The Action of Adrenoceptor Agonists and Antagonists on the Guinea Pig and Dog Trachea

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Abstract. The action of β- and α-adrenoceptor agonists (isoprenaline, orciprenaline, noradrenaline, phenylephrine and ephedrine) and antagonists (propranolol, metipranolol, exaprolol, BL 445 and phentolamine) on the resting tension and cAMP level of the guinea pig and the mechanical and electrical activities of the dog trachea were studied. By activating β_2-adrenoceptors, isoprenaline and orciprenaline relaxed the smooth muscle, elevated the membrane potential and attenuated the excitatory effect of histamine on membrane potential and muscle tension. Noradrenaline and phenylephrine, acting on α_1-receptors, did not affect the membrane potential and increased the basal tension of the dog trachea only insignificantly. Ephedrine, in high concentrations, however, hyperpolarized the smooth muscle membrane and relaxed the dog trachea, while it did not alter the cAMP level in the guinea pig preparations. It is, therefore unlikely that α_1-adrenoceptors play a major role in the excitation of the dog trachea under resting conditions whereas the participation of α_2-receptors in the mechanisms of adrenergic relaxation could not be ruled out. All the β-adrenoceptor antagonists studied enhanced the action of low isoprenaline concentrations and competitively antagonized it in high concentrations. The order of their antagonistic potency in the guinea pig trachea was as follows: metipranolol > propranolol $\doteq$ exaprolol $\geqslant$ BL 445. It was suggested that metipranolol and exaprolol are nonselective β-adrenoceptor antagonists, similarly as propranolol, whereas BL 445 shown some β_1-selectivity. In contrast to their antagonistic effects on the membrane activities and muscle tension, both histamine and isoprenaline increased the level of cAMP in smooth muscle cells and, when present simultaneously, their effect was additive. The mechanism of histamine-induced cAMP level elevation and the possible involvement of dif-

ferent subcellular compartments in the action of isoprenaline and histamine in relation to the contraction-relaxation cycle is discussed

Key words Trachea — Adrenoceptor agonists and antagonists

Introduction

The physiological and pharmacological basis of catecholamine actions on smooth muscle preparations has been studied intensively during the last decades The classification of adrenoceptors into α- and β-types (Ahlquist 1948) and their subtypes β_1-, β_2- (Lands et al 1967) and α_1-, α_2- (Langer 1974) has been widely accepted

Stimulation of α-adrenoceptors induces bronchoconstriction, and β-adrenoceptor stimulation induces bronchodilatation as has been repeatedly shown (Swedmyr 1970, 1971, 1977, Olsson et al 1979, Ohno et al 1981, Leff and Munoz 1981, Barnes et al 1983) Most β-adrenoceptor antagonists increase the basal tension of isolated respiratory smooth muscles and augment resistance of the airways (Hexheimer 1967, Chang et al 1978)

Sutherland and Robinson (1966) have suggested that adenylate cyclase is coupled to β-adrenoceptors in many tissues Although this hypothesis is plausible, many experimental data have indicated that the increase in the cAMP level may not always correlate with relaxation induced by agonists of β-adrenoceptors (for review, see Scheid et al 1979)

The aim of the present study was therefore to characterize the action of α- and β-adrenoceptor agonists on the mechanical and electrical activities, as well as on the level of cAMP in tracheal smooth muscle Since several new β--adrenoceptor antagonists were recently synthetized in Czechoslovakia and some of them have also been introduced into clinical practice, we were interested in studying the interactions of these new drugs with the effects of isoprenaline on the above mentioned activities of the tracheal smooth muscle, and to compare these interaction with those of propranolol and phentolamine

Materials and Methods

Experiments were performed on isolated guinea pig and dog smooth muscle preparations

Measurement of mechanical activity in guinea pig trachea

Male guinea pigs (300—450 g) were sacrificed and bled and the tracheae were quickly removed and placed into 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 inserted in a 30 ml organ bath After an equilibration period of 45 min at a tension of about 20 mN, the experiments were carried out under a tension of about 5 mN

The cumulative concentration-response curves (CRCs) for isoprenaline, orciprenaline and histamine were constructed by the methods of van Rossum and van den Brink (1963) Increasing concentrations of the drugs were applied at intervals, allowing a full development of the effects of each concentration tested After repeated washings, when the basal tension recovered, adrenoceptor antagonists were added to the bathing fluid for 15—20 min (the time required to reach a new steady state tension) and the CRCs to isoprenaline and histamine were repeated

Isometric 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 either sex (15—20 kg) were used The animals were anesthetized by intravenous administration of phenobarbital (30 mg kg ^{1}) Muscle strips were excized 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, the strips were then placed to a double sucrose gap chamber (Bauer and Zakhari 1977) The central part (0 7—0 8 mm in length) of the preparations 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 1 3 s in duration, 0 2 to 4 μA in intensity and a frequency of 0 07 Hz were applied through a series resistance of 50 MΩ Calomel electrodes were used for the stimulation and recording Any contact between the sucrose solution and electrolyte solutions was prevented by using latex membranes Simultaneously, the isometric muscle tension was recorded The experiments were carried out at 32 °C under an initial tension of about 5 mN

In a special series of experiments, designed to study the action of isoprenaline on the muscle tension of dog trachea, preparations were contracted by serotonin (5-HT) subsequently, isoprenaline was applied during sustained 5-HT-induced contraction

