The Effects of Methylxanthines, Ethymizol, Ephedrine and Papaverine on Guinea Pig and Dog Trachea

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Abstract. The study was aimed to compare the effects of pentoxyphylline, aminophylline, choline theophyllinate and ethymizol on guinea pig and dog trachea with those of theophylline, papaverine and ephedrine. The effects of these drugs on the basal tension, on dose-response curves for muscle contraction produced by histamine and on cAMP level were investigated in guinea pig trachea, together with their influence on the resting and histamine-evoked mechanical and membrane activities of dog trachea. Like papaverine, pentoxyphylline did not alter the resting membrane potential, although it relaxed both tracheal preparations, and it antagonised the effects of histamine and raised the cAMP level of the smooth muscle. The effects of ethymizol were similar to those of theophylline and its water soluble derivatives (aminophylline and choline theophyllinate). Whereas, ephedrine although it decreased the basal tension and inhibited histamine-evoked responses, also elicited substantial hyperpolarisation of the smooth muscle membrane with no effect on the cAMP level. These findings are consistent with the hypothesis that cAMP has an important role in the action of some bronchodilator drugs; however, it is concluded that the possibility of contributing of their action on membrane potential to their action needs to be considered. The similarity of the potencies of ethymizol and pentoxyphylline to that of classical bronchodilators in inhibiting contraction of guinea pig and dog tracheal smooth muscle suggests that they may have a therapeutic value.

Key words: Trachea — PDE inhibitors — Ethymizol — Ephedrine

Introduction

It has been suggested that certain pharmacological agents produce relaxation of airway smooth muscles as a result of their ability to increase the cAMP level (Triner et al. 1977).

Methylxanthines are widely used in the treatment of bronchial asthma,

mainly because of their inhibitory action on phosphodiesterase (PDE) and the resulting bronchodilator effect (Polson et al. 1979; Clarc 1982; Shenfield 1982).This inhibition of PDE activity and the consequent accumulation of cAMP is produced not only by methylxanthines but also by drugs of different chemical structures, such as papaverine (Kukovetz and Pöch 1970). The bronchodilator effect of methylxanthines may also involve mechanisms distinct from the effect on PDE activity (for review see Shenfield 1982 and Aubier et al. 1984). Because of the poor water solubility of theophylline, its more soluble complexes or salts, e.g. aminophylline and choline theophyllinate, were introduced into clinical practice.

The aim of the present study was to compare the action of aminophylline, choline theophyllinate, pentoxyphylline and ethymizol with that of the classical PDE inhibitors, theophylline and papaverine (Kukovetz and Pöch 1970; Kukovetz et al. 1983) and with that of an adrenoceptor agonist, ephedrine (Tattersfield 1982), on the resting and evoked tension, membrane characteristics and cAMP levels of the guinea pig and dog tracheal smooth muscle.

Materials and Methods

Experiments were performed on isolated smooth muscle preparations of guinea pigs and dogs

Measurement of mechanical activity in guinea pig preparations

Male guinea pigs 300—450 g were sacrificed and bled and the tracheae were quickly removed and placed in a modified Krebs solution The isolated preparations were prepared by cutting the trachea along its longitudinal axis as described earlier (Todorov 1977) The prepared tissues were placed in a 30 ml organ bath After an equilibration period of 45 min at about 20 mN tension, the actual experiments were carried out under a tension of about 5 mN

The cumulative concentration-response curves (CRCs) for histamine were prepared using the methods of van Rossum and van den Brink (1963) Increasing concentrations of histamine were applied at intervals which allowed the effects of each concentration to develop fully After repeated washings until the basal tension was restored, the smooth muscle relaxants were added to the bathing fluid for 5 min (the time required to reach a new steady state) and CRC of histamine was repeated

Isometric smooth muscle contractions were recorded with a strain gauge transducer The experiments were performed at 37 °C

