-5} (○) and 10^{-4} M (▽), and by glibenclamide (c and d) at concentrations of 3×10^{-7} (◇), 10^{-6} (□), and 10^{-5} M (○). The points are means of four to six values with vertical lines showing SEM.

prenaline (10^{-3} M) were 4.75 (2.64–8.53) $\times 10^{-7}$ M, n = 7 and 2.7 (0.64–11.5) $\times 10^{-6}$ M, n = 4 respectively. As with the guinea-pig trachealis, the relaxant effects of both agents were inhibited by prior incubation with either glibenclamide (10^{-5} M) or BRL 31660 (10^{-5} M) (Fig. 3). Thus, for BRL 38227, this concentration of BRL 31660 induced a six-fold rightward shift compared to a 114-fold rightward shift with glibenclamide (Fig. 3A). Similarly with pinacidil, BRL 31660 caused a ten-fold rightward shift of the concentration–response curve, although glibenclamide did not show the marked change observed with BRL 38227, causing only a 12-fold shift (Fig. 3).

Inhibition of $^{42/43}$K efflux in airway smooth muscle

Table 1 shows that BRL 38227 (10^{-7}–10^{-5} M) is able to stimulate potassium efflux in a concentration dependent fashion. Pinacidil caused efflux at concentrations of 10^{-5} M and above; lower concentrations were not tested. Pretreatment with either glibenclamide (10^{-5} M) or BRL 31660 (10^{-5} M) ablated the responses to both compounds (Fig. 4). At the concentrations used, neither glibenclamide nor BRL 31660 showed any effects on basal efflux of potassium; 101 ± 12% and 97 ± 4% of controls respectively (n = 4).

Relaxant effects of BRL 38227 and pinacidil in vivo

Histamine-induced bronchospasm

BRL 38227 (2.5 mg/kg po) and pinacidil (20 mg/kg po) prolonged the time to collapse following exposure of guinea-pigs to an aerosol of histamine, with the maximal effect occurring 30–60 min after dosing (Fig. 5). From subsequent dose–response studies conducted during this period of optimal activity, it was found that the dose of each compound required to protect half of the animals from the respiratory effects of the histamine aerosol during the 4 min observation time was 1.25–5 mg/kg and 10–20 mg/kg for BRL 38227 and pinacidil respectively (Fig. 6).

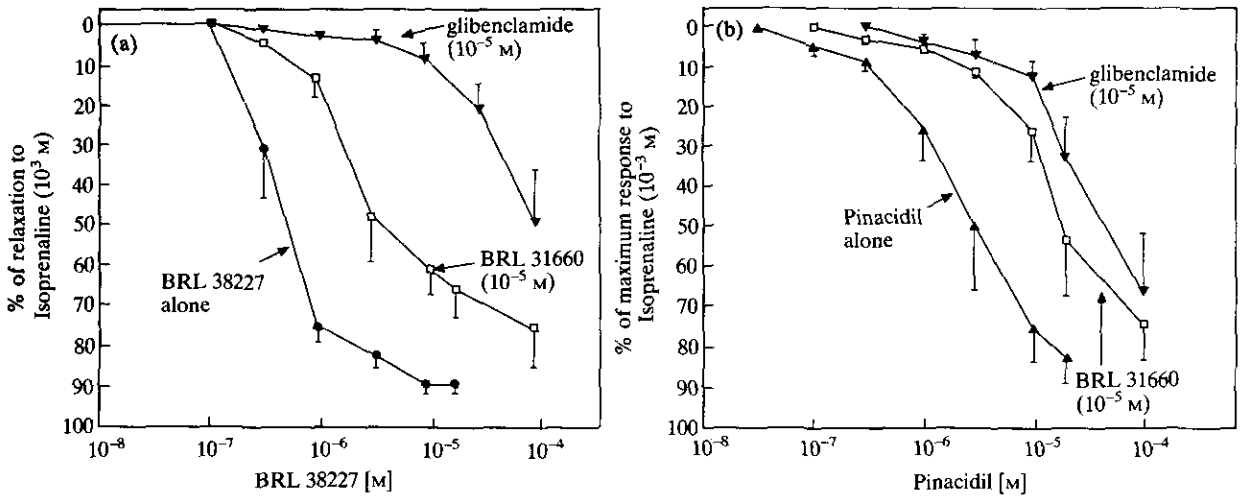

Fig. 3 Inhibition of histamine-induced tone in human bronchi by BRL 38227 (●, a) and pinacidil (▲, b) and blockade of these effects by BRL 31660 (10^{-6} M, □) and glibenclamide (10^{-6} M, ▼). The points are means of four values for tissues for four (BRL 38227) or three (pinacidil) patients, with vertical lines showing SE.