Cyclic AMP level determination

The entire trachea was rapidly removed and transferred into a dissecting tray soaked 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 preincubation was terminated by exchanging the incubation medium for fresh Krebs solution After another 30-min-incubation drugs were applied for 5 min The flasks were cooled and the smooth muscles were immediately taken and homogenized in ice-cold 50 % acetic acid with a tissue—acetic acid ratio of approximately 1 20 The homogenates were centrifugated and aliquots of supernatants dried in assay tubes The concentration of cAMP was determined by a modification of the protein binding assay according to Gilman (1970), following separation of the protein bound cAMP from the unbound nucleotide by adsorption of free cAMP on dextran coated charcoal (Kolena and Channing 1972) Cyclic AMP determination were performed in duplicate The protein content was determined in homogenates by the colorimetric method according to Lowry et al (1951) Results were expressed in pmoles cAMP/mg protein The modified Krebs solution contained (mmol l^{-1}) NaCl 123, KCl 6 2, $CaCl_2$ 2 7, $NaHCO_3$ 15 4, Na_2PO_4 1 2 and glucose 11 5, it was gassed with 95 % O_2 and 5 % CO_2

Following compounds were used cyclic ^{3}H AMP (Radiochemical Centre, Amersham), ephedrine (Merck), exaprolol (Spofa), histamine hydrochloride (Merck), isoprenaline hydrochloride (Spofa), metipranolol (Spofa), noradrenaline bitartrate hydrate (Calbiochem), orciprenaline sulphate (Polfa), phentolamine mesylate (Ciba), phenylephrine hydrochloride (Boehringer Ingelheim), propranolol hydrochloride (Galenika), serotonin creatine sulphate (Calbiochem), substance BL 445 (hydrochloride of buthylester of 4-(2-hydroxy-3-tert buthylamine) propoxycarbanilic acid), (Faculty

of Pharmacy, Comenius University, Bratislava)

The results were expressed as arithmetic means, with corresponding standard errors of the mean (SEM). Differences were tested by Student's *t*-test for paired observations

Results

Effects of adrenoceptor agonists on the guinea pig and dog trachea.

As has been reported repeatedly (Lohman et al. 1977; van der Heijden et al. 1984; Elguindi et al. 1985), cumulatively applied isoprenaline (0.001 to 100 μmol.l^{-1}), induced a concentration-dependent relaxation of the guinea pig tracheal muscle at basal tension (Fig. 3) and of the 5-HT (1 μmol.l^{-1}) precontracted dog tracheal smooth muscle (Fig. 1). In most experiments, maximal relaxation was even obtained with concentrations lower than 1 μmol.l^{-1} of isoprenaline in both guinea pig and dog trachea. The EC_{50} values (with 95 % confidence limits) for isoprenaline induced relaxation of the guinea pig and dog tracheal smooth muscle were 21.4 (8.5—53.7) nmol.l^{-1} and 39.8 (32.4—48.9) nmol.l^{-1} , respectively. Orciprenaline (0.18 μmol.l^{-1} to 1.8 mmol.l^{-1}) also relaxed dog trachea in a concentration-dependent manner, but its affinity to β-adrenoceptors was about 50-times lower than that of isoprenaline. The EC_{50} value for orciprenaline was 1.8 (1.0—3.2) μmol.l^{-1}.

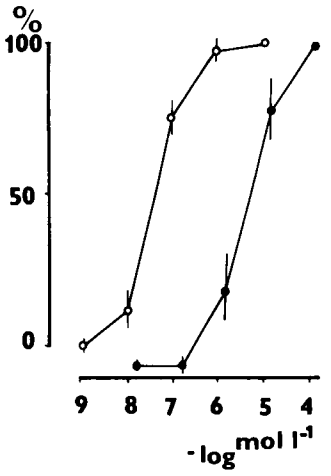

Fig. 1. Relaxing effect of cumulatively applied isoprenaline (○) and orciprenaline (●) on dog tracheal smooth muscle. The maximal contraction generated by serotonin (1 μmol.l^{-1}) served as control and relaxation was plotted as percent decrease of this value Each point represents the mean of at least 6 determinations, vertical lines show SEM.

Concentration-dependent contractions of guinea pig trachea induced by histamine (0.1 μmol.l^{-1} to 0.1 mmol.l^{-1}) were significantly (n = 19) suppressed with isoprenaline pretreatment after 5 min. In the presence of isoprenaline (0.01; 0.1; and 1 μmol.l^{-1}) even the effect of histamine in mmol.l^{-1} concentrations was reduced by 68 ± 15 %, 83 ±9 % and 92 ± 9 %, respectively.

At 30 °C and under an initial tension of about 10 mN, the dog trachea did not show spontaneous activity, and even electrical stimulation with up to 4 μA did not evoke spikes, and the voltage-current relationship did not show any pronounced rectification (Fig. 2). Isoprenaline (1 and 100 μmol.l^{-1}) enhanced the membrane potential by 0.8 $\pm$ 0.2 mV (n = 5) and 4.2 $\pm$ 0.4 mV (n = 16), and decreased the basal tension by 0.12 $\pm$ 0.03 mN and 0.18 $\pm$ 0.06 mN, respectively. In contrast, isoprenaline in concentrations used, either did not alter the membrane resistance or reduced it only slightly.

