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Comparison of the airways relaxant and hypotensive potencies of the potassium channel activators BRL 55834 and levcromakalim (BRL 38227) *in vivo* in guinea-pigs and rats

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1 BRL 55834, a novel potassium channel activator, has been compared with levcromakalim (BRL 38227) for its relaxant effects *in vivo* on the airways and vasculature of the guinea-pig and rat.

2 When administered intravenously 2 min prior to challenge, BRL 55834 and levcromakalim each inhibited histamine-induced increases in airways resistance (R_{aw}) in the anaesthetized guinea-pig, with BRL 55834 showing a 4.5 fold greater potency than levcromakalim ($ED_{25} = 2.5\ \mu g\ kg^{-1}$ and $11.3\ \mu g\ kg^{-1}$ respectively). By contrast, both compounds had similar hypotensive potencies ($ED_{18} = 8.5\ \mu g\ kg^{-1}$ and $6.5\ \mu g\ kg^{-1}$ respectively).

3 In the same guinea-pig model, intraduodenally administered BRL 55834 (100 and $250\ \mu g\ kg^{-1}$) and levcromakalim ($500\ \mu g\ kg^{-1}$) each protected against histamine-induced changes in R_{aw} and dynamic lung compliance (C_{dyn}), both compounds showing a rapid onset of action that persisted for more than 50 min. The lower dose of BRL 55834 had a similar bronchodilator effect to that of levcromakalim, yet both doses of BRL 55834 elicited substantially smaller effects than levcromakalim on mean arterial blood pressure.

4 In the anaesthetized rat, BRL 55834 and levcromakalim each evoked a dose-related inhibition of inhaled methacholine-induced changes in R_{aw} and C_{dyn} when given i.v., with BRL 55834 showing some four fold greater potency than levcromakalim (BRL 55834: R_{aw} $ED_{35} = 3.7\ \mu g\ kg^{-1}$, C_{dyn} $ED_{35} = 5.9\ \mu g\ kg^{-1}$; levcromakalim: R_{aw} $ED_{35} = 16\ \mu g\ kg^{-1}$, C_{dyn} $ED_{35} = 23.5\ \mu g\ kg^{-1}$). As in the guinea-pig, BRL 55834 had a reduced propensity to lower mean arterial blood pressure ($ED_{11} = 8\ \mu g\ kg^{-1}$ for BRL 55834, $11 \pm 3\%$ being its maximum effect; $ED_{11} = 16\ \mu g\ kg^{-1}$, maximum effect $= 34 \pm 6\%$ for levcromakalim.

5 When administered intraduodenally to anaesthetized rats, BRL 55834 (10, 20 and $100\ \mu g\ kg^{-1}$) evoked rapid and dose-related inhibitions of methacholine-induced R_{aw} and C_{dyn} changes which persisted for over 30 min. At the lower and middle dose there was little effect on mean arterial blood pressure (<10% fall). Levcromakalim ($500\ \mu g\ kg^{-1}$) by contrast elicited transient airways responses that diminished rapidly after 5 min, while the effects on blood pressure were well maintained (>20% at 65 min). Levcromakalim ($100\ \mu g\ kg^{-1}$) did not affect airways responses but also evoked a marked and sustained fall in blood pressure.

6 BRL 55834, administered *per os*, prolonged the time to histamine-induced dyspnoea in conscious guinea-pigs. The greatest effect of BRL 55834 was observed when it was administered 60 min prior to challenge, a dose of $0.20\ mg\ kg^{-1}$ doubling the mean time to collapse. A similar level of protection was afforded by levcromakalim ($1.25\ mg\ kg^{-1}$), with maximal activity occurring between 30 and 60 min.

7 The present studies in guinea-pigs and rats indicate that BRL 55834 is the first potassium channel activator to exhibit greater bronchodilator potency than levcromakalim but reduced tendency to lower arterial blood pressure. It is suggested that BRL 55834 may have greater potential than levcromakalim as a bronchodilator for therapeutic use in man.

Keywords: BRL 55834; levcromakalim; potassium channel activator; bronchodilator; airways; respiratory dynamics; blood pressure

Introduction

The potassium channel activators are potent and efficacious relaxants of spontaneous and induced tone in the airways of animals and man (Arch *et al.*, 1992). Studies *in vivo* have confirmed that when administered by a variety of different routes such compounds protect animals from the bronchospastic effects of a variety of stimuli (Arch *et al.*, 1988; Paciorek *et al.*, 1990a; Bowring *et al.*, 1991; Raeburn & Karlsson, 1991; Englert *et al.*, 1992). Furthermore, work with cromakalim has demonstrated that a single oral dose not only protects human volunteers against the effects of inhaled histamine (Baird *et al.*, 1988), but also, when given just prior to retiring, attenuates the morning dip in lung function in patients suffering from nocturnal asthma (Williams *et al.*, 1990).

In addition to bronchodilatation via smooth muscle relaxation, there is mounting evidence that the potassium channel activators will attenuate the release of neuropeptides (such as substance P and neurokinin A) from excitatory non-adrenergic, non-cholinergic (eNANC) unmyelinated nerves (C fibres) (Ichinose & Barnes, 1990; Lewis & Raeburn, 1990; Burka *et al.*, 1991; Good *et al.*, 1992). In particular, inhibition of the release of substance P, which is a potent inflammatory agent in human airways (Rogers *et al.*, 1989), may represent an additional beneficial effect of the potassium channel activators in asthma.

