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Evaluation of the potassium channel activator cromakalim (BRL 34915) as a bronchodilator in the guinea-pig: comparison with nifedipine

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1 The potential of the potassium channel activator, cromakalim (BRL 34915), as a bronchodilator has been evaluated in guinea-pig models in comparison with nifedipine. Some effects of the compounds on guinea-pig tracheal spirals have been studied in an attempt to elucidate their different efficacies *in vivo*.

2 When given by the intraduodenal route to anaesthetized guinea-pigs, cromakalim (3 and 10 mg kg^{-1}) inhibited 5-hydroxytryptamine (5-HT)-induced bronchospasm for at least 60 min. When given by the i.v. route, the dose of cromakalim producing 50% inhibition of the 5-HT response was 84 μg kg^{-1}. Nifedipine failed to show any protective effect up to 100 μg kg^{-1}, i.v. and was lethal at higher dose levels.

3 Cromakalim protected conscious guinea-pigs from asphyxic collapse in response to histamine aerosol. The maximal effect occurred 60 min following oral dosing, with 2.5 mg kg^{-1} providing complete protection for almost half of the animals. Nifedipine had only a weak protective effect even at a high dose level of 50 mg kg^{-1}, p.o.

4 Cromakalim prolonged the time before convulsive cough in response to an antigen challenge in actively sensitized guinea-pigs. Its minimum protective dose was 1 mg kg^{-1}, p.o. Nifedipine (50 mg kg^{-1}, p.o.) was ineffective.

5 Cromakalim inhibited both spontaneous and prostaglandin E_2-induced tone in guinea-pig isolated tracheal spirals with IC_{50} values, relative to the maximum inhibition achieved by isoprenaline (10^{-3} M), of 1.1×10^{-6} M and 8.9×10^{-7} M, respectively. Its maximal effect was 89% of that produced by isoprenaline. Removal of the epithelium did not influence its activity. Studies using the two enantiomers showed that the activity of cromakalim resided almost entirely in the (−)-enantiomer.

6 Nifedipine (2×10^{-5} M) achieved only 49% of the relaxant effect of 10^{-3} M isoprenaline in isolated tracheal spirals. Addition of cromakalim (10^{-5} M) at the end of the nifedipine concentration-response experiment caused further relaxation to 94% of the effect of isoprenaline.

7 It is concluded that cromakalim has greater potential than nifedipine as a bronchodilator. It appears that opening of potassium channels, with consequent hyperpolarization and stabilization of the membrane potential, prevents calcium entering the cytosol through routes that are unaffected by calcium entry blockers.

Introduction

Cromakalim (BRL 34915) is a novel relaxant of smooth muscle which is believed to act by enhancing potassium efflux from the cell and thereby hyperpolarizing the plasma membrane (Allen *et al.*, 1986; Hamilton *et al.*, 1986; Weir & Weston, 1986a,b; Coldwell & Howlett, 1987; Hollingsworth *et al.*, 1987; Quast, 1987). As a consequence of its relaxant activity in vascular smooth muscle (Clapham & Wilson, 1986; Hamilton *et al.*, 1986; Weir & Weston, 1986b; Quast, 1987) cromakalim is a potent antihypertensive agent in both laboratory animals (Buckingham *et al.*, 1986a) and man (VandenBurg *et al.*, 1986; 1987). The relaxant activity of cromakalim on spontaneous tone in guinea-pig isolated trachea

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(Allen et al., 1986) suggests that it may also be of value as a bronchodilator in the therapy of asthma, although Allen et al. (1986) have suggested that this use may be limited as judged by its poor inhibition of acetylcholine- and histamine-induced tone *in vitro*. Nonetheless, cromakalim is an effective inhibitor of histamine-induced bronchoconstriction in man (Baird et al., 1988).

