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Modulation by theophylline and enprofylline of the excitatory non-cholinergic transmission in guinea-pig bronchi

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ABSTRACT: The mechanism of action of xanthines in asthma remains controversial. Since sensory innervation may play a role in the pathogenesis of asthma, we investigated whether xanthines were capable of reducing the contractile response of the bronchi to nerve stimulation.

In guinea-pig bronchi *in vitro*, electrical field stimulation (EFS: 40 V, 16 Hz, 0.2 ms during 10 s) induces a rapid cholinergic contraction followed by a long-lasting contraction due to a local release of neuropeptides from C-fibre endings. We measured isometric neuronally-mediated contractions of bronchial smooth muscle and studied the effects of increasing concentrations of two xanthine derivatives, theophylline, an antagonist of adenosine receptors, and enprofylline, which has no effect on adenosine receptors.

Both enprofylline (1–50 μM) and theophylline (10–100 μM) inhibited, in a concentration-dependent manner, the peptidergic contraction, an effect which was more marked with enprofylline than theophylline (EC_{50}=9.6±0.7 μM and 62.0±4.7 μM, respectively). Conversely, the cholinergic response was unaffected. Contractions induced by exogenous substance P (0.03–3 μM) were also unaffected by theophylline and enprofylline at the above mentioned EC_{50}s.

Our results suggest that concentrations of theophylline, similar to those used therapeutically, reduce the release of sensory neuropeptides from C-fibre endings. This effect is unrelated to adenosine receptor blockade, since enprofylline had a similar inhibitory effect.

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The mechanism of action of xanthines in asthma is still controversial. In addition to relaxation of bronchial smooth muscle, other effects of xanthines may be clinically relevant, *e.g.* an anti-inflammatory effect. First, theophylline and enprofylline significantly inhibit leucocyte activation [1] and decrease allergen-induced plasma extravasation in animal airways [2, 3] and in human nasal mucosa [4]. Second, xanthines also inhibit the release of mast cell mediators, but only at high supratherapeutic doses [5, 6]. In asthmatic patients, both enprofylline and theophylline protect against the late-phase reaction, but have little preventive effect on the early response to allergen [7]. These anti-inflammatory effects of xanthines in asthma suggest inhibition of the local release of pro-inflammatory mediators in the airways.

Stimulation of bronchial C-fibres induces bronchoconstriction by means of central reflex pathways and local release of the sensory neuropeptides, substance P (SP), neurokinin A and calcitonin gene-related peptide [8–10]. These peptides cause multiple paracrine effects, including contraction of airway smooth muscle [11–13], mucus hypersecretion, increase in microvascular permeability, release of inflammatory mediators and inflammatory cell chemotaxis [8, 10, 14, 15]. These pro-inflammatory effects may play a role in the pathogenesis of asthma [8, 16], thereby suggesting that control of the local release of neuropeptides might be effective in the management of this disease. We hypothesized that the beneficial effect of xanthines in asthma might be explained, at least in part, by inhibition of the release of sensory neuropeptides.

Electrical field stimulation (EFS) of guinea-pig bronchi *in vitro* causes both a rapid cholinergic and a long lasting non-cholinergic (e-NC) contraction of bronchial smooth muscle due to release of sensory neuropeptides from C-fibre endings [17, 18]. We therefore studied the effects of the two xanthine derivatives, theophylline and enprofylline, on e-NC contraction of guinea-pig bronchi.

Material and methods

Organ bath studies

Male Dunkin-Hartley guinea-pigs (350–500 g) were killed by cervical dislocation. The thoracic content was rapidly removed and placed in Krebs-Henseleit (K-H) buffer, gassed with carbogen (95% O_2 and 5% CO_2).

