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Effect of nifedipine on constriction of human tracheal strips *in vitro*

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1 Autopsy specimens of human trachealis muscle were used to investigate the effect of the calcium channel blocker, nifedipine, on airway smooth muscle constriction. With these tracheal strips, two consecutive cumulative concentration-effect curves to histamine (0.1–100 μM) obtained at a 60 min interval were highly reproducible.

2 We examined the effects of adding nifedipine (2.9 μM) to the incubation medium before the second histamine response. The concentration-effect relationships determined after nifedipine incubation were significantly different from control: the response to 100 μM histamine was reduced by approximately one-half (to 2.53 ± 0.6 g; $P < 0.025$), and the concentration of histamine causing 40% of the maximal control contraction (EC_{40}) increased nearly ten fold (to 36.9 ± 10.5 μM; $P < 0.02$).

3 In two additional tracheal strips submaximally constricted with 10 μM histamine, nifedipine 2.9 μM caused complete relaxation to resting tension or below.

4 These results indicate a direct inhibitory effect of nifedipine on airway smooth muscle constriction and partial dependence of human trachealis muscle on calcium ion fluxes for initiation and maintenance of contraction. In addition, the results suggest a potential mechanism for the inhibitory effects of calcium channel blocking drugs in exercise-induced asthma.

Introduction

Cerrina and colleagues have previously demonstrated that administration of nifedipine to asthmatics before an exercise challenge inhibits the subsequent exercise-induced bronchoconstriction (Cerrina, Denjean, Alexandre, Lockhart & Duroux, 1981). However, these investigators and others (McFadden, 1981; Middleton, 1980) could only speculate as to the mechanism whereby nifedipine exhibited this action. They suggested a number of possible sites of action, including inhibition of release of mediators of immediate hypersensitivity by mast cells; impairment of neurotransmission along cholinergic (vagal) reflex pathways; and a direct inhibitory effect on airway smooth muscle. Evidence is available to indicate a role for calcium ion flux in initiating all three processes (Rubin, 1970; Coburn, 1977; Forman, Hallett & Mongar, 1977).

Although nifedipine is known to inhibit the contraction of airway smooth muscle *in vitro* and *in vivo* (Fanta, Venugopalan, Lacouture & Drazen, 1982), a direct inhibitory action of the drug on pharmacologically-induced constriction of human airway smooth muscle has not previously been demonstrated. In this study we found that nifedipine both inhibits histamine-induced constriction of human tracheal strips obtained from autopsy specimens and relaxes previously constricted tracheal strips.

Methods

Human tracheal strips were prepared from nine autopsy specimens, generally obtained within 8 h of death. No individual had a history of asthma or was receiving a calcium channel blocking drug or antihistamine before death.

To prepare the strips, the trachea was dissected from surrounding structures and then transected transversely such that individual tracheal rings were obtained. The trachealis muscle was cut free from each ring at its insertions into the cartilage. The tissue strips, approximately 2 cm in length, were suspended vertically in 10 ml glass organ baths. One end was tied by a silk thread to a glass hook at the bottom of the organ bath; the other end was similarly affixed by thread to a force transducer (Grass Instrument Co.,

Quincy, MA, Model FT 0.3 C). The strips were bathed in Tyrode solution at 37°C and continuously gassed with 95% O_2 and 5% CO_2.

In all but two cases the tracheal strips were held with an initial tension of 8.0 g. These strips then relaxed spontaneously over a period of approximately 1 h to a mean tension of 4.0 ± 0.3 g. Two additional strips set with an initial tension of 4.0 g developed maximal active tensions in response to histamine of 2.0 and 2.7 g, values within the range observed for those tissues set initially at 8.0 g (1.0–11.9 g).

In the first series of experiments (10 strips), two cumulative histamine concentration-effect curves were constructed sequentially to assess the viability and reproducibility of the tissues to repetitive constriction. The histamine concentration range used was 0.1–100 μM. After addition of the histamine to achieve each concentration, the strips were allowed to reach a plateau level of constriction (approximately 5 min) before adding the next highest concentration of histamine. After completing the sequence of concentration-effect measurements, the strips were rinsed by overflow with fresh Tyrode solution, and 60 min were allowed for strips to return to their previous baseline tension. Histamine concentration-effect curves were then generated a second time.

