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The effect of inhaled K^+ channel openers on bronchoconstriction and airway microvascular leakage in anaesthetised guinea pigs

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Abstract

Since orally administered K^+ channel openers may have cardiovascular side effects, it is possible that inhaled administration would be preferred for the treatment of asthma. We have investigated whether inhaled levcromakalim and HOE 234 inhibit histamine-induced bronchoconstriction and airway plasma exudation in anaesthetised guinea pigs. We have also investigated whether inhaled HOE 234 inhibits the bronchoconstriction and plasma exudation induced by vagus nerve stimulation, which is due to the release of tachykinins from sensory nerves. Lung resistance was measured by airway resistance (R_L) computed from airway and transpulmonary pressures and plasma exudation by measurement of Evans blue dye extravasation. Inhaled levcromakalim (25 μg/ml) had a short duration of action, being effective against histamine-induced bronchoconstriction 2 min after pretreatment, but not at 10 min. Inhaled HOE 234 (25 μg/ml) was similarly effective against histamine-induced bronchoconstriction but had a longer duration of action. Inhaled levcromakalim partially attenuated histamine-induced plasma extravasation in small airways, but not in the trachea or main bronchi, whereas inhaled HOE 234 had no effect. HOE 234 protected against non-adrenergic non-cholinergic nerve-induced bronchoconstriction, but had no effect on neurogenic- or substance P-induced plasma extravasation in the airway. Inhaled K^+ channel openers protect against induced bronchoconstriction, but provide little or no protection against plasma exudation, possibly because of an increase in airway blood flow. In addition, inhaled HOE 234 had no effect on neurogenic leakage, suggesting that its vagal inhibitory effect on bronchoconstriction was on airway smooth muscle, rather than on release of neuropeptides from sensory nerves.

Keywords: K^+ channel opener; Levcromakalim; HOE 234; Bronchoconstriction; Plasma exudation

1. Introduction

ATP sensitive K^+ channel openers are novel agents which relax smooth muscle, and may have therapeutic potential in the treatment of asthma (Black and Barnes, 1990). They exert their effect by opening the ATP sensitive K^+ channel (K_{ATP}) and causing hyperpolarisation of the cell membrane and inhibition of depolarisation (Quast and Baumlin, 1988). K^+ channel openers, such as cromakalim and its active enantiomer, levcromakalim (BRL 38227), inhibit the effect of induced constriction by histamine, carbachol and neurokinin A (Allen et al., 1986; Black et al., 1990; Miura et al., 1993) Levcromakalim and HOE 234 relax smooth muscle in animal (Englert et al., 1992) and human airways in vitro (Black et al., 1990; Miura et al., 1993). In vivo studies have shown that systemically administered K^+ channel openers inhibit histamine-induced bronchoconstriction (Arch et al., 1988; Chapman et al., 1992) but do not inhibit systemically administered histamine-induced airway microvascular leakage (Martin and Advenier, 1993). Similarly, K^+ channel openers reduce non-adrenergic non-cholinergic (NANC) nerve-induced bronchoconstriction (Stretton et al., 1992; Burka et al., 1991), but have less or no effect on tachykinin-induced bronchoconstriction in guinea pigs (Ichinose and Barnes, 1990; Stretton et al., 1992; Ishikawa et al., 1994). This suggests that K^+ channel openers inhibits the release of neuropeptides from sensory nerves, although other studies have shown no such differential effect (Aikawa et al., 1992)

In clinical studies in asthmatic patients, orally administered cromakalim attenuates nocturnal bron-

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choconstriction (Williams et al., 1990). This, combined with a prolonged plasma half life of 22 h (Davies et al., 1988), appeared to give this drug considerable potential as an oral bronchodilator. However, the oral dose may be limited by vasodilatation and a fall in blood pressure (Weston, 1989). Cromakalim is a racemic compound composed of the inactive isomer (BRL 38226) and an active (3*S*,4*R*)-isomer, levcromakalim.

We have previously shown that orally administered levcromakalim had no effect on airway calibre, as measured by FEV_1, and did not attenuate bronchoconstriction induced by histamine or methacholine, in mild and moderate asthma. The use of higher doses of levcromakalim (0.5 mg) was associated with a vasodilator type headache and episodic postural hypotension, a manifestation of vasodilatation. By comparison, salbutamol given at high doses significantly protected against histamine-induced bronchoconstriction (Kidney et al., 1993).

