Effects of Anti-Asthma Drugs and Potassium Channel Openers on Neurally-Mediated Contraction of Isolated Guinea Pig Trachea

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ABSTRACT—The effects of anti-asthma drugs, isoproterenol, aminophylline and hydrocortisone, and potassium channel openers on the contraction induced by electrical stimulation or exogenously applied acetylcholine were investigated in isolated guinea pig trachea. Isoproterenol and aminophylline non-selectively inhibited both the contraction evoked by vagus nerve- and that by transmural field-stimulation, but had no effect on the response induced by exogenously applied acetylcholine. Hydrocortisone and potassium channel openers, NIP-121 and cromakalim, preferentially inhibited vagus nerve-mediated response. These results suggest that anti-asthma drugs may have an ability to inhibit neurally-mediated contraction in the guinea pig trachea.

Keywords: Anti-asthma drugs, Neurally-mediated contraction, Trachea (guinea pig)

The airways are under the control of the autonomic nervous system. Abnormalities in autonomic regulation of the airway, such as an increase in cholinergic activity, may underlie bronchial hyperreactivity and contribute to the bronchoconstriction of asthma (1). β-Adrenoceptor agonist and methylxanthines have been widely used to treat asthma, because of their airway smooth muscle relaxant activity. Glucocorticoid therapy has been also applied in the treatment of asthma, based on its anti-inflammatory activity. However, actual involvement of these mechanisms in the treatment of asthma does not seem to be clear.

The potassium channel openers possess not only vasorelaxing activity but also bronchodilation activity in isolated guinea pig trachea (2). Taylor et al. (3) reported that cromakalim is an effective inhibitor of spontaneous tone in isolated human bronchioles. Recently, cromakalim was reported to modulate neurotransmission in the guinea pig trachea (4, 5), and its possible use in the treatment of asthma is now under investigation.

In this study, we investigated the effects of isoproterenol, aminophylline, hydrocortisone and potassium channel openers on the increase in intraluminal pressure (ILP) of isolated guinea pig trachea. The ILP was evoked by: 1) stimulation of the vagus nerve, 2) transmural field stimulation and 3) exogenously applied acetylcholine (ACh), and we compared the modes of action of these drugs.

The methods of Blackman and McCaig (6) were modified and used. Briefly, male Hartley guinea pigs (250–400 g) were killed by a blow to the head, and the trachea was excised with the right vagus nerve. The preparation was cannulated at each end, set up horizontally and maintained at 37°C in an 80-ml organ bath containing aerated modified Tyrode solution. The lumen of the trachea was filled with the Tyrode solution. The composition of the Tyrode solution was as follows: 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2, 20 mM NaHCO3, 0.32 mM NaH2PO4 and 11 mM dextrose. One end of the trachea was closed and the other end was attached to a pressure transducer (TP-400T, Nihon Kohden) to record ILP.

The trachea was equilibrated for 50 min and the response to 100 μM histamine was recorded and washed. Histamine increased ILP to 42 ± 2 mmHg (n = 7), and the responses of the following experiments were expressed as percentages of the histamine response. Fifty minutes after the washout of histamine, responses to vagus nerve and transmural field stimulation were obtained. The vagus nerve and transmural field stimulation were delivered by an electronic stimulator (SEN-
3301, Nihon Kohden) through bipolar platinum electrodes (pulses of 20 V, 1 msec duration) for 10 sec every 2 min at a frequency of 0.5 - 50 Hz. This procedure was performed twice at an interval of 50 min, and the drug or vehicle was added to the organ bath 30 min before the second procedure. In a similar experiment, the cumulative concentration in response to exogenous ACh was determined by adding ACh to the organ bath at concentrations from 0.01 - 10 μM using a separate preparation. The data were evaluated by calculating the area under the frequency-response curve or concentration-response curve for exogenously applied ACh, that is the area enclosed by the curves and the abscissa, as shown in Fig. 1-a,b,c. The area of the second response was expressed as a percentage of that of the first response. The results were expressed as means ± S.E.M., and statistical analyses were performed by the paired or Student's t-test, appropriate to the data. P values of less than 0.05 were considered to indicate a significant difference.

The contractile responses induced in the three different ways are shown in Fig. 1-a,b,c. Irrespective of the type of stimulation, the second control response was slightly smaller than the first response, and the area under the response curve was significantly reduced (Fig. 1-d). The response of the tissue in the presence of drug was, therefore, compared to the second response in the absence of the drug (Fig. 1-d, dotted column). All contractile responses were completely blocked by 0.1 μM atropine, indicating that these responses were mediated by muscarinic receptors. Hexamethonium at 250 μM also abolished the response to vagal stimulation, whereas it slightly inhibited the response obtained by transmural stimulation (second control response 84 ± 6%, hexamethonium treated 61 ± 6%, P < 0.05), but not that to applied ACh (data not shown). These results indicate that preganglionic nerves may be partly involved in the response to transmural stimulation.

