Adrenergic receptors in the guinea-pig trachea*

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The effects of adrenaline, noradrenaline and isoprenaline have been investigated on the guinea-pig isolated trachea in the presence of propranolol hydrochloride 10^{-5} and 10^{-6} g/ml. Adrenaline and noradrenaline were both shown to produce contractions of the tissue. The relative order of potency of the catecholamines (adrenaline > noradrenaline > isoprenaline), together with the antagonism exhibited by phenoxybenzamine, suggests that the contractions were due to α-adrenergic receptor involvement.

According to the classification of Ahlquist (1948, 1966), bronchodilatation by sympathomimetic amines is mediated via β-adrenergic receptors. There appears to be some controversy concerning the concomitant existence of excitatory α-adrenergic receptors in the respiratory tract. Castro de la Mata, Penna & Aviado (1962), demonstrated the existence of α-adrenergic receptors which were considered responsible for bronchoconstriction in the anaesthetized dog. The possible existence of similar receptors in cat isolated tracheal muscle has also been discussed by Türker & Kiran (1965). However, Foster (1966) could find no evidence for the presence of α-adrenergic receptors in the isolated trachea of the guinea-pig.

Propranolol has been shown to inhibit the action of β-agonist drugs on the isolated guinea-pig trachea (Foster, 1966). However, in the course of experiments made in the presence of propranolol, adrenaline was sometimes observed to produce contractions of the tissue. This observation suggested α-adrenergic receptor involvement, and further experiments were made to examine this effect in more detail. The ability of noradrenaline and isoprenaline to produce this response has also been investigated.

EXPERIMENTAL

Method

Guinea-pigs of either sex, weighing 400–700 g, were killed by dislocation of the neck. The trachea was removed, cut into a chain containing the equivalent of eleven rings, and suspended in a 25 ml organ bath containing Krebs-Henseleit solution maintained at 37°. The Krebs-Henseleit solution contained the following (per litre): NaCl, 6·9; KCl, 0·35; $CaCl_2$ 0·28; $MgSO_4 \cdot 7H_2O$, 0·11; KH_2PO_4, 0·14; D(+) glucose, 2·0; $NaHCO_3$, 2·1 g. The fluid reservoir and bath were gassed with a mixture of 5% carbon dioxide in oxygen. The tissue was allowed to stabilize to these conditions for 1 h. During this period the tissue was washed with pre-warmed and pre-gassed Krebs-Henseleit solution at 20-min intervals. Tissue responses (isotonic) were recorded on a Brush Mark 250 recorder via a semi-conductor strain gauge transducer. The tension on the tissue was 300 mg.

Initial experiments consisted of the recording of responses to adrenaline, nor-adrenaline and isoprenaline before and during exposure of the tissue to propranolol hydrochloride 10^{-6} g/ml added 30 min previously. In later experiments the initial relaxing responses to the three catecholamines were omitted and propranolol hydro-chloride 10^{-5} g/ml was in contact with the tissue throughout the duration of the experiment.

Catecholamine responses were recorded for 4 min after which the tissue was washed at 10 min intervals until it returned either to its original, or a steady, level. This usually resulted in an interval of 20–30 min between doses. L-(+)-Ascorbic acid 40 μg/ml and sodium edetate 40 μg/ml were included in the catecholamine solutions.

A maximum contraction and relaxation was produced by acetylcholine and amino-phylline, respectively, at the commencement and termination of each experiment with propranolol hydrochloride 10^{-5} g/ml. The effects of between five and nine different concentrations of a catecholamine were studied in each experiment. The sequential method, in which the tissue is washed to recovery between each dose, was used to record concentration-effect. The difference between concentrations was usually two-fold and the responses ranged from minimal to supra-maximal. Those concentrations producing responses were repeated at least once during each experiment. Responses have been expressed as % of maximum. Regression lines were fitted by the method of least squares.

Catecholamine concentrations refer to final bath concentration of the base in g/ml. Other drug concentrations have been expressed as final bath concentration of the salt in g/ml.

Drugs

Drugs used were acetylcholine chloride (Sigma), aminophylline B.P., (±)-iso-prenaline hydrochloride U.S.P., (—)-noradrenaline bitartrate monohydrate (Levo-phed, Winthrop), (—)-adrenaline tartrate (Parke Davis & Co.), propranolol hydro-chloride (Inderal, I.C.I.), atropine sulphate, diphenhydramine hydrochloride (Benadryl, Parke Davis & Co.), phenoxybenzamine hydrochloride (Dibenyline, S.K.F.) and dibenamine hydrochloride.

RESULTS

Effect of propranolol 10^{-6} g/ml

Isoprenaline, adrenaline and noradrenaline produced relaxations of the norma trachea. The concentration ranges producing minimal to maximal relaxations to these three catecholamines were as follows: isoprenaline: $5 \times 10^{-10} - 5 \times 10^{-9}$ g/ml; adrenaline: $4 \times 10^{-9} - 3 \times 10^{-8}$ g/ml; noradrenaline: $4 \times 10^{-8} - 2 \times 10^{-7}$ g/ml.

