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K^+ channel openers produce epithelium-dependent relaxation of the guinea-pig trachea

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Abstract

The relaxant effects of the K^+ channel openers, NIP-121, (+)-7,8-dihydro-6,6-dimethyl-7-hydroxy-8-(2-oxo-piperidin-1-yl)-6*H*-pyrano[2,3-*f*]benz-2,1,3-oxadiazole, and cromakalim, were investigated in epithelium-intact and -denuded tracheal spirals isolated from guinea-pigs. In the presence of 5 μM indomethacin, NIP-121 (0.01–1 μM) and cromakalim (0.1–10 μM) relaxed, in a concentration-dependent manner, epithelium-intact and -denuded trachea precontracted with a thromboxane A_2 mimetic, U46619, 9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$ (30 nM). The relaxations of epithelium-denuded trachea were significantly decreased as compared with those of epithelium-intact trachea. The relaxations induced by salbutamol or aminophylline were not affected by epithelium removal. In epithelium-intact trachea, the NIP-121- and cromakalim-induced relaxations were not modulated by the neutral endopeptidase inhibitor, phosphoramidon (10 μM), or the nitric oxide synthesis inhibitor, N^{ω}-nitro-L-arginine (100 μM). However, the guanylate cyclase inhibitor, methylene blue (100 μM), significantly reduced NIP-121- and cromakalim-induced relaxation of epithelium-intact trachea. Methylene blue also reduced sodium nitroprusside-induced relaxation but did not affect isoprenaline-induced relaxation. These findings suggest that the K^+ channel openers, NIP-121 and cromakalim, may induce, at least in part, epithelium-dependent and methylene blue-sensitive relaxation of the guinea-pig isolated trachea.

Keywords: K^+ channel opener; NIP-121; Cromakalim; Epithelium; Trachea, guinea-pig; Methylene blue; Phosphoramidon

1. Introduction

The bronchial epithelium has been reported to play an important role in modulating the responsiveness of the airway smooth muscle to drugs. Mechanical removal of epithelium from the isolated trachea increases the responsiveness to various bronchoconstrictors: histamine (Braunstein et al., 1988), acetylcholine (Holroyde, 1986), leukotrienes (Hay et al., 1987), adenosine (Advenier et al., 1988), substance P (Devillier et al., 1988) and endothelin (Hay, 1990), and also increases the responsiveness to the bronchodilators: isoprenaline (Lennart Lundblad and Persson, 1988) and sodium nitroprusside (Farmer et al., 1986). These reports suggest that some putative factors may be derived from epithelium and regulate the tracheal responsiveness to various agonists. Nitric oxide and vasoactive intestinal peptide are postulated to act as epithelium-derived inhibitory (relaxing) factors (Li and Rand, 1991; Lei et al., 1993). Thus, the presence or absence of the epithelium is very important to consider when examining the responsiveness of tracheal preparation to bronchoactive substances.

NIP-121, (+)-7,8-dihydro-6,6-dimethyl-7-hydroxy-8-(2-oxo-piperidin-1-yl)-6*H*-pyrano[2,3-*f*]benz-2,1,3-oxadiazole, is a potent K^+ channel opener (Masuda et al., 1991) and like both NIP-121 and the standard K^+ channel opener, cromakalim, preferentially suppresses the prostanoid-induced tone of the guinea-pig isolated trachea (Shikada et al., 1991). NIP-121 (0.1 μM) and cromakalim (1 μM), each of which submaximally reduces the spontaneous resting tone of guinea-pig trachea, enhance sodium nitroprusside-induced relaxation but do not affect the relaxation induced by isopren-

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aline, vasoactive intestinal peptide or prostaglandin E_2 (Shikada and Tanaka, 1992). This enhancement of sodium nitroprusside-induced relaxation was abolished in epithelium-denuded trachea. These findings suggest that the effect of K^+ channel openers on tracheal responsiveness to some agonists may be modulated by the presence of epithelium.

In the present study, we have investigated whether the relaxation of guinea-pig isolated trachea evoked by the K^+ channel openers, NIP-121 and cromakalim, is influenced by the removal of epithelium or by the modulation of the metabolism of nitric oxide or vasoactive intestinal peptide, and the effects were compared with those of other bronchodilators.

2. Materials and methods

2.1. Materials

The following agents were used: U46619, 9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$ (Cayman Chemical, USA); phosphoramidon (Peptide Institute, Japan); (−)-isoprenaline hydrochloride, aminophylline, indomethacin, salbutamol hemisulphate, methylene blue, N^{ω}-nitro-L-arginine (Sigma Chemical Co., USA); histamine hydrochloride, sodium nitroprusside dihydrate (Wako Pure Chemical Industries, Japan); NIP-121 and cromakalim were synthesized by the Nissan Chemical Industries, Central Research Laboratory, Japan.

