Br. J. Pharmacol. (1994), **113**, 889–897

Regional and species differences in glyburide-sensitive K^+ channels in airway smooth muscles as estimated from actions of KC 128 and levcromakalim

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1 The purpose of the present experiments was to elucidate the differences in actions of two K^+ channel openers, KC 128 and levcromakalim, on the carbachol-induced contraction, membrane potential and $^{86}Rb^+$ efflux of the dog tracheal and bronchial smooth muscles. Furthermore, we compared the effects of these agents on guinea-pig and human airway smooth muscles.

2 In the dog tracheal and bronchial smooth muscle tissues, levcromakalim induced a concentration-dependent relaxation of the carbachol-induced contraction. The IC_{50} values were 0.35 μM (pIC_{50}: 6.46 ± 0.10, $n = 9$) and 0.55 μM (pIC_{50}: 6.26 ± 0.07, $n = 5$), respectively. KC 128 relaxed bronchial smooth muscles precontracted by carbachol with an IC_{50} value of 0.19 μM (pIC_{50}: 6.73 ± 0.10, $n = 7$). However, KC 128 had almost no effect on the contraction evoked by carbachol in the trachea ($IC_{50} > 10$ μM). The relaxations induced by levcromakalim and KC 128 were antagonized by glyburide (0.03–1 μM) but not by charybdotoxin (100 nM).

3 Levcromakalim (1 μM) hyperpolarized the membrane of both dog tracheal and bronchial smooth muscle cells, whereas KC 128 (1 μM) hyperpolarized the membrane of bronchial but not of tracheal smooth muscle cells.

4 Levcromakalim (10 μM) increased $^{86}Rb^+$ efflux rate from both tracheal and bronchial smooth muscle tissues but KC 128 (10 μM) increased $^{86}Rb^+$ efflux rate only from bronchial and not tracheal smooth muscle tissues. Glyburide (1 μM) prevented the hyperpolarization and the $^{86}Rb^+$ efflux induced by these agents at the same concentration as observed for mechanical responses.

5 Both KC 128 and levcromakalim relaxed the guinea-pig isolated tracheal smooth muscles precontracted by carbachol (100 nM), histamine (3 μM) or U46619 (10 nM). KC 128 was approximately 10 times more potent than levcromakalim for each agonist.

6 In human bronchial smooth muscles, levcromakalim but not KC 128 induced a concentration-dependent relaxation of the carbachol-induced contraction.

7 It is concluded that KC 128 has relaxant and hyperpolarizing effects in the dog bronchial and guinea-pig tracheal smooth muscles, but not in the dog tracheal and human bronchial smooth muscles. On the other hand, levcromakalim acts consistently on all the above airway smooth muscle tissues. These results indicate that there are regional and species differences in distribution of K^+ channels, and at least two different K^+ channel opener- and glyburide-sensitive K^+ channels are present in the dog airway smooth muscles.

Keywords: Levcromakalim; KC 128; airway smooth muscle; K^+ channel opener (activator); membrane potential; Rb^+ efflux; glyburide

Introduction

Recently many K^+ channel openers have been developed for the treatment of diseases, such as hypertension, asthma and ischaemic heart disease (Edwards & Weston, 1990; 1993; Atwal, 1992; Quast, 1992; Small *et al.*, 1992). These drugs were thought to activate a glyburide- and ATP-sensitive K^+ channel that is not sensitive to charybdotoxin in vascular smooth muscle. However, until now there is no consensus on which K^+ channel is the common target. The target channel has been identified with a large or small conductance and as Ca^{2+}-sensitive or -insensitive in different vascular smooth muscle tissues (see reviews of Kajioka *et al.*, 1991; Edward & Weston, 1993).

For airway smooth muscles, it has also been reported that many ATP-sensitive K^+ channel openers relax the airway smooth muscles including human airway preparations (Taylor *et al.*, 1988; 1992; Black *et al.*, 1990; Black & Barns, 1990; Small *et al.*, 1992). When we examined the relaxant effect of bronchodilators including K^+ channel openers, we have often used guinea-pig tracheal smooth muscle tissues, because they have spontaneous tone that is similar to human airway preparations. However, the differences between guinea-pig and human airway preparations have often been pointed out. Concerning the K^+ channel openers, the relaxant effect of these agents on carbachol-induced contraction is reported to be weaker in guinea-pig than in human preparations (Taylor *et al.*, 1988; 1992; Black *et al.*, 1990; Imagawa *et al.*, 1993). Recently, we have reported that in the dog tracheal smooth muscle tissues, the action of K^+ channel openers shows greater similarity to human bronchi than to guinea-pig tracheal smooth muscle (Kamei *et al.*, 1994).

KC 128 (N-cyano-*N'*,*N''*,2,2-tetramethyl-6-nitro-2*H*-1-benzopyran-4-carboxamidine) has recently been synthesized in our laboratory based on computer-assisted analysis of the pharmacophore model of K^+ channel openers (Koga *et al.*, 1993a,b). We examined the effect of KC 128 and levcromakalim (Figure 1) on the electrical and mechanical properties of the dog tracheal and bronchial smooth muscles. Furthermore, we compared the effects of these compounds on the guinea-pig and human airway smooth muscles.

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Methods

Tissue preparation

Dog airway preparations Adult beagle dogs of either sex, (8–15 kg, CSK) were anaesthetized by i.v. administration of pentobarbitone (35 mg kg^{-1}) and exsanguinated. Segments of tracheal smooth muscle were prepared for all experiments as previously described (Kamei *et al.*, 1994). Briefly, circular muscle strips were excised from the cervical part of the trachea and immediately placed in Krebs-Henseleit solution. Tracheal smooth muscle tissues were dissected from both ends of the cartilage, then carefully freed of fat and connective tissue in a dissecting chamber under a binocular microscope. The preparation was cut into segments, 2–3 mm wide and 10 mm long, in the transverse direction. Bronchi were excised from the third or fourth branches (diameter; 1–3 mm) and were cleaned. These ring preparations were used to record the mechanical responses and the preparations, which were cut along the longitudinal axis, were used for measurement of $^{86}Rb^+$ efflux. For electrical response recording, following removal of cartilage and connective tissues the bronchial smooth muscle tissues were prepared and cut into segments, 3–5 mm wide and 5 mm long, in the transverse direction.

