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The effect of K^+ channel openers on submucosal gland function and epithelial transport of the ferret trachea, in vitro

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Abstract

The effects of three K^+ channel openers on lysozyme output from submucosal gland serous cells and epithelial albumin transport following maintained submaximal stimulation by the secretagogues methacholine and phenylephrine were examined in the ferret trachea in vitro preparation. The K^+ channel openers Ro 31-6930, 2-(6-cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-pyridine 1-oxide (10 nM–10 μM), levcromakalim, BRL38227 (10 nM–10 μM) and pinacidil (100 nM–10 μM) produced a concentration dependent inhibition of (20 μM) methacholine-induced lysozyme output, with pD_2 values of 7.64, 7.72 and 7.28 respectively. Ro 31-6930 (10 nM–10 μM), levcromakalim (10 nM–10 μM) and pinacidil (1 nM–10 μM) also produced a concentration dependent inhibition of (100 μM) phenylephrine-induced lysozyme output, with pD_2 values of 7.64, 6.55 and 9.16 respectively. Furthermore, glibenclamide (1 μM) produced a modest attenuation of the K^+ channel opener effects on secretagogue-induced lysozyme output. All three K^+ channel openers failed to produce any significant change in either methacholine or phenylephrine-induced albumin outputs. The K^+ channel openers exerted marked effects on airway secretion processes, suggesting that these compounds may have an antisecretory effect. The relevance of the use of the K^+ channel openers in airway disease remains to be determined.

Keywords: K^+ channel opener; Trachea; Submucosal gland; Epithelium

1. Introduction

The K^+ channel openers pinacidil, levcromakalim ((−)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-1-benzopyran-3-ol) and Ro 31-6930 (2-(6-cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-pyridine 1-oxide) have been shown to cause the relaxation of airway smooth muscle both in vivo and in vitro (Taylor et al., 1992; Paciorek et al., 1990). Owing to these effects, it has been suggested that K^+ channel openers may be useful as bronchodilators in the treatment of asthma (Black and Barnes, 1990) and cromakalim (structurally related to levcromakalim and Ro 31-6930) has been shown to alleviate nocturnal asthma in man (Williams et al., 1990). With the majority of research into K^+ channel openers in the airways being directed towards smooth muscle function and since asthma is now regarded as a chronic inflammatory disease affecting other cell types in the airways, it is important to investigate the effect of the K^+ channel openers on these other cells in the airways such as the surface epithelia and the cells of the submucosal glands.

Therefore in this study, the ferret whole trachea in vitro preparation (Webber and Widdicombe, 1987) was used to examine the effects of K^+ channel openers on submucosal gland secretion and airway epithelia function. Two markers of secretion, lysozyme and serum albumin, have been studied following stimulation by the secretagogues methacholine and phenylephrine. The enzyme lysozyme is secreted by submucosal gland serous cells and has an important role as an antibacterial agent in airway surface liquid (Basbaum et al., 1990). Serum albumin is actively transported across the trachea epithelia and also has a protective role in airway surface liquid (Webber and Widdicombe, 1989).

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The K^+ channel openers are a highly diverse group of compounds. In this study the compounds levcromakalim (BRL 38227) and Ro 31-6930, which are variations on the benzopyran nucleus and the structurally unrelated compound pinacidil (Weston and Edwards, 1992; Quast, 1992) have been used to get a broad view of the actions of distinct types of K^+ channel openers.

A preliminary account of this work has been reported to the British Pharmacological Society (Griffin and Webber, 1992).

2. Materials and methods

2.1. Ferret whole trachea in vitro

Ferrets of either sex were anaesthetized by intraperitoneal injection of sodium pentobarbitone (Sagatal; May and Baker; 50 $mg \cdot kg^{-1}$). The trachea was exposed, dissected from the thorax and placed in an organ bath as described by Webber and Widdicombe (1987). Briefly, the trachea was cannulated just below the larynx with a Perspex conical collecting well. The ferret was then killed by cardiac puncture and injection of an overdose of Sagatal (50 $mg \cdot kg^{-1}$) into the heart. Then the thoracic cavity was opened. The trachea was cut just above the carina and removed from the thoracic cavity, cleared of connective tissue and cannulated at the carina end.

The trachea, laryngeal end down, was mounted in an organ bath and bathed continuously with Krebs-Henseleit buffer (maintained at 37° C and gassed with 95% O_2/5% CO_2) on the submucosal side only, so that the trachea lumen remained air filled. The composition of the Krebs-Henseleit buffer was (mM): NaCl 120.8, KCl 4.7, KH_2PO_4 1.2, $MgSO_47H_2O$ 1.2, $NaHCO_3$ 24.9, $CaCl_2$ 2.4 and glucose 5.6.