Measurements of membrane and mechanical activity of the dog trachea

Dogs of both sexes 15—20 kg were used The animals were anesthetized by i v injection of phenobarbital (30 mg kg^{-1}) The muscle strips were excised from the cervical trachea The smooth muscle was freed from the fascia and cut into 1 to 2 mm wide and 10—15 mm long strips which were placed into a double sucrose gap chamber (Bauer and Zakhari 1977) The central part (0 7—0 8 mm length) of each preparation was perfused with modified Krebs solution Isotonic sucrose solution (270 mmol l^{-1}) and isotonic K_2SO_4 solution (77 5 mmol l^{-1}) were used for the double sucrose gap method Current pulses, usually of 1.3 s duration, intensity of 1 μA and frequency of 0 07 Hz were applied through a series resistance of 50 MΩ Calomel electrodes were used for stimulation and recording Any contact between the sucrose solution and the electrolyte solutions was prevented by using latex membranes Simultaneously the muscle tension was recorded isometrically The experiments were carried out at 32 °C under an initial tension of about 5 mN

Measurements of the cAMP level

The entire trachea was rapidly removed and transferred to a dissecting tray flooded with modified Krebs solution The tracheal smooth muscle was separated from the cartilaginous rings, cut into small pieces, and placed in flasks filled with modified Krebs solution at 37 °C The tissues were then preincubated for 30 min The preincubations was terminated by exchanging the incubation medium for fresh Krebs solution The drugs were applied for 5 min following a 30-min-incubation period Immediately after the flasks were cooled the smooth muscle was removed from the flasks and homogenized in ice-cold 50 % acetic acid with an approximately 1 20 ratio of tissue/acetic acid The homogenates were centrifuged and aliquots of supernatant dried in assay tubes The concentration of cAMP was determined by a modification of the protein binding assay described by Gilman (1970), when separation of protein bound cAMP from unbound nucleotide was achieved by adsorption of