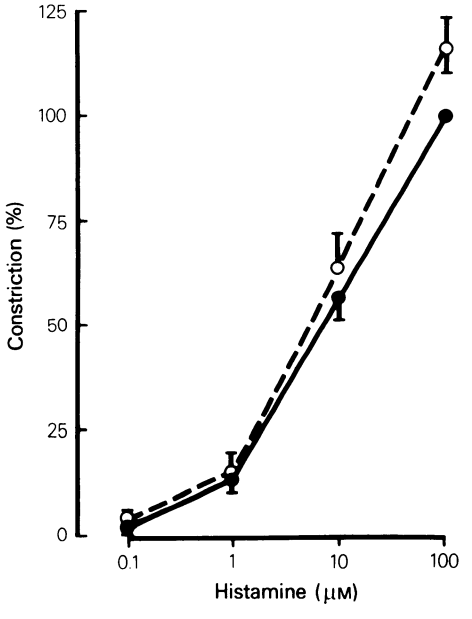

Figure 1 Preliminary study of the reproducibility of histamine concentration-effect relationships in human tracheal strips. Closed circles (●) and solid line indicate the first concentration-effect curve; open circles (○) and dashed line indicate the second curve generated 1 h later. Each point is the mean of observations from 10 tracheal strips and the bars represent one s.e.mean. The two curves do not differ significantly from each other at any point.

In six additional tracheal strips prepared from three separate autopsy specimens, nifedipine 2.9 μM (1 μg/ml) was added to the organ bath 10 min before the second histamine concentration-effect curve. Because of the sensitivity of nifedipine to photo-oxidation, nifedipine powder was refrigerated in a tinted-glass jar and fresh solutions, prepared each day from this stock, were protected from exposure to light. Nifedipine was dissolved in 0.1% ethanol in the organ bath. To test the effect of this solvent on the tracheal response to histamine, four additional strips were incubated with 0.1% ethanol alone for 10 min before repeating the histamine concentration-effect curves.

Finally, to test its smooth muscle relaxant effect, nifedipine (2.9 μM) was added to two tracheal strips already submaximally constricted with 10 μM histamine. To another submaximally constricted tracheal strip 0.1% ethanol was added, and a fourth constricted strip was simply observed for 30 min to assess the spontaneous fluctuations in tension.

Histamine was obtained from Sigma Chemical Co. (St Louis, Missouri). All drug weights are for the free base. Nifedipine was kindly supplied by Pfizer Pharmaceuticals (New York, New York). Statistical comparisons were made using Student's *t* test for paired and unpaired data.

Results

Cumulative histamine concentration-effect relationships were determined in 20 strips. The mean tension developed in response to 100 μM histamine was 3.78 ± 0.63 g. All strips gave greater than 1 g maximal tension. The concentration of histamine causing 50% of this response in each tissue was 7.0 ± 0.9 μM.

After washing the tracheas with Tyrode solution and allowing them to return to baseline tension, a second histamine concentration-effect curve was performed in 10 strips. Although there was a trend for greater constriction at the same histamine concentrations during the second concentration-effect curves, these differences were not significant (Figure 1).

In six other strips nifedipine (2.9 μM) was added to the incubation medium 10 min before a second histamine concentration-effect curve was obtained. In response to the nifedipine, the tracheal strips had a small, statistically insignificant relaxation from baseline tension. However, nifedipine significantly inhibited constriction of the tracheal strips to histamine (Figure 2). After pre-incubation with nifedipine, the contractile response to 100 μM histamine was only 50.4 ± 2.3% of the initial response