Indomethacin was dissolved in 100% ethanol (final concentration, 0.1%), NIP-121, cromakalim and N^{ω}-nitro-L-arginine were dissolved in 100% dimethyl sulphoxide (final concentration, 0.2%). Other drugs were dissolved in distilled water.

2.2. Methods

Tracheae were removed from male Hartley guinea-pigs (250–400 g) stunned by a blow to the head. Each trachea was cut spirally and divided into two or three segments (one acted as control). In some experiments, the epithelium was removed mechanically by gently rubbing the luminal surface with a cotton-tipped applicator. Individual tracheal segments were suspended under an applied tension of 1 g in a 10-ml organ bath containing 8 ml of modified Tyrode solution at 37°C and gassed continuously with 95% O_2 + 5% CO_2. The composition of the modified Tyrode solution was (mM): 137 NaCl, 2.7 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 0.3 $NaHPO_4$, 20 $NaHCO_3$ and 11 dextrose. The tracheal response was measured isotonically (type TD-112S, Nihon Kohden). After the tissues had equilibrated for 50–60 min, the maximal response to histamine (100 μM) was obtained. Subsequent contractile responses were expressed as percentages of the response to 100 μM histamine. The tissues were washed several times for 30 min to re-establish the baseline tension and were then incubated for an additional 30 min with 5 μM indomethacin before the addition of U46619 (30 nM). Inhibitors were added once the U46619 contraction had reached a plateau 30 min before constructing the cumulative concentration-relaxation curves for K^+ channel openers or other bronchodilators. Relaxant responses were expressed as percentages of the maximum relaxation obtained with 1 mM aminophylline added to the organ bath at the end of the experiment. All results are expressed as means ± S.E.M. Statistical significance ($P < 0.05$) was assessed by two-tailed paired t-test.

3. Results

3.1. Relaxations induced by NIP-121 and cromakalim in epithelium-intact and -denuded tracheal strips precontracted with 30 nM U46619

U46619 at a concentration of 30 nM caused sustained contraction of both epithelium-intact and -denuded tracheas. The contractile response of epithe-

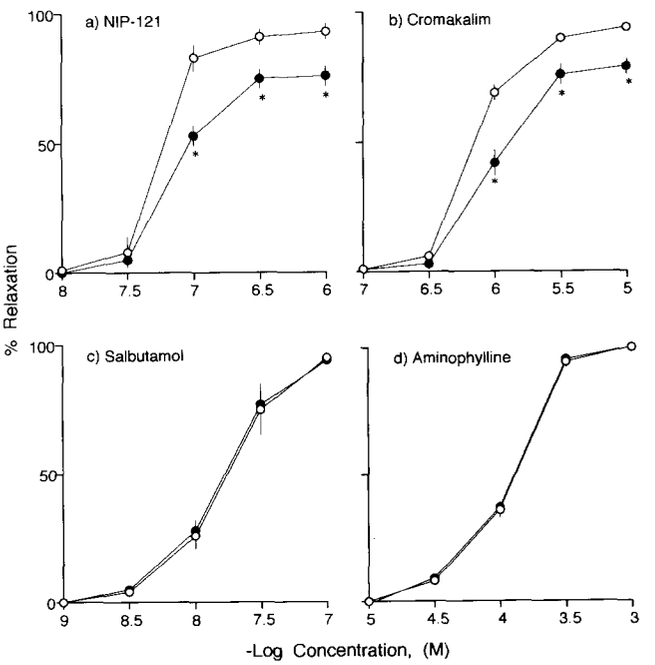

Fig. 1. Log concentration-relaxation curves for NIP-121 (a), cromakalim (b), salbutamol (c) and aminophylline (d) in epithelium-intact (open circles) and epithelium-denuded (closed circles) trachea isolated from guinea-pigs. Each point represents the mean ± S.E.M. of data from 4–5 preparations, paired control and test tissues. % Relaxation shows percentage of the response to 1 mM aminophylline. * Significant difference from the corresponding values for the paired epithelium-intact control tissues.

lium-denuded trachea to U46619 was not significantly different from that of the paired epithelium-intact trachea; for NIP-121-tested tissues contractile responses (percentage of maximal response to histamine) were 79 ± 2% denuded, 80 ± 3% intact, for cromakalim-tested tissues 78 ± 2% denuded, 84 ± 4% intact. NIP-121 (0.01–1 μM) and cromakalim (0.1–10 μM) relaxed both epithelium-intact and -denuded tracheas in a concentration-dependent manner but the extent of relaxation in epithelium-denuded trachea was significantly decreased compared with that observed in epithelium-intact trachea (Fig. 1a and b). However, epithelium removal did not affect the relaxation induced by salbutamol or aminophylline (Fig. 1c and d).

