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β_1-Adrenoceptors mediating relaxation of the guinea-pig trachea: experiments with prenalterol, a β_1-selective adrenoceptor agonist

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In the presence of 17β-oestradiol, prenalterol, a β_1-selective adrenoceptor agonist, caused a dose-dependent relaxation of the isolated, pilocarpine-contracted guinea-pig trachea. This effect was blocked by the antagonists propranolol (non-selective) and practolol (β_1-selective) but not by IPS 339 [(t-butylamino-3-ol-2-propyl)oximino-9-fluorene HCl] (β_2-selective). The relaxing effect of terbutaline, a β_2-selective adrenoceptor agonist, was more efficiently blocked by IPS 339 than by practolol. These data support the hypothesis that the guinea-pig trachea contains both β_1- and β_2-adrenoceptors mediating relaxation and that the β_1-adrenoceptors are selectively stimulated by prenalterol. The efficacy of prenalterol was less than that of terbutaline, thus confirming its partial agonistic activity. In the absence of 17β-oestradiol, the ability of prenalterol to relax the pilocarpine-contracted trachea was lost. It is suggested that 17β-oestradiol may act as a functional antagonist to pilocarpine as it caused a partial relaxation itself.

Prenalterol (H133/22), a partial and β_1-selective adrenoceptor agonist (Carlsson et al 1977; Hedberg et al 1980), did not relax the pilocarpine-contracted guinea-pig trachea (Johansson & Waldeck 1980). Using somewhat different experimental conditions, Kenakin & Beek (1980) demonstrated a significant relaxation of the guinea-pig trachea by prenalterol which led them to question the β_1-selectivity of this drug. But they did not attempt to block the effect by selective antagonists.

Although sympathomimetic-induced relaxation of the guinea-pig trachea is mediated mainly via β_2-adrenoceptors, more detailed studies show that both β_1- and β_2-adrenoceptors may be involved (Furchgott & Wakade 1975; O'Donnell & Wanstall 1979; Omini et al 1979; Iakovidis et al 1980). These findings are in agreement with the concept (Carlsson et al 1972) that there can be both β_1- and β_2-adrenoceptors in the same organ mediating the same effect.

When we tried to reproduce the experiments of Kenakin & Beek (1980) we noted that the presence of 17β-oestradiol was essential in order to detect the relaxing activity of prenalterol. We then used practolol (β_1-selective) and IPS339 (β_2-selective; Imbs et al 1977) as antagonists to characterize the effect. Attempts were also made to find out what role the 17β-oestradiol played.

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MATERIALS AND METHODS

Tracheae were removed from male guinea-pigs (Dunkin-Hartley, 200–400 g) under pentobarbitone anaesthesia (about 50 mg kg^{-1} i.p.) The tracheae were trimmed and cut transversally into rings each comprising two segments. Cotton threads were tied to each end of the cartilage bridges. The cartilage was then cut open in the middle. The preparation was mounted in an organ bath (40 ml) containing oxygenated Krebs' solution at 37 °C and was attached to a Grass FTO3C force transducer for isometric measurement. A basal tone of about 5 mN was applied and the preparation was allowed to stabilize for an hour.

Pilocarpine (2·6 μmol litre^{-1}) was added to the bath to induce a contraction about 50% of the maximum contraction produced by carbacholine. When the contraction was stable, terbutaline (2·3 μmol litre^{-1}) was added to test the sensitivity of the preparation. The preparation was rinsed and after recovery 17β-oestradiol or the vehicle, dimethylsulphoxide (DMSO), was added to the bath followed 1 h later by 2·6 μmol litre^{-1} of pilocarpine. When used, practolol and IPS339 were added together with 17β-oestradiol, and propranolol 30 min later. Prenalterol was then added cumulatively until a further addition yielded no further response. Finally, isoprenaline 10 μmol litre^{-1}, or in some experiments 100 μmol litre^{-1}, was added to determine the maximum relaxation of the trachea. The effects were calculated in per cent of this

maximum response. Individual concentration-response curves were constructed and the negative logarithm for the concentration of prenalterol giving half of its maximum response (the pD_2-value) was estimated graphically. Apparent pA_2 values for the antagonists were calculated according to the equation $pA_2 = \log (CR - 1) - \log I$, where CR is the agonist concentration-ratio and I represents the molar concentration of the antagonist (MacKay 1978). Student's *t*-test was used for statistical evaluation and the significance tests were usually based on the pD_2-values. Further details of the experiments are given in the results section.