First generation potassium channel activators such as cromakalim discriminate little between smooth muscle types and significantly lower blood pressure in anaesthetized animals as a consequence of vasodilatation (Arch *et al.*, 1988; Bowring *et al.*, 1991). As a result of reflex tachycardia such effects are less prominent in conscious animals including man

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(Baird *et al.*, 1988), but cardiovascular side-effects are nevertheless dose-limiting (Arch *et al.*, 1992). A potassium channel activator with an enhanced selectivity for the airways relative to the vasculature may offer considerable advantages over existing compounds, although no such agent has yet been described.

In this paper we describe the *in vivo* effects of BRL 55834 [(3S,4R)-3,4-dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-6-pentafluoroethyl-2H-1-benzopyran-3-ol] in the guinea-pig and rat following intravenous, oral and intraduodenal administration, and contrast the results with those found for levcromakalim (BRL 38227), the active enantiomer of cromakalim. Some of these results have been reported previously in preliminary form (Bowring *et al.*, 1992).

Methods

Anaesthetized guinea-pigs and rats

Guinea-pigs (male Dunkin-Hartley, 500–800 g) or rats (male Spraque-Dawley, 250–400 g) were anaesthetized with urethane (1.25 g kg^{-1}, i.p.). A jugular vein and carotid artery were cannulated for the administration of compounds and measurement of blood pressure respectively. The trachea was cannulated for the measurement of tidal airflow via a heated Fleisch pneumotachograph. The oesophagus was ligated above the gastro-oesophageal junction and cannulated to measure intrathoracic pressure. Airway resistance (R_{aw}) and dynamic lung compliance (C_{dyn}) were derived by use of a computer and software developed within our laboratories.

Guinea-pigs were challenged with histamine (2–4 $\mu g\ kg^{-1}$ histamine diphosphate, i.v.) every 10 min. The dose was adjusted so that R_{aw} increased by about 200% and C_{dyn} decreased by about 30%. Rats were challenged every 15 min with methacholine by inhalation according to the following procedure. A T-piece was attached to the pneumotachograph, and methacholine aerosol, generated using an ultrasonic nebulizer, was passed across the airway for 2 min. The concentration of methacholine solution was varied (0.5 to 2.5 mM) to produce an approximate increase in R_{aw} of 100% and a decrease in C_{dyn} of 40%. When stable responses were established in guinea-pigs or rats, BRL 55834 or levcromakalim was injected intravenously 2 min before each bronchoconstrictor challenge. The change in mean arterial blood pressure evoked by a given dose of BRL 55834 or levcromakalim was measured with respect to the mean pressure recorded immediately before the administration of that dose of the potassium channel activator. In general the maximal hypotensive effect of these agents occurred within 2 min of their intravenous injection. BRL 55834 (2–8 $\mu g\ kg^{-1}$) was given in increasing doses to guinea-pigs, each increasing dose being given when airways responses and blood pressure had returned to baseline. The 16 $\mu g\ kg^{-1}$ dose in guinea-pigs and each dose of BRL 55834 in rats were given to animals that received no other dose. Levcromakalim was given in increasing doses to guinea-pigs, but in rats the 3.1 and 200 $\mu g\ kg^{-1}$ doses were given to different animals from those receiving the 12.5 and 50 $\mu g\ kg^{-1}$ doses. When given intraduodenally, single doses of either BRL 55834 or levcromakalim were administered to each animal.

Conscious guinea-pigs

Guinea-pigs were placed individually in a perspex chamber of approximately 8 litres capacity at various times following the oral administration of BRL 55834 or levcromakalim and exposed for up to 240 s to an aerosol generated for 20 s from a 5 mM solution of histamine diphosphate. Those animals that collapsed were immediately removed from the chamber. They were allowed to recover and were not used for further experiments. The time from the introduction of the aerosol to collapse was recorded, with those animals not responding within the 4 min observation time being considered to be fully protected.

Drugs and solutions

BRL 55834 (Buckle *et al.*, 1992) and levcromakalim (BRL 38227) were synthesized in the laboratories of SmithKline Beecham Pharmaceuticals. Both compounds were administered i.v. in 0.9% NaCl and a maximum of 10% (w/v) dimethylsulphoxide and intraduodenally in a maximum of 1% (w/v) dimethylsulphoxide. The dosage volume was 0.1 ml 100 g^{-1} bodyweight for the i.v. route and 5 ml for the intraduodenal route.

Analysis of results

For experiments in anaesthetized animals, the response to the last bronchoconstrictor challenge prior to the administration of BRL 55834 or levcromakalim was used for calculation of the percentage inhibition of the bronchoconstrictor response. Statistical analysis was by single-sample *t* test. Potencies of BRL 55834 and levcromakalim have been compared for regions of the respective dose-response curves that overlap along the ordinate. Hence x in ED_x is not always a conventional number. Since ED_x values could in general not be estimated for individual animals, confidence intervals have been estimated by analysis of the linear part of the meaned dose-response curves using Fieller's theorem (Finney, 1980) and employing a pooled estimate of the residual variance from the entire curve. ED_{25} values for R_{aw} could be estimated for each guinea-pig given i.v. BRL 55834 or levcromakalim, however, and geometric means with 95% confidence intervals have in this instance been calculated in the conventional manner. For experiments in conscious animals, times of 240 s were allocated to animals that were fully protected for calculation of mean collapse times. Statistical analysis in these experiments was by one-tailed Mann-Whitney U-test.

