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Allergic responses reduce the relaxant effect of β-agonists but not potassium channel openers in guinea-pig isolated trachea

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Allergic responses reduce the relaxant effect of β-agonists but not potassium channel openers in guinea-pig isolated trachea. S. Houjou, K. Iizuka, K, Dobashi, T. Nakazawa. ©ERS Journals Ltd 1996.

ABSTRACT: This study was conducted to determine whether the relaxant effect of adenosine triphosphate (ATP)-sensitive potassium channel (K_{ATP}) openers on airway smooth muscle are reduced during the hyporesponsiveness of beta-adrenergic receptors.

Isometric tension was measured and dose-response curves were constructed for levcromakalim (a K_{ATP} opener), Y-26763 (another K_{ATP} opener), isoprenaline and theophylline in guinea-pig isolated trachea that was challenged with ovalbumin or pretreated *in vitro* with either isoprenaline or the K_{ATP} openers.

Antigen challenge *in vitro* significantly reduced the potency but not the efficacy of isoprenaline in tracheal strips from actively sensitized guinea-pigs. The concentration of drug that produced a half-maximum inhibition (IC_{50}) was 0.014±0.004 μM in the challenge group *versus* 0.006±0.001 μM in the control group ($p<0.05$; n=9). The IC_{50} of levcromakalim (1.73 ±0.17 μM; n=6) and of theophylline (110±3.1 μM; n=6) were unaffected. Exposure to 4 μM isoprenaline for 30 min evoked beta-adrenergic hyporesponsiveness: the IC_{50} of isoprenaline rose significantly from 0.010±0.001 to 0.017±0.002 μM ($p<0.01$; n=6). No effect was observed on the relaxant actions of levcromakalim and theophylline. In contrast, prior incubation with either levcromakalim or Y-26763 (300 μM for 30 min) significantly reduced the subsequent potency and efficacy of levcromakalim, but did not alter the effects of isoprenaline and theophylline.

We conclude that the relaxant effect of the adenosine triphosphate-sensitive potassium channel openers was independent of beta-adrenergic hyporesponsiveness in airway smooth muscle of guinea-pigs.

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In the last 10 yrs adenosine triphosphate (ATP)-sensitive potassium channel (K_{ATP}) openers, such as cromakalim, have been developed as antihypertensive agents [1–4]. These agents relax the smooth muscle of the airways as well as vascular smooth muscle [5] *via* membrane hyperpolarization [6–8], leading to the closure of the voltage-dependent Ca^{2+}-channels [9], a decrease in agonist-induced inositol-1,4,5 triphosphate production [10], and a reduction in the contractile apparatus sensitivity to Ca^{2+} [11]. Relaxation of airway smooth muscle occurs by K_{ATP} openers presumably mediated *via* a similar mechanism [12–14]. The deterioration of pulmonary function in asthmatics (morning dip) is prevented by the administration of levcromakalim [15], which is the active 3S, 4R enantiomer of cromakalim. This suggests that K_{ATP} openers may be useful in preventing asthma attacks. Indeed, levcromakalim relaxed the human bronchial smooth muscle *in vitro* [16, 17].

Although beta-adrenergic agonists are the most potent and widely-used bronchodilator agents, an allergic reaction and/or administration of a high dose of a beta-adrenergic agonist reduces their relaxant effects due to a decreased number of beta-adrenergic receptors [18–21], and a dysfunction of signalling at postreceptor sites in bronchial asthma [22, 23]. Whilst the relaxant effects of beta-adrenergic agonists on the basal and developed tone of airway smooth muscle have been extensively investigated, it is not known whether an allergic reaction would reduce the spasmolytic effect of the K_{ATP} openers, and whether the administration of a high dose of a beta-adrenergic agonist would modulate the action of the K_{ATP} openers.

Accordingly, we evaluated the efficacy and potency of levcromakalim administered after antigen-induced contraction *in vitro*, and compared its effects with those of the conventional bronchodilators, isoprenaline (ISO) and theophylline (THEO). Finally, we evaluated the heterologous or homologous tachyphylaxis of the K_{ATP} openers using tracheal muscle treated with high concentrations of ISO, levcromakalim, or Y-26763 (a K_{ATP} opener) [24, 25].