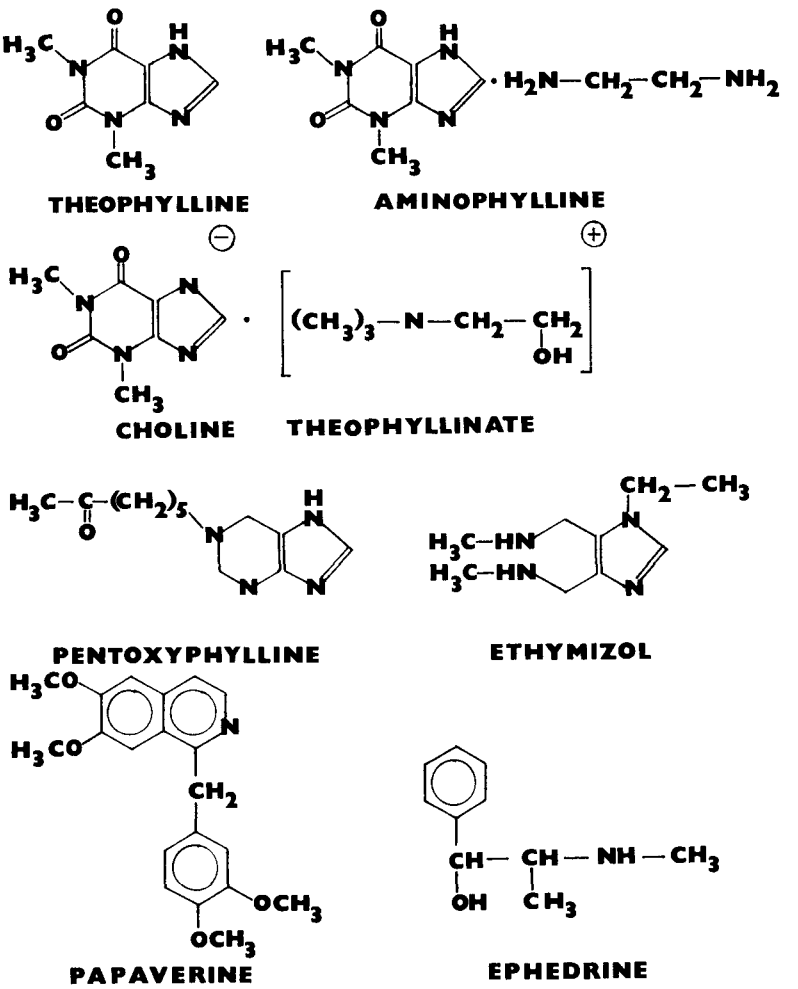

Fig. 1. Chemical structures of the relaxants studied

free cAMP on dextran coated charcoal (Kolena and Channing 1972) Each estimation of cAMP was performed in duplicate The protein content was determined in homogenates by the colorimetric method according to Lowry et al (1951) The values are given in pmoles cAMP/mg protein The modified Krebs solution of composition (mmol l^{-1}) NaCl 123, KCl 6 2, $CaCl_2$ 2 7, $MgSO_4$ 1 2, $NaHCO_3$ 15 4, NaH_2PO_4 1 2 and glucose 11 5 was gassed with 95% O_2 and CO_2

The following drugs were used cyclic (^3H) AMP (Radiochemical Centre, Amersham), aminophylline (Merck), choline theophyllinate (Spofa), ephedrine (Merck), ethymizol (Medexport), histamine hydrochloride (Merck), pentoxyphylline (Hoechst), papaverine hydrochloride (Merck), theophylline (Spofa) The chemical structures of the relaxants studied are shown in Fig 1

The results are expressed as arithmetic means, with standard errors of the mean (SEM) Differences were tested for significance by Student s *t*-test for paired observations

Results

Action of histamine on the guinea pig and dog trachea

Histamine (0 1 μmol l^{-1} — 1 mmol l^{-1}) elicited concentration-dependent contractions of the guinea pig trachea (Fig 2) with a mean pD_2 value of 5 45 ± 0 07 and at a concentration of 0 1 mmol l^{-1} also significantly enhanced the cAMP level (Table 1)

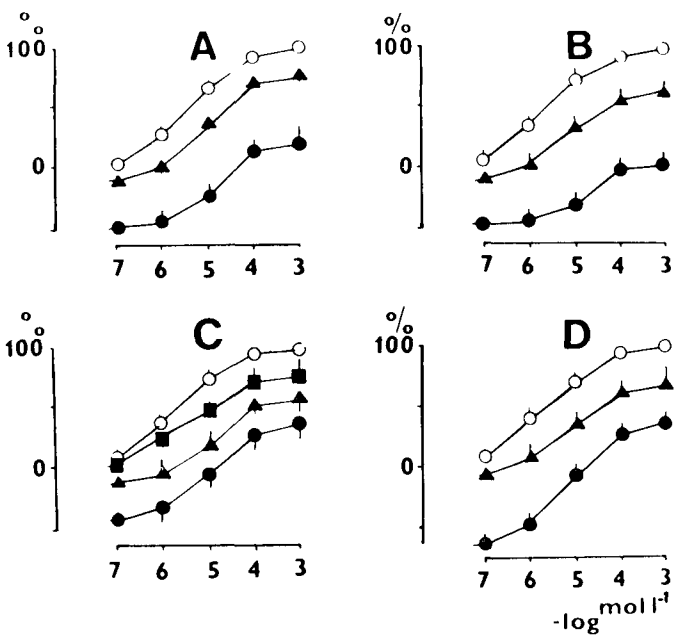

Fig. 2. Effect of theophylline (*A*), aminophylline (*B*), choline theophyllinate (*C*) and pentoxyphylline (*D*) on the contractile action of cumulatively applied histamine in guinea pig tracheal smooth muscle ○ — control response to histamine, responses to histamine after pretreatment with the methylxanthines studied are represented by ■ — 3 μmol l^{-1}, ▲ — 30 μmol l^{-1} and ● — 300 μmol l^{-1} Each point represents the mean of 6 to 10 trials, vertical lines show SEM

Table 1. Effects of drugs on cAMP levels in the guinea pig trachea

Compounds	Concentration mmol l^{-1}	Cyclic AMP pmoles mg proteins	n
Control		13 4 ± 1 1	15
Theophylline	3	16 7 ± 3 8	5
Choline theophyllinate	3	24 0 ± 4 3*	5
Pentoxyphylline	0 3	18 7 ± 1 6*	5
Pentoxyphylline	3	23 9 ± 3 6*	5
Ethymizol	3	29 3 ± 5 3*	5
Papaverine	3	26 3 ± 1 2*	5
Ephedrine	3	16 2 ± 1 0	5
Histamine	0 1	22 2 ± 2 3*	5