Tracheal secretions were carried by gravity and epithelial cilia activity to the laryngeal cannula, where they pooled during the collection time period. The secretions were withdrawn at regular intervals into polyethylene catheters which were inserted into the laryngeal cannula. The catheters were sealed with bone wax and stored frozen until required. Storage of up to 6 months does not affect the enzymatic activity of lysozyme in the samples (Webber and Widdicombe, 1987) but assessment of the enzymatic activity and albumin levels for this study were performed within 48 h of completion of the experiment.

When defrosted, the secretions were washed out of the catheters into Eppendorf vials with 1 ml of distilled water. The collected secretions were then sonicated to break up solidified components of the secretions. The total secretion volumes were established by assessing the differences between the weights of the dried and undried catheters, assuming that 1 mg of tracheal secretion was equivalent to 1 μl volume.

2.2. Assay for lysozyme

The concentration of lysozyme in each of the tracheal secretion samples was measured by the use of a turbidimetric assay (Webber and Widdicombe, 1987) which relies on the ability of lysozyme to disrupt the cell wall of the bacterium *Micrococcus lysodeikticus* causing a fall in the optical density of the sample. Briefly, a suspension of *M. lysodeikticus* (0.3 $mg \cdot ml^{-1}$) in 1.5 ml of phosphate buffer (50 mM, pH 7.4) containing sodium azide (1 $mg \cdot ml^{-1}$) and bovine serum albumin (1 $mg \cdot ml^{-1}$) was prepared. 20 μl of hen egg white lysozyme (0.5–100 $ng \cdot ml^{-1}$) and 20 μl of the tracheal secretion samples were incubated for 18 h at 37° C, after which the optical density of the mixture was measured at 450 nm on a spectrophotometer. A standard curve was obtained from the hen egg-white samples and so the concentration of lysozyme in the 20 μl sample could be estimated. Therefore, the lysozyme concentration ($ng \cdot \mu l^{-1}$) and lysozyme output ($ng \cdot min^{-1}$) for each original secretion sample could be calculated.

2.3. Albumin transport

To investigate the effect of K^+ channel openers on the active transport of albumin across the ferret trachea, bovine serum albumin was added to the buffer bathing the submucosal surface of the trachea at a concentration of 4 $mg \cdot ml^{-1}$. Fluorescein isothiocyanate (FITC) labelled bovine serum albumin (40 $\mu g \cdot ml^{-1}$) was also added to the buffer as a marker and enabled an estimation of the total albumin which appeared in the tracheal secretion samples. The fluorescence of the samples was measured with a fluorimeter, using excitation and emission wavelengths of 550 nm and 490 nm respectively. The fluorescent albumin concentration of the tracheal secretion samples was estimated from a standard curve relating fluorescence to the concentration of fluorescent bovine serum albumin (25 $ng \cdot ml^{-1}$ to 3 $\mu g \cdot ml^{-1}$). The total concentration of the albumin in the samples was obtained by multiplying the fluorescent albumin concentration by the ratio of unlabelled to labelled albumin used in the experiments. The rate of output of albumin was determined by dividing the total amount of albumin in a secretion sample by the time over which that sample accumulated.

2.4. Experimental protocol

Studies have previously shown that methacholine and phenylephrine produce concentration-dependent increases in lysozyme and albumin outputs from the ferret trachea (Webber and Widdicombe, 1987). After a 30 min period, methacholine (20 μM) or phenyl-

ephrine (100 μM) was added to the Krebs-Henseleit buffer bathing the trachea. These concentrations of secretagogues produce 80% of the respective maximum responses for lysozyme and albumin outputs (Webber and Widdicombe, 1987). Tracheal secretion samples were taken every 30 min until a steady maintained secretion output was observed (typically 2–3 h). After each 30 min collection period the Krebs-Henseleit buffer surrounding the trachea was replaced with fresh buffer containing the secretagogue.

Once a maintained secretagogue-induced secretion output was established, concentrations of K^+ channel openers were added, in ascending order, to the secretagogue-containing buffer surrounding the trachea. Each concentration of K^+ channel opener was left in contact with the trachea for 30 min. Then the secretagogue-containing was replaced with fresh buffer containing the next concentration of K^+ channel opener. Concentration-response curves for the effect of K^+ channel openers on maintained secretagogue-induced responses could be established.

In protocols where glibenclamide (1 μM) was used, it was present in the buffer for 30 min prior to the addition of any K^+ channel opener and was present in the buffer to the completion of the experiment.

2.5. Drugs

The following drugs were used and their source shown in brackets: Ro 31-6930 (2-(6-cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-pyridine-1-oxide) and levcromakalim ((−)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-1-benzopyran-3-ol) [Roche Products]; pinacidil [Leo Pharmaceuticals]; glibenclamide, methacholine and phenylephrine [Sigma]. Stock solutions (10 mM) were prepared in appropriate vehicles; Ro 31-6930, levcromakalim and pinacidil were prepared in absolute alcohol and glibenclamide in dimethyl sulfoxide (DMSO). Vehicle controls were performed randomly and had no effect on baseline or secretagogue-induced lysozyme or albumin outputs (data not shown, $n = 6$ for each vehicle).