Materials and methods

Active sensitization of guinea-pigs and assessment of passive cutaneous anaphylaxis (PCA) titre

Male Hartley guinea-pigs were actively sensitized with ovalbumin (OA), 1 mg·week^{-1}, *i.p.* ×4, in 5 mg $Al(OH)_3$. The 48 h PCA titre of the animals was more than 160 fold.

Preparation of tracheal strip

The trachea was removed from each animal anaesthetized with pentobarbitone sodium (40 mg·kg^{-1} *i.p.*) and dissected. The airways were first cut longitudinally at the centre of the cartilage opposite the smooth muscle, followed by slicing transversely into 10 strips approximately 3 mm wide. Strips were divided into two groups and tied with silk thread at the cartilage to minimize the difference in response to agonists due to the uneven distribution of receptors on the trachea. Thus, a pair of muscle chains consisting of five pieces of strips were obtained from one animal. Their contraction and relaxation characteristics were presumably identical. One chain was used for exposure to OA or drug treatment and the other served as control.

Isometric measurement of tension development

Strip chains were set between a hook and an isometric force transducer (strain gauge TB-612T, Nihon Kohden Ltd, Japan) connected to an amplifier (TB-611-T, Nihon Kohden Ltd, Tokyo, Japan) and a multipen recorder (R66, Rika Denki Ltd, Tokyo, Japan), and vertically mounted in a 10 mL Magnus tube filled with Tyrode's solution that was aerated continuously with 5% CO_2 in O_2. The composition of the solution was: NaCl 136.8 mM; KCl 2.7 mM; $CaCl_2$ 1.8 mM; $MgCl_2$ 1 mM; NaH_2PO_4 0.4 mM; $NaHPO_3$ 11.9 mM; glucose 5.6 mM; and temperature was kept at 37°C. At the beginning of each experiment, tissues were subjected to a tension of 1.0 g and allowed to equilibrate for 60 min. The solution was changed at 20 min intervals.

To obtain the maximal tension and to confirm stability of the preparations, the application of carbachol (100 µM) with wash-out was repeated three times. The first carbachol-induced contraction was always small. The second and third contractions showed a comparable amplitude that exceeded the first contraction. Indomethacin (2 µM), a cyclo-oxygenase inhibitor, was used throughout these experiments to prevent spontaneous tone development due to the release of cyclo-oxygenase products.

Study design

A pair of tracheal-strip chain preparations were contracted maximally by the addition of histamine (HIS), 3 µM). The amplitude was approximately 60% of the maximal contraction evoked by carbachol (100 µM). At the sustained phase of contraction induced by HIS, levcromakalim, ISO or THEO was cumulatively applied to the preparations to obtain dose-relaxant curves. After extensive washing with Tyrode's solution and a 1 h equilibration period, one tracheal segment was challenged with OA (0.1 mg·mL^{-1}) to initiate anaphylactic reaction (anaphylactic challenge group) for 60 min, whereas the other tracheal segment was challenged with HIS (3 µM) (control group) for the same duration. Preliminary experiments showed that 0.1 mg·mL^{-1} of OA produced the maximal response in which the peak amplitude was comparable to that of the maximal contraction induced by carbachol (100 µM). After extensive washing and readjustment of the basal tone at 1.0 g, the construction of dose-relaxant curves was repeated with the reagents against the HIS-induced contraction. Thus, comparisons between groups were performed.

In the second series of experiments, strips were incubated with high concentrations of ISO (4 µM), levcromakalim (300 µM) or Y-26763 (300 µM) for 30 min, followed by washing with Tyrode's solution for 5 min three times. Subsequently, basal tone was readjusted at 1.0 g, and HIS (3 µM) was applied to the muscle, followed by construction of dose-relaxant curves for levcromakalim, ISO or THEO. Control strips were added to the vehicle (distilled water or dimethylsulphoxide (DMSO)) of the relaxant agents for 30 min. Finally, ISO (10 µM) was applied to smooth muscle to determine the full relaxation. The chemical structures of the KATP openers are illustrated in figure 1.