In view of the side effects of systemic administration of levcromakalim, it is unlikely to be of benefit in the treatment of asthma. However, there is a need to evaluate K^+ channel openers as inhaled agents. Previous studies in guinea pigs have shown that inhaled levcromakalim and HOE 234 inhibit bronchoconstriction from systemically administered histamine (Englert et al., 1992). K^+ channel openers may have additional effects on the airways, such as inhibition of sensory neuropeptide release. Increased plasma exudation may occur in asthmatic airways in response to inflammatory mediators, and is due to contraction of endothelial cells in post-capillary venules which are the site of leakage. It is possible that K^+ channel openers prevent contraction of these endothelial cells and thereby reduce plasma exudation. There has been no assessment of the effect of inhaled K^+ channel openers, HOE 234 and levcromakalim, on induced airway microvascular leakage.

The purpose of these studies was to determine if levcromakalim and HOE 234 given by inhalation could protect against induced bronchoconstriction and airway microvascular leakage.

2. Materials and methods

2.1. Animal preparation

Pathogen-free female Dunkin-Hartley guinea pigs (350–600 g) were anaesthetised with urethane (1.5 g/kg) given by intraperitoneal injection with additional anaesthesia as required. Suxamethonium was given 1.5 mg/kg intravenously. The animals were tracheotomised and a cannula (8 mm in length and 2.7 mm internal diameter) was inserted into the cervical trachea, and secured with a suture. Polyethylene catheters were inserted into the left carotid artery for blood pressure measurement and the right internal jugular vein for drug administration. The animals were ventilated in the supine position with a volume constant mechanical ventilator (model 50-1718, Harvard Apparatus, Edenbridge, UK) with a tidal volume of 10 ml/kg and a frequency of 60 breaths per min. The study was approved under an Animal Project Licence.

2.2. Measurement of lung resistance

Trans-pulmonary pressure was measured using a pleural catheter which was inserted into the right pleural cavity and connected to one side of a pressure transducer and a side port of the tracheal cannula was connected to the other side of the transducer (model FCO-40, Furness Controls, Bexhill, UK) A second pressure transducer measured airflow through a pneumotachograph (model F1 L, Mercury Electronics, Glasgow, UK). The signals from the transducers were digitalised with a 1 2-bit NB-MIO analog-digital board connected to a Macintosh II computer (Apple Corporation, Cupertino, California, USA) and analysed with a LabVIEW software programme (National Instruments, Austin, Texas, USA) which give instant measurements of R_L by the method of Von Neergard and Wirz (1927).

2.3. Animal ventilation and drug administration

Anaesthetised animals were ventilated and any secretions in the airway were gently aspirated with a cannula attached to a 20 ml syringe. After aspiration the animals were hyperventilated by manually obstructing the outflow port for two breaths (to prevent atelectasis). No measurements were taken within 2 min of this manoeuvre. Drugs or saline control were nebulised for 2 min. The Lung resistance (R_L) was measured at 30 s intervals for 2 min. Evans blue dye was injected slowly i.v. (20 mg/kg) 1 min prior to bronchoconstrictor challenge. Histamine (10^{-3} M) was nebulised for 30 breaths.

2.4. Nebulised solutions

Solutions were nebulised with an ultrasonic nebuliser (model 2511, PulmoSonic, De Vilbiss, PA, USA) connected to a separate flow system which by-passed the pneumotachograph. The volume of this circuit was 50 ml. The output from the nebuliser, measured at the tracheal port was 73 μl/min, at a flow of 0.3 l/min. The particles generated had a mean diameter of 3.8 $\pm$ 1.3 μm, measured with a laser droplet and particle analyser (Model 2600C; Malvern Instruments, Malvern, UK).