This study investigates further the potential of cromakalim as a bronchodilator in *in vivo* and *in vitro* models believed to be relevant to asthma in man. The relaxant effects of cromakalim on spontaneous tone in guinea-pig isolated tracheal spirals is confirmed and interpreted in terms of its relaxation of prostaglandin-induced tone, and its inhibition of histamine-, 5-hydroxytryptamine (5-HT)- and antigen-induced bronchoconstriction in the guinea-pig *in vivo* is described. Throughout most of this study, cromakalim has been compared with the calcium entry blocker, nifedipine, since a consequence of potassium channel opening might simply be the inhibition of calcium entry through voltage-operated channels. The marked differences in activity between these two agents suggest that, in contrast to the calcium entry blockers, which are of little value in asthma (Löfdahl & Barnes, 1986; So et al., 1986; Hendeles & Harman, 1987), cromakalim may have considerable therapeutic potential.

We have also compared the potencies of the (+)- and (−)-enantiomers of cromakalim, and shown that activity resides almost exclusively in the (−)-enantiomer.

A preliminary account of some of these results has been given previously (Buckle et al., 1987).

Methods

Isolated tracheal spirals

Male Dunkin-Hartley guinea-pigs (300–600 g) were stunned by a blow to the head and bled from the carotid artery. Tracheal spiral strips (two per animal) were then prepared and suspended under isometric conditions in oxygenated Krebs solution. In some experiments the epithelium was removed from one of the two spiral strips by rubbing with a moistened cotton bud to produce a matched pair of rubbed/intact preparations. Removal of the epithelium was confirmed histologically. Tension was allowed to develop spontaneously, or it was induced in the presence of indomethacin (2.8×10^{-6} M) by 10^{-8} M prostaglandin E_2 (PGE_2), which was found in preliminary experiments to be an approximately EC_{70} concentration. Tension was maintained at 2 g. Compounds were added in a cumulative fashion and the inhibitory effects were calculated as a percentage of the relaxation induced by isoprenaline (10^{-3} M) added at the end of the experiment.

Bronchoconstriction in anaesthetized guinea-pigs

Male guinea-pigs (430–520 g) were anaesthetized with urethane (1.5 g kg^{-1}, i.p.). The left jugular vein was cannulated for administration of compounds, the right carotid artery for measurement of blood pressure, the trachea for artificial ventilation and, in some experiments, the duodenum for administration of cromakalim. Spontaneous respiration was abolished by injection of succinyl choline (2 mg kg^{-1}, i.v.) and the animals were artificially ventilated using a Palmer pump set at 73 strokes min^{-1} and a stroke volume of 10 ml kg^{-1}. Resistance to lung inflation was measured by the overflow technique of Konzett & Rössler (1940) in which air not entering the animals on inspiration overflowed to a Ugo Basile 7020 bronchospasm transducer.

After a 10 min stabilization period, the guinea-pigs were challenged at 5 min intervals with varying dose levels of 5-HT (3.3–5.2 μg free base kg^{-1}, i.v., given as the creatinine sulphate salt) to establish a dose which gave approximately 60% of the maximum overflow achieved by constricting the air flow to the animal. When reproducible bronchospasms had been established, i.v. cromakalim or nifedipine was administered one minute before subsequent 5-HT challenges, which were repeated at intervals of 5 min until a reproducible response had been established. Further doses of either cromakalim or nifedipine were then administered following the same schedule to establish a dose-response curve. When given intraduodenally, cromakalim was administered as a single dose to each animal 4.5 min before 5-HT challenge and challenges were repeated at intervals of 5 min for 1 h.

The bronchoconstrictor effect of the 5-HT challenge one minute after each i.v. dose of cromakalim or nifedipine, and at 5 min intervals after each intraduodenal dose of cromakalim, was expressed as a percentage of the mean effect of the two 5-HT challenges that preceded drug administration.