3.2. Effect of N^{ω}-nitro-L-arginine and phosphoramidon on NIP-121- and cromakalim-induced relaxations of epithelium-intact trachea

Neither N^{ω}-nitro-L-arginine (100 μM) nor phosphoramidon (10 μM) had any significant effect on U46619-induced contraction when each agent was applied to a trachea that had reached the plateau level. Neither N^{ω}-nitro-L-arginine nor phosphoramidon caused any significant change in the concentration-relaxation curves for NIP-121 and cromakalim (Fig. 2).

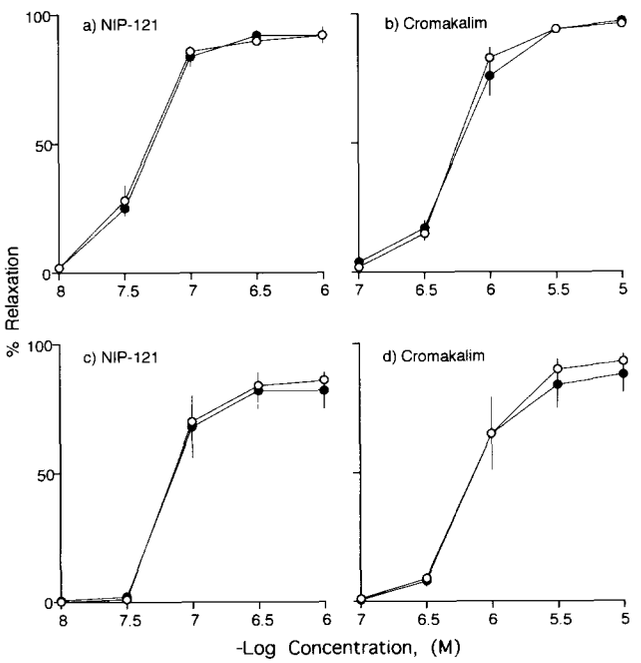

Fig. 2. Effect of 100 μM N^{ω}-nitro-L-arginine (a, b, closed circles) and 10 μM phosphoramidon (c, d, closed circles) on the relaxation induced by NIP-121 (a, c) and cromakalim (b, d) in epithelium-intact guinea-pig isolated trachea. Each point represents the mean ± S.E.M. of data from 4–5 preparations, paired control tissues and test tissues. % Relaxation shows percentage of the response to 1 mM aminophylline.

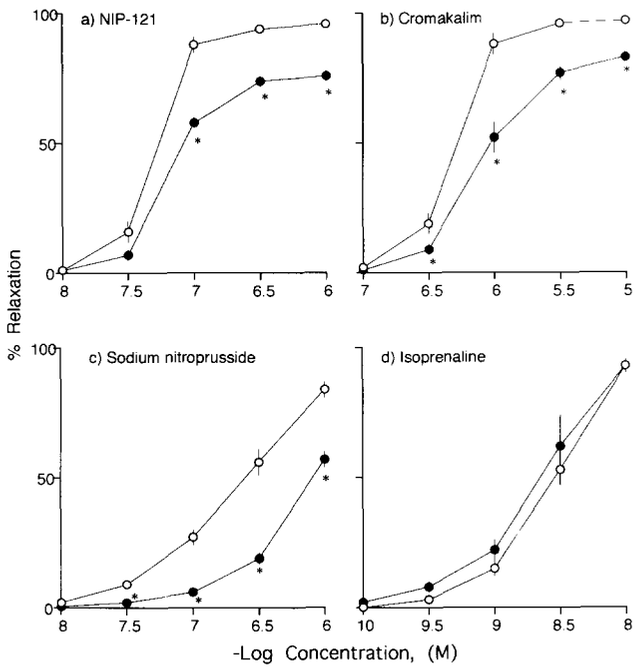

Fig. 3. Effect of 100 μM methylene blue (closed circles) on the relaxation induced by NIP-121 (a), cromakalim (b), sodium nitroprusside (c) and isoprenaline (d) in epithelium-intact guinea-pig isolated trachea. Each point represents the mean ± S.E.M. of data from 5–6 preparations, paired control tissues and test tissues. % Relaxation shows percentage of the response to 1 mM aminophylline. * Significant difference from the corresponding values for the paired control (open circles).

3.3. Effect of methylene blue on NIP-121- and cromakalim-induced relaxations of epithelium-intact trachea

The application of methylene blue (100 μM) to tracheal strips that had fully reached the plateau level caused transient contraction. However, this contractile response gradually declined and the response at the plateau level (30 min later) was not significantly different from that of vehicle-treated tissues. NIP-121- and cromakalim-induced relaxations were significantly decreased in methylene blue-treated trachea (Fig. 3a and b). The extent of the decrease in the relaxation caused by methylene blue was similar to those obtained in epithelium-denuded trachea (Fig. 1a and b). Methylene blue also significantly decreased sodium nitroprusside-induced relaxation but did not affect isoprenaline-induced relaxation (Fig. 3c and d).