2.5. Measurement of plasma exudation

After the animal was disconnected from the ventilator, the chest was opened and normal saline was perfused through the aorta at 100 mm Hg, until all venous fluid returning to the opened right atrium was clear. The lungs were removed and the parenchymal tissue was gently scraped, leaving the airway intact. The airway was cut into four segments (trachea, main bronchi, proximal intrapulmonary airway (IPA) and distal IPA). All tissues were weighed wet, and Evans blue dye was extracted in 2 ml of formamide kept in a water bath at 40°C for 24 h and the absorption of light at 620 nm was measured in a spectrophotometer (model 8480, Philips, Cambridge, UK). The dye extracted was quantified by interpolation against a standard curve of dye concentration in the range of 0.5–10 μg/ml and expressed as ng of dye/mg of wet tissue. This technique of Evans blue dye has been shown to correlate highly with extravasation of radiolabelled albumin in guinea-pig airways (Rogers et al., 1989).

2.6. Experimental protocols

2.6.1. The effect of inhaled K^+ channel openers on histamine-induced bronchoconstriction and airway microvascular leakage

Animals were administered either levcromakalim (25 μg/l) ($n = 18$) or vehicle control ($n = 26$) or HOE 234 (25 μg/l) ($n = 9$) or vehicle control ($n = 7$) for 2 min and challenges with 10^{-3} M histamine were performed 2 min later. In each case Evans blue dye was given 1 min prior to histamine administration.

2.6.2. Duration of effect of inhaled K^+ channel openers

Animals were pretreated with either levcromakalim or HOE 234 for 2 min. Measurements of R_L and mean arterial blood pressure were taken every 30 s. Histamine challenges were performed after 2 min ($n = 6$ levcromakalim, $n = 7$ HOE 234) and 10 min ($n = 6$ levcromakalim, $n = 6$ HOE 234) and where relevant 30 ($n = 6$, HOE 234) and 60 min ($n = 6$ HOE 234). The results were compared with saline-pretreated animals ($n = 9$).

2.6.3. The effect of inhaled HOE 234 on neurogenic stimulation

This was performed on animals pretreated with intravenous propranolol (1 mg/kg) and atropine (1 mg/kg). The vagi were carefully dissected and cut distal to the nodose ganglion (to avoid central nervous system stimulation). The distal ends were attached to platinum electrodes and liquid paraffin was applied to prevent desiccation. HOE 234 (25 μg/l) ($n = 8$) or inhaled saline ($n = 5$) were nebulised for 2 min. The vagi were stimulated with 5 V at a frequency of 5 Hz for 180 s (model S88 stimulator, Grass Instruments, Quincy, MA, USA). Levcromakalim was not used as it did not have a long enough duration of action to allow vagal stimulation.

2.6.4. The effect of inhaled HOE 234 on substance P responses

Slow intravenous injection (1 min) of substance P (10^{-6} mol/kg) was given 2 min after pretreatment with either inhaled HOE 234 (25 μg/ml) ($n = 3$) or saline ($n = 4$).