* significant differences from the control cAMP level $p < 0\ 05$

The membrane potential of the dog trachea under the double sucrose gap condition was 59 0 ± 1 4 mV ($n = 39$) Neither spontaneous nor evoked action potentials in dog trachea, nor spontaneous contractions of either the dog or guinea pig trachea were observed

Histamine (10 μmol l^{-1}) significantly depolarised the smooth muscle membrane, evoked fluctuations of the membrane potential and in some preparations the inward current pulses also elicited action potentials The smooth muscle tension was also significantly increased (Table 2)

Interactions between histamine and some relaxants of guinea pig and dog trachea

Exposure of the tracheal smooth muscle to the relaxants studied caused changes of the tension of the guinea pig trachea, its membrane potential and of the tension of the dog trachea which developed fully within 3—5 min Therefore their interaction with histamine was studied after 5 min pretreatment of the preparations with the drugs studied

Theophylline (0 03 and 0 3 mmol l^{-1}) relaxed the guinea pig trachea by 2 05 ± 0 28 mN ($n = 8$) and 7 78 ± 1 18 mN ($n = 6$), respectively The CRCs of histamine induced contractions were shifted to the right, the contraction amplitudes were reduced by 15 to 30 % and the maximal tension was reduced by 25 to 80 % (Fig 2*A*)

Theophylline (10 μmol l^{-1}) significantly enhanced the membrane potential and reduced the basal tension of the dog trachea but had no effect on its membrane resistance Histamine (10 μmol l^{-1}) after theophylline pretreatment depolarised the smooth muscle membrane to the same level as in untreated preparations but its effect on the basal tension was reduced by more than 50 % (Table 2)

Table 2. Action of the drugs on the mechanical and electrical activation of the dog trachea

Drug	Concentration mmol l^{-1}	Membrane potential ΔmV	Muscle tension ΔmN	Membrane resistance %	*n*
Histamine	0 01	−6 0 ± 0 8*	+1 3 ± 0 3*	103 7 ± 2 2	27
Theophylline	0 01	+3 6 ± 0 8*	−0 2 ± 0 03*	92 2 ± 4 3	4
Theophylline + Histamine	0 01 0 01	−6 2 ± 1 3o	+0 4 ± 0 02†o	103 0 ± 2 0	4
Pentoxyphylline	0 3	+0 3 ± 0 2	−0 2 ± 0 05*	104 6 ± 3 0	5
Pentoxyphylline + Histamine	0 3 0 01	−1 0 ± 0 1†o	+0 1 ± 0 03†o	96 0 ± 3 1	5
Ethymizol	0 03	+3 7 ± 1 2*	−0 4 ± 0 02*	93 6 ± 5 0	5
Ethymizol + Histamine	0 03 0 01	−2 4 ± 0 5†o	+0 3 ± 0 1†o	98 2 ± 4 4	5
Papaverine	0 01	−0 8 ± 0 7	−0 5 ± 0 2*	97 2 ± 3 5	5
Papaverine + Histamine	0 01 0 01	−4 5 ± 0 5o	+0 9 ± 0 4o	99 0 ± 3 8	5
Ephedrine	0 3	+4 4 ± 1 0*	−1 4 ± 0 1*	97 3 ± 4 7	7
Ephedrine + Histamine	0 3 0 01	−1 8 ± 0 6†o	−1 9 ± 0 3*	96 0 ± 2 8	4

− depolarisation and relaxation
+ hyperpolarisation and contraction
* significant differences from the control membrane potential or muscle tension $p < 0\ 05$
† significant differences from the response to histamine $p < 0\ 05$
o significant differences from the response to the respective antagonist alone $p < 0\ 05$

In contrast, theophylline even at a concentration of 3 mmol l^{-1} induced no significant changes in the cAMP level, although it tended to increase it (Table 1)

Aminophylline (0 03 and 0 3 mmol l^{-1}) reduced the initial tension of the guinea pig trachea by 3 1 ± 0 7 mN ($n = 8$) and 10 2 ± 0 9 mN ($n = 6$), respectively The CRC of histamine was not only shifted to the right but the maximal effect of histamine was also significantly reduced, hence the slope of the CRC was flattened (Fig 2*B*)