Bronchoconstriction in conscious guinea-pigs

Histamine-induced Male guinea-pigs (400–460 g) were dosed orally with cromakalim, nifedipine or the vehicle and placed in a perspex chamber of approximately 81 l capacity. At various times subsequent to dosing the animals were challenged for 20 s with a histamine aerosol generated from a 5 mM solution of histamine diphosphate using a Monaghan 675 ultrasonic nebulizer (power setting 7). If the animals collapsed they were immediately removed from the chamber and were capable of full recovery. However, they were not reused for further experiments. The time from the introduction of the aerosol to collapse was recorded, with those animals not collapsing

within the 4 min observation time being considered to be fully protected. The time between giving the compound and histamine that produced maximum protection, T_{max}, was determined from the time course experiments.

Antigen-induced Male guinea-pigs (250–300 g) were actively sensitized with ovalbumin by s.c. injection of 0.2 ml of a 0.5 mg ml^{-1} solution mixed with 0.3 ml of Freunds complete adjuvant, followed by an antigen booster injection of 0.1 ml of a 100 μg ml^{-1} solution of ovalbumin (i.d.) 14 days later. After a further 14 days, the animals were dosed orally with cromakalim, nifedipine or vehicle 60 min before challenge for 10 min with an aerosol of antigen generated from a 50 mg ml^{-1} solution of ovalbumin in water, using a Wrights nebulizer under a positive pressure of 10 p.s.i. The end point was taken as convulsive cough (Dawson & Sweatman, 1980). Those animals not reaching the stage of convulsive cough during the 10 min exposure period were considered to be fully protected.

Drugs and solutions

Cromakalim ((±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol was synthesized in Beecham Laboratories and was resolved into its enantiomers following the procedure described by Ashwood *et al.* (1986). Nifedipine was supplied by Bayer, U.K. Cromakalim was dissolved in 10% dimethylsulphoxide at 10^{-3} M for the *in vitro* work and at 1 mg ml^{-1} for i.v. administration. Nifedipine was dissolved in ethanol at 10^{-2} M for the *in vitro* work and at 2 mg ml^{-1} for i.v. administration. Stock solutions of nifedipine were protected from daylight and all *in vitro* experiments were performed under sodium light. Further dilutions of cromakalim and nifedipine were into water for the *in vitro* work or saline for i.v. administration. For oral or intraduodenal administration the compounds were suspended in 1% methylcellulose, the dose volume being 1 ml kg^{-1} body weight.

Statistical analysis

Results are expressed as arithmetic means ± s.e. or geometric means with 95% confidence limits. Statistical analysis of the effect of intraduodenal cromakalim in anaesthetized guinea-pigs was by two-tailed unpaired Student's *t* test. For the experiments in conscious animals, times of 240 s (histamine challenge) or 600 s (antigen challenge) 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

Isolated tracheal spirals

Cromakalim (10^{-7} to 2×10^{-5} M) inhibited both spontaneous- and PGE_2-induced tone in a concentration-dependent manner, with IC_{50} values relative to the inhibition achieved by 10^{-3} M isoprenaline of 1.1 (0.6–1.9) $\times 10^{-6}$ M ($n = 7$) for spontaneous tone and 8.9 (5.6–14.2) $\times 10^{-7}$ M ($n = 5$) for PGE_2-induced tone (Figure 1a). In time-matched control tissues to which the vehicle was added, the fall in tension relative to the effect of 10^{-3} M isoprenaline was 0.10 ± 0.02 over 30 min ($n = 8$). Removal of the epithelium did not affect the response of PGE_2-induced tone to cromakalim (IC_{50} values, $n = 4$: intact, 0.8 (0.6–1.0) $\times 10^{-6}$ M; epithelium removed, 1.1 (0.9–1.4) $\times 10^{-6}$ M). The activity of cromakalim was due almost entirely to its (−)-enantiomer. Thus, against spontaneous tone this enantiomer had a similar intrinsic activity to cromakalim and an IC_{50} value of 4.5 (1.8–10.9) $\times 10^{-7}$ M ($n = 4$), whereas the (+)-enantiomer at 2×10^{-5} M achieved only 0.28 ± 0.06 ($n = 4$) of the relaxant effect of 10^{-3} M isoprenaline (Figure 1b).