Reagents

Levcromakalim: (-)6-cyano-3,4-dihydro-2, 2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2H-1-6-benzopyran-3-ol was a gift from Smithkline Beecham Pharmaceuticals. ONO-1078: 8-(p-(4-phenylbutyloxy)benzoyl)amino-2-(tetrazol-5-yl)-4-oxo-4H-1-benzopyran was from ONO Yakuhin Co. Ltd. Y-26763: (-)-(3S, 4R)-4-(N-acetyl-N-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-3-ol was from Japan Tabaco Inc. KATP openers were dissolved in DMSO and stored at a concentration of 10 mM at 4°C in the dark. Prior to use, they were diluted with Tyrode's solution. Other reagents were dissolved with distilled water and immediately used in the experiments. Carbamylcholine chloride (carbachol), histamine chloride, theophylline and DMSO were purchased from Sigma Chemical Co., St Louis, Missouri, USA. Isoprenaline chloride was obtained from Nikken Chemicals Co. Ltd. The KCl, $CaCl_2$, $MgCl_2$, NaH_2PO_4, $NaHCO_3$, glucose and OA, were all obtained from Kanto Chemical Co. Inc., Tokyo, Japan.

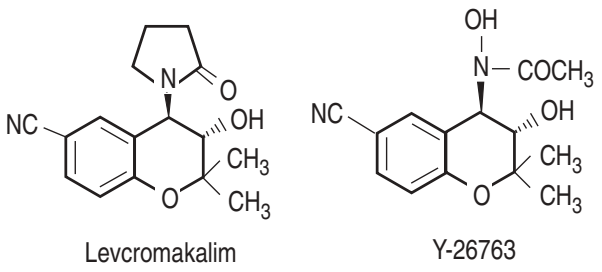

Fig. 1. – Chemical structures of adenosine triphosphate (ATP)-sensitive potassium channel openers.

Statistical method

The response to the relaxant reagents was normalized to the range from the initial basal tone to the peak contraction with HIS (3 µM), and expressed as mean±SEM. The concentration of drug required to produce the half-maximum inhibition (IC_{50}) value was obtained from individual dose-response curves of each strip. The negative logarithm of IC_{50} was used for statistical evaluation. Mann-Whitney U-test was used to evaluate differences. A p-value less than 0.05 were considered to be statistically significant.

Results

Antigen challenge in vitro

Challenge with OA rapidly produced a contraction that persisted for more than 60 min. The preparation was washed at least three times, 30 min after initiation of the OA challenge. Because the tension in some strips did not return to baseline even after such extensive washing, a leukotriene C4/D4 antagonist, ONO-1078 (1 µM), was added during the washing stage to allow the basal tension to be readjusted at 1.0 g. Control preparation was run in parallel except for contraction evoked by HIS but not by OA for 60 min. No tachyphylaxis to HIS (3 µM) was observed in control experiments.

Although dose-response curves for ISO were shifted to the right after the OA challenge without altering the maximal response (fig. 2a), relaxant efficacy and potency of levcromakalim did not change (fig. 2b). Since the maximal relaxation induced by ISO, levcromakalim and THEO were unchanged, only the IC_{50} values are summarized in table 1. The IC_{50} of levcromakalim and THEO were not affected by the OA provocation. Note that ISO was approximately 320 times more potent than levcromakalim, while THEO was 57 times less potent than levcromakalim. These results indicated that the relaxant action of levcromakalim was independent of the anaphylactic reaction *in vitro*, in contrast to the reduction of potency of ISO.

Table 1. – IC_{50} values for the relaxant potency of isoprenaline, levcromakalim and theophylline after antigen challenge inactively sensitized guinea-pig trachea

		Antigen challenge	
		Control	OA
Agent	n	µM	µM
Isoprenaline	9	0.006±0.001	0.014±0.004*
Levcromakalim	6	1.94±0.43	1.73±0.17
Theophylline	6	110±2.1	110±3.1

Values are expressed as mean±SEM. IC_{50}: concentration of drug producing half-maximum inhibition; OA: ovalbumin (1 mg·mL^{-1}). *: significantly different from control values, $p<0.05$. "Control" means data from time-matched experiments without OA challenge.