Table 1 Efflux of $^{42/43}K$ from guinea-pig trachealis in response to BRL 38227 and pinacidil.

Drug	Concentration M	Maximal efflux rate %/min	n
Control	0.0	1.30 ± 0.04	66
BRL 38227	10^{-7}	1.63 ± 0.05*	3
	3×10^{-7}	1.46 ± 0.11†	9
	10^{-6}	1.71 ± 0.08‡	8
	3×10^{-6}	1.74 ± 0.08‡	14
	5×10^{-6}	2.17 ± 0.10‡	32
	10^{-5}	2.15 ± 0.12‡	19
Pinacidil	10^{-5}	1.60 ± 0.16*	4
	3×10^{-5}	1.99 ± 0.12‡	21

Values quoted represent mean values ± SEM and are the maximum efflux occurring during the time that the drug was present. *($P < 0.05$), †($P < 0.01$), ‡($P < 0.001$) significantly different from time matched controls.

Respiratory dynamics in the anaesthetized guinea-pig

BRL 38227 (50 μg/kg iv) and pinacidil (500 μg/kg iv) did not alter the baseline levels of airway resistance or compliance, although systemic blood pressure was reduced by 38 ± 2.5% and 45 ± 3% respectively (Fig. 7). These doses reduced the subsequent histamine-induced changes in airway resistance and dynamic lung compliance (Fig. 7). Glibenclamide at 20 mg/kg iv transiently reduced baseline resistance and increased compliance in some animals, but by 15 min baseline values had returned to normal and glibenclamide did not attenuate responses to histamine. At this dose, however, glibenclamide increased systemic blood pressure by 45 ± 6% ($P < 0.001$, n = 13) 5 min after administration and this increase remained almost unchanged up to 30 min. When glibenclamide was given 15 min prior to BRL 38227 the reduction in blood pressure was inhibited and the protective effects to subsequent histamine challenge were ablated, but when given 30 min prior to BRL 38227, glibenclamide failed to influence the effect of BRL 38227 on the histamine challenge, whilst still reducing its effect on blood pressure (Fig. 7A). Significant inhibition of the effects of pinacidil was achieved only at 15 min and only with respect to blood pressure and airways resistance (Fig. 7B).

DISCUSSION

Potassium channel activators, notably cromakalim and pinacidil, have been extensively studied for their relaxant activity on vascular smooth muscle and have been shown to have potent antihypertensive effects in vivo.[10] The potential of the potassium channel activators in asthma requires further exploration, although preliminary reports concerning the effects of cromakalim on morning dip in lung function were encouraging,[15] and lend support to the protective effects shown with this compound in animal models of bronchoconstriction.[9,16]

Unlike other smooth muscle relaxants, potassium channel activators evoke steep concentration-dependent inhibition of spontaneous tone in guinea-pig isolated trachealis, which serves to help distinguish these compounds from other agents. Thus, in common with cromakalim,[9] both BRL 38227 and pinacidil show a characteristic inhibition of spontaneous tone in this tissue (Fig. 2), with BRL 38227 being some 8-fold more potent. In addition to the relaxation of guinea-pig airways, both BRL 38227 and pinacidil are potent relaxants of histamine-induced tone in isolated human bronchi (Fig. 3), with BRL 38227 being six-fold more potent. In both of these tissues BRL 38227 and pinacidil showed a similar efficacy to that of isoprenaline.