Nifedipine was significantly less effective than cromakalim as an inhibitor of both spontaneous and PGE_2-induced tone. For example, against spontaneous tone at 2×10^{-5} M, it achieved only 0.49 ± 0.05 ($n = 10$) of the relaxant effect of 10^{-3} M isoprenaline (Figure 1a). In time-matched controls the fall in tension relative to the effect of 10^{-3} M isoprenaline was 0.21 ± 0.09 over 60 min ($n = 6$). Addi-

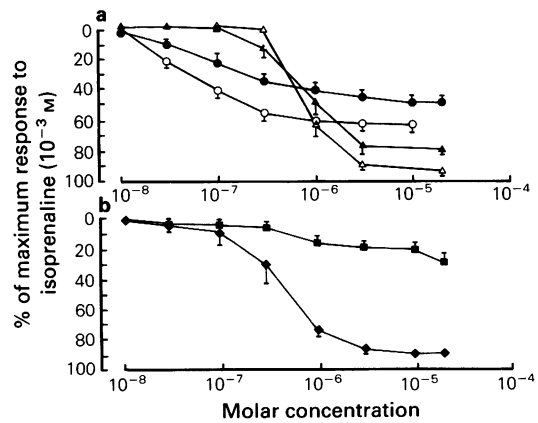

Figure 1 The effects of (a) cromakalim (▲, △) and nifedipine (●, ○) and (b) the (+)- (■) and (−)- (◆) enantiomers of cromakalim on spontaneous (closed symbols) and prostaglandin E_2-induced (open symbols) tone in guinea-pig tracheal spirals. The points are means of 4 to 10 values with vertical lines showing s.e.

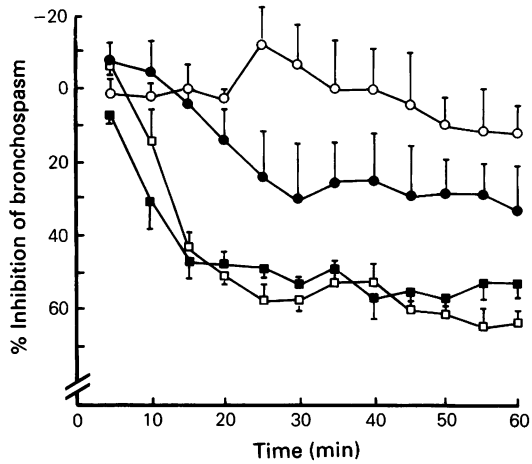

Figure 2 The effects of intraduodenal administration of cromakalim on 5-hydroxytryptamine (5-HT)-induced bronchospasm in anaesthetized guinea-pigs. Cromakalim was given at 0.5 min at dose levels of 1 (●, $n = 4$), 3 (□, $n = 4$) or 10 (■, $n = 6$) mg kg^{-1}. Controls (○, $n = 4$) were given the vehicle. The points are arithmetic means with vertical lines showing s.e. The effect of the 10 mg kg^{-1} dose was significant ($P < 0.05$) at the 10 min time point. The effects of both the 3 and 10 mg kg^{-1} dose levels were significant at the $P < 0.001$ level from 15 min to 60 min.