Drug-induced desensitization

Prior incubation with a high concentration of ISO, 4 µM for 30 min, reduced the subsequent ISO relaxant potency and efficacy (fig. 3a). Neither the IC_{50} nor the maximal response of levcromakalim were affected by this treatment (fig. 3b). Table 2 summarizes the IC_{50} values.

By contrast, prior incubation with levcromakalim, 300 µM for 30 min, markedly reduced its subsequent relaxant action (fig. 4a), but not the effects of ISO (fig 4b) and THEO (fig 4c). Pretreatment with another K_{ATP} opener, Y-26763, 300 µM for 30 min, also reduced its relaxant action (fig. 5a), but not the action of ISO and THEO (Fig. 5b and c).

Cross-tachyphylaxis between levcromakalim and Y-26763 was observed in this study (fig. 6). Treatment with Y-26763 markedly reduced the effect of levcromakalim.

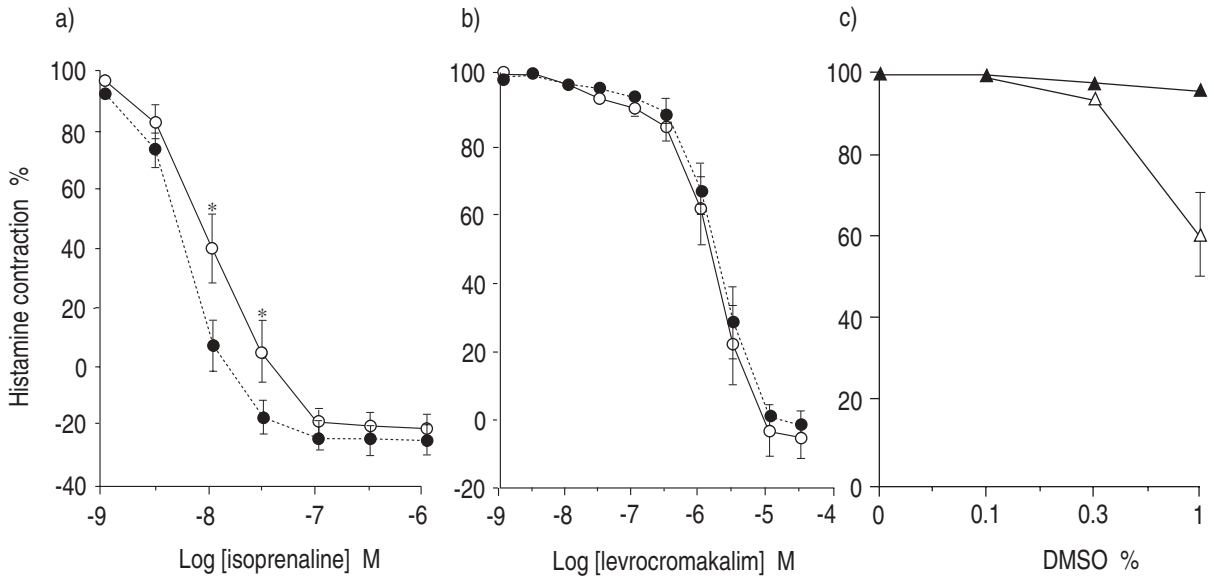

Fig. 2. – Reduction of relaxant potency of isoprenaline, but not of levcromakalim, by antigen challenge. The contraction induced by histamine (3 µM) was relaxed by either: a) isoprenaline; b) levcromakalim, without (·····●·····) and with (——○——) antigen challenge (values are expressed as a percentage of initial histamine-induced contraction (mean±SEM) (n=6–9)); and c) relaxation of histamine (Δ) and carbachol (▲) induced contraction by the dimethylsulphoxide (DMSO) used as solvent for levcromakalim (n=3). *: $p<0.05$, significant difference (Mann Whitney U-test).