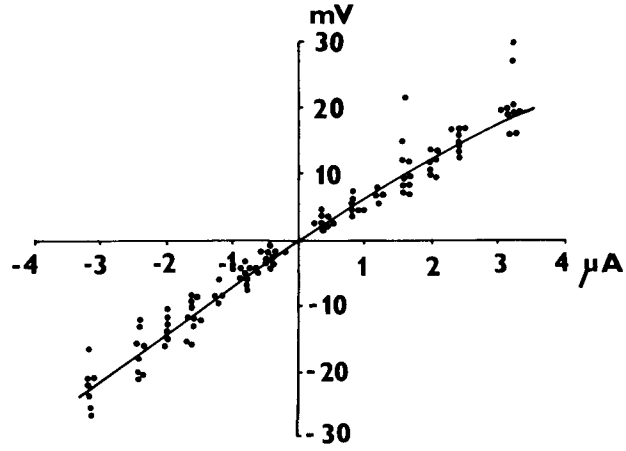

Fig. 2. Current-voltage relationship in the dog tracheal smooth muscle under double sucrose gap.

Histamine (10 μmol.l^{-1}) depolarized the smooth muscle membrane of the dog trachea by 6.4 $\pm$ 1.8 mV and induced local responses, i.e. spontaneous fluctuations of the membrane potential. Moreover during histamine presence in the bathing fluid, depolarizing pulses of high intensity also induced action potentials. Isoprenaline (0.1 mmol.l^{-1}) slightly reduced the histamine induced depolarization, abolished contractions and completely prevented the development of membrane potential fluctuations and the generation of action potential.

Orciprenaline (0.1 mmol.l^{-1}) similarly to isoprenaline, hyperpolarized the membrane (by 4.3 $\pm$ 0.3 mV), relaxed the smooth muscle (by 0.31 $\pm$ 0.07 mN) and reduced the membrane resistance of the dog trachea by 21.0 $\pm$ 2.9 % (n = 9). In contrast, noradrenaline (10 μmol.l^{-1}, n = 5), phenylephrine (10 μmol.l^{-1}, n = 8) and ephedrine (10 μmol.l^{-1}, n = 9) did not alter significantly the membrane potential, membrane resistance and contracted the smooth muscle only insignificantly by 0.08 $\pm$ 0.03 mN (n = 5), 0.05 $\pm$ 0.04 mN (n = 7) and 0.02 $\pm$ 0.02 mN (n = 7), respectively.

High concentration of ephedrine (0.3 mmol.l^{-1}), however, significantly

hyperpolarized the smooth muscle membrane by 4.4 ± 1.0 mV and relaxed it by 1.42 ± 0.06 mN ($n = 7$).

Interaction between isoprenaline and adrenoceptor antagonists on the guinea pig and dog trachea.

The adrenoceptors were applied 15—20 min before the cumulative application of isoprenaline and were left in contact with the preparation throughout the experiments.

Phentolamine (1 μmol.l^{-1}) elevated the smooth muscle tension by 3.9 ± 0.9 mN ($n = 7$). As it is illustrated in Fig. 3 pretreatment of guinea pig trachea with phentolamine reduced the effect of low concentrations of isoprenaline (up to 0.01 μmol.l^{-1}), whereas relaxation induced by concentrations higher than 0.01 μmol.l^{-1} were unaffected.

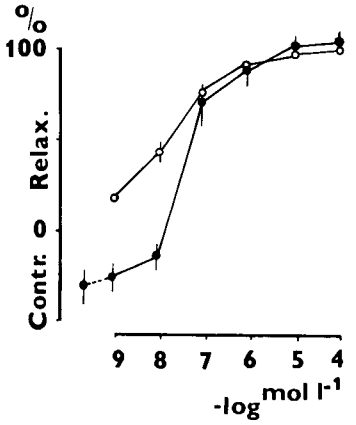

Fig. 3. Effect of phentolamine (1 μmol l^{-1}) on the relaxing action of cumulatively applied isoprenaline in the guinea pig tracheal smooth muscle at basal tension. ○ — control response to isoprenaline, ● — response after pretreatment by phentolamine Each point represents the mean of at least seven determinations, vertical lines show SEM.

Propranolol, metipranolol, exaprolol and BL 445 (0.01 to 1 μmol.l^{-1}) had no significant effects on the basal tension.

Propranolol (0.01 μmol.l^{-1}) did not influence significantly the action of low isoprenaline concentrations (up to 0.1 μmol.l^{-1}) and enhanced the effect of isoprenaline concentrations higher than 0.1 μmol.l^{-1} by about 50 %. In contrast, 0.1 and 1 μmol.l^{-1} of propranolol produced parallel, concentration-dependent shifts to the right of the CRC induced by isoprenaline (Fig. 4). The pA_2 value of propranolol at postjunctional β-adrenoceptors of the guinea pig trachea activated by isoprenaline was 7.83 ± 0.24 (mean ± SEM).