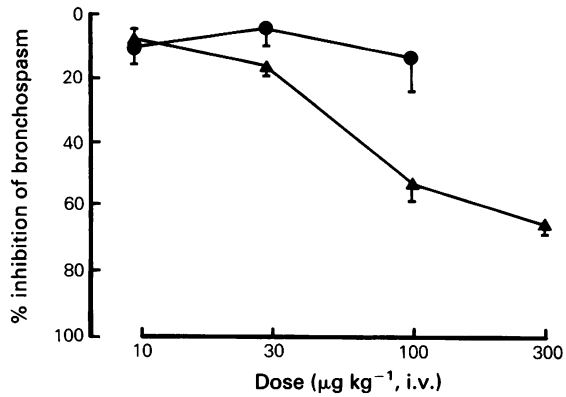

Figure 3 The effect of intravenous administration of cromakalim (▲, $n = 17$) and nifedipine (●, $n = 5$) on 5-hydroxytryptamine (5-HT)-induced bronchospasm in anaesthetized guinea-pigs. Points are arithmetic means with vertical lines indicating s.e.

tion of cromakalim (10^{-5} M) at the end of the nifedipine concentration-response experiment caused further relaxation to 0.94 ± 0.01 ($n = 10$) of the relaxant effect of 10^{-3} M isoprenaline.

Bronchoconstriction in anaesthetized guinea-pigs

When given by the intraduodenal route to anaesthetized guinea-pigs, cromakalim (3 and 10 mg kg^{-1}) inhibited 5-HT-induced bronchospasm within 9.5 to 14.5 min (Figure 2). Maximal inhibition was reached by about 30 min and was sustained until at least 60 min after dosing. Maximal inhibition (about 60%) was similar at the 3 and 10 mg kg^{-1} dose levels. The lower dose level of 1 mg kg^{-1} also appeared to cause some inhibition of the 5-HT-induced response but the effect did not reach statistical significance.

When given by the i.v. route, cromakalim (10–300 μg kg^{-1}) caused a dose-dependent inhibition of 5-HT-induced bronchospasm (Figure 3). The effect of cromakalim was usually maximal by 1 min after dosing, but there was sometimes a greater effect on the response to the 5-HT dose given 6 min after dosing. At 1 min post-dosing, the dose producing 50% inhibition of the 5-HT response was 84 (61–114) μg kg^{-1} ($n = 17$).

In contrast to cromakalim, nifedipine within the dose range 10–100 μg kg^{-1}, i.v. failed to inhibit the bronchoconstrictor effect of 5-HT (Figure 3). Attempts to inhibit bronchoconstriction using higher doses of nifedipine resulted in death at 300 μg kg^{-1}, i.v.

Both cromakalim and nifedipine lowered blood pressure in anaesthetized guinea-pigs. When given by the intraduodenal route, cromakalim caused decreases in diastolic blood pressure of 18.8 ± 1.9% (40 min, $n = 4$), 37.7 ± 2.5% (35 min, $n = 4$) and 39.4 ± 3.8% (25 min, $n = 6$) at 1, 3 and 10 mg kg^{-1}, respectively, from baselines of approximately 40 mmHg. When given by the intravenous route, cromakalim caused a 25% decrease in diastolic blood pressure at a dose level of 28 (20–39) μg kg^{-1}, and nifedipine caused a similar decrease at a dose level of 35 (20–45) μg kg^{-1}.

Bronchoconstriction in conscious guinea-pigs

Cromakalim (2.5 mg kg^{-1}, p.o.) prolonged the time before conscious guinea-pigs collapsed in response to histamine aerosol (Figure 4). It had a statistically significant effect when given 30 to 120 min before the histamine, the maximum effect (T_{max}) being after 60 min. In a dose-response study conducted at T_{max}, 2.5 mg kg^{-1} cromakalim protected 2 out of 6 animals from collapse during the 4 min observation period (Figure 5). Combining these results with those from the time course experiment at the 60 min time point (Figure 4), 5 out of 12 animals were protected by 2.5 mg kg^{-1} of cromakalim.