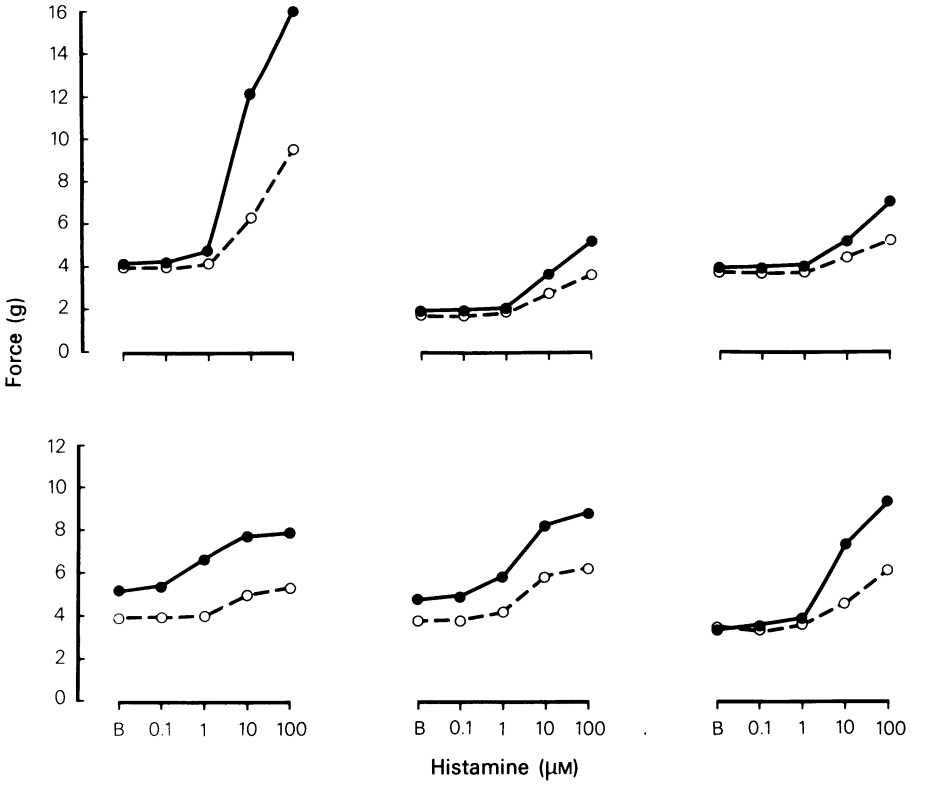

Figure 2 Effect of nifedipine on the histamine concentration-effect relationships. The concentration-effect curves with (○) and without (●) nifedipine (2.9 μM) pre-incubation are given for each of the six individual tracheal strips tested. Mean values were significantly different at histamine concentrations of 10 μM and 100 μM ($P<0.005$). B indicates baseline tension before the addition of histamine.

(5.16 ± 1.4 g without nifedipine vs 2.53 ± 0.6 g with nifedipine; $P<0.025$). Since some tissue strips gave less than 50% contraction after nifedipine, we calculated the concentration of histamine giving 40% of the maximal control contraction (to 100 μM histamine) before and after incubating with nifedipine. Before nifedipine was added, 4.3 ± 1.4 μM histamine was required and after nifedipine, 36.9 ± 10.5 μM histamine ($P<0.05$). The mean dose-ratio for histamine before and after incubation with nifedipine (EC_{40} after/EC_{40} before) was 10.5 ± 2.7.

Pre-incubation of four tracheal strips with 0.1% ethanol alone had no effect on the subsequent histamine concentration-effect curve (Figure 3).

The smooth muscle relaxant effect of nifedipine is demonstrated in Figure 4. Both the control and the ethanol-treated strips remained constricted in response to 10 μM histamine for the 30 min observation period. The nifedipine-treated strips, on the other hand, relaxed to below their initial tensions.

Discussion

The first studies of human isolated bronchi were performed approximately 30 years ago (Rosa & McDowall, 1951; Hawkins & Schild, 1951). Bronchial spirals and chains of bronchial rings cut from lung specimens obtained at surgery have been studied fresh or after overnight refrigeration. Use of autopsy specimens to investigate the response of human trachealis muscle has also been previously described, although studies have been limited (Townley, Honrath & Guirgis, 1972; Richardson & Beland, 1976; Kneussl & Richardson, 1978). We found that trachealis tissue obtained at autopsy within 8 h of death can give reproducible contractile responses. Because of the larger amount of smooth muscle tissue and more uniform muscle fibre orientation in trachealis strips compared to bronchial spirals, considerably larger tensions are generated by the autopsy preparations. In all other respects, however,

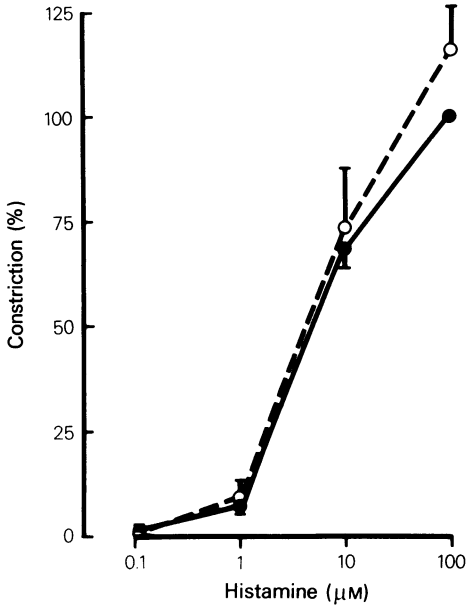

Figure 3 Control experiment comparing histamine concentration-effect curves with (○) and without (●) prior incubation of tracheal strips with 0.1% ethanol, the solvent used for nifedipine. Each point is the mean from 4 tracheal strips, and the bars represent one s.e.mean. At no point do the two curves differ significantly.

the trachealis muscle appeared to respond to broncho-constrictor stimulation in the same manner as surgically-obtained smooth muscle preparations.