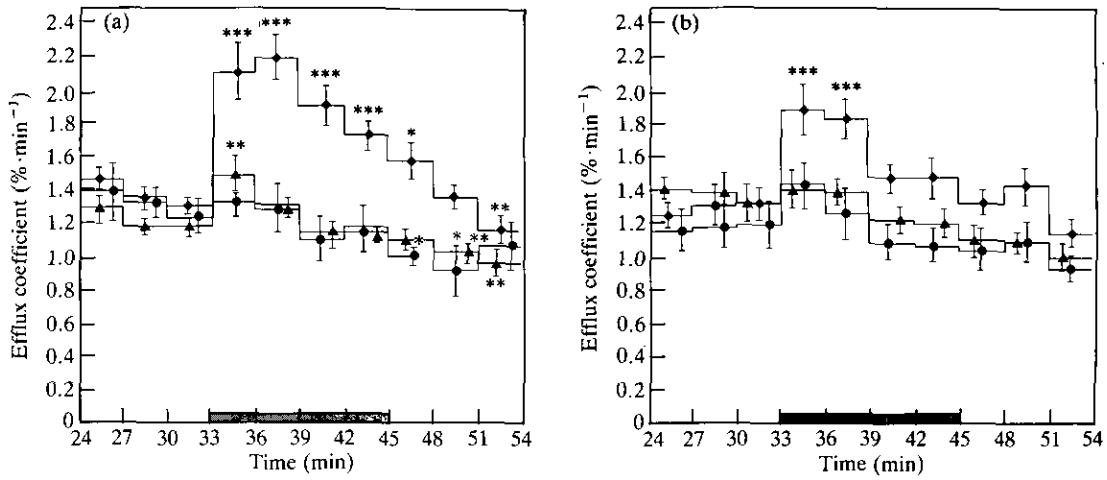

Fig. 4 Efflux of $^{42/43}K^+$ from guinea-pig trachealis was measured as described in the methods section. Drugs were added during the period indicated by the hatched bar. (A) ◆ 5×10^{-6} M BRL 38227 (n = 21); ● 5×10^{-6} M BRL 38227 + 10^{-6} M BRL 31660 (n = 5); ▲ 5×10^{-6} M BRL 38227 + 10^{-6} M Glibenclamide (n = 11). (B) ◆ 3×10^{-5} M Pinacidil (n = 21); ● 3×10^{-5} M Pinacidil + 10^{-6} M BRL 31660 (n = 16); ▲ 3×10^{-5} M Pinacidil + 10^{-6} M Glibenclamide (n = 5). Each point is the mean of the number of determinations given with the bar denoting the SEM. Significant differences were assessed relative to the mean efflux occurring in the three tubes immediately prior to drug addition using the Student's *t*-test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

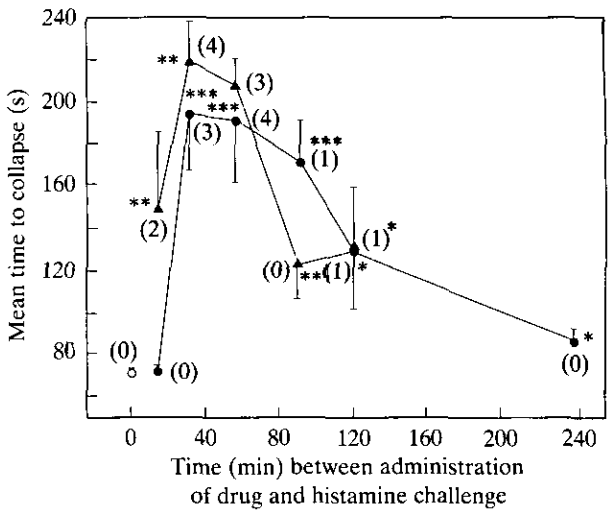

Fig. 5 Time course of the effects of BRL 38227 (●, 2.5 mg/kg, po) and pinacidil (▲, 20 mg/kg, po) on the time to collapse following administration of a histamine aerosol to conscious guinea-pigs. The collapse time of the controls (○) was 71 ± 4 s (*n* = 6) in the BRL 38227 experiment and 70 ± 5 s (*n* = 5) in the pinacidil experiment. The values shown are means with vertical lines indicating SE $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared to the collapse time for controls. The figures in parentheses indicate the numbers of animals protected during the 4 min observation time out of six (BRL 38227) or five (pinacidil).