Nifedipine 5 mg kg^{-1}, p.o., failed to protect guinea-pigs from histamine challenge (results not

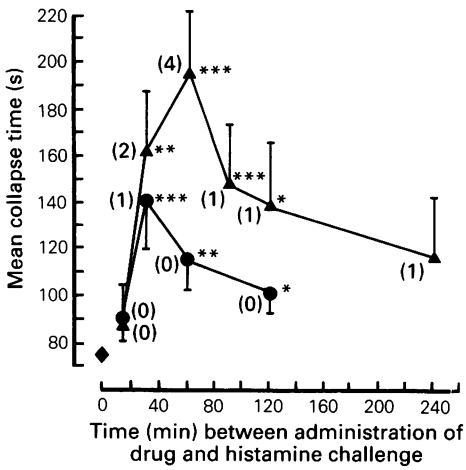

Figure 4 Time course of the effects of cromakalim (▲, 2.5 mg kg^{-1}, p.o.) and nifedipine (●, 50 mg kg^{-1}, p.o.) on the time to collapse following administration of an histamine aerosol to conscious guinea-pigs. The collapse time of the controls (◆) was 77 ± 3 s in the cromakalim experiment and 74 ± 6 s in the nifedipine experiment ($n = 6$). The values shown are means with vertical lines indicating s.e. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to the collapse times for controls. The figures in parentheses indicate the numbers of animals protected during the 4 min observation time, the numbers of animals in the treatment group being 6, except for nifedipine at 15 min ($n = 4$) and 120 min ($n = 3$).

shown) and at the high dose of 50 mg kg^{-1}, p.o., its protective effect, though statistically significant, was weaker than that of cromakalim 2.5 mg kg^{-1}, p.o. (Figure 4).

Cromakalim also prolonged the time before convulsive cough in response to an antigen challenge (Figure 6). A time course was not conducted for these experiments, it being assumed that maximal protection would be 60 min post-dosing, as it was for the histamine challenge. The minimum protective dose level of cromakalim was 1 mg kg^{-1}, p.o.; 5 mg kg^{-1}, p.o., protecting half the animals from convulsive cough over the 10 min exposure period. Nifedipine (50 mg kg^{-1}, p.o.) offered no protection from bronchospasm when given 30 min before an antigen challenge (mean time to cough: controls, 203 s ($n = 6$); nifedipine, 303 s ($n = 6$), $P = 0.44$).

Discussion

Cromakalim, a novel potassium channel activator drug, protected anaesthetized and conscious guinea-pigs from the bronchoconstrictor effects of 5-HT and histamine respectively, suggesting that it may have therapeutic potential as a bronchodilator. Since a number of different spasmogenic mediators are believed to be involved in human asthma, cromakalim was also evaluated against antigen-induced dyspnoea in conscious guinea-pigs, which is widely used as a model of asthma (Payne & Nucci, 1987). In this

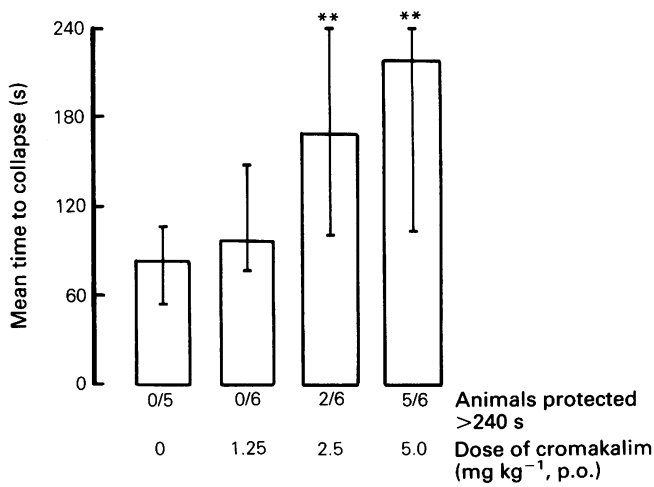

Figure 5 Dose-response relationship for the protection of conscious guinea-pigs from histamine aerosol by cromakalim given 60 min before challenge. The bars indicate the range of collapse times recorded, with 240 s indicating total protection. **$P < 0.01$ compared to controls.