Propranolol in a low concentration (0.01 μmol.l^{-1}) hyperpolarized the smooth muscle membrane of the dog trachea by 1.8 ± 0.9 mV ($n = 8$), while in a high concentration (10 μmol.l^{-1}) it decreased the membrane potential by

1.8 ± 0.1 mV ($n = 8$). Yet, propranolol did not affect the basal tension and membrane resistance of the tracheal muscle. As in the case of guinea pig trachea, propranolol in a low concentration (0.01 μmol . l^{-1}) augmented the isoprenaline induced relaxation in the dog trachea, but simultaneously decreased the effect of isoprenaline on the membrane potential and membrane resistance. A high concentration of propranolol (10 μmol . l^{-1}) not only completely prevented the isoprenaline induced membrane hyperpolarization and smooth muscle relaxation, but even reversed them into a small membrane depolarization and smooth muscle contraction (Fig. 5).

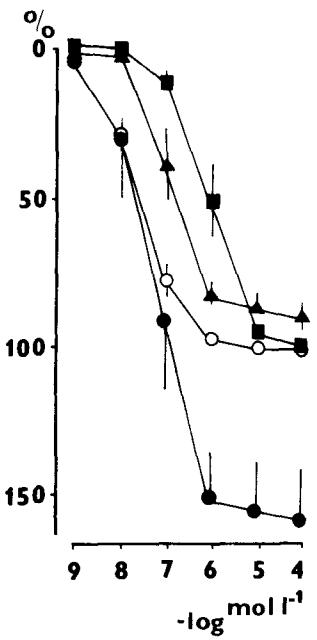

Fig. 4. Effect of propranolol on the relaxing action of cumulatively applied isoprenaline in the guinea pig tracheal smooth muscle at basal tension ○ — control response to isoprenaline, responses to isoprenaline after pretreatment by propranolol in concentrations of 0 01 μmol l^{-1} (●), 0 1 μmol l^{-1} (▲) and 1 μmol l^{-1} (■) Each point represents the mean of 6 to 7 trials, vertical lines show SEM

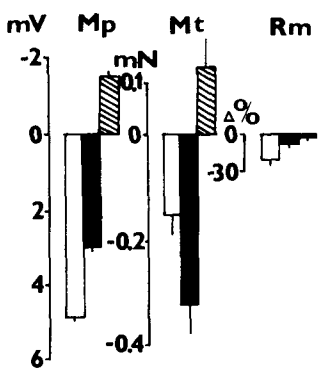

Fig. 5. Effect to propranolol on isoprenaline induced hyperpolarization, relaxation and increased membrane conductance in the dog tracheal smooth muscle Changes in membrane potential (*Mp*), basal tension (*Mt*) and membrane resistance (*Rm*) are illustrated Negative values mean depolarization, relaxation and decreased membrane resistance □ — control response to isoprenaline (0 1 mmol l^{-1}), responses to isoprenaline after pretreatment by propranolol in concentrations of 0 01 μmol l^{-1} (■) and 10 μmol l^{-1} (▨) Each column represents the mean of 5 to 9 determinations, vertical lines show SEM

Metipranolol (0.01; 0.1 and 1 μmol . l^{-1}) displaced the CRC for isoprenaline induced relaxation to the right in a concentration-dependent manner. The action of metipranolol in the concentration of 0.1 μmol . l^{-1} was biphasic, i.e. it reduced the effect of low isoprenaline concentrations (0.001 to 0.1 mmol . l^{-1}) and augmented the effect of the two highest concentrations used by 15.2 $\pm$ 7.7 % and 19.5 $\pm$ 4.8 %, respectively (Fig. 6). The pA_2 value for metipranolol with isoprenaline used as an agonist to the postjunctional β-adrenoceptors of the guinea pig trachea, was 8.18 $\pm$ 0.19 (mean $\pm$ SEM).

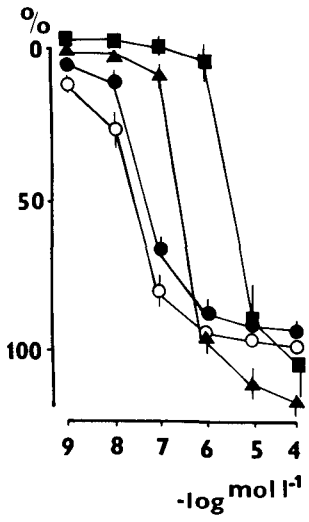

Fig. 6. Effects of metipranolol on the relaxing action of cumulatively applied isoprenaline in the guinea pig tracheal smooth muscle at basal tension ○ — control response to isoprenaline, responses to isoprenaline after pretreatment by metipranolol in concentrations of 0 01 μmol . l^{-1} (●), 0 1 μmol l^{-1} (▲) and 1 μmol l^{-1} (■) Each point represents the mean of 5 to 6 trials, vertical lines show SEM

The low concentration of exaprolol (0.01 μmol . l^{-1}) slightly and insignificantly reduced the action of low, and substantially enhanced the action of high isoprenaline concentrations. Higher concentrations of exaprolol (0.1 and 1 μmol . l^{-1}), however, inhibited the action of isoprenaline in the whole concentration range (Fig. 7), and the pA_2 value for exaprolol was 7.72 $\pm$ 0.11 (mean $\pm$ SEM).

Substance BL 445 (0.01 and 0.1 μmol . l^{-1}) had only minimal effects on isoprenaline induced relaxation of guinea pig trachea. However, a tendency to biphasic change of the isoprenaline CRC, was also noticed. A higher concentration of BL 445 (1 μmol . l^{-1}) produced a parallel shift of the CRC of isoprenaline giving a pA_2 value of 7.12 $\pm$ 0.19 (Fig. 8).