Results

Baseline values

Mean resting R_{aw}, C_{dyn} and arterial blood pressure in guinea-pigs and rats are shown in Table 1. Administration of vehicle had no significant effect on baseline R_{aw}, C_{dyn} or arterial blood pressure or on the response of R_{aw} and C_{dyn} to histamine or methacholine.

Bronchoconstriction in anaesthetized guinea-pigs

Intravenous route When administered by the i.v. route to freely respiring anaesthetized guinea-pigs, BRL 55834 (2 to 16 $\mu g\ kg^{-1}$) elicited a dose-dependent inhibition of the histamine-induced increase in R_{aw}, providing 66% inhibition at the highest dose tested (Figure 1a). The dose of BRL 55834 providing a 25% inhibition (ED_{25}) of the histamine response was 2.5 (2.1–3.9) $\mu g\ kg^{-1}$ (geometric mean and 95% confidence interval). Inhibition of the histamine-induced change in R_{aw} was smaller 12 min after giving BRL 55834 than after 2 min in most animals (4 $\mu g\ kg^{-1}$: 56 ± 6%

Table 1 Mean resting values for R_{aw}, C_{dyn} and mean blood pressure

	R_{aw} ($cmH_2O\ ml^{-1}\ s^{-1}$)	C_{dyn} ($ml\ cmH_2O^{-1}$)	*Mean blood pressure* (mmHg)
Guinea-pigs	0.049 ± 0.022	1.50 ± 0.05	51.7 ± 8.0
Rats	0.017 ± 0.003	0.60 ± 0.13	81.1 ± 8.6

Results are shown as mean ± s.d. for at least 30 values.

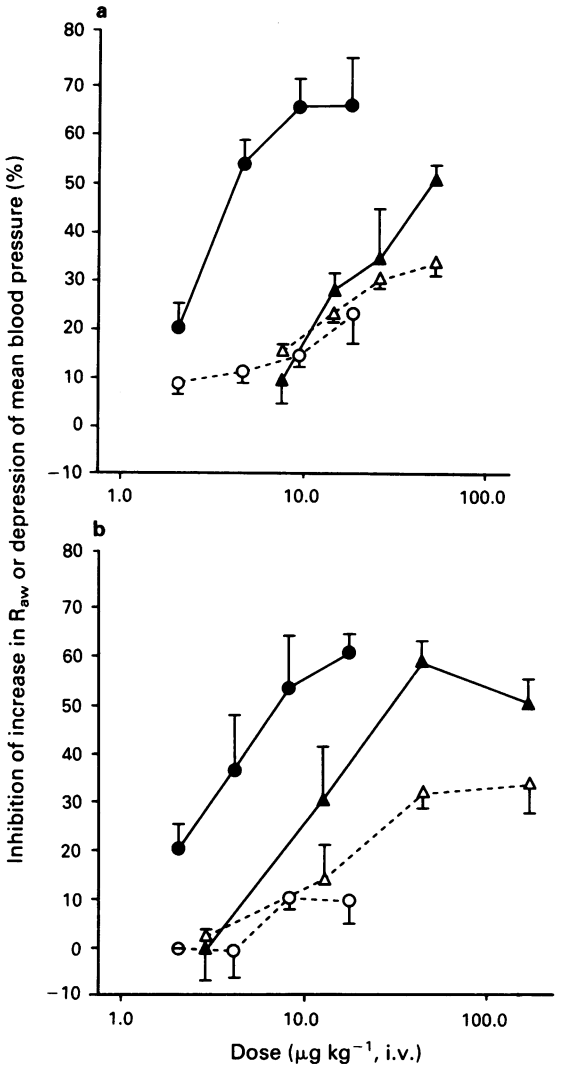

Figure 1 The effects of i.v. BRL 55834 and levcromakalim on baseline mean blood pressure and histamine-induced increases in R_{aw} in anaesthetized guinea-pigs (a) or methacholine-induced increases in R_{aw} in anaesthetized rats (b). Effects of BRL 55834 (●) and levcromakalim (▲) on airways; effects of BRL 55834 (○) and levcromakalim (△) on blood pressure. Responses were measured 2 min after the administration of BRL 55834 or levcromakalim. Details of whether cumulative dose-response curves were constructed or whether animals were given single doses of BRL 55834 or levcromakalim are given in the Methods section.