Guinea-pig tracheal preparations Male guinea-pigs (500–1000 g, Hartley, CRJ) were killed by a blow to the head and exsanguinated. The trachea was removed and immediately placed in Krebs-Henseleit solution and carefully freed of fat and connective tissue. The trachea was cut along the longitudinal axis directly opposite the smooth muscle layer for tension recording (Imagawa *et al.*, 1993).

Human bronchus preparations Human lung tissues were obtained from patients undergoing surgery for carcinoma of the lung and maintained at 4°C overnight in Krebs-Henseleit solution before use. Bronchi (diameter; 1–5 mm) were dissected free of adhering fat and connective tissue and cut into ring preparations (Imagawa *et al.*, 1993).

Mechanical response recording

Dog airways Dog tracheal smooth muscle tissues were suspended in organ baths containing Krebs-Henseleit solution (35°C) and mounted vertically with the ends tied by silk thread. Ring preparations of dog bronchial smooth muscle were cannulated and fixed by a pair of fine L-shaped needles. One end of each preparation was tied to an isometric mechanotransducer (TB-911T, Nihon Kohden, Tokyo, Japan) and the other end to the bottom of the bath. Resting tension was maintained at 0.5 g in both tissues. Tension recordings were started after repetitively applied 60 mM K^+ produced a consistent amplitude of contraction (about 2–3 h were required after placing the tissues in the bath). For experiments on the inhibitory effects of KC 128 or levcromakalim on precontracted smooth muscles, tracheal and bronchial smooth muscle were exposed to 100 nM and 300 nM carbachol, respectively, which induced about 70% of the maximum contraction of each preparation. When a stable amplitude of tone had been achieved, inhibitory, concentration-response curves were constructed for cumulative doses of KC 128 or levcromakalim. To observe the effects of glyburide, charybdotoxin or RbCl on the KC 128- or levcromakalim-induced relaxation (of the carbachol-induced contraction), these agents were applied 15 min before application of KC 128 or levcromakalim and throughout the experiments. Maximum relaxation was obtained by application of 1 mM aminophylline at the end of each experiment.

Guinea-pig trachealis Guinea-pig tracheal smooth muscle tissues were suspended in organ baths (capacity 10 ml) containing Krebs-Henseleit solution (35°C) and incubated with indomethacin (3 μM) for 60 min to inhibit spontaneous tone generated by prostaglandins (Farmer *et al.*, 1974). The tissues were placed under 1 g initial tension and were connected to TB-911T (Nihon Kohden). Following the 60–90 min stabilization period, tone was generated by the application of carbachol (100 nM), histamine (3 μM) or U46619 (10 nM). These concentrations induced about 70% of the maximum contraction for each agonist. When a stable amplitude of tracheal tone had been achieved, inhibitory concentration-response curves were constructed with cumulative doses of KC 128 or levcromakalim. Maximum relaxation was obtained by application of 1 mM aminophylline given at the end of each experiment.

Human bronchi Ring preparations of human bronchial smooth muscles were suspended in organ baths in a similar way to the dog bronchial smooth muscle preparations and resting tension was maintained at 1 g. After a 90 min stabilization period, the bronchi were exposed to 300 nM carbachol which also induced about 70% of the maximum contraction. When a stable amplitude of the tone had been achieved, inhibitory concentration-response curves were constructed with cumulative doses of KC 128 or levcromakalim. The maximum relaxation was obtained by application of 1 mM aminophylline at the end of each experiment.

Electrophysiological response recording

The membrane potential from single smooth muscle cells was recorded with glass capillary microelectrodes (Glass Filament 1.0X100, 6010, A-M System, Inc. WA, U.S.A.) filled with 3 M KCl and with tip resistance of 30–50 MΩ. The microelectrode was held in an electrode-holder (MEH-1S10, World Precision Instruments, Inc., New Haven, U.S.A.) connected to a high input impedance preamplifier (NEZ-7200, Nihon Kohden). Signals from the preamplifier were recorded on a chart recorder (model VP-6538, Panasonic, Yokohama, Japan). The tissue was pinned onto a rubber plate in a chamber (2 ml bath volume) and superfused with Krebs-Henseleit solution (35°C) at a flow rate of 3 ml min^{-1}. The electrode was inserted into smooth muscle cells from the inner surface of the tracheal tissue and from the outer surface of bronchial tissue. To observe the effect of KC 128 or levcromakalim on the membrane potential in normal Krebs-Henselit solution ($[K^+]_{out}$ = 6.0 mM), the solution containing the agent was superfused for longer than 15 min before measurements of the membrane potential were made. When the effects of KC 128 or levcromakalim on the membrane potential in low K^+ solution were examined, low K^+ solution ($[K^+]_{out}$ = 1.2 mM) was superfused up to 20 min before application of the agent. After a stable level of the membrane potential had been achieved, superfusion of the solution containing the agent was started. To observe the effects of glyburide on the KC 128- or levcromakalim-induced membrane potential changes, glyburide was added to the superfusing fluid 15 min before application of the solution containing KC 128 or levcromakalim with glyburide.

Measurement of $^{86}Rb^+$ efflux rate

Segments of tracheal or bronchial smooth muscles were loaded with $^{86}Rb^+$ (74 MBq ml^{-1}) at 35°C for 180 min in Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2. After loading with $^{86}Rb^+$, the tissue was pinned onto a rubber plate in a chamber filled with 2 ml Krebs-Henselit solution, and the solution was replaced with fresh Krebs-Henseleit solution every 3 min. The 8th–13th replacing solutions contained vehicle, KC 128 or levcromakalim. When the effect of glyburide on KC 128- or levcromakalim-induced $^{86}Rb^+$ efflux was examined, a solution containing glyburide (1 μM) was used as all the replacing solutions. The radioactivity of each replacing solution was counted by a liquid scintillation counter. At the end of experiments, the tissues

were solubilized with Soluen 350 (Packard) overnight at 35°C, then the radio activity of $^{86}Rb^+$ remaining in the tissues was measured.