Changes in cAMP level in the guinea pig trachea.

Isoprenaline (0.01 and 0.1 mmol . l^{-1}) induced a concentration-dependent increase in the level of cAMP; this increase was significant at the highest

isoprenaline concentration used. The effect of orciprenaline (0.1 mmol . l^{-1}) was less expressed than that of isoprenaline.

Ephedrine, even in a very high concentration (0.5 mmol . l^{-1}), did not affect the cAMP level. The isoprenaline induced increase in cAMP level was significantly attenuated by propranolol (0.01 μmol . l^{-1}) or metipranolol (10 μmol . l^{-1}) pretreatment, and only marginally affected by phentolamine (1 μmol . l^{-1}) pretreatment. Histamine (0.1 mmol . l^{-1}), which in contrast to isoprenaline substantially contracted the guinea pig trachea, also significantly increased the cAMP level. Moreover, the increase in cAMP level, with histamine and isoprenaline applied simultaneously, was as large as the sum of their separate effects (Table 1). This contrasted with the opposite effects on the smooth muscle tension and membrane properties as described above.

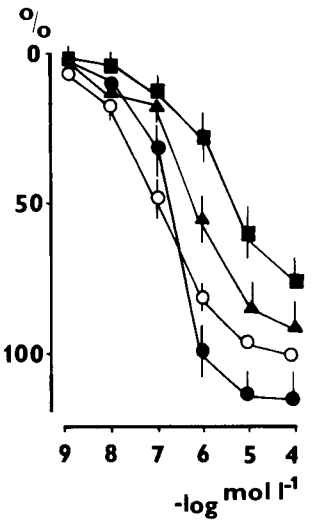

Fig. 7. Effects of exaprolol on the relaxing action of cumulatively applied isoprenaline in the guinea pig tracheal smooth muscle at basal tension ○ — control response to isoprenaline, responses to isoprenaline after pretreatment by exaprolol in concentrations of 0 01 μmol l^{-1} (●), 0 1 μmol l^{-1} (▲) and 1 μmol l^{-1} (■) Each point represents the mean of 6 to 10 trials, vertical lines show SEM

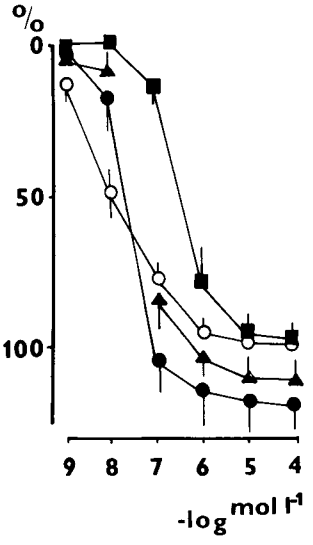

Fig. 8. Effects of substance BL 445 on the relaxing action of cumulatively applied isoprenaline in the guinea pig tracheal smooth muscle at basal tension ○ — control response to isoprenaline , responses to isoprenaline after pretreatment by BL 445 in concentrations of 0 01 μmol l^{-1} (●), 0 1 μmol 1 ^{1} (▲) and 1 μmol 1 ^{1} (■) Each point represents the mean of 6 to 7 trials, vertical lines show SEM

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

Compounds	Concentration $-\log$ mol l^{-1}	Cyclic AMP pmoles/mg proteins	*n*
Control		13 4 ± 1 1	15
Isoprenaline	5	15 7 ± 1 2	5
Isoprenaline	4	27 7 ± 2 9*	5
Orciprenaline	4	17 8 ± 2 9	5
Ephedrine	2 3	16 2 ± 1 0	5
Histamine	4	22 2 ± 2 3*	5
Isoprenaline	4		
+		39 5 ± 3 0*×●	5
Histamine	4		
Phentolamine	6		
+		24 9 ± 3 8*	5
Isoprenaline	4		
Propranolol	8		
+		20 0 ± 0 9*×	10
Isoprenaline	4		
Metipranolol	5		
+		14 5 ± 1 5×	10
Isoprenaline	4		

* — significant ($p < 0\ 05$) differences from the control cAMP level
× — significant ($p < 0\ 05$) differences from the control response to isoprenaline (100 μmol l^{-1})
● — significant ($p < 0\ 05$) difference from the control response to histamine

Discussion

There is a controversy about the functional role of α-adrenoceptors in airway smooth muscles. Some authors deny the existence of α-receptors (Foster 1966; Cabezas et al. 1971), whereas others have shown the presence of α-receptors in the guinea pig airways (Mathé et al. 1971; Vornanen 1982; Barnes et al. 1983). This controversy results partly from species differences and partly from the existence of adrenoceptor subtypes. The α-adrenoceptors mediating excitation of airway smooth muscles were repeatedly demonstrated in human and rat tissues (Mathé et al. 1971; Vornanen 1982), while, as also shown in our study, their presence in the guinea pig and canine preparations was not so obvious (Foster 1966; Cabezas et al. 1971). Several investigators, however, found α-agonist-mediated contractions in canine tracheal smooth muscle after precontraction by different spasmogens (Ohno et al. 1981; Leff and Munoz 1981; Barnes et al. 1983). Barnes et al. (1983) using the radioligand binding technique found higher densities of α_2- than α_1-adrenoceptors in the canine trachea. Since phen-