(mean ± s.e.mean) at 2 min, 29 ± 15% at 12 min, $n = 8$; 8 μg kg^{-1}: 67 ± 7% at 2 min, 46 ± 11% at 12 min, $n = 8$; 16 μg kg^{-1}: 66 ± 10% at 2 min, 63 ± 11% at 12 min). At 16 μg kg^{-1} the protective effect of BRL 55834 persisted for at least 22 min (17 ± 4%, $n = 5$). BRL 55834 evoked only a small, albeit significant ($P < 0.01$ at all dose levels), reduction of mean arterial blood pressure (Figure 1a), the dose inducing an 18% fall (ED_{18}) being 8.5 (5.1–15.3) μg kg^{-1}. To provide assurance that no tachyphylaxis occurred in response to BRL 55834, a single dose of 4 μg kg^{-1} was administered to a separate set of animals. In this instance the protection provided (54 ± 5%, $n = 11$) was the same as that achieved by the cumulative dosage regimen shown in Figure 1a.

Levcromakalim (6.25 to 50 μg kg^{-1}) also caused a dose-related inhibition of the histamine-induced changes in R_{aw} in this model (Figure 1a), with ED_{25} = 11.3 (8.7–14.6) μg kg^{-1}. Like BRL 55834, levcromakalim lowered mean arterial blood pressure ($P < 0.001$ at each dose level), but, its ED_{18} value [6.5 (4.7–9.1) μg kg^{-1}] was lower than its ED_{25} value for inhibiting changes in R_{aw} (Figure 1a).

Essentially similar results were obtained for the inhibitory effects of both BRL 55834 and levcromakalim on the histamine-induced decrease in C_{dyn}, although the data (not shown) were generally more variable than those with R_{aw} changes.

Intraduodenal route BRL 55834 and levcromakalim were also compared in this model when given by the intraduodenal route. Doses of BRL 55834 (100 μg kg^{-1}) and levcromakalim (500 μg kg^{-1}) were chosen to provide similar levels of protection based on intravenous potencies, and the fact that earlier studies (not published) had shown little protective effect of levcromakalim at a dose of 150 μg kg^{-1}. A higher dose of BRL 55834 (250 μg kg^{-1}) was included to ensure that the response to the lower dose was not supramaximal.

Both compounds showed a rapid onset of anti-bronchoconstrictor activity, with the lower dose of BRL 55834 providing similar protection to levcromakalim against the histamine-induced changes in R_{aw} and the protective effect of both compounds persisting over the 50 min observation period (Figure 2a). The higher dose of BRL 55834 was substantially more effective than the lower dose over the early time period, although by 50 min the two doses had similar effects. The onset of hypotensive activity with levcromakalim was also rapid and prolonged (Figure 2b). BRL 55834, by contrast, lowered arterial blood pressure at a slower rate than levcromakalim, even at the higher 0.25 mg kg^{-1} dose (Figure 2b). Furthermore, at least at the lower dose, the extent of blood pressure lowering was less with BRL 55834 than levcromakalim, since an experiment over a 2 h time course showed that the peak effect of BRL 55834 was achieved by 60 min. The effects of BRL 55834 on C_{dyn} (not shown) followed a similar trend to those on R_{aw} but were more variable.

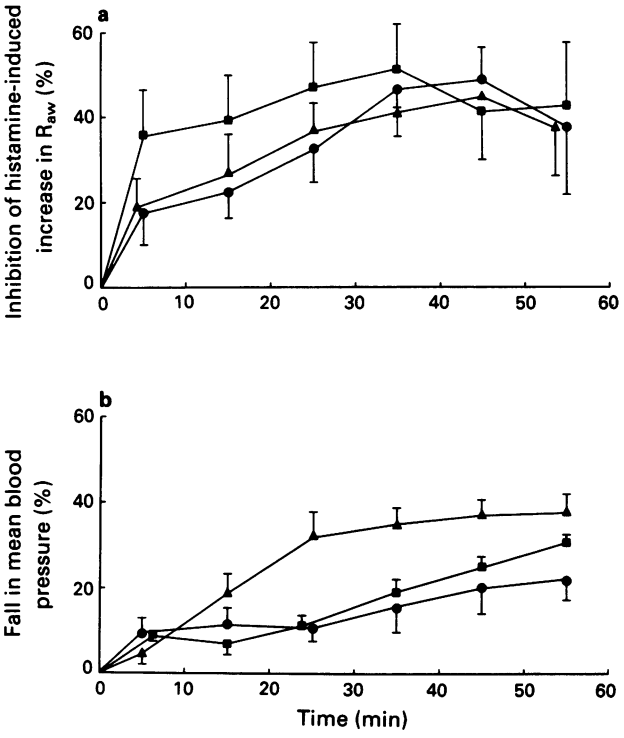

Figure 2 The effects of intraduodenal BRL 55834 (●, 100 μg kg^{-1}; ■, 250 μg kg^{-1}) and levcromakalim (▲, 500 μg kg^{-1}) on (a) histamine-induced increases in R_{aw} and (b) baseline mean blood pressure in anaesthetized guinea-pigs. The points are means of 6–8 values, ± s.e. All the points represent statistically significant effects, except for the effect of BRL 55834 (100 μg kg^{-1}) on blood pressure at 5 min.