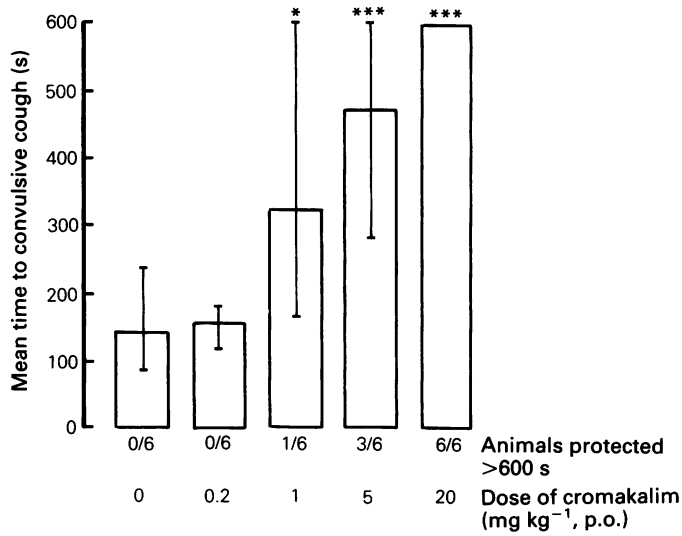

Figure 6 Dose-response relationship for the protection of actively sensitized conscious guinea-pigs from antigen challenge by cromakalim. The bars indicate the range of collapse times recorded, with 600 s indicating total protection. $*P < 0.05$; $***P \leqslant 0.001$ compared to controls.

model, cromakalim inhibited dyspnoea at doses similar to those at which it inhibited histamine-induced bronchospasm (Figures 5 and 6), suggesting that it is an effective functional antagonist of those mediators involved in the allergic response. The efficacy of cromakalim in this model, its prolonged bronchodilator activity against histamine-induced collapse in the guinea-pig and its long biological half-life in man (Davies *et al.*, 1988) all suggest that it should prove beneficial as an oral bronchodilator in man. Although cromakalim is a potent anti-hypertensive agent and had marked hypotensive activity in anaesthetized guinea-pigs, it appears to show little hypotensive activity in normotensive volunteers (Fox *et al.*, 1987). It is therefore unlikely that hypotension would be a complication of its use in normotensive asthmatics.

The potency and efficacy of cromakalim in these animal models is in contrast to that of the calcium entry blocker, nifedipine. Thus, nifedipine was ineffective against antigen- or 5-HT-induced bronchoconstriction in the guinea-pig and provided only partial protection against histamine-induced bronchospasm at doses which would be expected to elicit pronounced hypotensive effects (50 mg kg^{-1}, p.o.). Our results for nifedipine differ from those of Fanta *et al.* (1982, 1987) and Rounding & Towart (1985), who obtained protective effects for nifedipine *in vivo* against histamine- and antigen-induced bronchospasm using different protocols. Nonetheless, nifedipine and other calcium entry blockers have proved to have limited efficacy in the treatment of asthma (Löfdahl & Barnes, 1986) and this may be a reflection of the poor activity shown in the models described here.

One possible explanation for the protective effects of cromakalim against bronchospasm *in vivo* is that they are due to an action on the vascular system. However, nifedipine, although not as potent as cromakalim as an anti-hypertensive agent (Buckingham *et al.*, 1986a), lowered blood pressure in anaesthetized guinea-pigs and would be expected to have a pronounced hypotensive effect at the doses used in conscious animals. Since nifedipine was only weakly effective in providing protection from bronchospasm, it seems probable that cromakalim exerts its inhibitory effects directly on respiratory smooth muscle.