In an endeavour to increase our understanding of the mechanism by which potassium channel activators exert their smooth muscle relaxant effects, the inhibitory action of two structurally and mechanistically distinct blocking agents, glibenclamide and BRL 31660, on the airway relaxant effects of BRL 38227 and pinacidil have been evaluated. Glibenclamide and BRL 31660 differ in that glibenclamide is believed to exert its effects through the direct blockade of ATP-dependent and possibly other potassium channels and is a potent hypoglycaemic agent in vivo.[12,17-22] In contrast, BRL 31660 presumably effects blockade through its action as a membrane stabilizing agent, since it is known to have both class I and class IV anti-arrythmic activity.[23] It was considered therefore that these two agents might elicit differential inhibitory effects against the two unrelated potassium channel activators.

These two agents were preferred to classical blockers, such as 4-aminopyridine and procaine,[24] because the induction of phasic activity in trachealis muscle by the earlier blockers limits their value as mechanistic tools.[13] Glibenclamide and BRL 31660 do not suffer from this disadvantage, although glibenclamide (10^{-5} M) has tended to accelerate the fade in tension prior to addition of the potassium channel activators [25](and data not shown), possibly as a consequence of thromboxane A_2 receptor antagonism.[26] Our results show that at concentrations which did not block the relaxant effects of isoprenaline (10^{-9}–10^{-6} M), sodium nitroprusside (10^{-8}–10^{-5} M) or indomethacin (10^{-8}–10^{-5} M),[13] both glibenclamide and BRL 31660 markedly attenuated the relaxation induced by either BRL 38227 or pinacidil in guinea-pig and human airways (Figs 2 and 3), which adds support to other evidence presented here that these compounds owe their relaxant activity in these tissues to potassium channel activation. Consistent with the results of Black et al[27] on human tissue, higher concentrations of glibenclamide suppressed the maximum response to BRL 38227 but not to that of pinacidil. Similar observations have been reported with cromakalim[28-30] and with pinacidil[29] in the guinea-pig,

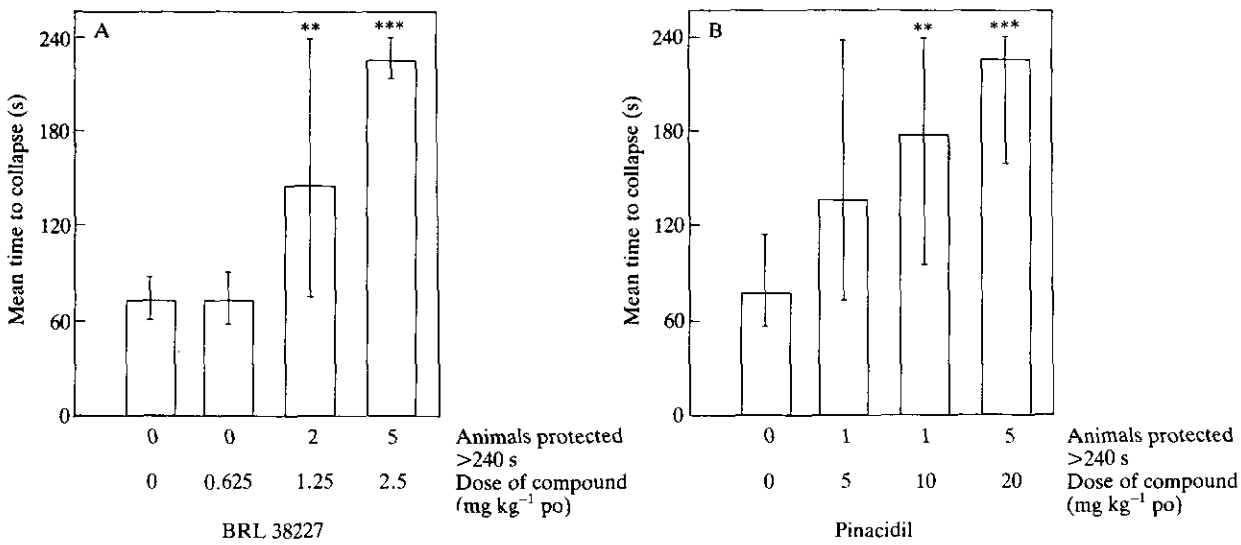

Fig. 6 Dose–response relationship for the protection of conscious guinea-pigs from histamine aerosol by (A) BRL 38227 given 60 min before and (B) pinacidil given 60 min before challenge. The bars indicate the range of collapse times recorded with 240 s indicating total protection. $^{**}P<0.01$; $^{***}P<0.001$ compared to controls.