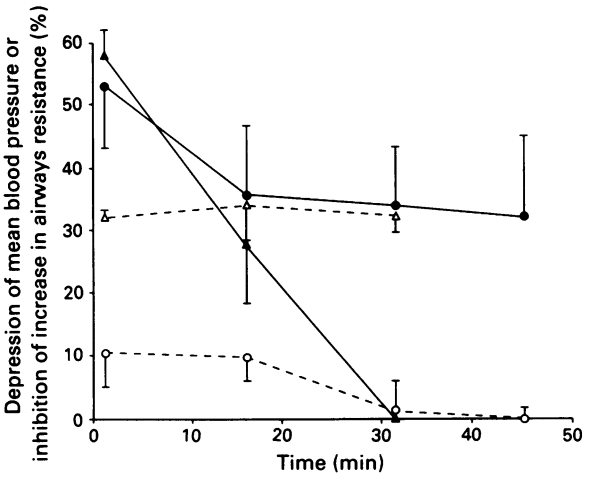

Figure 3 Time course of the effects of i.v. BRL 55834 (8 μg kg^{-1}) and levcromakalim (50 μg kg^{-1}) on methacholine-induced increases in R_{aw} and mean baseline blood pressure in anaesthetized rats. BRL 55834 (●) and levcromakalim (▲) on airways; BRL 55834 (○) and levcromakalim (△) on blood pressure. The points are means of 5 values, ± s.e.

Bronchoconstriction in the anaesthetized rat

Intravenous route Peak effects of BRL 55834 and levcromakalim on both the airways and the vasculature were observed 2 min after their i.v. administration to freely respiring, anaesthetized rats. BRL 55834 (2 to 16 μg kg^{-1}) evoked a dose-related inhibition of the increase in R_{aw} in response to inhaled methacholine (ED_{35} = 3.7 [1.5–6.7] μg kg^{-1}), providing a 60% inhibition at the highest dose (Figure 1b). Similarly, between 2 and 8 μg kg^{-1}, it evoked a dose-related inhibition of the decrease in C_{dyn} (ED_{35} = 5.9 [4.0–10.9] μg kg^{-1}), the maximum inhibition being 44% (data not shown). BRL 55834 was without effect on arterial blood pressure at the lower doses and its maximum effect was only 11 ± 3% (at 8 μg kg^{-1}, Figure 1b). In a similar experiment, levcromakalim showed a lower potency (ED_{35} = 16 [10–26] μg kg^{-1} resistance; ED_{35} = 23.5 [10.9–43.4] μg kg^{-1} compliance) on the airways, causing dose-related inhibitions over the range 3.1 to 50 μg kg^{-1}. Unlike BRL 55834, however, levcromakalim (ED_{11} = 8 [3–18] μg kg^{-1}) had a significant effect on arterial blood pressure at all doses which showed a respiratory protective effect (Figure 1b).

Evaluation of the time courses of the effects of BRL 55834 and levcromakalim in the rat demonstrates an even clearer distinction between their selectivities. BRL 55834 (8 μg kg^{-1}) provided a prolonged airways protectant effect, lasting over the duration of the experiment (>40 min) (Figure 3). Its effects on arterial blood pressure, however, had returned to control levels at 30 min and were minimal at all time points. The airways protectant effects of an equieffective dose of levcromakalim (50 μg kg^{-1}) by contrast were short-lived, returning to baseline values at 30 min, whereas the effects of this dose of levcromakalim on arterial blood pressure were well maintained (Figure 3).

Intraduodenal route BRL 55834 was a potent inhibitor of inhaled methacholine-induced R_{aw} and C_{dyn} changes in the anaesthetized rat when given by the intraduodenal route. BRL 55834 (10, 20 and 100 μg kg^{-1}) caused a dose-related inhibitory effect which persisted for over 30 min at the lower and middle doses, and for the duration of the experiment (65 min) at the higher dose (Figure 4a and b). Arterial blood pressure was unaffected by BRL 55834 at the lower and middle doses (the transient effect at the middle dose was not statistically significant), but was reduced by approximately 15% at the high dose (Figure 4c). Levcromakalim (100 μg kg^{-1}) afforded no protective effects against R_{aw} and C_{dyn} changes at any time point, whereas a 500 μg kg^{-1} dose resulted in a transient response which diminished rapidly after 5 min (Figure 4a and b). Consistent with the intravenous data, both doses of levcromakalim evoked a pronounced and prolonged reduction in arterial blood pressure which persisted for the duration of the experiment (65 min) (Figure 4c).