Results from the present study indicate that nifedipine can directly inhibit pharmacologically-induced constriction of human isolated tracheal strips. Autopsy specimens were obtained from patients without a history of asthma or other related allergic diseases and presumed free of sensitized airway mast calls. Vagal efferent pathways were no longer intact. Nonetheless, nifedipine significantly blocked histamine-induced contraction of the trachealis muscle, causing an approximately ten fold increase in the histamine concentration needed to produce 40% of maximal constriction. Furthermore, nifedipine induced marked relaxation of constricted tracheal strips.

Previous animal experiments by us (Fanta *et al.*, 1982) and others (Himori & Taira, 1980) had suggested a direct action of the calcium channel blocking drugs on tracheal and parenchymal strips *in vitro*. In these animal models, nifedipine and other calcium channel blockers (Coburn, 1977; Kitamura & Ishihara, 1980) have been shown to block airway smooth muscle constriction induced by a variety of agents, including histamine, carbachol, prostaglandin $F_{2\alpha}$, 5-hydroxytryptamine, and potassium. Thus, the observed effect on human tracheal tissue is unlikely to be due to specific antagonism of histamine H_1-receptors. Furthermore, nifedipine has not been

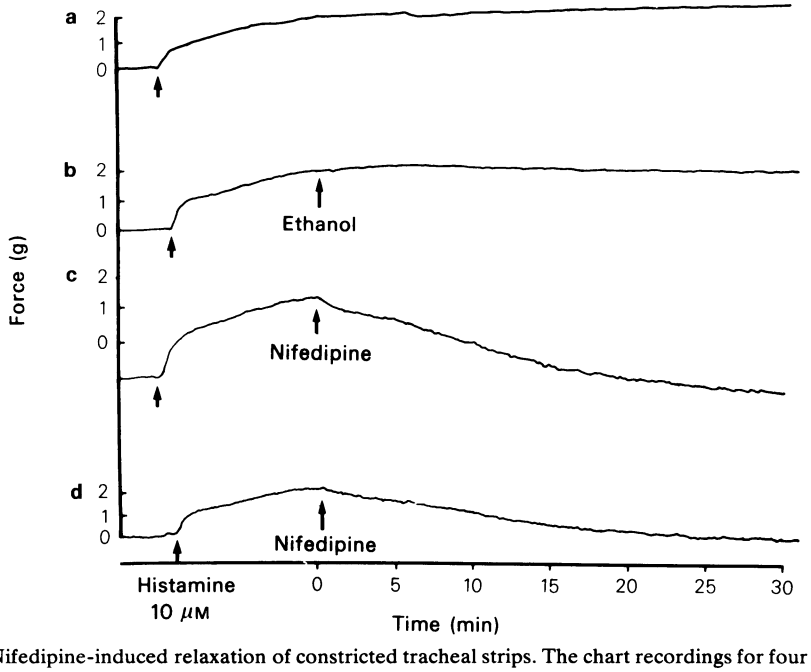

Figure 4 Nifedipine-induced relaxation of constricted tracheal strips. The chart recordings for four tracheal strips are shown. All strips were initially constricted with 10 μM histamine. The first strip (a) received no intervention; ethanol (0.1%) was added to (b); and (c) and (d) were treated with 2.9 μM nifedipine.

found to have any β-agonist actions in other systems (Kroneberg, 1975).

It appears from other studies that nifedipine directly impedes smooth muscle constriction by its competitive inhibition of transmembrane calcium flux, thereby uncoupling the process by which pharmacological (or electrical) stimulation is translated into mechanical contraction (Coburn, 1977). The ability of nifedipine to inhibit histamine-induced constriction of human tracheal strips supports the concept that contraction of human airway smooth muscle is likewise dependent on trans-membrane calcium ion movements. Failure of nifedipine to abolish completely all constriction in our experiments may be related to the concentration of nifedipine used or may result from histamine-induced release of calcium stores not influenced by the calcium channel blocker.

From these experiments we would predict that nifedipine would inhibit histamine-induced bronchoconstriction in man. In fact, Williams and colleagues recently demonstrated that nifedipine, 20 mg by mouth, caused a three fold increase in the concentration of histamine producing a 20% fall in the one-second forced expiratory volume in ten asthmatics (Williams, Varnes, Vickers & Rudolf, 1981). Just as we found no significant relaxation of baseline tension in tracheal strips when nifedipine was added to the bath, these authors (Williams *et al.*, 1981) and others (Cerrina *et al.*, 1981) have not found nifedipine to cause significant bronchodilatation in asymptomatic subjects with obstructive airway diseases. However, our findings would lead us to predict that in persons with acute bronchoconstriction, nifedipine in doses sufficient to achieve serum concentrations in the order of 2.9 μM would have significant bronchodilator action. This hypothesis has yet to be tested clinically.

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