Choline theophyllinate (0 003, 0 03 and 0 3 mmol l^{-1}) relaxed the guinea pig trachea by 0 07 ± 0 29 mN ($n = 10$), 2 9 ± 0 8 mN ($n = 8$) and 9 2 ± 1 2 mN ($n = 8$), respectively Pretreatment of the preparations by this xanthine derivative even at the lowest concentration, which did not alter basal tension, signifi-

cantly suppressed the maximal contractions elicited by histamine. In spite of the high concentration of choline theophyllinate (0.3 mmol . l^{-1}) and significant reduction of the basal tension, histamine still produced smooth muscle contractions, which however could not reach levels higher than 40 % of those in untreated tissues (Fig. 2*C*). In other experiments choline theophyllinate (3 mmol . l^{-1}) significantly enhanced the cAMP level of guinea pig tracheal smooth muscle Table 1.

Pentoxyphylline (0.03 and 0.3 mmol . l^{-1}) reduced the initial tension of the guinea pig tracheal preparations by 2.2 ± 0.6 mN ($n = 6$) and 10.9 ± 0.9 mN ($n = 6$), respectively. Bathing the strip in pentoxyphylline containing solution for 5 min did not significantly affect the amplitude of the histamine-induced contractions or the slope of its CRC. The tension reached was however lowered, and this corresponded with the extent of the relaxation caused by pentoxyphylline alone (Fig. 2*D*). Likewise, pentoxyphylline (0.3 mmol . l^{-1}) relaxed the dog trachea but the membrane potential and membrane resistance remained unaltered. The effects of histamine (10 μmol . l^{-1}) on the membrane potential and muscle tension were also attenuated by pentoxyphylline pretreatment (Table 2). Consistently with the smooth muscle relaxation, pentoxyphylline (0.3 and 3 mmol . l^{-1}) enhanced the cAMP level in a concentration-dependent manner (Table 1).

Exposure of the guinea pig tracheal smooth muscle to ethymizol (0.03 and 0.3 mmol . l^{-1}) resulted in smooth muscle relaxations of 5.1 ± 0.7 mN ($n = 6$) and 8.1 ± 2.0 mN ($n = 8$), respectively. At lower concentration (0.03 mmol . l^{-1}) ethymizol shifted the histamine CRC to the right without affecting its maximum, whereas in the higher concentration (0.3 mmol . l^{-1}) it also reduced the amplitude of the histamine evoked contractions, thus flattening the slope of its CRC (Fig. 3*A*). Ethymizol (3 mmol . l^{-1}) also significantly elevated the cAMP level of the guinea pig tracheal muscle (Table 1). The action of ethymizol (0.03 mmol . l^{-1}) on the dog trachea was characterized by membrane hyperpolarisation, muscle relaxation and significant reduction of the action of histamine (Table 2).

Papaverine (1, 3 and 10 μmol . l^{-1}) induced concentration-dependent relaxation of the guinea pig trachea of 3.2 ± 0.8 mN ($n = 6$), 6.8 ± 1.5 mN ($n = 6$) and 8.8 ± 1.2 mN ($n = 8$), respectively. At low concentration (1 μmol . l^{-1}) papaverine did not change significantly either the slope of the histamine CRC of the amplitude of histamine induced contractions. In contrast, concentrations of papaverine above 3 μmol . l^{-1} suppressed the amplitude of the contractions elicited by histamine (Fig. 3*B*). Exposure of the dog trachea to papaverine (10 μmol . l^{-1}) resulted in smooth muscle relaxation but the membrane potential, membrane resistance and amplitude of the histamine induced depolarisation and contraction remained unaltered. In contrast to the effect of histamine in

untreated preparations, in the presence of papaverine the level of membrane potential reached and muscle tension tended to be higher and lower, respectively, although without statistical significance (Table 2). Papaverine (3 mmol . l^{-1}) also significantly increased the cAMP level in guinea pig trachea (Table 1).