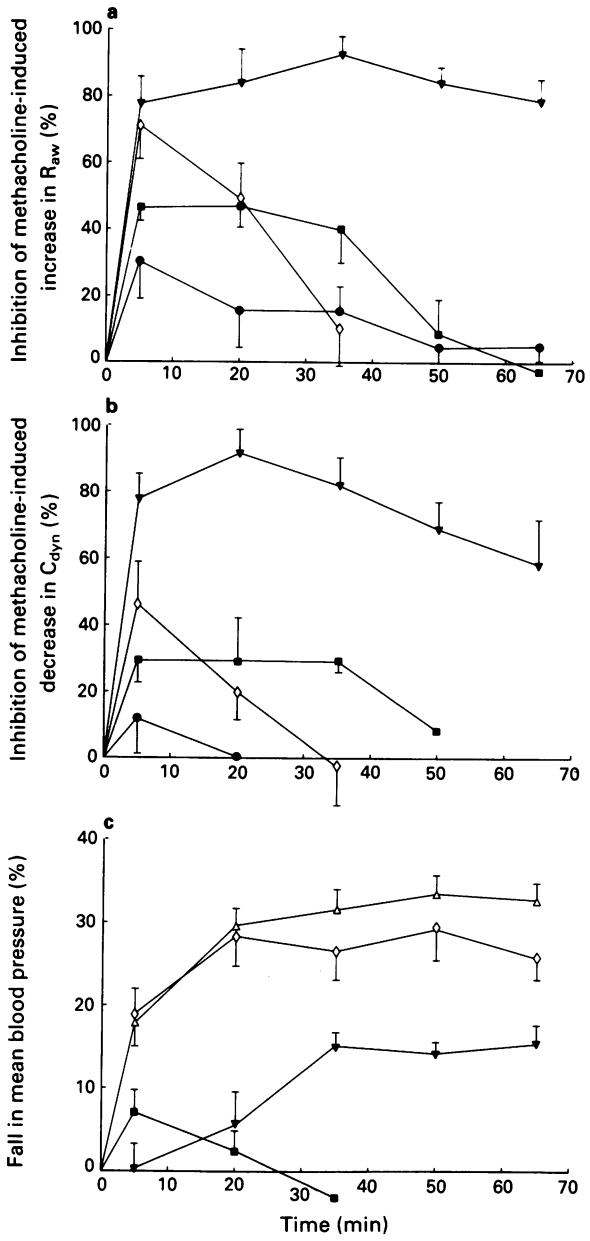

Figure 4 The effects of intraduodenal BRL 55834 (●, 10 μg kg^{-1}; ■, 20 μg kg^{-1}; ▼, 100 μg kg^{-1}) and levcromakalim (△, 100 μg kg^{-1}; ◇, 500 μg kg^{-1}) on (a) methacholine-induced increases in R_{aw}, (b) methacholine-induced decreases in C_{dyn} and (c) baseline mean blood pressure in anaesthetized rats. Levcromakalim, 100 μg kg^{-1}, had no effect on R_{aw} or C_{dyn} and BRL 55834, 10 μg kg^{-1}, had no effect on blood pressure. The points are means of 5 values with s.e. Statistically significant effects ($P<0.05$) were for BRL 55834 (100 μg kg^{-1}): all times for R_{aw} and C_{dyn} and from 35 min for blood pressure; BRL 55834 (20 μg kg^{-1}): to 35 min for R_{aw} and C_{dyn}; levcromakalim (100 μg kg^{-1}): all times for blood pressure; levcromakalim (500 μg kg^{-1}): 10 and 20 min for R_{aw}, 10 min only for C_{dyn}, all times for blood pressure.

Bronchoconstriction in the conscious guinea-pig

Orally administered BRL 55834 (0.5 mg kg^{-1}) and levcromakalim (2.5 mg kg^{-1}) protected conscious guinea-pigs from

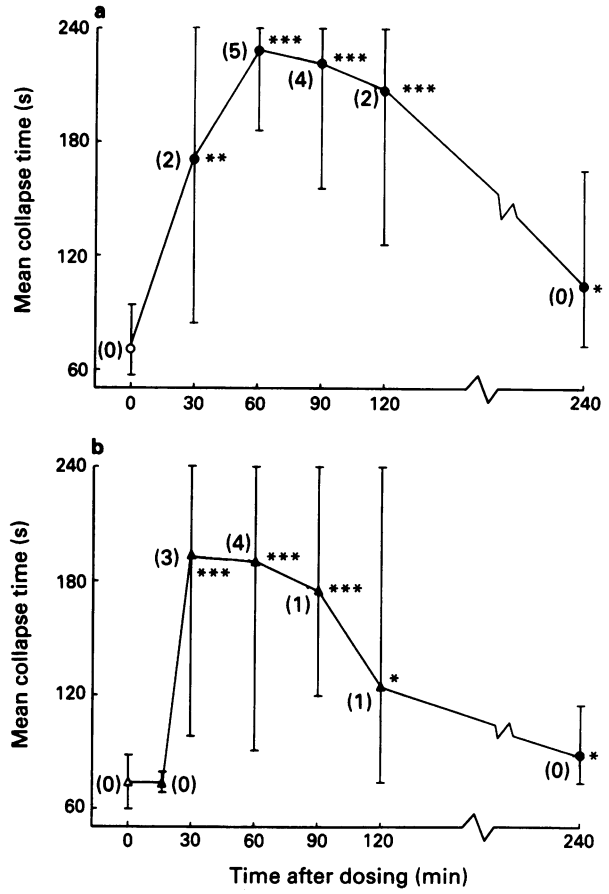

Figure 5 Time course of the effects of (a) BRL 55834 (0.5 mg kg^{-1}, p.o.) and (b) levcromakalim (2.5 mg kg^{-1}, p.o.) on the time to collapse following administration of an histamine aerosol to conscious guinea-pigs. Mean collapse times for controls are shown as the time 0 points. The points are means of 6 values and the vertical lines show the range of collapse times, with 240 s indicating full protection. The numbers in parentheses indicate the number of animals that were protected for 240 s out of the 6 challenged at each time. $*P<0.05$; $**P<0.01$; $***P<0.001$ compared to collapse times for controls.