Krebs' solution had the following composition in mmol litre^{-1}: NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·16; $NaHCO_3$, 25; KH_2PO_4, 1·18; D-glucose, 11·1. A stream of 5% CO_2 in oxygen was bubbled through the organ bath and the bulk of solution. All drugs were dissolved in 0·9% NaCl except the steroids which were dissolved in DMSO, final concentration about 0·2%. The drugs used and their sources were: Budesonide (AB Draco), 17β-oestradiol (Sigma), hydrocortisone (Apoteksbolaget), IPS339 (t-butyl-amino-3-ol-2-propyl)oximino-9-fluorene HCl (AB Hässle), metanephrine HCl (Calbiochem), pentobarbitone (ACO), pilocarpine HCl (Apoteksbolaget), practolol (ICI), prenalterol HCl (H133/22, AB Hässle), propranolol (ICI), and terbutaline sulphate (AB Draco).

RESULTS

Relaxation of the pilocarpine-contracted trachea by prenalterol in the presence of 17β-oestradiol

In the presence of 50 μmol litre^{-1} 17β-oestradiol, prenalterol caused a concentration-dependent relaxation of the pilocarpine-contracted trachea (Fig. 1). The mean $pD_2 \pm$ s.e. of five experiments was 7·03 ± 0·06 and the corresponding intrinsic activity (α-value) was 0·58 ± 0·06. Propranolol, 0·1 μmol litre^{-1}, shifted the concentration-response curve of prenalterol to the right ($P < 0·001$). In the absence of 17β-oestradiol or in the presence of DMSO, the solvent used for the hormone, the effect of prenalterol was slight and difficult to quantify (Fig. 1).

The next experiments, aimed to characterize the effect of prenalterol, were all performed in the presence of 50 μmol litre^{-1} 17β-oestradiol. Practolol, 2 μmol litre^{-1}, shifted the concentration-response curve for prenalterol to the right by a factor of 8 ($P < 0·001$) whereas 20 nmol litre^{-1} IPS339 was without effect (Fig. 2A). None of the inhibitors

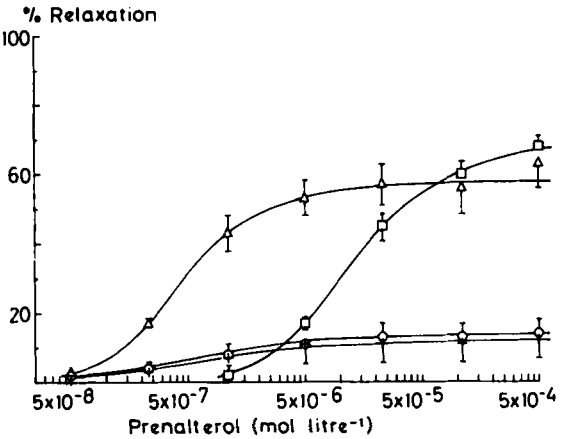

FIG. 1. Relaxation of the pilocarpine-contracted trachea by prenalterol. Cumulative concentration-response curves in the presence of 50 μmol litre^{-1} 17β-oestradiol alone (△) or together with 0·1 μmol litre^{-1} propranolol (□). Control experiments in the absence of 17β-oestradiol (+) or in the presence of the solvent of 17β-oestradiol, DMSO (○), were run in parallel. Each point represents the mean ± s.e. from 5 experiments.

changed the maximum tracheal relaxation obtained with prenalterol. When the β_2-selective agonist, terbutaline, was substituted for prenalterol, IPS339 was much more efficient than practolol as an antagonist ($P < 0·005$) (Fig. 2B).

From the data presented in Figs 1 and 2, apparent pA_2 values for propranolol, practolol and IPS339 were calculated. These values are shown in Table 1 and will be discussed below.

Interaction between prenalterol and steroids with respect to relaxation of the guinea-pig trachea

The ability of 17β-oestradiol to enhance the relaxing effect of prenalterol on the pilocarpine-contracted trachea was dose-dependent. At 25 μmol litre^{-1} of 17β-oestradiol the maximum relaxation by 1 μmol litre^{-1} prenalterol was almost three times that achieved at 5 μmol litre^{-1} of the hormone ($P < 0·005$). However, a further increase in 17β-oestradiol did not cause a further enhancement (Table 2).