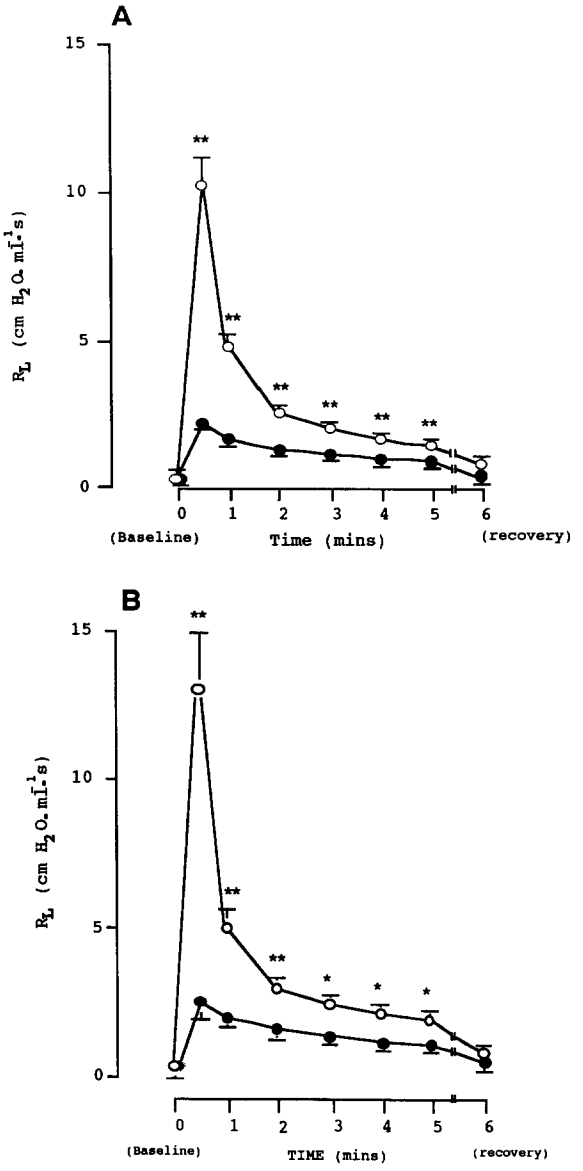

Fig. 1. Time course of histamine (30 breaths 10^{-3} M) induced lung resistance (R_L). Open circles represent saline pretreatment and filled circles represent inhaled K^+ chanel openers (25 μg/ml for 2 min, 120 breaths). Panel A represents the values for levcromakalim- and panel B for HOE 234-pretreated animals. Values are mean ± S.E.M. (** $P < 0.01$, * $P < 0.05$).

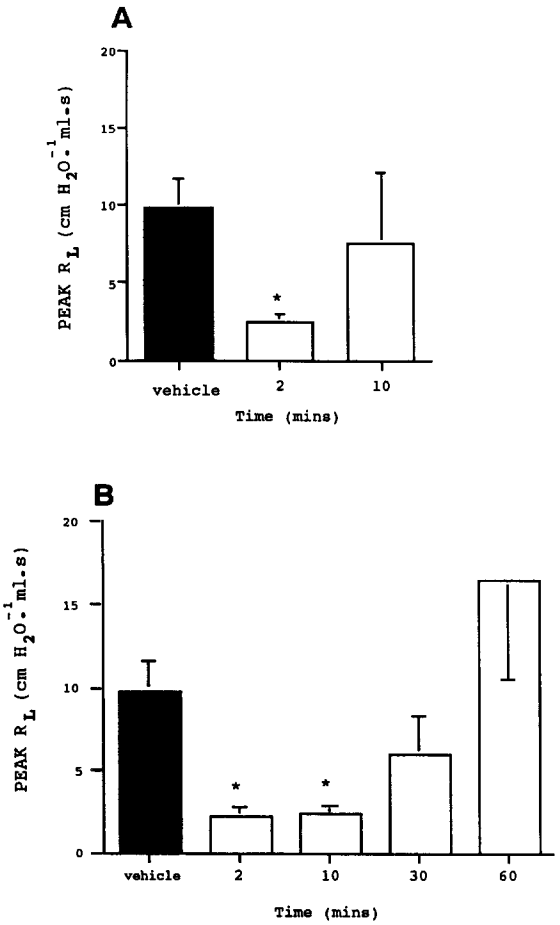

Fig. 2. Duration of protection of inhaled K^+ chanel openers against histamine-induced bronchoconstriction. Solid bars represent saline pretreatment, open bars represent levcromakalim in panel A, and HOE 234 in panel B. $^*P < 0.05$, compared with saline-treated animals.

2.7. Drugs

Levcromakalim was kindly donated by Smith-Kline Beecham (Great Burgh, Epsom, UK) and HOE 234 by Hoechst AG (Frankfurt am Main, Germany). Both compounds were dissolved in normal saline. The solutions were sonicated with a probe sonicator, at a concentration of 25 μg/ml. Histamine (Sigma, Poole, UK) was dissolved in normal saline to a concentration of 10^{-3} M. Evans Blue dye (Sigma) was dissolved in normal saline to a concentration of 20 mg/ml. Substance P (Sigma) was dissolved to a concentration of 10^{-6} M, kept frozen at −24°C and was thawed immediately prior to use each day. Propanolol (Inderal, ICI, Cheshire, UK) and atropine (Phoenix Pharmaceuticals, Gloucester, UK) were purchased in solutions for intravenous use.

2.8. Data analysis

Data are reported as means ± SEM. Comparison of groups was performed using an unpaired t-test. Significance was accepted as $P < 0.05$. Data were analysed with a Macintosh computer (Apple Computer) using a standard statistical package (Statview, Abacus Concept).