To study the effects of xanthines on bronchial contractions induced by EFS, hilar bronchi were dissected out and suspended between platinum electrodes in tissue baths containing 10 ml oxygenated K-H solution at 37°C, as described previously [19]. The platinum electrodes were linked to a Grass S88 stim-ulator connected to an amplifier (R. Willetts, Electronics Dept, Hammersmith Hospital, London, UK). Current flowing through the electrodes was monitored on an oscilloscope face throughout the study. Isometric contractions of bronchial smooth muscle were measured with Myograph F-60 transducers connected to MKIV Physiographs (Narco Bio-System, USA). An initial force of 500 mg was applied. After a 45–60 min equilibration period, a stable baseline was obtained (319±16 mg (mean±SEM), n=16) and experiments commenced. Bronchial rings were electrically stimulated by square wave pulses of 40 V, 16 Hz, 0.2 ms for 10 s. At least three consistent and similar EFS-induced contractions were obtained to check the reproducibility of the response. Increasing concentrations of theophylline (10–100 μM) or enprofylline (1–50 μM), or solvent alone, were added to the bath when isometric force had returned to baseline. A 10 min incubation period was allowed before the next EFS (fig. 1).

To study the effect of xanthines on contractions induced by exogenous substance P (SP), two ring segments of the left main bronchi of the same animals as above were used. An initial force of 800 mg was applied and bronchial rings were equilibrated for 60 min in the organ bath with 1 or 2 readjustments of the tension. After obtaining a stable baseline (658±13 mg (mean±SEM), n=12), theophylline (50 μM) or enprofylline (10 μM) at concentrations inhibiting about 50% of the e-NC contraction determined in two EFS experiments, or solvent, was preincubated with the tissue for 10 min before SP was added in a cumulative fashion (0.03–3 μM).

Reagents and buffer

K-H buffer had the following composition (mM): NaCl 120; KCl 4.75; $CaCl_2$ 1.25; $MgSO_4$ 1.2; glucose 10; KH_2PO_4 1.15; $NaHCO_3$ 25. Substance P (Sigma, St-Louis, USA) and theophylline (Laboratoires Bruneau, France) were used. Enprofylline was a gift from C. Persson (Astra-Draco, Lund, Sweden). A 5% solution of 1 M NaOH was used as a solvent for enprofylline. Solvent alone, used as control, had no effect on the bronchial contractile responses.

Expression of the results and statistical analysis

Baseline force and induced contractions were expressed as means±SEM (mg). Since the initial contractions were not significantly different between groups, inhibition of the contractile responses to EFS was expressed as percentage reduction of the initial contraction. Comparison between initial e-NC contractions (mg) was made by unpaired t-test. Xanthines-induced inhibitions of e-NC contractions, and SP-curves in the presence and absence of the xanthines were compared by a two-way analysis of variance.

Results

Figure 1 is a representative example of the inhibitory effect of both theophylline and enprofylline as compared with solvent on the long-lasting e-NC contraction to EFS.

Initial e-NC contractions were similar for the three groups of experiments, *i.e.* theophylline, enprofylline, and solvent (table 1). Neither theophylline nor enprofylline modified resting tension or the force of cholinergic contraction at any of the concentrations used.