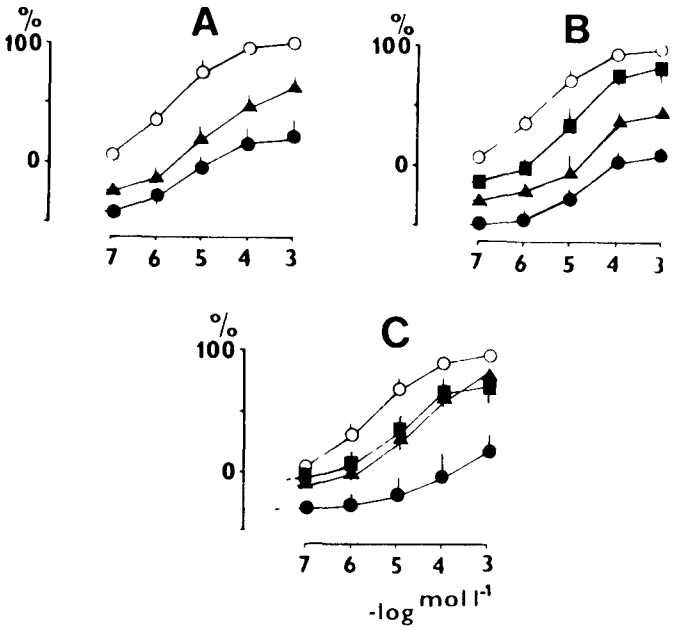

Fig. 3. Effect of ethymizol (*A*, 30 and 300 μmol l^{-1}), papaverine (*B*, 1, 3 and 10 μmol l^{-1}) and ephedrine (*C*; 3, 30 and 300 μmol . l^{-1}) on the contractile action of cumulatively applied histamine in the guinea pig tracheal smooth muscle. ○ — control response to histamine; responses to histamine after pretreatment by the respective relaxants in ascending order of concentration represented by (■), (▲) and (●). Each point represents the mean of 6 to 8 trials, vertical lines show SEM

Ephedrine (0.003, 0.03 and 0.3 mmol . l^{-1}) decreased the basal tension of the guinea pig trachea by 1.5 ± 0.5 mN (n = 7), 2.7 ± 0.4 mN (n = 6) and 6.1 ± 0.6 mN (n = 6), respectively. In concentrations up to 30 μmol . l^{-1} it shifted the CRC of histamine to the right without significant alteration in its slope and with only a small 5 to 30 % reduction of the amplitude of histamine induced contractions. At high concentration (0.3 mmol . l^{-1}) ephedrine significantly flattened the slope of the histamine CRC due to the pronounced reduction of the amplitude of histamine evoked contractions (Fig. 3*C*). In the dog trachea ephedrine at low concentration (10 μmol . l^{-1}) was ineffective. At a high concentration (0.3 mmol . l^{-1}), however, ephedrine hyperpolarised the membrane and relaxed the smooth muscle. In the presence of this high concentration of ephedrine histamine still depolarised the smooth muscle membrane, although to a lower extent than under control conditions, but its ability to contract the smooth

muscle was abolished (Table 2) Ephedrine (3 mmol l^{-1}) tended to increase the cAMP level of the guinea pig trachea but not significantly (Table 1)

Discussion

The tracheal smooth muscles did not exhibit regenerative electrical and spontaneous mechanical activities under resting conditions as previously described by Kirkpatrick (1975), Suzuki et al (1976) and Coburn and Yamaguchi (1977) on the bovine, canine, and guinea pig tracheal smooth muscles, respectively

The guinea pig tracheaobronchial smooth muscles react in a similar way to those of human asthmatics (Herxheimer 1967) Histamine is among the endogenous bronchoactive agents liberated also in allergy in man (Assen and Shild 1968) Its action on the electrical and mechanical activities of the tracheal smooth muscle was exitatory, while like some other bronchoconstrictor agents, e g acetylcholine and carbachol, it elevated the cAMP level (Murad and Kimura 1974, Creese and Denborough 1980, Bilčikova et al 1987) This apparent contradiction between the change in the tension and cAMP level may result from a) the presence of both excitatory and inhibitory histaminergic receptors on the tracheobronchial tree of different animal species, including guinea pigs and dogs (Krell and Charkin 1977, Okpako et al 1978), b) the proposed existance of distinct subcellular cAMP compartments coupled in a different way with the contraction-relaxation cycle (Bilčikova et al 1987) and/or c) the simultaneous histamine induced rise of cytoplasmic calcium ion concentration and release of bronchodilator prostaglandins, which stimulate adenylate cyclase (Creese and Denborough 1980)