2.6. Analysis

Any change in methacholine or phenylephrine induced lysozyme or albumin output produced by a K^+ channel opener was calculated as the difference in output obtained between the period immediately before the K^+ channel opener was added, i.e. at steady-state for secretagogue-induced tracheal secretion and the period when the K^+ channel opener was present in the buffer surrounding the trachea, expressed as a percentage. Means ± standard error of the mean (S.E.M.) are given throughout. Comparisons to the control periods were made using Student's paired and unpaired *t*-tests (two-tailed), where appropriate. A probability level (P) of 0.05 or less was required for statistical significance. The negative $\log_{10}$ concentration of K^+ channel opener which produced 50% of the maximal response (pD_2) was calculated from the concentration-response curves which had been normalised so that the maximum response was defined as 100%.

3. Results

3.1. Baseline values

In order to ensure that no hypersecretion state existed in the trachea, secretions were collected for 30 min prior to any addition of secretagogues. The total tracheal secretions in this period were minute (< 0.1 μl) or undetectable, therefore lysozyme and albumin outputs were not assessed.

3.2. Effect of secretagogues on total tracheal secretion

Secretagogue-induced tracheal secretion reached a steady-state (maintained) after 2–3 h. The mean levels for this maintained secretion volume were 9.7 ± 0.7 μl ($n = 28$) and 11.8 ± 2.1 μl ($n = 24$) for methacholine (20 μM) and phenylephrine (100 μM) respectively. These values were obtained in 30 min periods prior to the application of the K^+ channel openers.

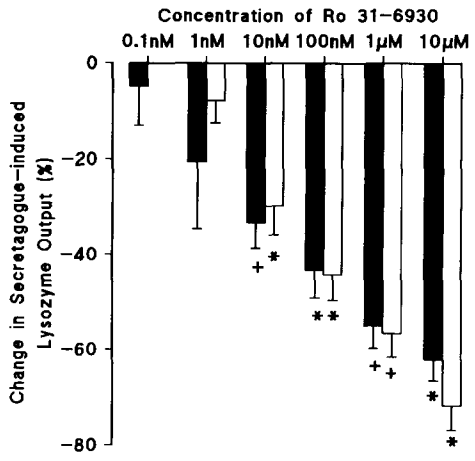

Fig. 1. Actions of the K^+ channel opener Ro 31-6930 on secretagogue-induced lysozyme output. Bar charts showing the effect of the K^+ channel opener Ro 31-6930 on maintained secretagogue-induced lysozyme output produced by (black columns) 20 μM methacholine or (open columns) 100 μM phenylephrine. Columns represent the means of 4–10 determinations, vertical bars represent the S.E.M. * Indicates significant ($P < 0.05$) inhibition of maintained secretagogue-induced lysozyme output and + indicates significance ($P < 0.01$).

3.3. Effect of K^+ channel openers on secretagogue-induced tracheal secretion

The K^+ channel openers investigated produced significant inhibition of the secretagogue-induced tracheal secretion but not in a concentration dependent manner. The volume of methacholine-induced tracheal secretion was inhibited by $45.2 \pm 6.6\%$, $50.1 \pm 2.3\%$ and $40.3 \pm 6.2\%$ by 10 μM Ro 31-6930, levcromakalim and pinacidil respectively. Similarly, the volume of phenylephrine-induced tracheal secretion was inhibited by $57.4 \pm 6.6\%$, $50.9 \pm 5.0\%$ and $48.8 \pm 10.3\%$ by 10 μM Ro 31-6930, levcromakalim and pinacidil respectively.

3.4. Effect of secretagogues on lysozyme output

In the 30 min period immediately after addition of the secretagogues methacholine or phenylephrine, the lysozyme output increased from baseline levels to mean levels of 349 ± 43 ng · min^{-1} ($n = 28$) and 449 ± 41 ng · min^{-1} ($n = 26$) respectively. With continued application of methacholine or phenylephrine the lysozyme output fell to a constant level after approximately 3 h. The mean levels of maintained output for the secretagogues methacholine and phenylephrine were 103.4 ± 15 ng · min^{-1} ($n = 28$) and 129.7 ± 11 ng · min^{-1} ($n = 26$) respectively.

3.5. Effect of K^+ channel openers on secretagogue-induced lysozyme output

All the K^+ channel openers caused a concentration dependent inhibition of the maintained methacholine or phenylephrine-induced lysozyme outputs, although there were differences in the order of potency depending on the secretagogue. Both Ro 31-6930 (10 nM–10 μM) and levcromakalim (10 nM–10 μM) produced significant inhibition of maintained lysozyme output in the presence of either secretagogue, Fig. 1 and Fig. 2 respectively. In contrast, pinacidil (1 nM–10 μM) produced a significant inhibition of phenylephrine-induced lysozyme output (Fig. 3) but only produced significant inhibition of methacholine-induced output at concentrations 100 nM–10 μM.