4. Discussion

We previously reported that the K^+ channel openers, NIP-121 and cromakalim, preferentially relaxed the prostanoid-induced tone of the guinea-pig isolated

trachea and the concentration-relaxation curves of NIP-121 and cromakalim for the tone induced by the thromboxane A_2 mimetic, U46616, were shifted rightward by the ATP-sensitive K^+ channel blocker, glibenclamide (Shikada et al., 1991). These data indicate that both NIP-121 and cromakalim relax the U46619-induced tone of the guinea-pig trachea by activating ATP-sensitive K^+ channels. The present study showed that both NIP-121 and cromakalim relaxed, in a concentration-dependent manner, epithelium-intact and -denuded trachea contracted with U46616. In comparison with those observed in epithelium-intact tissues, the relaxation in epithelium-denuded trachea was significantly decreased. However, epithelium removal had no significant effect on the relaxation induced by salbutamol or aminophylline. The lack of effect of epithelium removal on the relaxation induced by salbutamol and aminophylline is consistent with the earlier report of Farmer et al. (1986) and Lennart Lundblad and Persson (1988). Our present findings suggest that the K^+ channel openers, NIP-121 and cromakalim, may cause relaxation of the guinea-pig isolated trachea by mechanisms which, at least in part, depend on the presence of the tracheal epithelium.

Some putative inhibitory factor(s) have been postulated to be released from airway epithelium and regulate the responsiveness of airway smooth muscle tone (Barnes et al., 1985; Hay et al., 1987; Fernandes et al., 1989). If the K^+ channel openers, NIP-121 and cromakalim, affect the epithelium and cause relaxation by stimulating the release of an inhibitory factor(s), their relaxant effects could be attenuated when the epithelium is removed. This possibility was suggested by our previous finding that NIP-121 and cromakalim prevent the contraction of the guinea-pig isolated trachea elicited by a phospholipase A_2 activator, melittin, and that the inhibition effect is abolished by the removal of tracheal epithelium (Shikada and Tanaka, 1993). It is, therefore, postulated that the action of the K^+ channel openers, NIP-121 and cromakalim, may involve the release of inhibitory factor(s) from the tracheal epithelium.

Because the present study was carried out in the presence of the cyclooxygenase inhibitor, indomethacin, bronchodilator prostaglandins may not be involved in the relaxation induced by NIP-121 or cromakalim. Li and Rand (1991) have proposed that nitric oxide and vasoactive intestinal peptide are possible mediators of nonadrenergic noncholinergic relaxation of the guinea-pig tracheal muscle. In this study, the relaxations induced by NIP-121 and cromakalim were not affected by N^{ω}-nitro-L-arginine (100 μM), a nitric oxide synthase inhibitor, or by phosphoramidon (10 μM), a neutral endopeptidase inhibitor. It is reported that N^{ω}-nitro-L-arginine (100 μM) inhibits nonadrenergic noncholinergic relaxation (Tucker et al., 1990) and phosphoramidon (10 μM) potentiates the relaxation caused by exogenously applied vasoactive intestinal peptide (Farmer and Togo, 1990) in the guinea-pig isolated trachea. It seems that, in the present study, secondarily released nitric oxide or vasoactive intestinal peptide may not be involved in the relaxation induced by NIP-121 and cromakalim. Further experiments are needed to explain the interaction between K^+ channel opener-induced relaxation and the putative epithelium-derived inhibitory factor(s) in the guinea-pig trachea.

The relaxations induced by NIP-121 and cromakalim were significantly attenuated by the guanylate cyclase inhibitor, methylene blue. Since the concentration used (100 μM) did not affect the relaxation induced by isoprenaline but clearly attenuated that induced by sodium nitroprusside, the inhibition effect of methylene blue did not result from the non-specific effect of methylene blue. Allen et al. (1986) have already reported that 100 μM of methylene blue inhibits the relaxation induced by sodium nitroprusside in the guinea-pig trachea. It is a possible interpretation that the K^+ channel openers, NIP-121 and cromakalim, may relax the guinea-pig trachea by mediating a methylene blue-sensitive pathway. Lei et al. (1993) recently reported that nonadrenergic noncholinergic bronchoconstriction in the guinea-pig was regulated by nitric oxide and possibly by vasoactive intestinal peptide as well as activation of soluble guanylate cyclase. Our present finding supports the possibility of the involvement of the guanylate cyclase pathway in K^+ channel opener-induced relaxation of the guinea-pig trachea.

In conclusion, we suggest that the K^+ channel openers, NIP-121 and cromakalim, may induce, at least in part, epithelium-dependent and methylene blue-sensitive relaxation of the guinea-pig isolated trachea.

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