Drugs and solutions

KC 128 (*N*-cyano-*N'*,*N'*,2,2-tetramethyl-6-nitro-2*H*-1-benzopyran-4-carboxamidine) and levcromakalim were synthesized in our laboratory (Fuji-Gotemba research Labs, Gotemba, Japan) (Figure 1). KC 128, levcromakalim and glyburide were dissolved to 10–20 mM in DMSO (dimethylsulphoxide). The maximum final concentration of DMSO was 0.5%. Carbachol (carbamylcholine chloride, Tokyo Kasei Co., Tokyo, Japan), charybdotoxin (Peptide Institute Inc., Osaka, Japan) and aminophylline (Sigma Chemical Co., St. Louis, MO, U.S.A.) were dissolved in Krebs-Henseleit solution. Solutions were freshy prepared before each experiment except for the solution containing 100 μM charybdotoxin which was stored at − 20°C and used within one week.

The Krebs-Henseleit solution used in all experiments had the following composition (mM): Na^+ 143.8, K^+ 6.0, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.8, HCO_3^- 24.8, $H_2PO_4^-$ 1.2 and glucose 10.0. Solutions containing various concentrations of K^+ were prepared by replacing NaCl with KCl isosmotically. All these solutions were bubbled with 95% O_2 and 5% CO_2, and their pH maintained at 7.4.

Statistical analysis

The concentration of drug required to produced half-maximum inhibition (IC_{50}) was calculated by linear regression analysis applied to the linear portion of each concentration-response curve. The negative logarithm of the IC_{50} value (pIC_{50}) is expressed as mean ± s.e.mean of the number (n) of observations. The significance of differences between means was assessed by Student's t test. Values of $P<0.05$ were considered statistically significant.

Results

Relaxant effects on dog tracheal and bronchial smooth muscles

Figure 2 shows the relaxant effects of levcromakalim and KC 128 on the dog tracheal and bronchial smooth muscles precontracted by carbachol. Levcromakalim induced concentration-dependent relaxation in both tracheal and bronchial preparations in the same concentration range. The IC_{50} values were 0.35 μM (pIC_{50}; 6.46 ± 0.10, $n=9$) and 0.55 μM (pIC_{50}; 6.26 ± 0.07, $n=5$), and the maximum relaxations were 96.5 ± 0.6% and 93.9 ± 2.1%, respectively. However, KC 128-induced relaxation occurred to a greater extent in bronchial (IC_{50}; 0.19 μM, pIC_{50}; 6.73 ± 0.10, maximum relaxation; 90.9 ± 2.9%, $n=7$) than in tracheal smooth muscle ($IC_{50}>10$ μM). The relaxant effect of KC 128 was observed in epithelial cell-denuded bronchial smooth muscle (data not shown). In the tracheal smooth muscle, high concentration of KC 128 (30 μM) produced only a transient relaxation.

The relaxation induced by levcromakalim in bronchial smooth muscle was antagonized by glyburide (Figure 3a). The IC_{50} values measured in the absence and presence of glyburide (0.03, 0.1, 0.3, 1 μM) were 0.55 μM (pIC_{50}; 6.26 ± 0.04, $n=5$), 0.91 μM (pIC_{50}; 6.04 ± 0.11, $n=4$), 2.34 μM (pIC_{50}; 5.63 ± 0.12, $n=4$), 8.91 μM (pIC_{50}; 5.05 ± 0.09, $n=5$) and 30.9 μM (pIC_{50}; 4.51 ± 0.10, $n=7$), respectively. The maximum relaxations reached 85% of that induced by aminophylline (1 mM). Glyburide produced a rightward parallel shift of the concentration-relaxation curves of levcromakalim on the carbachol-induced contraction in bronchial smooth muscles ($pA_2=7.50$). Glyburide also inhibited the relaxant action of KC 128 in a concentration-dependent manner (Figure 3b). However the inhibitory action was different from that of the levcromakalim-induced relaxation, since the maximum relaxation induced by KC 128 was markedly reduced by glyburide, but the levcromakalim-induced maximum relaxation was not. Charybdotoxin (100 nM), a blocker of the large conductance Ca^{2+}-activated K^+ channel, had no effect on the relaxant actions of either levcromakalim or KC 128. The IC_{50} values of levcromakalim

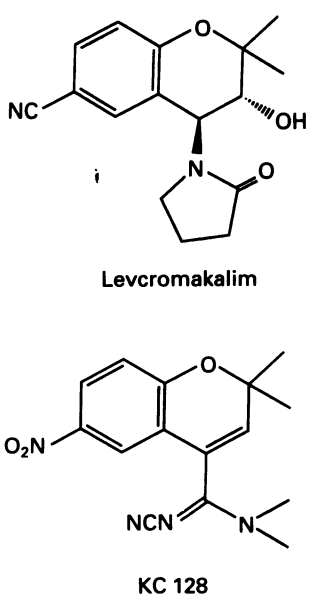

Figure 1 Chemical structures of levcromakalim and KC 128.

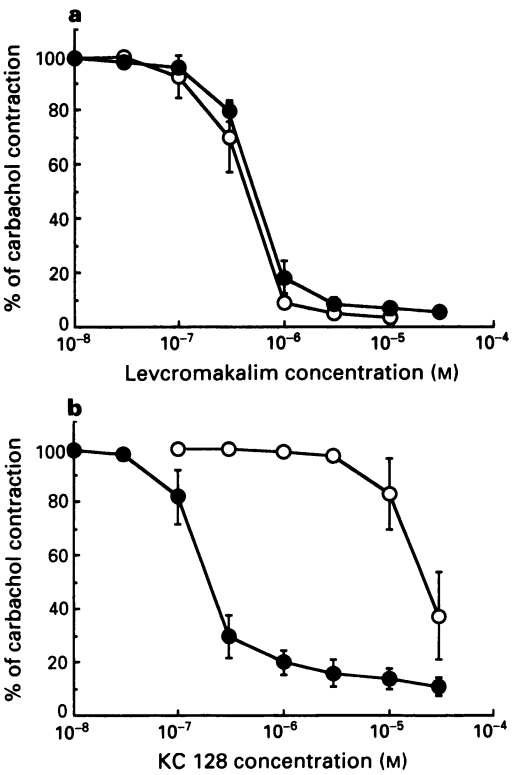

Figure 2 Concentration-dependent relaxation induced by levcromakalim (a) and KC 128 (b) in dog tracheal (○) or bronchial (●) smooth muscle tissues precontracted by carbachol (0.1 μM (tracheal), 0.3 μM (bronchial)). Each point represents the mean ± s.e.mean from 5 to 9 preparations.