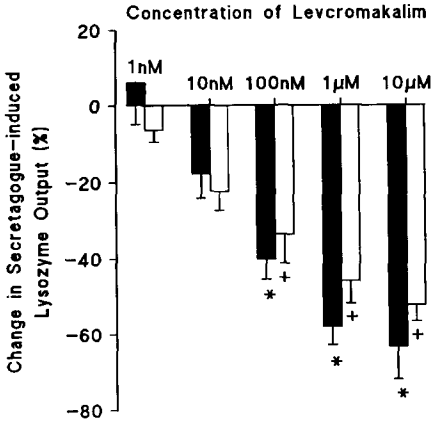

Fig. 2. Actions of the K^+ channel opener levcromakalim secretagogue-induced lysozyme output. Bar charts showing the effect of the K^+ channel opener levcromakalim on maintained secretagogue-induced lysozyme output produced by (black columns) 20 μM methacholine or (open columns) 100 μM phenylephrine. Columns represent the means of 4–10 determinations, vertical bars represent the S.E.M. * Indicates significant ($P < 0.05$) inhibition of maintained secretagogue-induced lysozyme output and + indicates significance ($P < 0.01$).

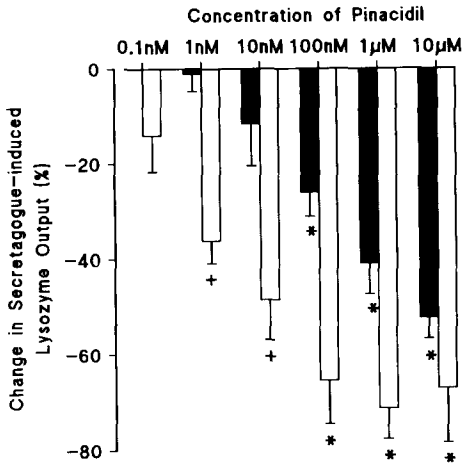

Fig. 3. Actions of the K^+ channel opener pinacidil on secretagogue-induced lysozyme output. Bar charts showing the effect of the K^+ channel openers pinacidil on maintained secretagogue-induced lysozyme output produced by (black columns) 20 μM methacholine or (open columns) 100 μM phenylephrine. Columns represent the means of 4–10 determinations, vertical bars represent the S.E.M. * Indicates significant ($P < 0.05$) inhibition of maintained secretagogue-induced lysozyme output and + indicates significance ($P < 0.01$).

The maintained methacholine-induced lysozyme output was inhibited maximally by mean percentages of $62.2 \pm 4.3\%$, $63.3 \pm 8.5\%$ and $52.3 \pm 4.3\%$ by Ro 31-6930 (10 μM), levcromakalim (10 μM) and pinacidil (10 μM) respectively. By plotting concentration-response curves for each K^+ channel opener the calculated pD_2 values for the inhibition of the maintained methacholine-induced lysozyme output by Ro 31-6930, levcromakalim and pinacidil were 7.64, 7.72 and 7.28, respectively (Table 1). In contrast, the maintained phenylephrine-induced lysozyme output was inhibited maximally by percentages of $63.1 \pm 3.5\%$, $52.2 \pm 4.4\%$ and $67.0 \pm 11.4\%$ by Ro 31-6930 (10 μM), levcromakalim (10 μM) and pinacidil (10 μM), respectively. The pD_2 values for the inhibition of phenylephrine-induced lysozyme output by Ro 31-6930, levcromakalim and pinacidil were 7.64, 6.55 and 9.16, respectively (Table 1).

Therefore it is apparent that pinacidil is significantly more potent against phenylephrine-induced lysozyme output than the cromakalim derived compounds Ro

Table 1
The effects of the K^+ channel openers Ro 31-6930, levcromakalim and pinacidil on methacholine or phenylephrine-induced lysozyme output from in vitro ferret trachea

	Methacholine		Phenylephrine	
	pD_2	E_{max}	pD_2	E_{max}
Ro 31-6930	7.64 (6.89–8.39)	62.2 ± 4.3	7.64 (7.01–8.23)	63.1 ± 3.5
Levcromakalim	7.72 (7.30–8.14)	63.3 ± 5.8	6.55 (5.04–8.06)	52.2 ± 4.4
Pinacidil	7.28 (6.41–8.14)	52.3 ± 4.3	9.16 (8.48–9.84)	67.0 ± 11.3

A maintained secretagogue-induced lysozyme output was produced by methacholine (20 μM) or phenylephrine (100 μM). The negative $\log_{10}$ of the concentration of K^+ channel opener (pD_2) which produced 50% inhibition of the maximal response (E_{max}) was calculated from concentration-response curves which had been normalised so that the maximal response was defined as 100%. The E_{max} values were calculated from the bar charts as shown in Figs. 1–3. Values shown are means, with 95% confidence limits (for pD_2) in brackets or S.E.M. (for E_{max}). Values are the mean of 4–10 determinations.