3. Results

3.1. Effect on bronchoconstriction

There was significant attenuation of the increase in R_L induced by histamine after inhaled levcromakalim

Table 1
The effect of inhaled K^- channel openers on baseline lung resistance (R_L)

	Baseline R_L (cm $H_2O \cdot ml^{-1} \cdot s$)				
	Pre	30 s	60 s	90 s	120 s
Levcromakalim	0.18 ± 0.03	0.43 ± 0.06	0.44 ± 0.06	0.44 ± 0.06	0.44 ± 0.06
Vehicle	0.17 ± 0.02	0.43 ± 0.04	0.40 ± 0.04	0.40 ± 0.04	0.40 ± 0.04
HOE 234	0.25 ± 0.03	0.56 ± 0.09	0.58 ± 0.09	0.52 ± 0.06	0.52 ± 0.07
Vehicle	0.26 ± 0.05	0.47 ± 0.08	0.43 ± 0.08	0.47 ± 0.08	0.46 ± 0.08

Values shown are the means ± S.E.M. R_L before and every 30 s after inhalation of levcromakalim ($n = 18$), its vehicle ($n = 24$) or HOE 234 ($n = 9$) or its vehicle ($n = 7$).

Table 2
Effect of inhaled K^+ channel openers on blood pressure

	Mean arterial blood pressure (mm Hg)				
	Pre	30 s	60 s	90 s	120 s
Levcromakalim	51.9 ± 3.5	46.1 ± 3.7	47.9 ± 3.6	48.6 ± 3.4	51.1 ± 3.4
Vehicle	52.9 ± 2.9	50.6 ± 2.9	51.6 ± 2.9	51.5 ± 2.6	51.6 ± 3.0
HOE 234	47.6 ± 6.8	43.0 ± 6.9	43.8 ± 6.8	42.9 ± 6.8	43.8 ± 6.6
Vehicle	42.1 ± 2.3	40.6 ± 3.4	41.4 ± 3.3	41.6 ± 3.3	43.1 ± 3.8

Values shown are the means ± S.E.M. arterial blood pressure before and every 30 s after inhalation of levcromakalim ($n = 18$), its vehicle ($n = 24$) or HOE 234 ($n = 9$) or its vehicle ($n = 7$).

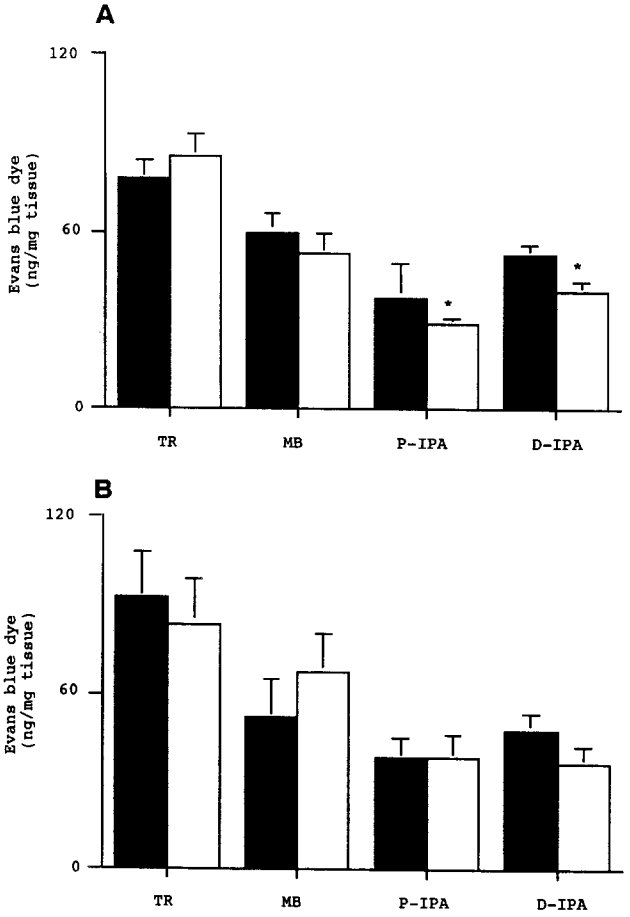

Fig. 3. Effect of inhaled K^+ chanel openers on airway microvascular leakage. Filled bars represent saline pretreated group. In the upper panel the open bars represent leakage seen at various airway levels in levcromakalim-treated animals. In the lower panel open bars represent HOE 234-pretreated group. TR = trachea, MB = main bronchi, PIPA = proximal intrapulmonary airways, DIPA = distal intrapulmonary airways. * $P < 0.05$ compared with vehicle-treated animals.