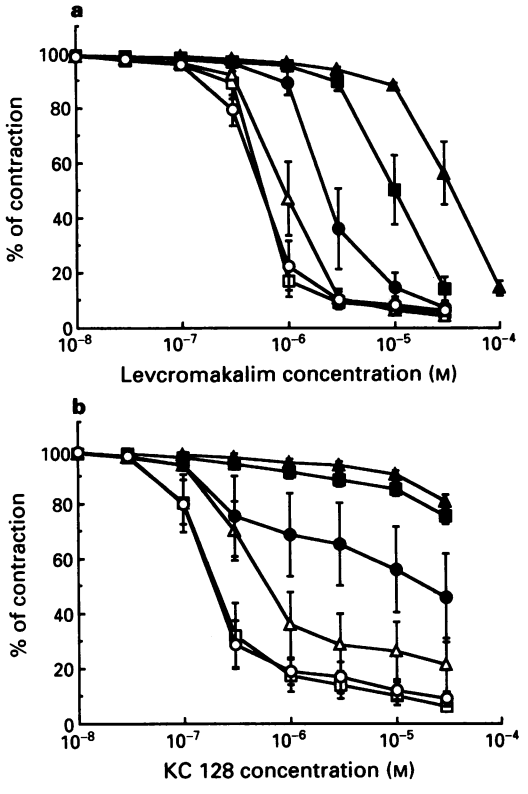

Figure 3 Concentration-dependent relaxation induced by levcromakalim (a) and KC 128 (b) in the dog bronchial smooth muscle tissues precontracted by carbachol (0.3 μM) in the absence (○) or presence of charybdotoxin (□, 0.1 μM) or glyburide (△, 0.03 μM; ●, 0.1 μM; ■, 0.3 μM; ▲, 1 μM). Each point represents the mean ± s.e.mean from 4 to 6 preparations.

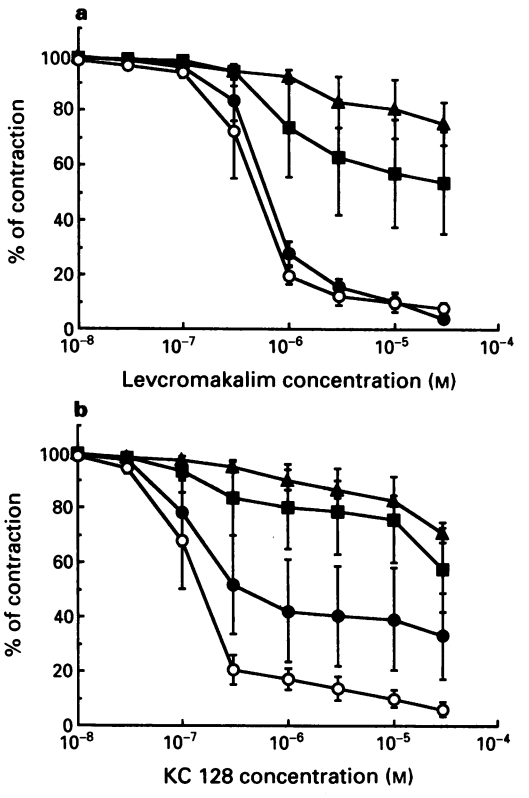

Figure 4 Effects of various concentrations of RbCl (○, vehicle; ●, 1 mM; ■, 3 mM; ▲, 5 mM) on levcromakalim- (a) and KC 128- (b) induced relaxation in dog bronchial smooth muscle precontracted by carbachol (0.3 μM). Each point represents the mean ± s.e.mean from 4 to 6 preparations.

and KC 128 in the presence of charybdotoxin were 0.55 μM (pIC_{50}; 6.26 ± 0.04, $n = 5$) and 0.21 μM (pIC_{50}; 6.67 ± 0.12, $n = 6$), respectively. These IC_{50} values were not significantly different from those observed in the absence of charybdotoxin ($P > 0.05$).

Application of RbCl (1, 3 and 5 mM) to bath solution containing 6.0 mM KCl slightly enhanced the carbachol (300 nM)-induced contraction in dog bronchial smooth muscles (102.6 ± 0.6%; $n = 11$, 106.2 ± 1.2%; $n = 11$ and 107.8 ± 1.4%; $n = 9$, respectively). The concentration-dependent relaxations induced by levcromakalim and KC 128 in dog bronchial smooth muscles were inhibited by application of RbCl in a concentration-dependent manner (Figure 4), but such inhibitions were not observed with application of additional KCl (1–5 mM). The inhibitory effect of RbCl on the concentration-relaxation curves of KC 128 was not different from that of levcromakalim, but the inhibitory effects of RbCl on the KC 128-induced relaxation was more potent than on that of levcromakalim.

Effects on the membrane potential of dog airway smooth muscle cells

The membranes of the dog tracheal and bronchial smooth muscle cells were electrically quiescent. In normal Krebs-Henseleit solution ($[K^+]_{out} = 6.0$ mM), the mean resting membrane potential of tracheal smooth muscle cells was − 60.2 ± 0.8 mV ($n = 21$) and that of bronchial smooth muscle cells was − 70.0 ± 0.7 mV ($n = 16$). The resting membrane potential of bronchi was significantly higher than trachea by about 10 mV ($P < 0.05$). In low K^+ solution ($[K^+]_{out} = 1.2$ mM), the membrane potential did not change in tracheal smooth muscle cells (− 60.8 ± 0.8 mV, $n = 10$). However, a small but significant hyperpolarization was observed (− 75.4 ± 1.7 mV, $n = 8$) in bronchial smooth muscle cells ($P < 0.05$).