In the next experiment, 17β-oestradiol was compared with budesonide, a new and highly potent glucocorticoid (Thalén & Brattsand 1979), and hydrocortisone with respect to the enhancement of relaxation brought about by 1 μmol litre^{-1} prenalterol (Table 2). At 50 μmol litre^{-1}, budesonide and hydrocortisone were much less efficient than 17β-oestradiol ($P < 0·001$). In some experiments, metanephrine, 50 μmol litre^{-1}, was substituted for the steroids. This compound did not enhance the effect of prenalterol.

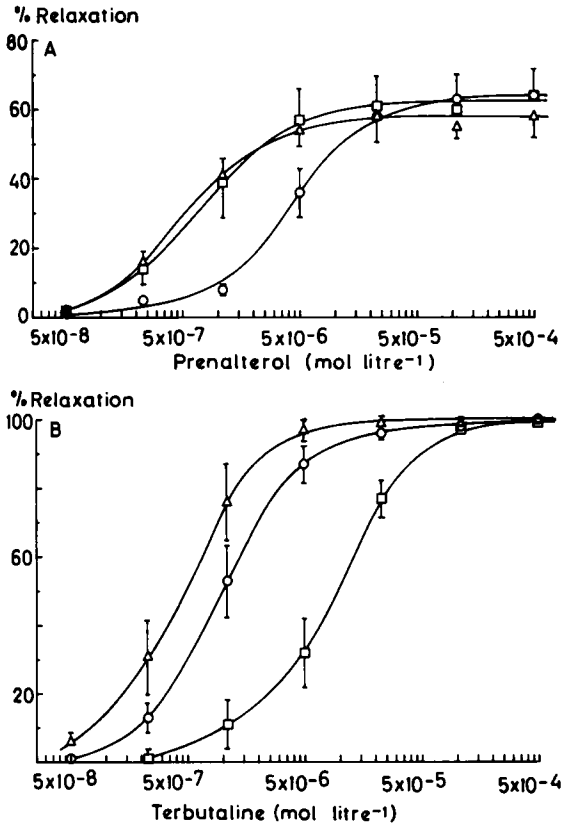

FIG. 2. Inhibition of the effects of prenalterol (A) and terbutaline (B) on the pilocarpine-contracted guinea-pig trachea by selective antagonists. All experiments were performed in the presence of 50 μmol litre^{-1} 17β-oestradiol. Control ($\triangle$), 2 μmol litre^{-1} practolol ($\bigcirc$), 20 nmol litre^{-1} IPS 339 ($\square$). Each point represents the mean $\pm$ s.e. from 4–6 experiments.

The effect of 1 μmol litre^{-1} of prenalterol was tested also on tracheal preparations with intrinsic tone and in the absence of steroids. The mean relaxation $\pm$ s.e. in 4 experiments was 57 $\pm$ 4% of the maximum effect elicited by isoprenaline. When tracheal tone was induced by 0·5 μmol litre^{-1} pilocarpine, the mean relaxation by 1 μmol litre^{-1} of prenalterol in the absence of 17β-oestradiol was 29 $\pm$ 2% in 3 experiments.

Table 1. Apparent pA_2 values (mean $\pm$ s.e.) for three adrenoceptor antagonists estimated on the guinea-pig trachea using prenalterol or terbutaline as agonist. The number of experiments is shown in parentheses.

Agonist	Antagonist: Propranolol	Antagonist: Practolol	Antagonist: IPS 339
Prenalterol	8·42 $\pm$ 0·05 (5)	6·52 $\pm$ 0·09 (6)	$\leq$ 7·7 (6)
Terbutaline	—	5·71 $\pm$ 0·19 (6)	8·93 $\pm$ 0·06 (6)

Table 2. Effects of 17β-oestradiol, budesonide and hydrocortisone on the relaxation by prenalterol of the pilocarpine-contracted guinea-pig trachea. The values are the means $\pm$ s.e. and the number of experiments in parentheses.

Steroid, μmol litre^{-1}	Prenalterol, 1 μmol litre^{-1} per cent relaxation
17β-Oestradiol, 5	17 $\pm$ 5 (6)
17β-Oestradiol, 25	48 $\pm$ 6 (5)
17β-Oestradiol, 125	51 $\pm$ 5 (5)
17β-Oestradiol, 50	66 $\pm$ 9 (7)
Budesonide, 50	20 $\pm$ 5 (8)
Hydrocortisone, 50	10 $\pm$ 3 (7)

In other experiments, 17β-oestradiol, 50 μmol litre^{-1}, or only DMSO was added after the trachea had been contracted by pilocarpine. 17β-oestradiol, but not DMSO, caused a slowly progressing relaxation of the trachea. After about 40 min, the percentage relaxation (relative to isoprenaline) was 31 $\pm$ 4 and 8 $\pm$ 4 respectively (mean $\pm$ s.e. of 6 experiments). The difference was highly significant ($P < 0.001$).