tolamine (an $\alpha_{1,2}$-antagonist) contracted and did not relax the guinea pig trachea, and noradrenaline (an $\alpha_{1,2}$-agonist), phenylephrine (an α_1-agonist) and ephedrine (an α_2-agonist) (Bauer 1981), even in relatively high concentrations, did not increase the basal tension to an appreciable extent and did not alter the membrane potential, it could be suggested that α-adrenoceptors do not play a physiologically significant role in the elevation of the basal tension. The different effects of noradrenaline on membrane potential observed in the present study and by Suzuki et al. (1976) were probably due to the presence of propranolol in the experiments of the latter authors and, consequently, to the blockade of β-adrenoeptors. In contrast to the effect of clonidine, high ephedrine concentrations were inhibitory to basal tension in precontracted preparations (Barnes et al. 1983) and were not associated with increased intracellular cAMP concentration, thus supporting the presence of α_2-adrenoceptors in canine trachea.

The subclassification of β-adrenoceptors to β_1- and β_2-subtypes was first suggested by Lands et al. (1967), on the basis of relative responses to adrenoceptor agonists in different tissues. Further support to this hypothesis has come from the use of selective antagonists. The coexistence of β_1- and β_2-receptors in the heart, gut and adipose tissue (Wagner et al. 1981; Bauer 1982; William-Olsson et al. 1979) as well as in airways has been clearly demonstrated (Zaagsma et al. 1979), and the relative ratio of β_1-:β_2-adrenoceptive binding sites was found to be 15:85 % (Carswell and Nahorski 1983). The present study confirmed and extened the earlier observations (Lohman et al. 1977; Olsson et al. 1979; van der Heijden et al. 1984; Elguindi et al. 1985) showing that the relaxation of tracheal smooth muscle of the guinea pig and dog elicited by catecholamines is mediated by β-adrenoceptors. As described earlier in the guinea pig trachea (Olsson et al. 1979), orciprenaline (a β_2-agonist) and isoprenaline (a $\beta_{1,2}$-agonist) also are full agonists in the dog trachea, and in the latter preparation the pD_2 value of orciprenaline also is significantly smaller than that of isoprenaline. Such differences in the potency were also noticed in the effects of these drugs on the cAMP level.

When, however, supramaximal concentrations of isoprenaline and orciprenaline were present at resting conditions, the dog trachea was hyperpolarized and relaxed to the same extent by both of them, suggesting an important role for the β_2-adrenoceptor subtype in the relaxation of the airways. This is in accordance with earlier reports (Carswell and Nahorski 1983).

The interaction of low β-adrenoceptor antagonist concentrations with isoprenaline represents an overadditive synergism of the sequential type (Pöch and Holzman 1980). Such a synergism between antagonists of β-adrenoceptors and isoprenaline was also described in the sympathetic ganglion (Machová et al. 1980). This synergism is not due to the intrinsic sympathomimetic activity of the β-adrenoceptor antagonists studied, since they did not affect the basal tension

of the preparations in an appreciable extent. Kenakin and Black (1978) suggested that the enhancement of the effect of isoprenaline by practolol might result from the suppression of catechol-*o*-methyl-transferase activity and of uptake$_2$. Since the action of low isoprenaline concentrations was not potentiated by the β-adrenoceptor antagonists studied, it is unlikely that such a mechanism should be responsible for the overadditive synergism observed. Further experiments are necessary to clarify the mechanism of the above described action of low concentrations of β-adrenoceptor antagonists. In contrast to the effect of the above mentioned low concentrations, the higher concentrations of the drugs studied antagonized the actions of isoprenaline, on both the smooth muscle tension and the membrane potential as well as on cAMP content. Based on the pA_2 values, the order of their potencies was as follows: metipranolol > propranolol ≐ exaprolol ⩾ BL 445. It is widely accepted that propranolol (for review, see Patil and Ruffolo 1980), and metipranolol (Zakhari 1974; Bauer and Zakhari 1977) are non-selective β-receptor antagonists. The present results confirmed these findings. Since the differences between the antiisoprenaline pA_2 values of exaprolol in the heart (Dřímal et al. 1978) and in the trachea were found to be small in the present study, exaprolol is also considered to belong to nonselective antagonists of β-receptors. The effect of BL 445 exhibit some selectivity to myocardial β-adrenoceptors, since its antiisoprenaline action on the myocardial β-receptors was almost of the same potency as that of metipranolol (Béderová 1981) in contrast to its significantly lower affinity to tracheal β-adrenoceptors found in the present experiments.

It has been suggested that changes in the tissue levels of cAMP may be important in the regulation of smooth muscle tension and in the action of β-adrenoceptor agonists (for review, see Andersson et al. 1975; Will-Shahab and Krause 1980). There is a general hypothesis to suggest than an increase in the cAMP level due to the β-adrenoceptor activation promotes smooth muscle relaxation (Murad and Kimura 1974; Triner et al. 1977; Wong and Buckner 1978). This, however, was not found to be an obligatory step in the relaxant action of most smooth muscle relaxants (for review, see Diamond 1978). The present study showed that not only drugs such as β-adrenoceptor agonists which relax the guinea pig trachea but also excitatory substances, such as histamine, which contracted the guinea pig trachea, were able to increase the cAMP level.