31-6930 and levcromakalim, the order of potency was pinacidil > Ro 31-6930 > levcromakalim. In contrast, the potencies of the K^+ channel openers against methacholine-induced lysozyme output are similar, the compounds were equipotent against this secretagogue. In addition, none of the K^+ channel openers produced more than 70% inhibition of maintained secretagogue-induced lysozyme output.

3.6. Effect of glibenclamide on the inhibition of secretagogue-induced lysozyme output by the K^+ channel openers

Preliminary experiments showed that glibenclamide (1 nM–10 μM) produced no significant change in either baseline or secretagogue-induced lysozyme output, although maintained methacholine-induced lysozyme output was 88.1 ± 9.7 ng · min^{-1}, which decreased slightly to 74.2 ± 16.5 ng · min^{-1} ($n = 6$) in the presence of 10 μM glibenclamide.

The presence of glibenclamide (1 μM) significantly attenuated the concentration-response of the K^+ channel openers against secretagogue-induced lysozyme output, causing a shift to the right of the concentration-response curves for the effect of each K^+ channel opener on secretagogue-induced lysozyme output. Fig. 4A and B shows the effect of glibenclamide (1 μM) on the inhibition of secretagogue-induced lysozyme output by Ro 31-6930, an example of the attenuation produced by glibenclamide. Fig. 5A and B shows the attenuation by glibenclamide of the levcromakalim response and Fig. 6A and B shows the effect of glibenclamide on the pinacidil responses.

3.7. Effect of secretagogues on albumin output

A steady-state of secretagogue-induced albumin output was reached after 2–3 h. The mean levels for this maintained secretagogue-induced albumin output were 32 ± 6 ng · min^{-1} ($n = 26$) and 98 ± 28 ng · min^{-1} ($n = 22$) for methacholine (20 μM) and phenylephrine (100 μM) respectively.

3.8. Effect of K^+ channel openers on secretagogue-induced albumin output

All the K^+ channel openers investigated here failed to produce any significant change in either methacholine or phenylephrine-induced albumin output, over the concentration range of 1 nM to 10 μM (Fig. 7A and B). Although, Ro 31-6930 (10 μM) inhibited

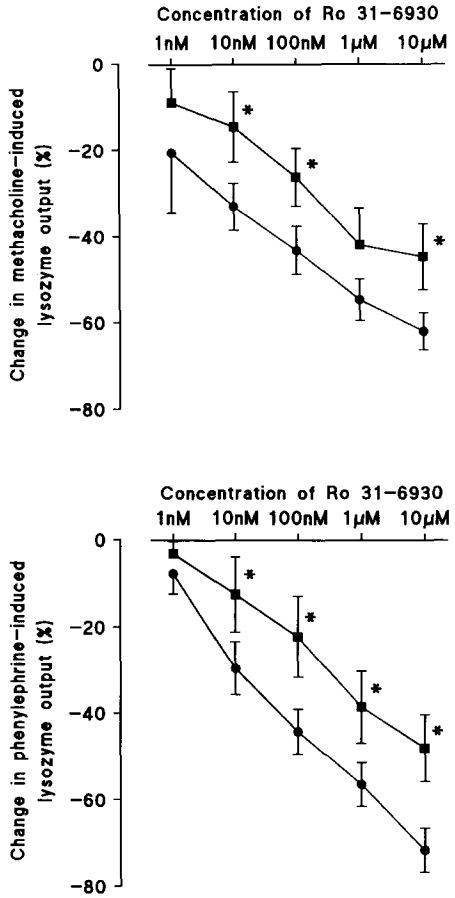

Fig. 4. Glibenclamide attenuates the actions of Ro 31-6930 on lysozyme output. Concentration-response curves showing the effect of 1 μM glibenclamide on the response to the K^+ channel opener Ro 31-6930 on maintained secretagogue-induced lysozyme output, produced by (A) 20 μM methacholine or (B) 100 μM phenylephrine. The K^+ channel opener Ro 31-6930 alone (●) and in the presence of 1 μM glibenclamide (■). Data points represent the means of 4–10 determinations, vertical bars represent the S.E.M. * Indicates a significantly different ($P < 0.05$) response of Ro 31-6930 with glibenclamide present compared to the response with Ro 31-6930 alone.