In tracheal smooth muscle, levcromakalim (> 0.1 μM) hyperpolarized the membrane in a concentration-dependent manner in both normal and low K^+ solution (Figure 5a and b, closed circle and Figure 6). The levcromakalim-induced hyperpolarization in the low K^+ solution was larger than that in normal K^+ solution. However, in the case of KC 128, hyperpolarization was observed in neither normal nor low K^+ solution (< 10 μM). On the other hand, in bronchial smooth muscles, KC 128 (> 0.3 μM) hyperpolarized the membrane to a small extent in normal K^+ solution and markedly hyperpolarized it in low K^+ solution, and effect similar to that produced by levcromakalim (Figure 5c and d and Figure 6). Glyburide (1 μM) depolarized the membrane of bronchial smooth muscle cell from − 75.4 mV to − 69.5 mV ($n = 2$). The hyperpolarizations induced by KC 128 (1 μM) or levcromakalim (1 μM) were inhibited with application of glyburide (1 μM) (Figure 6b). However, the levcromakalim (1 μM)-induced hyperpolarization was not suppressed by the pretreatment of KC 128 (1, 3 μM, Figure 6).

Effects on $^{86}Rb^+$ efflux from dog airway smooth muscles

Figure 7 shows the effects of KC 128 (10 μM) and levcromakalim (10 μM) on $^{86}Rb^+$ efflux rate in the absence or presence of glyburide (1 μM) from the dog tracheal and bronchial smooth muscles. The efflux rate settled to a low and almost constant value about 20 min after starting to replace the solution. The averages of $^{86}Rb^+$ efflux rate before application of drugs (basal value, 24 min) were 0.65 ± 0.04% min^{-1} ($n = 18$) and 0.99 ± 0.02% min^{-1} ($n = 18$) from tracheal and bronchial smooth muscle tissues, respectively.

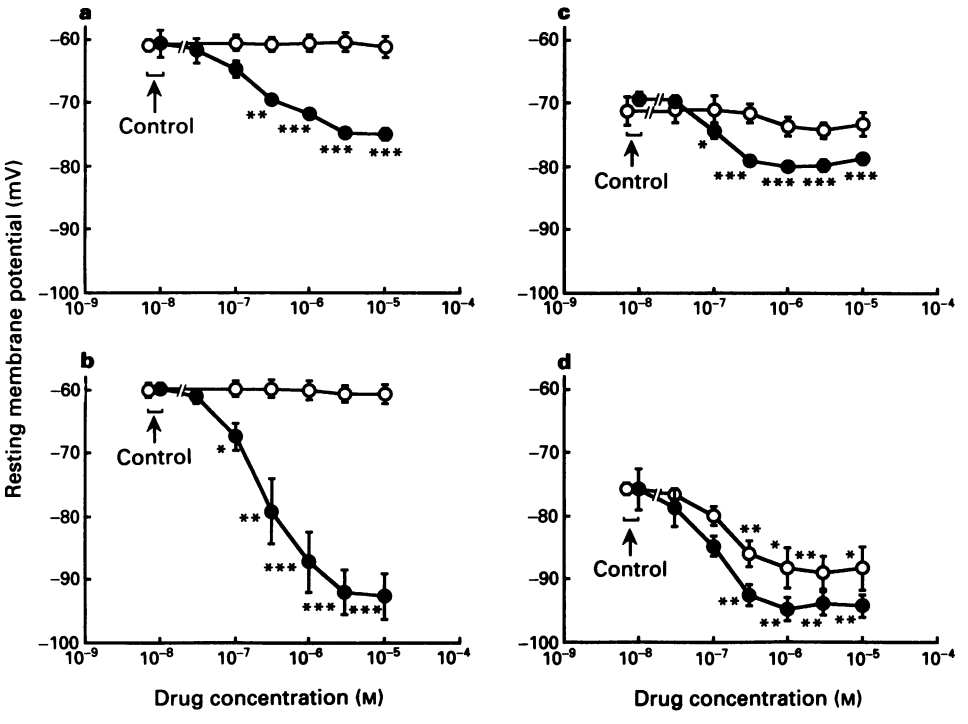

Figure 5 Effects of levcromakalim (●) and KC 128 (○) on the membrane potential of the dog tracheal (a and b) or bronchial smooth muscle cells (c and d) in normal ($[K^+]_{out}$ = 6.0 mM, a and c) or low K^+ ($[K^+]_{out}$ = 1.2 mM, b and d) solution. Each point represents the mean ± s.e.mean from 4 to 6 preparations. Control values represent the membrane potential before treatment with drugs. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ for comparison with the control value.

The basal value of the efflux rate from tracheal smooth muscle tissues was significantly larger than that from bronchial smooth muscle tissues ($P<0.05$).

Levcromakalim caused a significant increase of $^{86}Rb^+$ efflux rate from both tracheal and bronchial smooth muscle tissues (2.16 ± 0.22 and 1.55 ± 0.04 fold respectively at the peak compared to vehicle, $n = 6$) within 6 min of exposure to the drug. However, KC 128 produced a significant increase in efflux only from bronchial smooth muscle tissues (1.33 ± 0.07 fold, $n = 6$) within 9 min of exposure to the drug. This was similar to levcromakalim, but the increase induced by KC 128 was lower than induced by levcromakalim ($P<0.05$). On the other hand, there was no change in the efflux rate in tracheal smooth muscle tissues exposed to KC 128 for 15 min.

In the presence of glyburide (1 μM), the basal values of $^{86}Rb^+$ efflux rate were 0.59 ± 0.04% min^{-1} ($n = 6$) and 0.97 ± 0.04% min^{-1} ($n = 6$) from tracheal and bronchial smooth muscle tissues, respectively. There was no difference between tissues in the absence and presence of glyburide. However, glyburide inhibited the increase of $^{86}Rb^+$ efflux rate induce by KC 128 and levcromakalim in both tissues.