Creese and Denborough (1980) suggested that the action of histamine on the cAMP level did not appear to be mediated by the release of catecholamines and did not involve stimulation of β-adrenoceptors, as also found in the present study, but resulted from the release of prostaglandins triggered due to activation of H_1-receptors and the contractile response.

The antagonistic action of histamine and isoprenaline on membrane properties and muscle tension, and their additive effect on the cAMP level, suggest the

possibility of distinct subcellular cAMP compartments, coupled differently to the contraction-relaxation cycle and undetectable by total tissue cAMP measurements. Isoprenaline (Ito and Tajima 1982; Wong and Buckner 1978) and also orciprenaline hyperpolarized the smooth muscle membrane increased the cAMP level by the proposed cAMP-dependent increase in the sodium pump activity in smooth muscles (Watanabe 1976; Bauer and Rusko 1982). The activation of the sodium pump could thus be responsible for membrane potential changes induced by stimulation of β-adrenoceptors.

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References

Ahlquist P A (1948) A study of adrenotropic receptors Amer J Physiol **153**, 586—600

Andersson R G G, Nilsson K, Wikberg J, Johansson S, Mohme-Lundholm E, Lundholm L (1975) Cyclic nucleotides and contraction of smooth muscle In Advances in Cyclic Nucleotide Research (Eds G I Drumond, P Greengard, G A Robison), vol 5, pp 491—518, Raven Press, New York

Barnes P J, Skoogh B E, Nadel J A, Roberts J M (1983) Postsynaptic alpha$_2$-adrenoceptors predominate over alpha$_1$-adrenoceptors in canine tracheal smooth muscle and mediate neuronal and hormonal alpha-adrenergic contractions Mol Pharmacol **23**, 570—575

Bauer V (1981) Distribution and types of adrenoceptors in the guinea pig ileum The action of α- and β-adrenoceptor agonists Brit J Pharmacol **72**, 201—210

Bauer V (1982) Inhibition of guinea-pig taenia coli mediated by α_1-, β_2-adrenoceptors and ATP-receptor activation Gen Physiol Biophys **1**, 175—188

Bauer V, Zakhari S (1977) Pharmacological studies with beta-adrenoceptor blocking agents I Effect on the smooth muscle of the taenia coli of the guinea pig Life Sci **21**, 683—694

Bauer V, Rusko J (1982) TEA-sensitive potassium conductance changes induced by α_1-adrenoceptor and ATP-receptor activation in guinea pig taenia coli Gen Physiol Biophys **2**, 89—102

Bederova E (1981) Pharmacological evaluation of a series of new synthetized arylaminopropyl derivatives Farm Obzor **50**, 443—451 (in Slovak)

Cabezas G A, Graf O P, Nadel J A (1971) Sympathetic versus parasympathetic nervous regulation of airways in dogs J Appl Physiol **31**, 651—655

Carswell H, Nahorski S R (1983) β-adrenoceptor heterogenity in guinea-pig airways comparison of functional an receptor labelling studies Brit J Pharmacol **79**, 965—971

Chang L T C, Wong J C Y, Roberts M B (1978) A study of the interaction of practolol with acetylcholine, histamine, serotonin and bradykinin in the guinea-pig and with bean-sprout juice in the mouse Clin Exp Pharmacol Physiol **5**, 99—105

Creese B K, Denborough M A (1980) The effect of histamine on cyclic AMP levels in guinea-pig tracheal smooth muscle Eur J Pharmacol **66**, 95—101

Diamond J (1978) Role of cyclic nucleotides in control of smooth muscle contraction In Advances in Cyclic Nucleotide Research (Eds W J George and L J Ignarro), vol 9, pp 327—340, Raven Press, New York

Dřimal J , Seginko J , Gibala P , Strižova K (1978) Pharmacology of the new β-adrenoceptor antagonists II Bronchopulmonal and cardiovascular effects of VUL 111 and trimepranol Brat Lek Listy **70**, 429—439 (in Slovak)

Elgundi A S , Smith J K , Speir W A (1985) Facilitation of isoproterenol induced airway smooth muscle relaxation by nifedipine Life Sci **36**, 75—81

Foster R W (1966) The nature of the adrenergic receptors of the trachea of the guinea pig J Pharm Pharmacol **18**, 1- 12

Gilman A C (1970) A protein binding assay for adenosine 3 , 5 -cyclic monophosphate Proc Nat Acad Sci USA **67**, 305 –312

Hexheimer H (1967) The bronchoconstrictor action of propranolol aerosol in guinea-pig J Physiol (London) **190**, 41P—42P

Ito Y Tajima K (1982) Dual effects of catecholamines on pre- and post-junctional membranes in the dog trachea Brit J Pharmacol **75**, 433—440

Kenakin T P , Black S W (1978) The pharmacological classification of practolol and chlorpractolol Mol Pharmacol **14**, 607—623

Kolena J , Channing C P (1972) Stimulatory effect of LH, FHD and prostaglandins upom cAMP levels in porcine granulosa cells Endocrinology **90**, 1543 1550

Lands A M , Arnold A , McAuliff J P , Luduena F P , Brown T G (1967) Differentation of receptor systems activated by sympathomimetic amines Nature **214**, 597— 598

Langer S Z (1974) Presynaptic regulation of catecholamine release Biochem Pharmacol **23**, 1793 —1800