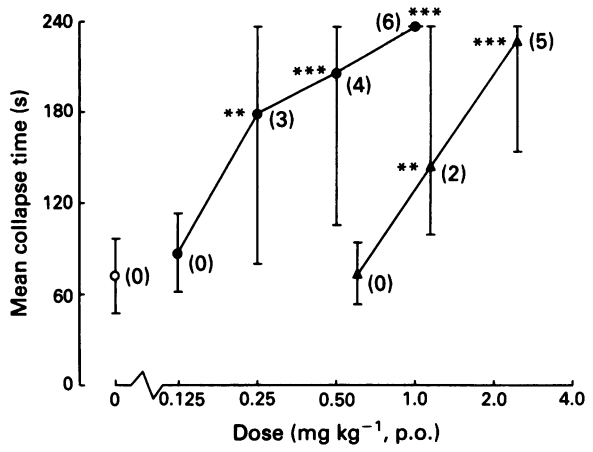

Figure 6 Dose-response relationships for the protection of conscious guinea-pigs from histamine aerosol by BRL 55834 (●) and levcromakalim (▲) given 60 min before challenge. The points are means of 6 values (12 for controls) and the vertical lines show the range of collapse times, with 240 s indicating full protection. The numbers in parentheses indicate the number of animals that were protected for 240 s. $**P<0.01$; $***P<0.001$ compared to collapse times for controls.

dyspnoea in response to a subsequent histamine aerosol challenge, with maximum protection occurring 60 min after dosing with BRL 55834 and between 30 and 60 min after levcromakalim (Figure 5). From subsequent dose-response studies carried out at the 60 min time point both BRL 55834 (0.125 to 1.00 mg kg^{-1}) and levcromakalim (0.625 to 2.5 mg kg^{-1}) inhibited dyspnoea in a dose-dependent fashion. ED_{100} doses (doubling of mean time to collapse) were 0.20 mg kg^{-1} and 1.25 mg kg^{-1} for BRL 55834 and levcromakalim respectively (Figure 6).

Discussion

The smooth muscle relaxant activity of the potassium channel activators has been extensively demonstrated in a wide range of tissues, suggesting that such compounds may have a potential utility in many diseases (Longman & Hamilton, 1992). It is this same diversity, however, that may restrict their clinical usefulness in certain disorders, particularly where competing smooth muscle events may be dose-limiting. This situation is especially evident with first generation potassium channel activators in which potent vasodilator activity is manifested as marked antihypertensive activity in animal models (Arch *et al.*, 1988; Paciorek *et al.*, 1990a; Mondot *et al.*, 1988). Cromakalim is relatively free of blood pressure lowering activity in normotensive human volunteers and in asthmatic patients (Baird *et al.*, 1988; Williams *et al.*, 1990), but cerebral vasodilatation, resulting in headache, is dose-limiting (Arch *et al.*, 1992).

Despite the structural diversity of the many known potassium channel activators, BRL 55834, a compound developed from the benzopyran series, is the first compound for which airway selectivity *in vivo* has been described. In contrast, a preliminary report from our laboratory (Taylor *et al.*, 1992c) has failed to demonstrate that BRL 55834 is airways-selective *in vitro*, in that it has similar IC_{50} values for inhibition of 30 mM KCl-induced tone in guinea-pig portal vein and for inhibition of spontaneous tone and tone induced by various spasmogens in guinea-pig tracheal spirals. However, levcromakalim differs from BRL 55834 in being less potent in tracheal spirals than in portal vein. It is for this reason that BRL 55834 has been extensively evaluated for its bronchodilator activity in animal models. It is quite possible, however, that the differing *in vitro* profiles of BRL 55834 and levcromakalim are of no relevance to the *in vivo* selectivity of BRL 55834.

In order to compare the anti-bronchoconstrictor and vasodilator potencies of BRL 55834 *in vivo* with those of the reference compound levcromakalim, experiments have been carried out in freely respiring anaesthetized guinea-pigs and rats. The guinea-pig, in particular, can be used as a model of human asthma because of the similarities that exist between the two in their response to various agonists and to known relaxants (Kallos & Kallos, 1984). A second species, the rat, was chosen to confirm and support the selectivity that was subsequently established in the guinea-pig. In both species, urethane anaesthesia largely abolishes the reflex tachycardia evoked in response to vasodilatation and lowered blood pressure. Consequently, the fall in blood pressure in these models provides a sensitive index of vasodilator activity. Anti-bronchoconstrictor activity was assessed in terms of agonist-induced changes in both R_{aw} and C_{dyn}, which are measures of large and small airway changes, respectively.

Largely in accord with its *in vitro* potencies relative to levcromakalim (Taylor *et al.*, 1992c),BRL 55834 (i.v.) was 4.5 times more potent than levcromakalim as an inhibitor of histamine-induced increases in R_{aw}, yet had similar potency as a blood pressure lowering agent in the anaesthetized guinea-pig (Figure 1a). Essentially similar effects were seen on both R_{aw} and C_{dyn}, a result which parallels those found earlier for levcromakalim (Bowring *et al.*, 1991; Paciorek *et al.*, 1991; Buckle *et al.*, 1993), cromakalim (de Souza *et al.*,

1989) and Ro 31-6930 (Paciorek *et al.*, 1991). In contrast, the β_2-adrenoceptor agonist, salbutamol, showed greater potency on the larger relative to the smaller airways in this model (Bowring *et al.*, 1991), although no difference has been shown for salmeterol on these parameters in either the guinea-pig or the cat (Paciorek *et al.*, 1991). Thus, BRL 55834 shows similar potency on both the large and small airways in the guinea-pig, a result which parallels that found earlier with levcromakalim. Whether these differences seen between the potassium channel activators and salbutamol have any clinical relevance remains to be established.