Triner et al (1977) found that the bronchodilator potency and ability to enhance cAMP formation were similar in the case of papaverine, theophylline and catecholamines in dog bronchi and assumed that the cAMP system was one of the mechanisms mediating respiratory smooth muscle relaxation In the present study we attempted to find an association between the membrane potential or cAMP level changes on the one hand which may be involved in the effect of drugs in the trachea, and the smooth muscle tension changes on the other hand

Theophylline, one of the methylxanthines widely used in the treatment of bronchial spasm, has poor water solubility Its solubility is much enhanced by the formation of complexes such as aminophylline (theophylline and ethylendiamine) or true salts such as choline theophyllinate (Cushley and Holgate 1984) There were some differences between the action of theophylline (no more than a tendency to increase) and choline theophyllinate (significant increase) on the cAMP level, and between the more pronounced suppression of histamine induced contractions by aminophylline and choline theophyllinate than theophylline in low concentrations These differences were probably due to the

different solubility of the drugs studied and to their relatively short contact time with the preparations.

The nature of theophylline action on the basal tension and histamine evoked contractions was similar to that of papaverine. There were, however, also differences between the actions of these two PDE inhibitors, i.e. in contrast to theophylline, papaverine did not alter significantly the resting membrane potential. Moreover, papaverine was more effective in the guinea pig trachea than theophylline on a molar basis, whereas in the dog trachea they both antagonized the histamine effects almost equieffectively.

Pentoxyphylline, a water soluble theobromine derivative, had similar effects to those of choline theophyllinate in the guinea pig trachea. Similarly as papaverine, it did not affect the resting membrane potential even in high concentrations but significantly suppressed the action of histamine.

Ephedrine, an adrenoceptor agonist (Tattersfield 1982) which possesses rather selective α_2-receptor agonist properties (Bauer 1982), was devoid of β-adrenotropic actions because of its lack of ability to enhance significantly the cAMP level in contrast to β-adrenoceptor agonists (Bilčíková et al. 1987). The pronounced hyperpolarising effect of ephedrine and its efficacy in suppressing or abolishing the contractile actions of histamine on the guinea pig and dog trachea suggest that in addition to raising the cAMP level also membrane hyperpolarisation might be responsible for the inhibitory action of the drug studied on the respiratory smooth muscle. The membrane hyperpolarisation produced by PDE inhibitors found in the present experiments and that caused by β-adrenoceptor agonists (Ito and Tajima 1982; Bilčíková et al. 1987) do not necessarily result from an enhanced cAMP level, since papaverine and pentoxyphylline which significantly elevated the cAMP level did not alter the membrane potential.

Ethymizole, a xanthine-related respiratory analeptic (Strukov 1973; Mashkovskyj 1977), which lacks the PDE inhibitory effects and enhances the cAMP level as a consequence of activation of adenylate cyclase (Migas and Bulion 1974), substantially relaxed the tracheal smooth muscles of guinea pigs and dogs.

As previously concluded (Murad and Kimura 1974; Katsuki and Murad 1977; Creese and Denborough 1980; Ito and Tajima 1982) and also confirmed by the results presented here the relaxation of the respiratory smooth muscles may result at least in part from an elevation in the cAMP level and may also partly involve other mechanisms, such as hyperpolarisation of the smooth muscle membrane. Of the drugs studied, papaverine and pentoxyphylline relaxed the smooth muscle mainly as a consequence of their elevation of the cAMP level, ephedrine mainly due to its action on the membrane potential and theophylline and ethymizol through both of the above mechanisms.

The results of our study also indicate that pentoxyphylline, in addition to its

vasodilator properties (Mansfeld 1972, Stefanovich 1973), and ethymizol, in addition to its respiratory stimulant effects (Strukov 1973, Mashkovskij 1977), possess substantial broncholytic properties These findings suggest that they might also be useful in the therapy of bronchial spasms

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