Comparison of the relaxant effects on the guinea-pig and human airway tissues contracted by carbachol

Levcromakalim relaxed the guinea-pig tracheal smooth muscles precontracted by carbachol (100 nM), histamine (3 μM) and U46619 (10 nM) in a concentration-dependent manner with IC_{50} values of 1.58 μM, 0.66 μM and 0.83 μM, respectively (Table 1). KC 128 also relaxed the guinea-pig tracheal smooth muscle with IC_{50} values of 0.19 μM, 0.07 μM and 0.08 μM, respectively (Table 1). The potency of KC 128 was approximately 10 times higher than that of levcromakalim as estimated from the IC_{50} value on any agonist-induced contraction. The maximum relaxations occurring on application of either levcromakalim or KC 128 to tissues contracted by U46619 were roughly the same ($P>0.05$), and were greater than the effects on the carbachol and histaime-induced contractions ($P<0.05$). However, the maximum relaxations induced by KC 128 on the carbachol- and histamine-induced contractions were less than those induced by levcromakalim.

Levcromakalim caused concentration-dependent relaxation in the dog tracheal, bronchial, guinea-pig tracheal and human bronchial smooth muscles contracted with carbachol (Figure 8a). Concentrations of carbachol in each preparation induced about 70% of maximum contraction (see Methods). Both the potency and maximum relaxation of levcromakalim in the dog tracheal smooth muscles were almost equal to those in dog bronchial smooth muscles. In the human bronchial smooth muscles, the potency was similar to that in dog airway preparations. In the guinea-pig tracheal smooth muscles, both the potency and maximum relaxation were significantly weaker than those in dog tracheal and bronchial preparations.

KC 128 caused concentration-dependent relaxation only in the dog bronchial and the guinea-pig tracheal smooth muscle tissues but not in other preparations, but the maximum relaxation was smaller in the guinea-pig tracheal smooth muscle than in the dog bronchial smooth muscle (Figure 8b).

Discussion and conclusions

Differences in electrical properties and action of K^+ channel openers between tracheal and bronchial smooth muscle cells

Although the membranes of both dog tracheal and bronchial smooth mucle cells were electrically quiescent, the resting membrane potential of the bronchial smooth muscle cells was more negative than that of tracheal smooth muscle cells by about 10 mV in normal Krebs-Henseleit solution. Moreover the basal $^{86}Rb^+$ efflux from the bronchial smooth muscle tissues was also larger than that from the tracheal smooth muscle tissues. Xie *et al.* (1992) reported that the resting

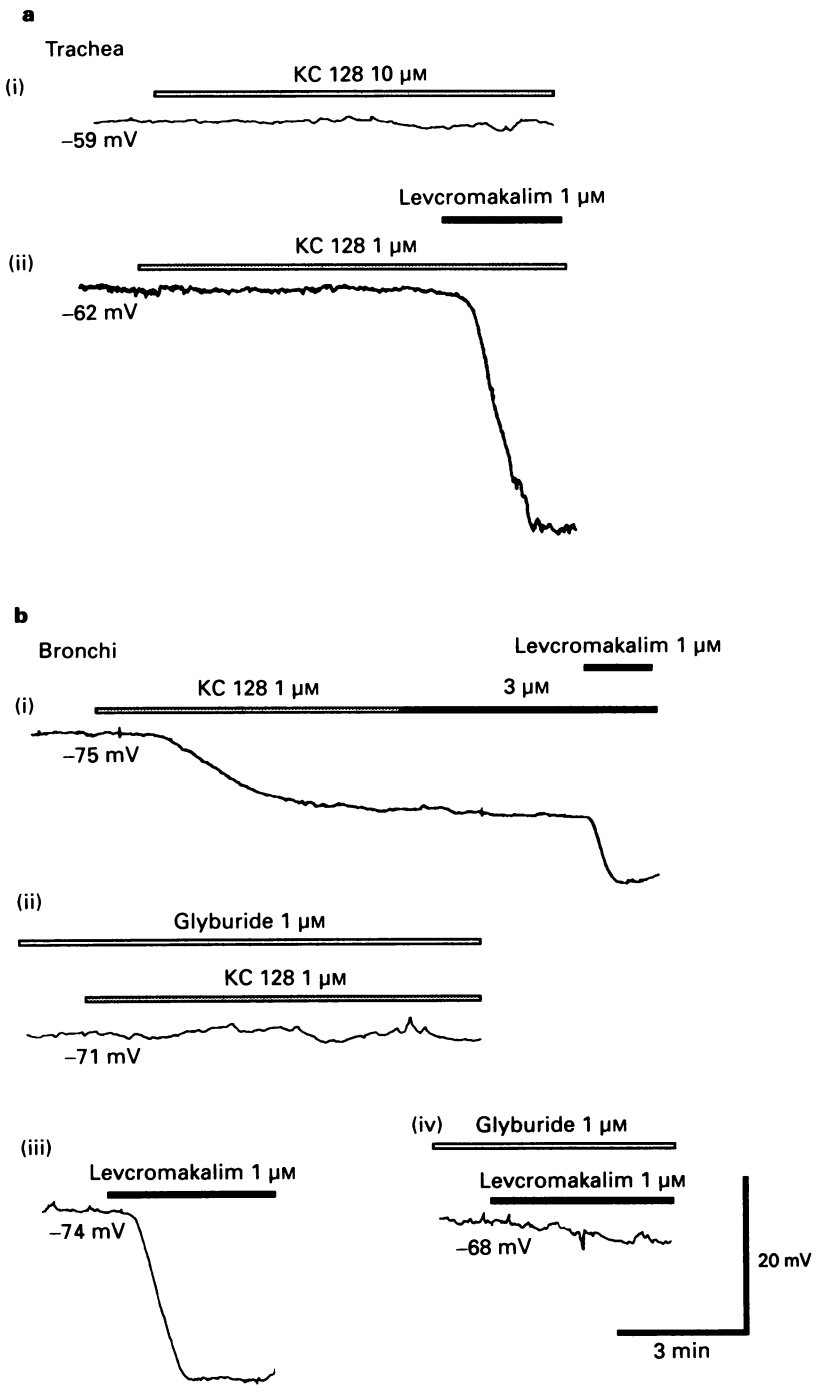

Figure 6 Effects of levcromakalim and KC 128 on the membrane potential of dog tracheal (a) and bronchial (b) smooth muscle cells in low K^+ solution ($[K^+]_{out}$ = 1.2 mM). Bars indicate application of levcromakalim, KC 128 or glyburide. The resting membrane potential before application of levcromakalim or KC 128 is indicated in each trace.

membrane potential of the dog bronchiole with epithelium was higher than that without epithelium, and they postulated that a hyperpolarizing factor is released from the epithelial cells. The bronchial preparation we used in this study also had intact epithelial cells, so the higher resting membrane potential of bronchial smooth muscle cells may be due to an epithelial-drived hyperpolarizing factor that activates the K^+ channels.