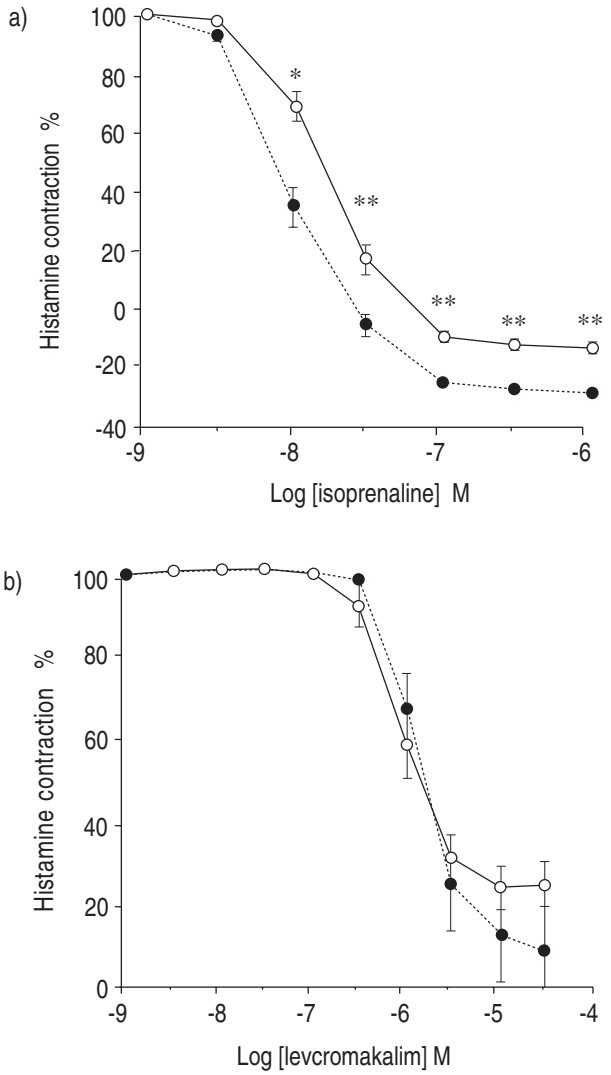

Fig. 3. – Tachyphylaxis of beta-adrenergic system in guinea-pig isolated trachea evoked by prior incubation with isoprenaline. Either: a) isoprenaline; or b) levcromakalim was cumulatively applied to the 3 µM histamine-contracted trachea without (·····●·····) and with (——○——) prior incubation with isoprenaline (3 µM) for 30 min. Values are expressed as a percentage of initial histamine-induced contraction (mean±SEM) (n=6). *: $p<0.05$; **: $p<0.01$ *vs* without preincubation with isoprenaline (Mann Whitney U-test).

Table 2. – IC_{50} values for the relaxant potency of isoprenaline, levcromakalim and theophylline before and after incubation with isoprenaline (4 µM) for 30 min in guinea-pig trachea

		Prior isoprenaline treatment	
Agent	n	(+) µM	(-) µM
Isoprenaline	6	0.017±0.002	0.010±0.001**
Levcromakalim	6	0.95±0.15	1.28±0.33
Theophylline	6	105±9.6	120±8.0

Values are expressed as mean±SEM. IC_{50}: concentration of drug producing half-maximum inhibition. **: significantly different from control values, $p<0.01$