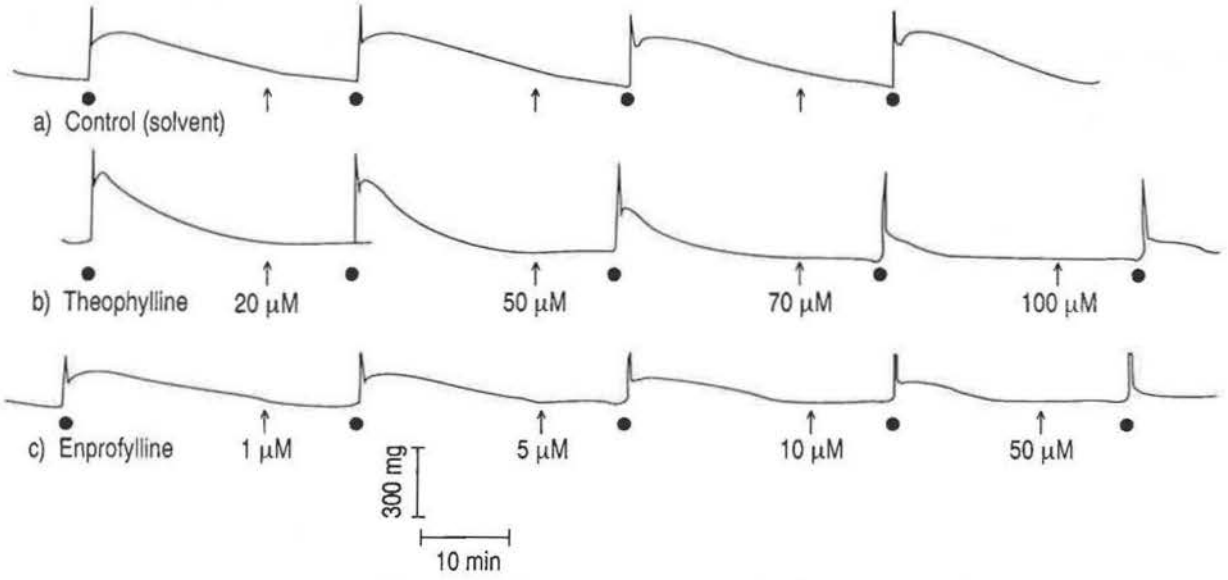

Fig. 1 – Typical experiments showing the effect of: a) solvent compared to b) theophylline and c) enprofylline on electrical field stimulation (EFS)-induced (40 V, 16 H, 0.2 ms for 10 s; dots) contraction of guinea-pig bronchi. Theophylline, enprofylline or solvent were added 10 min before each EFS when force had returned to baseline.

Table 1. – Effect of theophylline (Theo), enprofylline (Enpro), and Solvent, on the e-NC contraction of guinea-pig bronchi induced by EFS

Theo Dose μM	0	10	20	50	75	100		
e-NC mg	203 ±57	187 ±45	148 ±37	117 ±25	73 ±18	37 ±12		
Enpro Dose μM	0	1	2	5	7	10	20	50
e-NC mg	132 ±25	122 ±23	100 ±26	92 ±21	80 ±18	66 ±17	46 ±13	14 ±9
Solvent								
e-NC mg	130 ±34	130 ±34	129 ±33	128 ±34	124 ±34	122 ±34	122 ±33	119 ±33

Results are expressed in mg of e-NC contraction±SEM of 6 (Theo), 5 (Enpro) and 4 (Solvent) experiments. e-NC: Long-lasting non-cholinergic; EFS: electrical field stimulation.

Conversely, there was a significant and concentration-dependent reduction of the e-NC contraction both with theophylline (10–100 μM) and enprofylline (1–50 μM): EC_{50}=62.0±4.7 μM and 9.6±0.7 μM, respectively (table 1, fig. 2). The lowest concentration that caused a significant inhibition was 5 μM for enprofylline ($p<0.01$) and 20 μM for theophylline ($p<0.05$). Experiments run in parallel, with solvent added to the bath at the time theophylline and/or enprofylline were added, showed only a slight variation of e-NC contraction during time (fig. 2).

Theophylline and enprofylline at both concentrations close to the above mentioned EC_{50}s (50 and 10 μM, respectively) had no effect on the contractile response to exogenous SP (0.03–3 μM) in the guinea-pig main bronchi (fig. 3).