Levcromakalim induced concentration-dependent relaxations in the dog tracheal and bronchial preparations precontracted by carbachol in the same concentration-range and was able to relax each preparation almost completely. These effects of levcromakalim on the dog were more potent than on the guinea-pig tracheal smooth muscles. In the dog bronchial smooth muscle, there was a parallel shift of the concentration-relaxation curve to levcromakalim to the right on application of glyburide in a concentration-dependent manner. Furthermore, the levcromakalim-induced hyperpolarization in both tracheal and bronchial tissues occurred in a concentration-dependent manner and this hyperpolarization was enhanced in low K^+ solution. These hyperpolarizing actions were inhibited by glyburide. The increases of $^{86}Rb^+$ efflux rate from both tissues induced by levcromakalim was inhibited by glyburide. These results indicate that dog tracheal and bronchial smooth muscle possess glyburide-sensitive K^+ channels and these are opened by levcromakalim.

In contrast, only in bronchial smooth muscle was the relaxant effect of KC 128 observed in the same

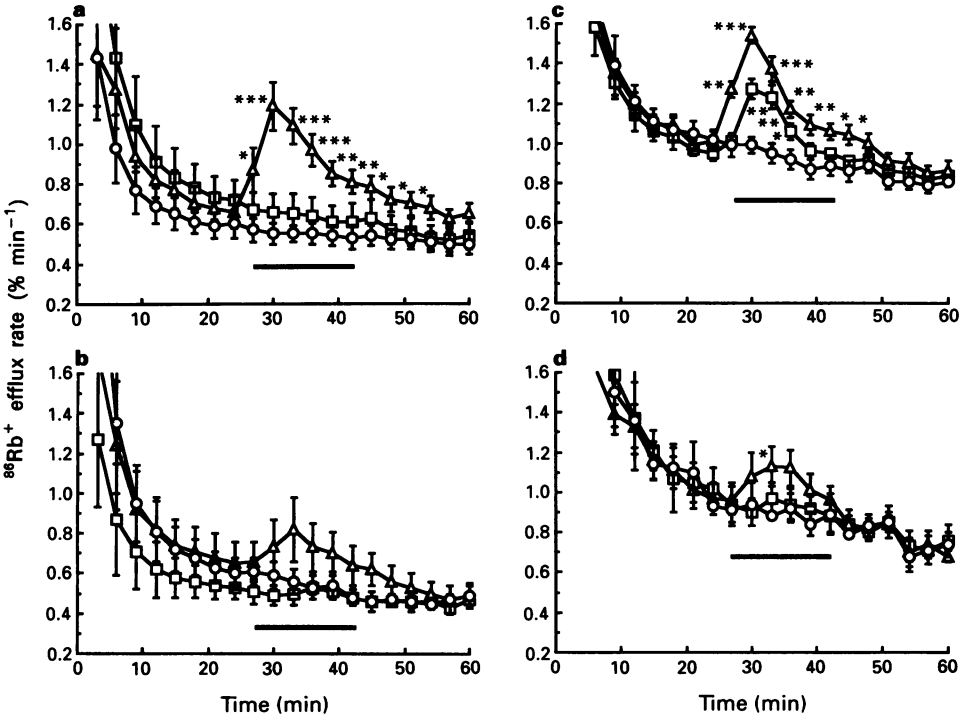

Figure 7 Effects of vehicle (○), levcromakalim (△) and KC 128 (□) on the $^{86}Rb^+$ efflux rate from the dog tracheal (a.b) and bronchial (c,d) smooth muscle cells in the absence (a and c) and presence of glyburide (b and d). Bars indicate application of levcromakalin or KC 128. Each point represents the mean ± s.e.mean from 4 to 6 preparations. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ for comparison with value of vehicle only for each point.

Table 1 The effects of levcromakalim and KC 128 on agonist-induced contraction in guinea pig tracheal smooth muscle

	Levcromakalim			*KC 128*		
Agonist	pIC_{50} (−log[M])	*Max* (%)	(n)	pIC_{50} (−log[M])	*Max* (%)	(n)
Carbachol	−5.80 ± 0.06	69.1 ± 4.5	(7)	−6.73 ± 0.10***	49.8 ± 4.9*	(6)
Histamine	−6.18 ± 0.10	76.0 ± 3.4	(7)	−7.16 ± 0.07***	57.2 ± 3.1*	(6)
U46619	−6.05 ± 0.08	91.5 ± 1.5	(7)	−7.11 ± 0.07***	89.7 ± 3.9	(6)

The concentration of each agonist induced about 70% of maximum contraction. Each pIC_{50} value is the negative logarithm of the IC_{50} value. Maximum relaxation (Max) is expressed as a percentage of relaxation induced by aminophylline (1 mM). Values are expressed as the mean ± s.e.mean of 6 or 7 preparations (*n*). $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to the response to levcromakalim for each agonist.

concentration-range as in the guinea-pig tracheal smooth muscle. The dog tracheal smooth muscle was less sensitive to KC 128, but high concentrations (10–30 μM) of KC 128 induced a transient relaxation in tracheal smooth muscle precontracted by carbachol. Presumably, high concentrations of KC 128 may be nonspecific relaxing actions. KC 128 hyperpolarized the membrane of bronchial smooth muscle cells but not of tracheal smooth muscle cells. Furthermore, a KC 128-induced increase of $^{86}Rb^+$ efflux was observed only from bronchial smooth muscle tissues. The relaxation, hyperpolarization and increase of $^{86}Rb^+$ efflux rate were all attenuated by the presence of glyburide. These findings suggested that KC 128 relaxed selectively the bronchial smooth muscle mainly by opening the glyburide-sensitive K^+ channel.