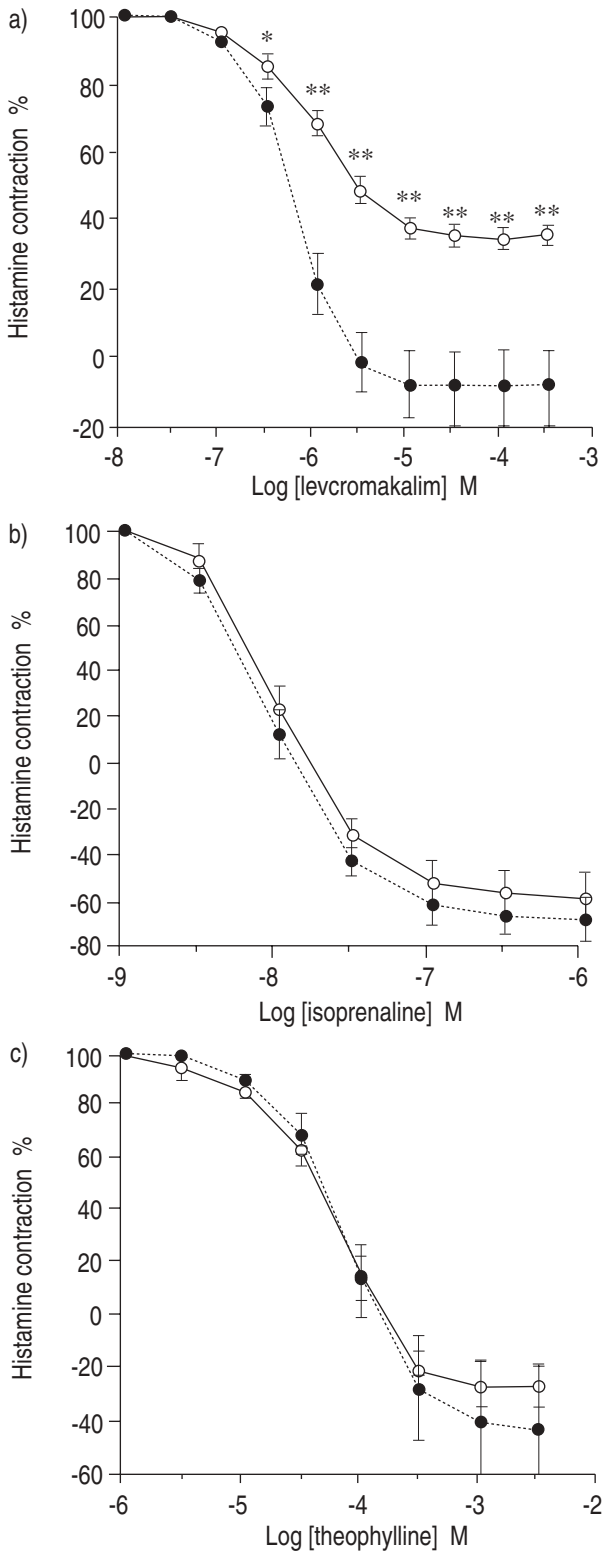

Fig. 4. – Tachyphylaxis of levcromakalim, but not of isoprenaline and theophylline, induced by prior incubation with levcromakalim. Levcromakalim, isoprenaline and theophylline were cumulatively applied to the 3 µM histamine-contracted trachea without (·····●·····) and with (——○——) prior incubation with levcromakalim (300 µM) for 30 min. Values are expressed as a percentage of initial histamine-induced contraction (mean±SEM) (n=6). *: $p<0.05$; **: $p<0.01$, *vs* without preincubation with levcromakalim (Mann Whitney U-test).