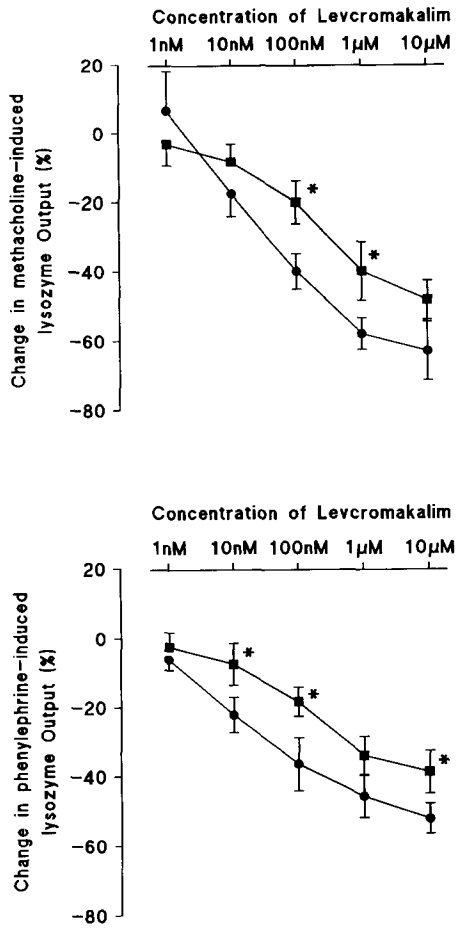

Fig. 5. Glibenclamide attenuates the actions of levcromakalim on lysozyme output. Concentration-response curves showing the effect of 1 μM glibenclamide on the response to the K^+ channel opener levcromakalim on maintained secretagogue-induced lysozyme output, produced by (A) 20 μM methacholine or (B) 100 μM phenylephrine. The K^+ channel opener levcromakalim alone (●) and in the presence of 1 μM glibenclamide (■). Data points represent the means of 4–10 determinations, vertical bars represent the S.E.M. * Indicates a significantly different ($P < 0.05$) response of levcromakalim with glibenclamide present compared to the response with levcromakalim alone.

methacholine or phenylephrine-induced albumin output by $28.0 \pm 9.7\%$ and $25.4 \pm 10.4\%$, respectively. Levcromakalim (10 μM) inhibited methacholine or phenylephrine-induced albumin output by $31.3 \pm 14.3\%$ and $3.2 \pm 5.3\%$, respectively. Pinacidil (10 μM) inhibited the maintained methacholine or phenylephrine-induced albumin output by $6.0 \pm 12.1\%$ and $14.4 \pm 6.4\%$, respectively.

4. Discussion

In this study, K^+ channel openers, which have previously been shown to cause the relaxation of bronchial smooth muscle (Taylor et al., 1992; Paciorek et al., 1990) have been investigated in an in vitro model of secretion of airway surface liquid (mucus), lysozyme and albumin from the air filled ferret trachea. Various techniques have been used to investigate the control and regulation of airway secretion, such as the use of radiolabelled precursors of mucins (Gashi et al., 1987), study of isolated submucosal glands (Shimura et al., 1988) and the morphometric measurement of secretory product loss from various secretory cell types in the airways (Tokuyama et al., 1990).

The air filled isolated ferret trachea in vitro preparation used in this investigation allows the study of three markers of airway secretion: the volume of airway surface liquid secreted, the lysozyme output and albumin output. The ferret trachea contains an abundance of submucosal glands but, in contrast, lacks significant numbers of goblet cells within the airway epithelia (Robinson et al., 1986). So the secretion of

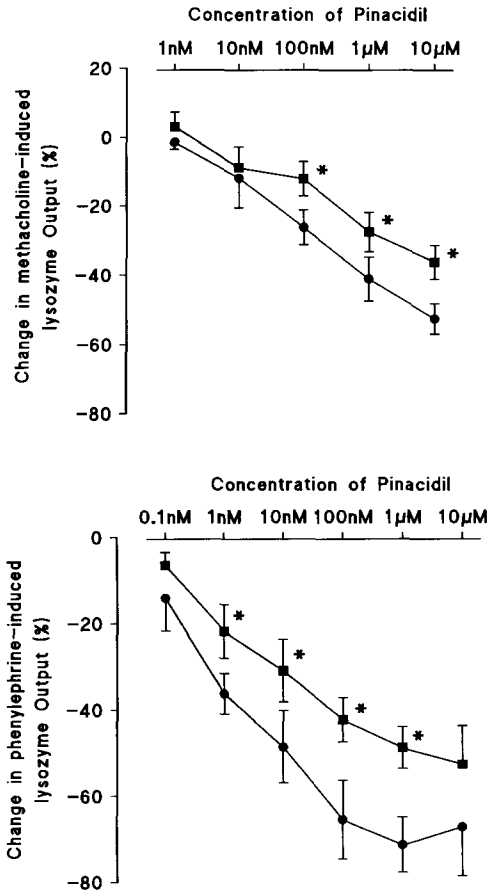

Fig. 6. Glibenclamide attenuates the actions of pinacidil on lysozyme output. Concentration-response curves showing the effect of 1 μM glibenclamide on the response to the K^+ channel opener pinacidil on maintained secretagogue-induced lysozyme output, produced by (A) 20 μM methacholine or (B) 100 μM phenylephrine. The K^+ channel opener pinacidil alone (●) and in the presence of 1 μM glibenclamide (■). Data points represent the means of 4–10 determinations, vertical bars represent the S.E.M. * Indicates a significantly different ($P < 0.05$) response of pinacidil with glibenclamide present compared to the response with pinacidil alone.