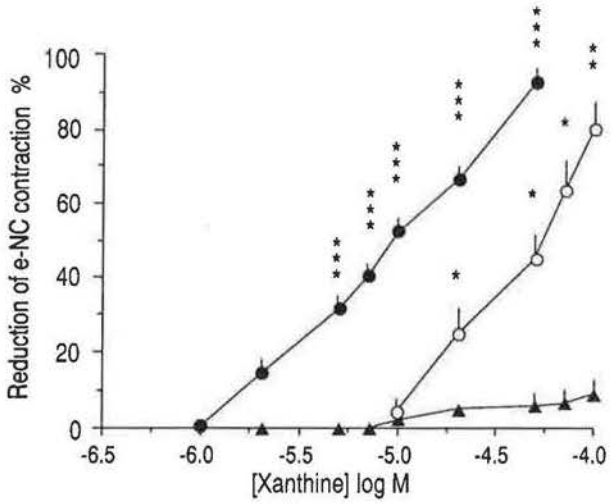

Fig. 2. Effects of increasing concentrations of theophylline (○), enprofylline (●) and solvent (▲) on the e-NC contractile response to EFS in guinea-pig bronchial rings. Values are mean±SEM (bars) of 6 (theophylline), 5 (enprofylline) and 4 (solvent) experiments and are expressed as percentage of reduction of initial e-NC contraction. *: $p<0.05$, **: $p<0.02$; ***: $p<0.01$. e-NC: long-lasting non-cholinergic; EFS: electrical field stimulation.

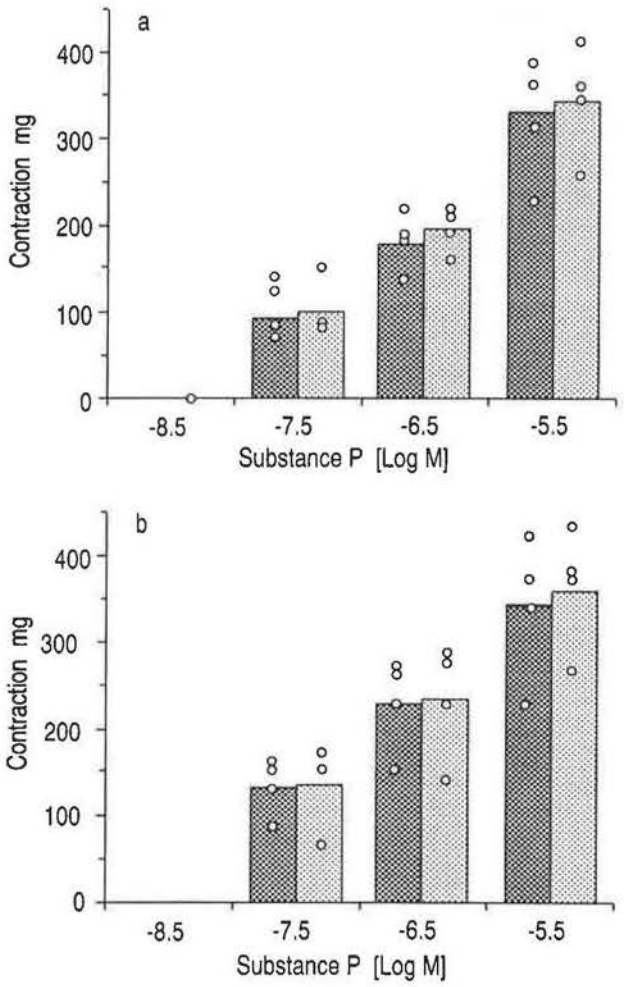

Fig. 3. – Contractile responses of guinea-pig main bronchi to increasing concentrations of substance P in the presence of: a) 50 μM theophylline; or b) 10 μM enprofylline; or solvent. Individual values of four experiments are indicated by open circles and group average values are height of column. ▨: xanthine; ▩: solvent.

Discussion

Our results show that both theophylline and enprofylline at clinically relevant concentrations significantly reduce the e-NC contraction of guinea-pig bronchi. This effect is not due to a reduced contractile response of bronchial smooth muscle, since the cholinergic contraction was unaffected. Moreover, the contraction to exogenous SP, a neuropeptide of the e-NC nerves, was also unaffected, suggesting that neither affinity nor responsiveness of SP receptors was modified by xanthine derivatives. Altogether, these results suggest that the two xanthines used reduce the release of neuropeptides from e-NC nerve endings. Since enprofylline, a xanthine devoid of effect on adenosine receptors, is active, this effect is independent of adenosine receptors.