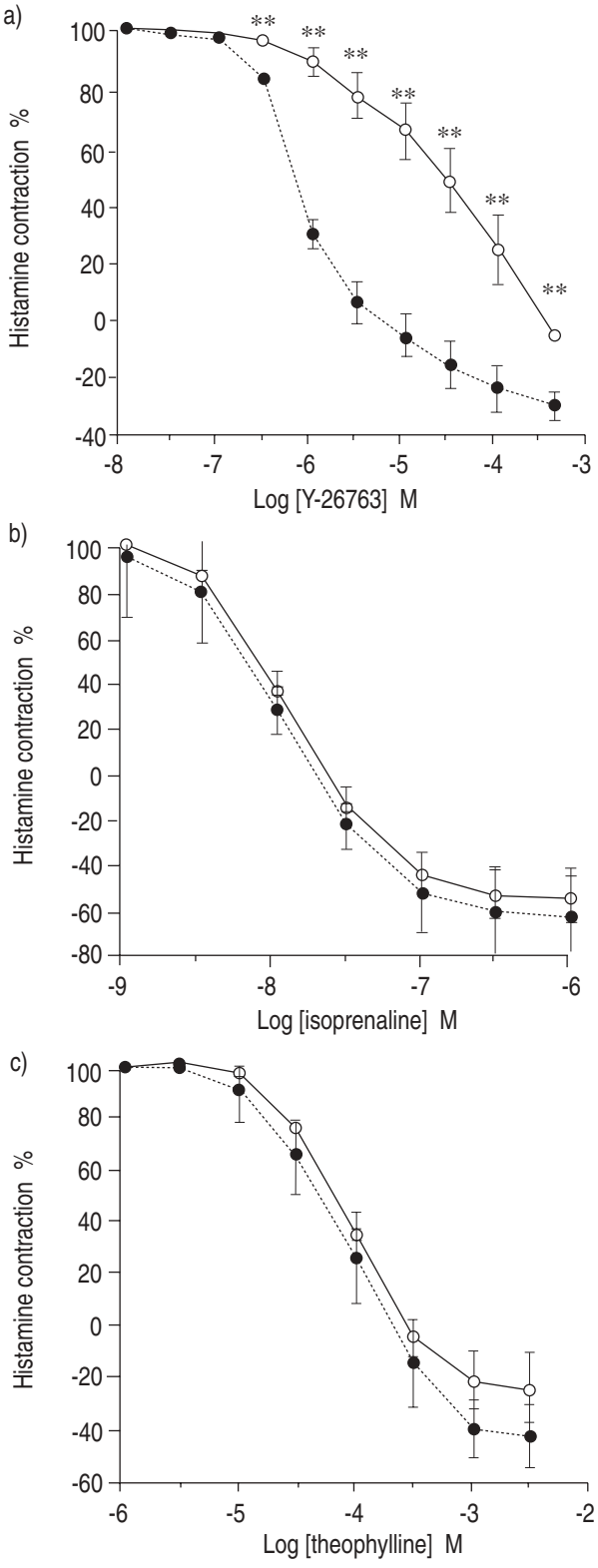

Fig. 5. – Tachyphylaxis of Y-26763, but not isoprenaline and theophylline induced by prior incubation with Y-26763 treatment. Y-26763, isoprenaline or theophylline was cumulatively applied to the 3 µM histamine-contracted trachea without (·····●·····) and with (——○——) prior incubation with Y-26763 (300 µM) for 30 min. Values are expressed as a percentage of initial histamine-induced contraction (mean±SEM) (n=6). **: $p<0.01$, *vs* without preincubation with Y-26763 (Mann-Whitney U-test).

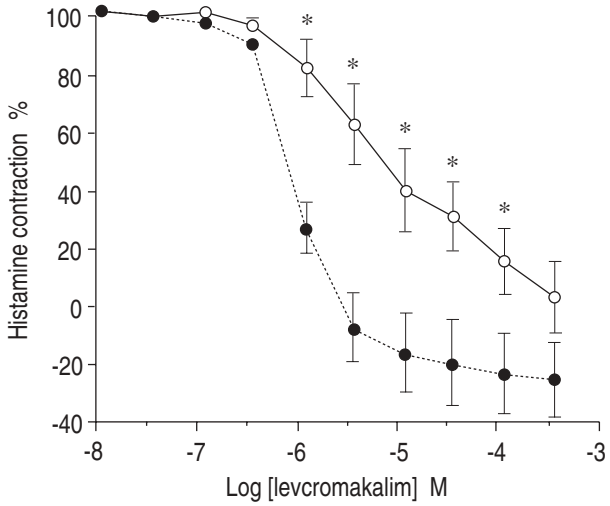

Fig. 6. – Cross-tachyphylaxis between levcromakalim and Y-26763. The relaxant action of levcromakalim was studied without (·····●·····) and with (——○——) prior incubation with Y-26763 (300 µM, 30 min). Values are expressed as a percentage of initial histamine-induced contraction (mean±SEM) (n=6). *: $p<0.05$ significant difference *vs* without preincubation with Y-26763 (Mann Whitney U-test).

Discussion

In the present study, the IC_{50} of levcromakalim was in agreement with other reports using vascular and airway smooth muscle [9, 26–29]; it was more potent than THEO. However, levcromakalim cannot be a first-line regimen for bronchodilatation, since the β-adrenergic agonist, ISO, was much more potent than levcromakalim in this study. The relationship between the three bronchodilators, β-adrenergic agonist, K_{ATP} openers and THEO supports the results reported by PACIOREK *et al.* [27], where the order of guinea-pig tracheal relaxants was always salbutamol > Ro 31-6930 (a potassium channel opener) > cromakalim > THEO.