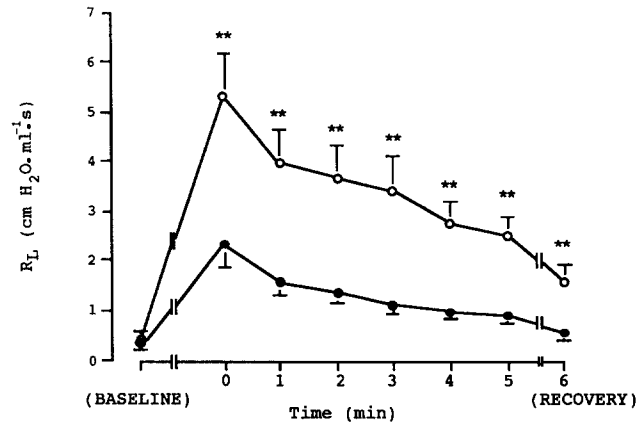

Fig. 4. The effect of inhaled HOE 234 on the time course of vagus nerve-induced increase in lung resistance (R_L). Vagal stimulation was given 5 V, 5 Hz, 5 ms for 180 s. Filled circles represent HOE 234 and the open circles represent saline-treated animals. ** $P < 0.01$, compared to vehicle-treated animals.

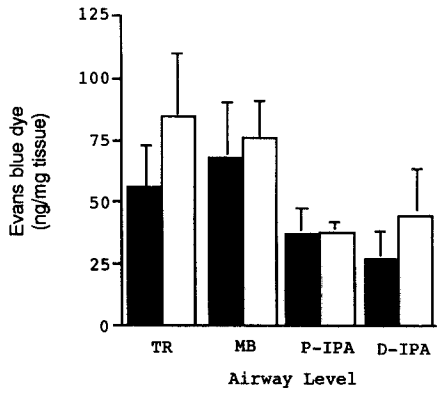

Fig. 5. The effect of vagus nerve stimulation on airway microvascular leakage. The solid bars represent saline-pretreated animals, the open bars represent HOE 234-pretreated animals. TR = trachea, MB = main bronchi, PIPA = proximal intrapulmonary airways, DIPA = distal intrapulmonary airways.

and HOE 234 (Fig. 1). Neither levcromakalim or HOE 234 had any effect on R_L prior to histamine administration, indicating that there was no bronchodilator effect (Table 1). Inhaled levcromakalim and HOE 234 had no significant effect on blood pressure (Table 2).

When histamine was given 2 min after inhaling levcromakalim there was a significant reduction in peak R_L, compared to saline-pretreated animals. However, there was no reduction in R_L when histamine was administered 10 min after pretreatment (Fig. 2). This indicates a relatively short duration of action of levcromakalim when given by inhalation. HOE 234 had a longer duration of action with significant reduction in R_L at 2, 10 and a small but insignificant reduction 30 min after pretreatment. There was no effect at 1 h (Fig. 2).

3.2. Effect on plasma exudation

There was a small but significant reduction in Evans blue dye leakage in response to histamine in the proxi-

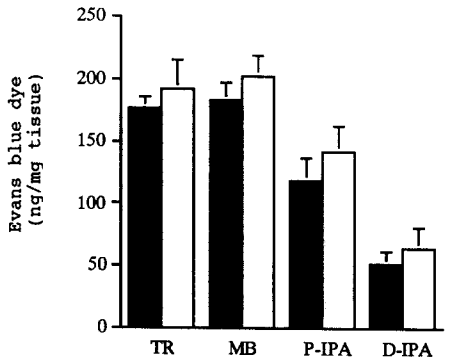

Fig. 6. The effect of inhaled HOE 234 on substance P (10^{-6} mol/kg)- induced airway microvascular leakage. The solid bars represent saline and the open bars represent HOE 234-pretreated animals.

mal and distal intrapulmonary airways after inhaled levcromakalim, but there was no significant effect on the trachea or main extrapulmonary airways. With inhaled HOE 234 there was no reduction in plasma extravasation at any airway level (Fig. 3).