Our results, showing that both theophylline and enprofylline attenuate the release of the e-NC neuropeptides induced by EFS, are in accordance with the inhibition by theophylline of capsaicin-induced bronchoconstriction in the guinea-pig *in vivo* [20]. Capsaicin releases sensory neuropeptides from C-fibre endings [21]. In the guinea-pig airways, e-NC nerves are probably the afferent sensory C-fibres. Their neuromediators are the neuropeptides substance P, neurokinin A and calcitonin gene-related peptide, capable of causing neurogenic bronchoconstriction, mucosecretion, vasodilatation and increase in microvascular permeability, leading to plasma exsudation and inflammatory cell infiltrate in the airways in animals [14, 17, 22–24]. In humans, although no effect of exogenous tachykinins is observed in the airways of healthy subjects, airway obstruction is induced by inhalation or nebulization of tachykinins in patients with allergic asthma [25] or rhinitis [15, 26]. SP-induced nasal obstruction is associated with influx of albumin, protein and inflammatory cells, polymorphonuclear cells and eosinophils, in the nasal lavage fluid [15, 27]. Sensory neuro-peptides are released by nerve endings in the airways in response to local mechanical, chemical or pharmacological irritation [16, 22], and it has been suggested that structural changes in asthmatic subjects may facilitate this release. Therefore, our finding that xanthines reduce the release of sensory neuropeptides in guinea-pig airways may be relevant to their anti-inflammatory effect in human asthma [28]. Direct anti-inflammatory effects of xanthines, that may also account for their therapeutic effect in asthma, have been described, *e.g.* decrease in permeability oedema induced by various mediators in animal [2, 3] and human airways [4, 28], increase in mucociliary clearance [29] and inhibition of the activation of polymorphonuclear neutrophils by inhibition of the production of oxygen metabolites [1].

The primary molecular mechanism involved in the effect of xanthines on e-NC contraction remains obscure. Both enprofylline and theophylline, at the therapeutic doses used (15–20 $\mu g \cdot ml^{-1}$), are weak phosphodiesterase inhibitors. Indeed, total phosphodiesterase activity in human lung extracts is only reduced by about 5–10% at therapeutic levels of theophylline in plasma [30, 31]. In human bronchioles *in vitro*, inhibition of one of the four isoforms of phosphodiesterase was only 50% at a concentration of 100 μM of theophylline [32]. An antagonism of theophylline at adenosine receptor level has been suggested as an explanation for its anti-asthmatic effect at therapeutic plasma concentration [2, 33]. However, in our study, enprofylline, a xanthine derivative devoid of adenosine antagonism, was a more potent inhibitor of e-NC contraction than theophylline. Moreover, adenosine itself inhibits e-NC contractions of guinea-pig bronchi [34]. It is, therefore, unlikely that adenosine receptor antagonism is involved in the observed effects of xanthines. Another effect of xanthines is translocation of intracellular calcium. Both theophylline and enprofylline influence Ca^{++} fluxes and redistribution, at therapeutic concentrations similar to those used in our study, and do not modify cyclic adenosine monophosphate (AMP) levels [35, 36]. Whether these effects of xanthines on calcium account for the inhibition of e-NC contraction that we observed in the airways has still to be demonstrated.

In conclusion, our results suggest that both theophylline and enprofylline are capable of reducing the release of sensory neuropeptides from C-fibre endings. We therefore submit that e-NC neurotransmission itself is a target for xanthines in the airways and that inhibition of the release of e-NC neuropeptides is a possible mechanism of action for xanthines in asthma. The anti-inflammatory effect of xanthines in the airways may be linked to this inhibition of release of the pro-inflammatory neuropeptides. Therefore, further research on xanthine-induced control of neurogenic inflammation in asthma might be useful.

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