Leff A , Munoz N M (1981) Interrelationship between alpha- and beta-adrenergic agonists and histamine in canine airways J Allergy Clin Immunol **68**, 300—309

Lohman S M , Ralph P , Butcher R (1977) Effects of isoproterenol, theophylline and carbachol on cyclic nucleotide levels and relaxation of bovine tracheal smooth muscle Biochim Biophys Acta **499**, 238—250

Lowry O H , Rosenbrough N J , Lewis Farr A , Randall R J (1951) Protein measurement with the folin phenol reagent J Biol Chem **193**, 265—275

Machova J , Belan V , Greguškova M (1980) Pharmacological study of beta-adrenoceptors on neuro-neuronal synapse Čs Fysiol **29**, 61 (in Slovak)

Mathe A A , Åstrom A , Persson N Å (1971) Some bronchodilating responses of human isolated bronchi evidence for existence of α-adrenoceptors J Pharm Pharmacol **23**, 905—910

Murad F , Kimura H (1974) Cyclic nucleotide levels in incubations of guinea-pig trachea Biochim Biophys Acta **343**, 275—286

Ohno Y , Watanabe M , Kasuya Y (1981) Manifestation of latent alpha-excitatory response in the canine tracheal smooth muscle preparation relation to basal tone Arch Int Pharmacodyn Ther **251**, 205—216

Olsson O A T , Swaberg E , Svedinger I , Waldeck B (1979) Effects of β-adrenoceptor agonists on airway smooth muscle on slow-contracting skeletal muscle In vitro and in vivo results compared Acta Pharmacol Toxicol **44**, 272—276

Patil P N , Ruffolo P R (1980) Evaluation of adrenergic alpha- and beta-receptor activators and adrenergic alpha- and beta-receptor blocking agents In Adrenergic Activators and Inhibitors (Eds L Szekeres), pp 89—134, Springer Verlag, Berlin and Heidelberg, New York

Poch G , Holzman S (1980) Quantitative estimation of overadditive and underadditive drug effects by mean of theoretical, additive dose-response curves J Pharmacol Methods **4**, 179—188

Scheid C R , Honeyman T W , Fay F S (1979) Mechanism of β-adrenergic relaxation of smooth muscle Nature **277**, 32—36

Sutherland E W , Robison G A (1966) The role of cyclic 3′, 5′- AMP in response to catecholamines and other hormones Pharmacol Rev **18**, 145—161

Suzuki H , Morita K , Kuriyama H (1976) Innervation and properties of the smooth muscle of the dog trachea Jpn J Physiol **26**, 303—320

Svedmyr N (1970) Bronchodilating effect in vitro Scand J Resp Dis Suppl **74**, 15

Svedmyr N (1971) A comparison of the effect of isoprenaline and salbutamol on different adrenergic β-receptors in asthmatic patiens Postgrad Med J **47**, Suppl 44—46

Svedmyr N (1977) Treatment with beta-adrenostimulants Scand J Rep Dis Suppl **101** 59—69

Triner L Vullemoz Y , Verosky M (1977) Cyclic 3 , 5 -adenosine monophosphate and bronchial tone Eur J Pharmacol **41**, 37—46

Todorov S (1977) New method for in vitro studies of the histaminergic receptors in tracheal smooth muscle of male and female guinea-pigs Acta Physiol Pharmacol Bulg **3**, 77—84

Van Rossum J M , van den Brink F G (1963) Cumulative dose- β-response curves I Introduction to the technique Arch Int Pharmacodyn **143**, 240—266

Van der Heijden P J C M , van Amsterdam J G C , Zaagsma J (1984) Desenzitization of smooth muscle and mast cell β-adrenoceptors in the airway of the guinea-pig Eur J Resp Dis **65**, Suppl **135**, 128—135

Vornanen M (1982) Adrenergic response in different sections of rat airways Acta Physiol Scand **144**, 587—591

Wagner J , Nick B , Rohm R , Schumann H J (1981) On the coexistence of β_1- and β_2-adrenoceptors in various organs Arch Int Pharmacodyn Ther **249**, 26—38

Watanabe H (1976) Inhibitory mechanisms of isoprenaline in the guinea-pig taenia coli Jpn J Pharmacol **26**, 217—226

William-Olsson T , Fellenius E , Bjorntorp P , Smith U (1979) Differences in metabolic responses to β-adrenergic stimulation after propranolol on metoprolol administration Acta Med Scand **205**, 201—206

Will-Shahab L , Krause E G (1980) Effects on myocardial metabolism In Adrenergic Activators and Inhibitors (Eds L Szekeres), pp 824—852, Springer Verlag, Berlin, Heidelberg New York

Wong S K , Buckner C K (1978) Studies on β-adrenoceptors mediating changes in mechanical events and adenosine 3 , 5 -monophosphate levels, guinea-pig trachea Eur J Pharmacol **17**, 273—280

Zaagsma J , Oudhof R , van der Heijden P J C M , Plantje J F (1979) Subheterogenity of β-adrenoceptors in the pulmonary and the cardiac system of the guinea-pig In Catecholamines Basic and Clinical Frontiers (Eds E Usdin, J J Kopin, J Barchas), pp 435—437, Pergamon Press, Oxford

Zakhari S (1974) Structure selectivity relationship of β-adrenoceptor antagonist in cats Eur J Pharmacol **29**, 22—29

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