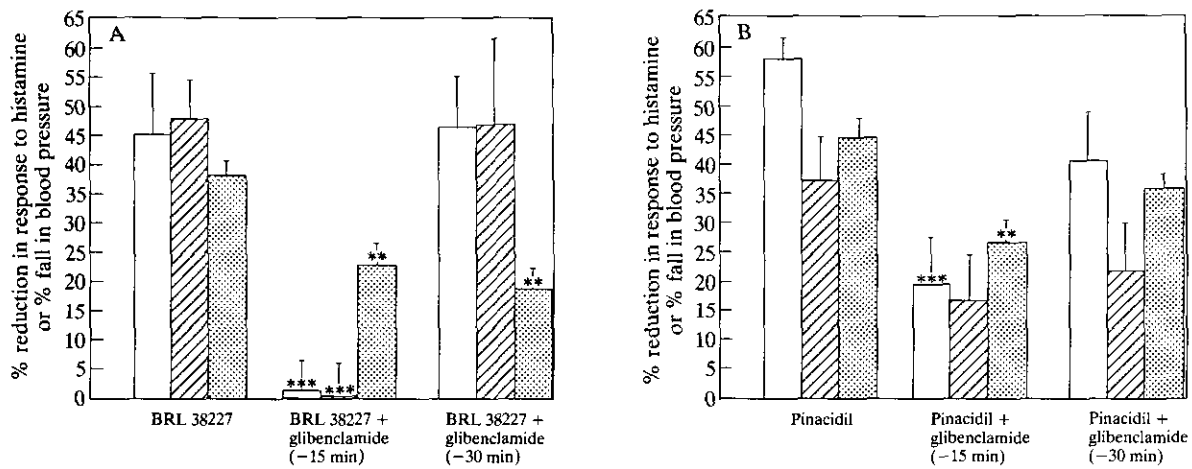

Fig. 7 Effects of BRL 38227 (A: 50 μg/kg iv) and pinacidil (B: 500 μg/kg iv) in the absence and presence of glibenclamide (20 μg/kg iv) on mean blood pressure (spotted columns) and on histamine-induced increases in resistance (open columns) and decreases in compliance (hatched columns). Results are means of at least six values with vertical lines showing SEM. Significance of effects of glibenclamide: $^{**}P<0.01$; $^{***}P<0.001$.

though Lewis et al[25] found only a rightward shift with cromakalim in guinea-pig trachealis.

It seems probable that glibenclamide and BRL 31660 do not compete for the same binding site as BRL 38227 and pinacidil and that the failure of both BRL 31660 and glibenclamide to inhibit the relaxant effects of high concentrations of pinacidil (Fig. 2B and D) is due to other activities.[31] Thus, these agents only remove that part of the action of pinacidil that is due to the opening of potassium channels.[32–35] The ability of glibenclamide and BRL 31660 to completely block the effects of BRL 38227 in guinea-pig trachealis (Fig. 2A and C), yet only effect rightward shifts of the concentration–response curve in human airways (Fig. 3A), is possibly due to BRL 38227 being a more potent relaxant in the airways of man than those of the guinea-pig, an effect which might reflect an increased number of BRL 38227 sensitive potassium channels in this tissue. By analogy with classical receptor theory, there may be sufficient spare receptors (potassium channels) in the human tissue that a non-competitive antagonist fails to suppress the maximum response to the agonist.[36]

The efflux of potassium ions from smooth muscle cells incubated in the presence of potassium channel activators serves as an additional indicator of the mechanism by which these compounds exert their relaxant activity and ^{86}Rb has been extensively used as a stable surrogate to ^{42}K in vascular tissue.[37–39] Rb^+ is a less efficient marker of potassium movement in

airway smooth muscle, however, since the rate of efflux is low and BRL 38227 may open some potassium channels that are not permeable to Rb^+.[24,40,41] As a consequence, we have used guinea-pig trachealis labelled with the mixed radioisotopes $^{42/43}K$ and shown concentration-dependent stimulation of efflux in the presence of BRL 38227. Pinacidil also enhanced efflux (Table 1) and the effects of both agents were ablated on preincubation with either glibenclamide or BRL 31660 (Fig. 4A,B).