The concentration-dependent inhibition by glyburide of the KC 128-induced relaxation differed from the inhibition of the levcromakalim-induced relaxation. The levcromakalim-induced relaxation was antagonized by glyburide apparently in a competitive manner in bronchial smooth muscles, whereas the inhibition by glyburide of the KC 128-induced relaxation was not competitive and the maximum relaxation induced by KC 128 was suppressed by glyburide. KC 128-induced hyperpolarization and increase of $^{86}Rb^+$ efflux rate were smaller than levcromakalim. However, in the dog airway smooth muscle pretreated by KC 128 (1 or 3 μM), the levcromakalim (1 μM)-induced hyperpolarization was observed to the same extent as that in untreated cells in the low K^+ solution (Figure 5 and 6). Furthermore in preliminary experiments, we confirmed that levcromakalim-induced relaxation was not modified by KC 128 (0.3, 1, 3 μM) in tracheal smooth muscles. Thus KC 128 does not antagonize the action of levcromakalim and KC 128 may not be a 'partial K^+ channel opener'.

Recently, it was reported that the K^+ channel opener and glyburide bind to different sites on the ATP-sensitive K^+ channel and were coupled in a negatively allosteric manner (Gopalakrishnan *et al.*, 1991; Bray & Quast, 1992; Schwanstecher *et al.*, 1992). Therefore, it is plausible to postulate that in the dog bronchial smooth muscle, a glyburide-sensitive K^+ channel may have more than one binding site for K^+ channel openers. The glyburide-sensitive K^+ channel in bronchial smooth muscle may differ from that in the dog tracheal smooth muscle. The K^+ channel activated by KC 128 may also be different from that activated by levcromakalim. Both levcromakalim- and KC 128-sensitive and also glyburide-

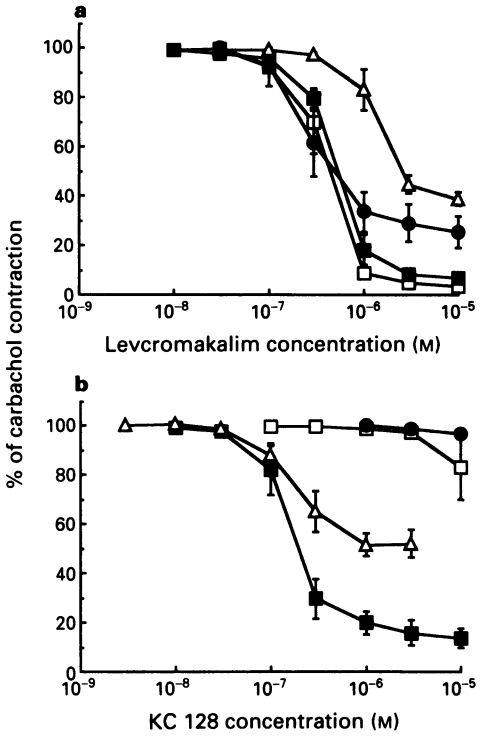

Figure 8 Comparison of the relaxant effects of levcromakalim (a) and KC 128 (b) on the bronchial smooth muscle tissues of dog (■) and human (●), and on the tracheal smooth muscle tissues of dog (□) and guinea-pig (△). Each point represents the mean ± s.e.mean from 3 to 7 preparations.

sensitive K^+ channels may be distributed in bronchial smooth muscle, but in tracheal smooth muscle there may be no KC 128- and glyburide-sensitive K^+ channels.

Foster *et al.* (1992) have reported that cromakalim opens two populations of potassium channels in the guinea-pig tracheal smooth muscle, one of which is susceptible to blockade by Rb^+ and one of which is not. Both KC 128- and levcromakalim-induced relaxations were inhibited by RbCl in a concentration-dependent manner (1–5 mM) in dog bronchial smooth muscle tissues. The inhibition was similar to each, therefore it is difficult to distinguish these two channels by the sensitivity to Rb^+ in the present experiments.

Distribution of KC 128-sensitive K^+ channel in various airway tissues

Generally, ATP-sensitive K^+ channel openers, including levcromakalim, have been reported to be not very effective on cholinoceptor agonist-induced contraction in the guinea-pig tracheal smooth muscle (Allen *et al.*, 1986; Nielsen-Kudsk *et al.*, 1988; Paciorek *et al.*, 1990; Taylor *et al.*, 1992; Imagawa *et al.*, 1993). It has also been reported that the relaxant effects of K^+ channel openers in airway tissues were largely dependent on the concentration of agonist used (Raeburn & Brown, 1991). Therefore in the present experiments the concentration of each agonist used was chosen to induce 70% of the maximum contraction. Under these conditions, we confirmed that the relaxant effects of levcromakalim on the carbachol-induced contraction in the guinea-pig tracheal smooth muscle were less potent that those on the histamine- and U46619-induced contraction.

Glyburide-sensitive K^+ channels have been reported to be widely distributed in airway smooth muscle of many species, including guinea-pig, dog, human, bovine and others (Allen *et al.*, 1986; Black *et al.*, 1990; Longmore *et al.*, 1991; Taylor *et al.*, 1992). Levcromakalim and other K^+ channel openers have been reported to relax almost any airway smooth muscles. In our experiment, levcromakalim relaxed the dog tracheal, bronchial, guinea-pig tracheal and human bronchial smooth muscle tissues precontracted by carbachol. However, KC 128 relaxed only the guinea-pig tracheal and dog bronchial smooth muscles in the same concentration-range. These results suggested that the dog tracheal and human bronchial muscles possess only the levcromakalim-sensitive K^+ channel but not the KC 128-sensitive K^+ channel.

In summary, KC 128 and levcromakalim showed different actions on airway smooth muscles: KC 128 produced relaxation, hyperpolarization and increase in $^{86}Rb^+$ efflux in the dog bronchial smooth muscles but not in tracheal smooth muscles, whereas levcromakalim consistently relaxed both tracheal and bronchial smooth muscles. Furthermore, KC 128 relaxed precontracted guinea-pig tracheal smooth muscles but not human bronchial smooth muscles. In contrast, levcromakalim consistently relaxed both tissues. Thus, it is estimated that at least two different glyburide-sensitive K^+ channels occur in the dog airway smooth muscle.

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(Received February 4, 1994
Revised June 16, 1994
Accepted June 22, 1994)