DISCUSSION

In the presence of 17β-oestradiol, prenalterol caused a concentration-dependent relaxation of the pilocarpine-contracted trachea. This effect appears to be mediated via β_1-adrenoceptors since it is blocked by propranolol (non-selective) and practolol (β_1-selective) but not by IPS339 (β_2-selective). This conclusion is further supported by the results obtained with the β_2-selective agonist terbutaline. The relaxation brought about by this compound was much more efficiently inhibited by IPS339 than by practolol. Thus both β_1- and β_2-adrenoceptors mediate relaxation of the guinea-pig trachea (see above).

A more quantitative evaluation of the selectivity is offered by the pA_2-values. Although our estimations of pA_2 are based on one inhibitor concentration only and thus are of limited validity (cf O'Donnell & Wanstall 1980; Holmberg et al 1980), our values are interesting to compare with data found in the literature. In a study on isolated trachea (mainly β_2-adrenoceptors) and right auricle (mainly β_1-adrenoceptors) from guinea-pigs, Miesch et al (1978) estimated the pA_2 for a number of antagonists using isoprenaline (non-selective) as agonist. When prenalterol was used as an agonist, the pA_2-values estimated for practolol and IPS339, respectively, (the present data) were close to those obtained on the auricle when isoprenaline was used (Miesch et al 1978). Conversely, the pA_2 values obtained with

terbutaline were comparable with those obtained on the trachea with isoprenaline. The pA_2 for propranolol was less dependent on the tissue or agonist used.

In our previous study on prenalterol (Johansson & Waldeck 1980) we were not able to detect a significant relaxation of the trachea. In fact, prenalterol inhibited the effect of terbutaline competitively. Rohm et al (1980) found that prenalterol inhibited the relaxing effect of noradrenaline on the guinea-pig trachea. This is compatible with the view that prenalterol is a non-selective β-adrenoceptor ligand with absolute β_1-selective, partial, agonistic activity (Hedberg et al 1980). The inclusion of 17β-oestradiol in the incubation medium markedly changed the responsiveness of the trachea to prenalterol. Kenakin & Beek (1980) added 17β-oestradiol in order to inhibit the extraneuronal uptake of isoprenaline in their experiments. However, the inhibition of extraneuronal uptake does not explain the effect of 17β-oestradiol in the present experiments and for two reasons. Firstly, another established inhibitor of extraneuronal uptake, metanephrine (see Trendelenburg 1972), was without effect. Secondly, in the absence of 17β-oestradiol, prenalterol would be expected to produce the same effect as in its presence but at a higher concentration. This was not the case.

In guinea-pig trachea, the maximum degree of relaxation that can be elicited by β-adrenoceptor agonists depends upon the degree of contraction of the smooth muscle induced by cholinergic agonists (Buckner & Saini 1975). In our experiments the maximum degree of relaxation produced by prenalterol increased with the concentration of 17β-oestradiol and was inversely related to the concentration of pilocarpine. Furthermore, 17β-oestradiol itself relaxed the pilocarpine-concentracted trachea. Thus 17β-oestradiol appears to act as a *functional* antagonist to pilocarpine. This explanation is supported by data showing that the maximum relaxation of the trachea produced by prenalterol is inversely related to the concentration of carbachol (Kenakin & Beek 1980). Similar observations have been made for other partial β-adrenoceptor agonists, structurally related to prenalterol (Keh et al 1978; Iakovidis et al 1980).

The relaxing effect of 17β-oestradiol is intriguing and warrants further studies. But 17β-oestradiol was more potent than both budesonide and hydrocortisone in enhancing the relaxing effect of prenalterol. Thus the choice of steroid in experiments with tracheal smooth muscle may be critical for the estimation of potency and efficacy of bronchodilators. This is illustrated by the failure to detect the relaxing effect of prenalterol on the carbachol contracted (1 μmol litre^{-1}) trachea when corticosterone is substituted for 17β-oestradiol (compare Rohm et al 1980 with Kenakin & Beek 1980).

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