Under these conditions, the effects of isoproterenol (Iso, β-agonist), aminophylline (AP, ethylenediamine salt of theophylline) and hydrocortisone (glucocorticoid) on these contractile responses were examined. We chose two effective concentrations (low and high) of Iso and AP from the results of the separate experiment. That is a low concentration of Iso (1 nM) and AP (30 μM) induced relaxation to 32% and 36% of the maximum response on spontaneous tone of the tracheal spirals, and high concentrations (10 nM and 100 μM)

![Fig. 1.](image_url)

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**Fig. 1.** Increase in intraluminal pressure of isolated guinea pig trachea evoked by vagus nerve stimulation (a), transmural field stimulation (b) and exogenously applied acetylcholine (c). Open symbols indicate the first frequency- or concentration-response curve and closed symbols indicate the second ones. Comparison of the area under the frequency- or concentration-response curve evoked by each stimulation method (d). The area under the second response curve was expressed as a percentage of the first response. Each point represents the mean ± S.E.M. of six to seven experiments. *P < 0.05, by the paired t-test. □ 1st, □ 2nd. Exo.: Exogenous.
relaxed it to 93% and 83%, respectively. Iso (10 nM) and AP (30 and 100 μM) significantly attenuated the responses evoked by both vagus nerve stimulation and field stimulation (Fig. 2). They had no significant effect on the response to exogenous ACh. Further high concentrations of Iso (30 nM) and AP (300 μM) significantly inhibited the response to it (data not shown). Hydrocortisone (100 μM) significantly inhibited the vagus nerve-mediated response, but had no effect on the response to field stimulation. A higher concentration of hydrocortisone (300 μM) largely inhibited the vagus nerve-mediated response and had a small but significant effect on the response to field stimulation. The novel potassium channel opener NIP-121 (7) and cromakalim, at concentrations that abolished spontaneous tone of the tracheal spiral preparation to a submaximal extent (2), preferentially suppressed the vagus nerve-mediated response over the response induced by field stimulation. The potency of NIP-121 was about ten times that of cromakalim. They did not inhibit ACh-induced contraction.

Kamikawa and Shimo (8) reported that the adrenoceptor agonist modulates cholinergic neurotransmission of guinea pig tracheal smooth muscle induced by field stimulation. In this study, we examined the effect of anti-asthma drugs on the response to the increase in ILP using a guinea pig vagus nerve-tracheal preparation. Our results showed that Iso and AP inhibited the responses induced by both vagus nerve stimulation and field stimulation, suggesting that they inhibit neurally-mediated responses. We also showed that hydrocortisone selectively inhibited the vagus nerve-evoked response, and it is suggested that hydrocortisone preferentially inhibits the preganglionic mechanism in the present study. Intravenous administration of hydrocortisone has been used in acute asthma therapy (9) even though its therapeutic mechanism has not been clarified. The concentration of hydrocortisone examined in the present study is higher than the effective blood concentration during the treatment of acute severe asthma (10). It is difficult to apply the present results directly to explain the therapeutic efficacy of hydrocortisone, but they provide a possible interpretation for the mechanism of the immediate clinical effectiveness of hydrocortisone.

McCaig and De Jonckheere (4) reported that cromakalim modulated cholinergic transmission in guinea pig trachea and concluded that the inhibition occurred mainly by a preganglionic mechanism. Burka (5) reported that the inhibitory effect of cromakalim on cholinergic contraction is not specific to a preganglionic mechanism. However, in our calculation of the area under the frequency-response curve, cromakalim (0.3 μM) showed selective and significant inhibition against vagus nerve-evoked responses, a finding that is in agreement with the study of McCaig and De Jonckheere.

Fig. 2. The effect of several anti-asthma drugs and potassium channel openers on the area under the frequency- or concentration-response curve. Each point represents the mean ± S.E.M. of three to seven experiments. *P < 0.05, compared with the second control response by Student’s t-test. Vagus nerve, Field, Exogenous ACh.
Our novel potassium channel opener NIP-121 was reported to be 5 times stronger than cromakalim in suppressing spontaneous tone in guinea pig trachea (2). In the present study, NIP-121 was about 10 times more potent than cromakalim. So it seems possible that the preferential inhibitory effects of NIP-121 and cromakalim on the response to vagus nerve stimulation are mediated by the opening of potassium channels in tracheal ganglia and modify ganglionic transmission, as suggested by MacCaig and De Jonckheere (4).

It is interesting that hydrocortisone and potassium channel openers similarly inhibited the vagus nerve-mediated response and, the actions of these drugs were different from the those of Iso and AP. Further study is necessary to clarify the involvement of hydrocortisone in ganglionic transmission.

In our experiment, the preganglionic nerves may have been stimulated to a slight extent in the response evoked by field stimulation, since hexamethonium inhibited not only nerve-evoked responses but also field stimulated responses. These observations are in disagreement with the study of McCaig and De Jonckheere (4). However, Richardson (10) reported that airway ganglia are present on the airway wall; and in ferret trachea ganglia, they are present along the tracheal muscle (11). It seems difficult to distinguish completely postganglionic nerve stimulation from preganglionic ones.

In conclusion, clinically used anti-asthma drugs have preferential inhibitory effects on neurally-mediated contractile response, and potassium channel openers also have an inhibitory activity on vagus nerve-evoked response in the guinea pig trachea.

REFERENCES