As previously shown for the racemic material, cromakalim,[9] BRL 38227 was an effective and persistent inhibitor of histamine-induced bronchoconstriction in the conscious guinea-pig when administered orally (Fig. 5), and was eight times more potent than pinacidil when each drug was given at the optimal time prior to challenge (Fig. 6).

The blocking effects of glibenclamide and BRL 31660 in vivo were not investigated in conscious animals since glibenclamide is a highly potent hypoglycaemic agent and BRL 31660 has profound antiarrythmic effects. In anaesthetized animals the hypoglycaemia induced by glibenclamide may be compensated by the coadministration of glucose. With BRL 31660, however, the anti-arrythmic effects are not easily reversed and investigative blocking studies were unsuccessful because of its short action in vivo, and because given alone (at 4 mg/kg iv) it caused large falls in heart rate and blood pressure and inhibited bronchospasm. However, glibenclamide has been shown to inhibit the antihypertensive effects of BRL 38227 in the anaesthetized rat.[42] The blocking effects of glibenclamide were best studied using respiratory dynamics measurements in the anaesthetized guinea-pig. Thus, at doses which were shown to have no effects on baseline airways responses, BRL 38227 and pinacidil given intravenously were effective inhibitors of histamine-induced changes in resistance and dynamic lung compliance (Fig. 7). Since urethane anaesthesia abolishes reflex tachycardia in the guinea-pig, similar doses also caused a significant fall in systemic mean arterial blood pressure. Administration of a bolus intravenous dose of glibenclamide (20 mg/kg) was shown to have only a transient effect on baseline respiratory parameters, and no effect on the respiratory effects of histamine, but did significantly increase systemic blood pressure. This dose given 15 min prior to BRL 38227 ablated the protective effects seen against histamine challenge, but when given 30 min prior to BRL 38227 it had no inhibitory effect (Fig. 7A). That the effects of glibenclamide persisted in vivo for the longer duration experiments was evident from the prolonged elevation of baseline blood pressure and the inhibitory effects seen against the hypotensive action of BRL 38227 at 30 min. These results concur with those recently reported by Ichinose and Barnes[43] where glibenclamide was shown to attenuate the protective action of cromakalim on non-adrenergic, non-cholinergic excitatory bronchoconstriction in the anaesthetized guinea-pig at 15 min, but no other in vivo studies of the effects of glibenclamide on respiratory parameters have been reported. Against pinacidil, glibenclamide was significantly effective at the 15 min time point only and, in contrast to the BRL 38227 experiment, it had no effects against blood pressure at 30 min (Fig. 7B).

These differences between the airways and blood pressure responses, and between BRL 38227 and pinacidil, might be explained by differences in the sensitivities of potassium channels in blood vessels and the lung to glibenclamide. Alternatively, they may reflect differences in the pharmacokinetics of glibenclamide in the two tissues, coupled with minor differences in the mechanism of action of the two potassium channel activators in blood vessels. An action of pinacidil independent of potassium channels has been alluded to above, but evidence also exists that it differs somewhat from cromakalim, and possibly therefore BRL 38227, in its effects on potassium channels.[44,45]

That the mechanistically distinct potassium channel blockers, glibenclamide and BRL 31660, do not appear to differentiate between BRL 38227 and pinacidil at low concentrations in vitro adds credence to the supposition that both compounds elicit airway smooth muscle relaxant effects via essentially a common mechanism, although in vitro results obtained at high concentrations and the in vivo effects of glibenclamide suggest some differences in their mechanism of action. Whilst these results support the potential of BRL 38227 and pinacidil as novel bronchodilator agents, and confirm the greater potency of the former in animal models, recent clinical data suggests that BRL 38227 does not achieve sufficient separation of respiratory effects from those on the vasculature to evoke significant bronchodilatation in man.[46,47] Potassium channel activators having greater selectivity for the airways should provide compounds having a more suitable profile for the treatment of respiratory disorders.

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