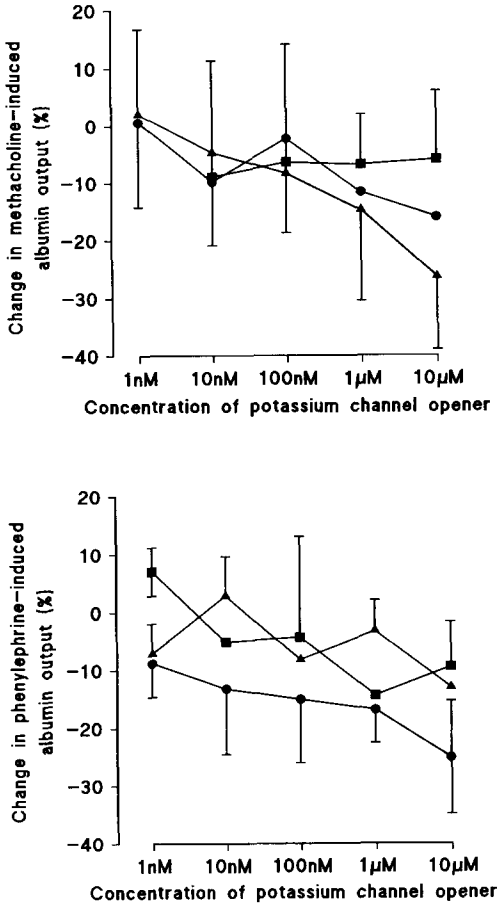

Fig. 7. K^+ channel openers do not affect secretagogue-induced albumin output. Concentration-response curves showing the non-significant effect of Ro 31-6930 (●), levcromakalim (▲) and pinacidil (■) on maintained secretagogue-induced albumin output produced by (A) 20 μM methacholine or (B) 100 μM phenylephrine. Data points represent the means of 4–10 determinations, vertical bars represent the S.E.M.

airway surface liquid composed of ions, macromolecules and water into lumen of the ferret trachea comes from the submucosal gland cells and the surface epithelial cells. The submucosal gland of the ferret contains both mucous and serous secretory cells, 25% of the gland being mucous cells and 65% being the serous cell type (Gashi et al., 1986). At present, there is not a specific marker for secretion from the mucous cell although these cells do contribute to the total tracheal secretion volume. The secretion from the serous cell type does contain the specific marker lysozyme which permits the study of the control and regulation of these cells.

Lysozyme in airway surface liquid provides the bacterial protection to the airways, in addition to the antibacterial enzyme lactoferrin which is also secreted by the serous cell of the airway submucosal gland. Lysozyme is only found within the secretatory granules of serous submucosal gland cells of the ferret trachea (Tom-Moy et al., 1983) whilst being absent from mucous, epithelia and cartilage tissue. Furthermore, immunocytochemistry in human trachea tissue (Bowes and Corrin, 1977) identified the serous submucosal gland cell as the sole source of lysozyme in the airway surface liquid. Therefore, since lysozyme can be easily assayed (Lorenz et al., 1957) it makes it an ideal biochemical marker for the pharmacological regulation of the submucosal gland serous cell.

The secretagogues methacholine and phenylephrine cause the discharge of serous cell granules (Basbaum et al., 1981). Autoradiographic studies of distribution of the receptors for these agonists indicate that muscarinic receptors are present on mucous and serous cells of the ferret submucosal gland but α-adrenoceptors are present only on the serous cells of the glands (Basbaum et al., 1983). Pharmacological (Webber and Widdicombe, 1987) and rheology (De Sanctis et al., 1993) studies suggest that methacholine produces a largely watery mucus from the ferret trachea which is a consequence of stimulation of secretion from both serous and mucous cells, to a similar degree. In contrast, the distribution of α-adrenoceptors in the ferret submucosal gland (Basbaum et al., 1983) and pharmacological studies (Culp et al., 1990; Webber and Widdicombe, 1987) suggests that phenylephrine stimulates secretion from serous cells to a much greater degree than mucous cells.

The K^+ channel openers investigated all produced a concentration-dependent inhibition of both methacholine and phenylephrine-induced lysozyme output ($ng \cdot min^{-1}$). The compounds Ro 31-6930 and levcromakalim, which are derivatives of cromakalim, were equipotent (not significantly different pD_2 values) in the inhibition of both methacholine and phenylephrine-induced lysozyme output. A potency difference between these two compounds has been previously shown in other systems; Ro 31-6930 has been shown to be 2–5 times more potent than levcromakalim as a bronchodilator in guinea-pig airways (Paciorek et al., 1990) and in electrical field stimulated guinea-pig trachea (Gater et al., 1993).