In order to demonstrate that BRL 55834 had the potential to protect against provoked bronchospasm when administered orally, experiments were carried out in which the compound was introduced directly into the duodenum via an indwelling cannula. In this study BRL 55834 showed a rapid onset of protective action, comparable to that of levcromakalim, and was again some five fold more potent than levcromakalim administered in the same manner (Figure 2a). That selectivity for the airways was retained was evident from the finding that an equi-bronchoprotective dose of BRL 55834 showed a significantly reduced effect on arterial blood pressure (Figure 2b). Whether an intraduodenal dose of BRL 55834 that does not affect blood pressure would elicit a useful bronchodilator effect was not determined in the guinea-pig. Studies in conscious guinea-pigs confirmed the ability of BRL 55834 to protect against a histamine challenge when given orally (Figures 5 and 6). In comparative experiments, BRL 55834 was six fold more potent than levcromakalim. In the guinea-pig therefore, BRL 55834 retains selectivity as an airways relaxant relative to levcromakalim, whether administered intravenously or intraduodenally.

In the anaesthetized rat, methacholine was chosen as the respiratory spasmogen since preliminary studies showed that the rat was insensitive to histamine challenges at sub-toxic doses. In this model i.v. BRL 55834 was found to be at least four fold more potent than BRL 38227 at protecting rats from the ventilatory effects of inhaled methacholine, yet elicited similar hypotensive activity (Figures 1b and 4). It is particularly significant that by both i.v. and intraduodenal routes BRL 55834 shows effects on airways parameters at doses that do not affect blood pressure and that those hypotensive effects that do occur are less than those observed with doses of levcromakalim required to provide an equal degree of respiratory protection. In addition to its greater potency, BRL 55834 showed a prolonged effect on the airways of rats when administered either intravenously or intraduodenally, whereas the effects of levcromakalim were relatively short (Figures 3 and 4). Since the duration of the effects of BRL 55834 and levcromakalim on arterial blood pressure were reversed (Figures 3 and 4), it was evident that BRL 55834 showed a preferential and more beneficial action on the airways of both the guinea-pig and rat than levcromakalim.

Few published data exist on previously described potassium channel activators which would allow a definitive assessment of the intrinsic airway selectivity of these agents. From our own experience, however, most compounds show either a profile similar to that of levcromakalim *in vitro* or exhibit a greater effect on the vasculature (unpublished results). BRL 55834 is to our knowledge the first potassium channel activator for which a selectivity for the airways has been unequivocally demonstrated *in vivo*.

If the *in vivo* selectivity of BRL 55834 is related to it having a different *in vitro* profile from levcromakalim (see above), it is appropriate to search for an explanation for this selectivity at a molecular level. Available physiological evidence supports the notion that BRL 55834 exerts its relaxant activity through potassium channel activation (Taylor *et al.*, 1992b). Furthermore, preliminary electrophysiological studies with BRL 55834 in bovine isolated trachealis have shown that in addition to the low conductance, ATP-inhibited and glibenclamide-sensitive potassium channel (30 pS) opened by levcromakalim, BRL 55834 also opens a high conductance potassium channel (247 pS) having characteristics similar to those of the calcium-activated, glibenclamide-insensitive channel (Ward *et al.*, 1992). Some workers have claimed that levcromakalim also opens the high conductance potassium channel, but others have failed to reproduce these findings (see Noack *et al.*, 1992). The fact that one group (Ward *et al.*, 1992) has found a difference in the abilities of levcromakalim and BRL 55834 to open this channel serves to highlight a difference between the compounds at the molecular level. Whether this difference is responsible for the airway selectivity of BRL 55834 *in vivo* remains to be established.

Based on the concentrations of cromakalim required to relax tone in human isolated bronchi (Taylor *et al.*, 1992a), it has been argued that the clinical effects of this agent cannot be due to direct smooth muscle relaxation since insufficient plasma concentrations are achieved (Small *et al.*, 1992). One suggestion is that cromakalim inhibits neural mechanisms underlying airways hyper-responsiveness, since available data suggest that cromakalim and other potassium channel activators affect hyper-responsiveness at doses that have no bronchoprotective effect in normal airways (Chapman *et al.*, 1991; 1992; Burka *et al.*, 1991). However, irrespective of the effects of the potassium channel activators on airways hyper-responsiveness, they are unequivocally relaxants of normal airways smooth muscle. In addition to its high potency on the isolated trachealis of the guinea-pig (Taylor *et al.*, 1992c), BRL 55834 is a potent relaxant of tone in human isolated bronchi (unpublished). Taken together with the selectivity found *in vivo* in the guinea-pig and rat, these data suggest that BRL 55834 may provide useful bronchodilatation in man.

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