The reasons for the greater efficacy of cromakalim relative to nifedipine *in vivo* may be elucidated by comparing their effects *in vitro*. Cromakalim inhibited spontaneous- and PGE_2-induced tone by an action independent of the presence of the epithelium. Its potency and maximum activity were similar to those found by Allen *et al.* (1986), and were shown to reside predominantly in the (−)-enantiomer, as previously found for its effect on blood vessels both *in vitro* and *in vivo* (Buckingham *et al.*, 1986b; Bray *et al.*, 1987). Nifedipine caused only a small relaxation of spontaneous tone and this result is in accord with those of others, who observed little or no effect (see Ahmed *et al.*, 1985). Since cromakalim was able to inhibit residual spontaneous tone in tissues where

nifedipine had induced its maximal inhibitory effect, these results, taken together, suggest that cromakalim has effects additional to the prevention of calcium entry through voltage-operated calcium channels. This conclusion has been expressed by others (Bray *et al.*, 1988a,b; Cook, 1988).

In accord with its purported mechanism of action, cromakalim stimulates rubidium efflux from trachealis smooth muscle and hyperpolarizes the plasma membrane towards the potassium equilibrium potential. Blockade of potassium channels by procaine (5 mM) abolishes not only the hyperpolarization induced by cromakalim (1 μM), but also its inhibition of spontaneous tone (Allen *et al.*, 1986). Whilst it might be supposed that hyperpolarization and stabilization of the plasma membrane potential by cromakalim would lead merely to the prevention of calcium entry through the same channels blocked by nifedipine, it appears that it is this mechanism of action of cromakalim that leads to its additional effects.

The explanation for these additional effects may be that some receptor-operated channels, such as those opened by prostaglandins, which mediate spontaneous tone (Farmer *et al.*, 1974), may be influenced by voltage (Bolton, 1979). Alternatively, hyperpolarization of the plasma membrane potential may prevent the opening of a calcium channel involved in refilling calcium stores (Bray *et al.*, 1988b) or promote calcium efflux via the plasma membrane electrogenic Na^+/Ca^{2+} exchange mechanism (Cook, 1988; Quast & Cook, 1988). Conceivably, membrane hyperpolarization might cause some inhibition of the release of calcium from intracellular stores (Creese & Denborough, 1981; Foster *et al.*, 1983). However, attempts to demonstrate an effect of cromakalim on intracellular calcium release in rabbit vascular muscle have been unsuccessful (Chui *et al.*, 1987; Howlett, 1987). Finally, the possibility that membrane hyperpolarization reduces the sensitivity of the contractile apparatus to cytosolic calcium is difficult to reconcile with the finding that cromakalim is more effective against spontaneous (or PGE_2-induced) tone than histamine- or acetylcholine-induced tone (Allen *et al.*, 1986).

Whilst cromakalim is more effective than nifedipine in inhibiting spontaneous- and PGE_2-induced tone, and this may suggest why cromakalim is the more effective bronchodilator *in vivo*, the poor inhibitory effects of cromakalim against histamine *in vitro* (Allen *et al.*, 1986) are in contrast to those found in the present study *in vivo*. Guinea-pig trachealis is thought to have high intracellular calcium stores and the mechanism by which histamine induces release of these stores may not be affected by cromakalim. *In vivo*, cromakalim would be expected to exert its effects on small as well as large airways, and these small airways may not have such high intracellular calcium stores. If cromakalim is more effective against histamine in small rather than large airways this would be manifest as an increase in compliance. Such an effect may be essential for protection from exposure to histamine because this spasmogen has a more prolonged effect on compliance than on resistance (Stein *et al.*, 1961).

In summary, the potassium channel activator cromakalim is more effective than the calcium entry blocker nifedipine both as an inhibitor of guinea-pig spontaneous and PGE_2-induced tracheal tone *in vitro* and as an inhibitor of 5-HT-, histamine- or antigen-induced bronchospasm *in vivo*. It is concluded that hyperpolarization and stabilization of the membrane potential by cromakalim (Allen *et al.*, 1986) prevents calcium entering the cytosol through routes that are unaffected by calcium entry blockers. Consequently, cromakalim has far greater potential than nifedipine as a bronchodilator in the treatment of asthma.

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