In contrast, pinacidil (a member of the cyanoguanidine group, rather than benzopyran as the cromakalim derivatives described above) showed differential effect on the secretagogue-induced lysozyme output depending on the agonist used. Pinacidil was more potent in the inhibition of phenylephrine than methacholine-induced lysozyme output, the respective pD_2 values being 7.28 and 9.16. Furthermore, pinacidil was the most potent of the K^+ channel openers in respect to the inhibition of phenylephrine-induced lysozyme output. Previous studies in other systems (Paciorek et al., 1990; Taylor et al., 1992) showed a potency order for the K^+ channel openers as observed in this study: Ro 31-6930 > levcromakalim > pinacidil. In contrast, phenyl-

ephrine-induced lysozyme output was more sensitive to pinacidil than the benzopyran compounds. It has been suggested that pinacidil at high concentration may be acting via mechanisms additional to opening K^+ channels (Nielsen-Kudsk et al., 1990), such as by the inhibition of Ca^{2+} influx (Masuzawa et al., 1990).

The present study has shown that the K^+ channel opener induced inhibition of secretagogue-induced lysozyme output was not fully reversed by the putative antagonist glibenclamide (1 μM). This suggests that these K^+ channel openers have effects on sites other than a glibenclamide-sensitive channel or site.

The K^+ channel openers cause an increase in the permeability of the smooth muscle membrane to potassium (K^+), which leads to the hyperpolarisation of the cell membrane and indirect inhibition of calcium (Ca^{2+}) influx via voltage-activated Ca^{2+} channels (Quast and Cook, 1989). When the mechanism of action of the K^+ channel openers was investigated further it was noted that 3–5 times greater concentration of levcromakalim was required to open K^+ channels (measured by $^{42}K^+$ efflux) than to cause smooth muscle relaxation (Quast and Baumlin, 1988), suggesting that the mechanism of vasorelaxation was in part not associated with the opening of cell membrane K_{ATP} channels. Furthermore, recent evidence from smooth muscle preparations have shown that K^+ channel openers interact with Ca^{2+} and $InsP_3$ second messenger systems, which are involved in the stimulus secretion coupling in the airway submucosal gland cell. Lemakalim (the previous name for levcromakalim) inhibited the noradrenaline-induced synthesis of $InsP_3$ in smooth muscle strips from rabbit mesenteric artery (Ito et al., 1991) and this effect was also shown to occur with pinacidil (Itoh et al., 1992). These studies also showed that the K^+ channel openers inhibited agonist-stimulated Ca^{2+} mobilization from intracellular stores. Also Okada et al. (1993) showed that levcromakalim reduced the Ca^{2+} sensitivity of the contractile elements of vascular smooth muscle.

The K^+ channel openers can inhibit the neurally induced contraction of tracheal smooth muscle tone by reducing neurotransmitter release (Wessler et al., 1993). Furthermore, Kuo et al. (1992) showed that K^+ channel openers cause the inhibition of neurally mediated goblet cell secretion in response to vagal stimulation or acute inhalation of cigarette smoke but not to methacholine-induced goblet cell secretion.

Therefore it is apparent that there are various sites and mechanisms of actions of the K^+ channel openers, which would help explain the effect of these compounds on secretagogue-induced lysozyme output.

Electrophysiological data (Griffin and Scott, 1994) have shown that a voltage-activated outward K^+ conductance was expressed in cultured airway submucosal gland cells, which was increased by 1 μM Ro 31-6930. This effect of Ro 31-6930 was shown to be sensitive to glibenclamide, which as shown above augments the effect of this K^+ channel opener on secretagogue-induced lysozyme output.

In contrast, to their effects on secretagogue-induced lysozyme output, the K^+ channel openers produced no significant change in secretagogue-induced albumin output. It is now established that the movement of albumin across the trachea is not a passive process but involves active transport across the trachea epithelia which is modulated by muscarinic and adrenergic activation (Webber and Widdicombe, 1989; Price et al., 1990). The function of albumin in the tracheal mucus is to contribute to general rheology of mucus and to provide antioxidant and anti-inflammatory function. The lack of effect of the K^+ channel openers on the active transport of albumin in the airway epithelia suggests that the K^+ channel openers are not modulating this process either directly or indirectly.

Where a state of hypersecretion exists, which has been reported to be the case in asthma (Persson, 1991), the K^+ channel openers may inhibit the function of submucosal glands causing a reduction of lysozyme output from the serous cells and total secretion volume. It should be noted that since no specific marker for mucous cells secretion exists, the effect of the K^+ channel openers on the full function of the submucosal glands is unknown and therefore does reduce the significance of these findings. A potential therapeutic use for these compounds was suggested by Kuo et al. (1992), from a study of neurally stimulated mucus secretion from goblet cells, stating that the K^+ channel openers could be useful in blocking airway hypersecretion in bronchial diseases such as chronic bronchitis.

In conclusion, we have shown that K^+ channel openers previously shown to be bronchodilators also exert marked effects on other cells in the airways, producing inhibition of secretagogue-induced lysozyme output but no effects on epithelia active transport of albumin. The mechanism of action of the K^+ channel openers that produces the inhibition of lysozyme output from airway submucosal gland cells has yet to be fully elucidated. Furthermore, the full therapeutic implications of these findings have yet to be assessed.

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