3.3. Effect on neurogenic responses

HOE 234 significantly attenuated the increase in R_L due to vagal stimulation at all time points (Fig. 4). However, HOE 234 had no effect on plasma exudation due to vagus nerve stimulation (Fig. 5). HOE 234 had no effect on plasma exudation induced by substance P (Fig. 6), or on substance P-induced bronchoconstriction (R_L 0.2 ± 0.1 before and 0.4 ± 0.1) after substance P in HOE 234 group, and 0.2 ± 0.04 before and 0.5 ± 0.1 after vehicle, $P > 0.05$).

4. Discussion

This study shows that the inhaled K^+ chanel openers levcromakalim and HOE 234 inhibited histamine-induced bronchoconstriction. Levcromakalim had a short duration of action, which is in marked contrast to its prolonged duration of action when given systemically (Davies et al., 1988) This suggests that when given by inhalation it may not be retained in the airways. Pretreatment with levcromakalim caused a small but statistically significant reduction in airway microvascular leakage in response to inhaled histamine in the intra-pulmonary airways but there was no effect in the trachea or main bronchi. Cromakalim when given systemically in previous studies attenuated the effect the bronchoconstrictor effect of parenterally administered histamine, but did not affect airway microvascular leakage (Martin and Advenier, 1993). However, the main purpose of this study was to assess levcromakalim as an inhaled agent, as oral administration appeared to have no effect on lung function or airway responsiveness in asthma, and the doses administered were accompanied by cardiovascular side effects (Kidney et al., 1993). Also, previous studies have shown attenuation of neural-induced bronchoconstriction, when cromakalim is given parenterally (Ichinose and Barnes, 1990). However, in this study the duration of action of inhaled levcromakalim is probably too short to inhibit neurogenic responses in the airways.

HOE 234 had a longer duration of action and this may be due to its greater potency (Englert et al., 1992; Miura et al., 1993). This length of duration of action indicates that this agent may have prospects as an inhaled bronchodilator. HOE 234 did not attenuate histamine-induced airway microvascular leakage. It is possible that this is because the vasodilator effect of this drug overcomes any inhibitory effect on plasma exudation by increasing blood flow to leaky vessels. Although there were no changes in systemic blood pressure after inhaled K^+ chanel openers, this does not preclude a local effect on airway blood flow.

Inhaled HOE 234 significantly attenuated bronchoconstrictor NANC responses in agreement with previous studies with K^+ chanel openers administered systemically (Ichinose and Barnes, 1990; Ishikawa et al., 1994). Since cromakalim did not inhibit the bronchoconstrictor responses to intravenous substance P this suggests inhibition of tachykinin release from airway sensory nerves. This has also been demonstrated in vitro (Stretton et al., 1992; Burka et al., 1991). Vagus nerve stimulation also causes plasma exudation which is due to the release of tachykinins from sensory nerves. Surprisingly, we found that there was no inhibitory effect of inhaled HOE 234 on neurogenic leakage, which may indicate that the concentration of drug at the site of leakage is insufficient to inhibit the release of substance P. This suggests that the effect of inhaled HOE 234 on NANC bronchoconstriction is likely to be due to inhibition of the effects of tachykinins, rather than their release.

In conclusion, the inhaled K^+ channel openers levcromakalim and HOE 234 attenuated histamine-induced bronchoconstriction, with HOE 234 having a longer duration of action than levcromakalim. Inhaled levcromakalim reduced airway microvascular leakage in the intrapulmonary airways, but not in the trachea or main bronchi, whereas inhaled HOE 234 did not attenuate histamine-induced airway microvascular leakage. Inhaled HOE 234 inhibited NANC bronchoconstriction, but not NANC plasma exudation, suggesting that it counteracts the effect of tachykinin on airway smooth muscle rather than release from airway nerves.

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