Although levcromakalim was a more potent bronchodilator than THEO (about 57 times in this study), systemic application of the K_{ATP} openers brought some side-effects, such as headache, tachycardia and oedema [4]. Indeed, in patients with asthma, levcromakalim failed to show bronchodilator or protective effect against bronchoconstrictor challenge with HIS or methacholine at maximally tolerated systemic dose [30, 31]. Therefore, other drug delivery methods, such as inhalation, will be required to increase the concentration of levcromakalim up to a sufficient dose for bronchodilatation without systemic side-effects [31, 32]. In this case, K_{ATP} openers may have a clinical advantage as a new bronchodilator, because their relaxant action was resistant to allergic response (fig. 2).

Either passively or actively (spontaneously) sensitized human bronchus revealed a decrease in relaxant efficacy of levcromakalim on basal tone through ion channel modulation [33]. In developed tone, however, the action of levcromakalim was not affected by sensitization itself [33], and by allergic response (this study). We observed that neither active nor passive sensitization influenced the relaxant effect of levcromakalim on HIS-induced contraction (data not shown). In addition to this, there was no cross-tachyphylaxis between ISO and levcromakalim

or Y-26763 in guinea-pig tracheal muscle (figs. 4b and 5b), whereas one-way cross-tachyphylaxis between salbutamol and levcromakalim was reported in rat uterus smooth muscle [34].

The effect of THEO was totally independent of antigen challenge (table 1) and of ISO- or KATP opener-induced tachyphylaxis (table 2, and figs. 4c and 5c). It is likely that tissue and species-related differences of KATP channels may exist [29]. Thus, levcromakalim may be suitable for additional treatment of severe asthma attack, such as "status asthmatics", when β-adrenergic agonist and THEO, even used in combination, do not reverse airway narrowing. Until airway inflammation and increased airway exudate are improved by anti-inflammatory drugs (*e.g.* corticosteroids), which usually requires several hours for relief, KATP openers may be beneficial to support the conventional bronchodilatation therapy.

Some loss of sensitivity to the KATP openers was observed following incubation with the openers at a high dose (figs. 4, 5 and 6). After the relaxant response to levcromakalim (300 μM) had peaked, addition of Y-26763 (300 μM) produced a small but significant additional relaxation (data not shown). However, this did not imply an additive effect of Y-26763, because the same dose of DMSO also decreased the tone further. It should be noted that DMSO itself (more than 1%) relaxed the muscle (fig. 2c). Hence, we could not exclude the possibility that the effect of DMSO was involved in the relaxant action of the KATP openers at the high concentrations (>30 μM) (figs. 2b, 3b, 4a, 5a and 6).

The chemical structures of levcromakalim and Y-26763 are similar (fig. 1). However, there was a difference in the tachyphylaxis pattern between levcromakalim and Y-26763. The tachyphylaxis induced by levcromakalim mainly decreased the efficacy of the drug (fig. 4a). In contrast, the treatment with Y-26763 caused a prominent reduction of potency rather than efficacy (fig. 5a). It is likely that this observation was not due to technical error, because similar rightward shifts of the dose-response curves for levcromakalim were obtained after the treatment with Y-26763 (fig. 6). Thus, the mechanisms of tachyphylaxis may be different between levcromakalim and Y-26763. Further studies are required to clarify this point. Nevertheless, these tachyphylaxis data suggest that physicians have to pay attention to desensitization. One needs to be aware of the possibility of desensitization in this class of compounds, especially when high doses of KATP openers are inhaled as a bronchodilators. Perhaps their use could be rotated with other conventional bronchodilators, such as β-adrenergic agonists and THEO.

In conclusion, the effect of levcromakalim on the histamine-induced contraction of isolated tracheal muscle was independent of β-adrenergic desensitization induced by allergic response and by the administration of a high dose of β-adrenergic agonist, although desensitization of adenosine triphosphate-sensitive potassium channels occurs by the adenosine triphosphate-sensitive potassium channel openers themselves. Additive studies are required for selective control of adenosine triphosphate-sensitive potassi-um channels on airway smooth muscle and for exploration of more airway specific potassium channel activators.

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