A parallel shift to the right of the isoprenaline, adrenaline and noradrenaline log concentration: response (relaxation) lines occurred in the presence of propranolol 10^{-6}. However, contractions of the tissue were observed in response to concentra-tions of adrenaline and noradrenaline below those required to produce relaxations as a result of antagonism of the propranolol β-adrenergic receptor blockade. A similar effect was not observed with isoprenaline.

The size of the contractions was insufficient to permit estimations of the relative potency of adrenaline and noradrenaline to be made. In view of this, it was decided to increase the concentration of propranolol ten-fold in an attempt to enhance the

contraction size, and also to increase the concentration range within which these catecholamine contractions could be studied.

Effect of propranolol 10^{-5} g/ml

The use of propranolol at this concentration qualitatively increased both the contraction size and concentration range within which contractions to adrenaline and noradrenaline occurred.

A typical record of a section of an experiment made with adrenaline in the presence of propranolol 10^{-5} g/ml is shown in Fig. 1. The responses shown are the result of

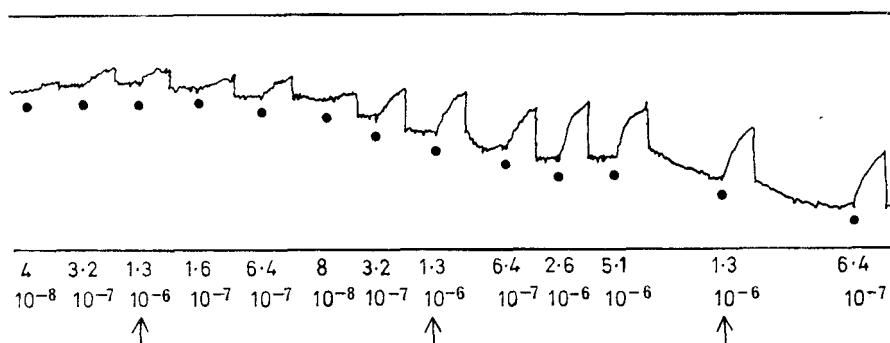|												
4	3·2	1·3	1·6	6·4	8	3·2	1·3	6·4	2·6	5·1	1·3	6·4
10^{-8}	10^{-7}	10^{-6}	10^{-7}	10^{-7}	10^{-8}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-6}	10^{-6}	10^{-7}

Fig. 1. Guinea-pig isolated trachea suspended in Krebs-Henseleit solution at 37° containing propranolol hydrochloride 10^{-5} g/ml. Responses shown are due to adrenaline at bath concentrations (g/ml) indicated. Adrenaline contact time: 4 min. Interval between doses: 20–30 min. Line above the responses was drawn between the two maximum acetylcholine contractions; line below was drawn between two maximum aminophylline relaxations. Note the influence of loss of inherent tone on the three responses to adrenaline $1·3 \times 10^{-6}$ g/ml indicated by arrows (↑).

various concentrations of adrenaline indicated in the figure. The line above the responses was drawn between the two maximum acetylcholine contractions; the line below was drawn between the two points of maximum relaxation to aminophylline. This procedure was carried out in each experiment. The distance between these two lines was essentially the same at both the beginning and end of all experiments indicating that the tissues were still able to produce their full range of response as exhibited by aminophylline and acetylcholine. With these two lines, it is possible to assess the inherent tone of the tissue at any stage of an experiment. The catecholamine contractions may also be expressed in terms of the maximum acetylcholine response of the tissue. A gradual loss of tone occurred during all experiments. This effect, illustrated in Fig. 1, usually occurred within 2–4 h of commencement of an experiment. The rate of loss of tone between preparations was variable. In some preparations a sudden, and relatively rapid, loss occurred over a 2 h period, whilst in others the effect was much slower and a period of 6 h was required before a steady level was reached. At this point, further relaxation could be produced by aminophylline in all tissues.

The tone of the tissue was found to influence the size of the response to a standard dose of adrenaline. This is illustrated also in Fig. 1 where the arrows indicate the responses produced by a standard dose $(1·3 \times 10^{-6}$ g/ml) of adrenaline at various times during the same experiment. A large increase in the size of the response was observed as inherent tone was lost; however, when the tone of the tissue had reached a

steady level, the responses to the same concentration of adrenaline were constant. Hence, the increase in contraction amplitude to a standard dose of adrenaline is dependent upon the loss of tone of the tissue.   Very similar results were obtained in the experiments made with noradrenaline.

The ability of isoprenaline to produce contractions in the presence of propranolol 10^{-5} g/ml was also investigated.   Contractions were not observed at concentrations below those antagonizing the β-adrenergic receptor blockade.

The dose-response relation for adrenaline and noradrenaline is shown in Fig. 2. Six experiments were made with each catecholamine.   The regression lines were
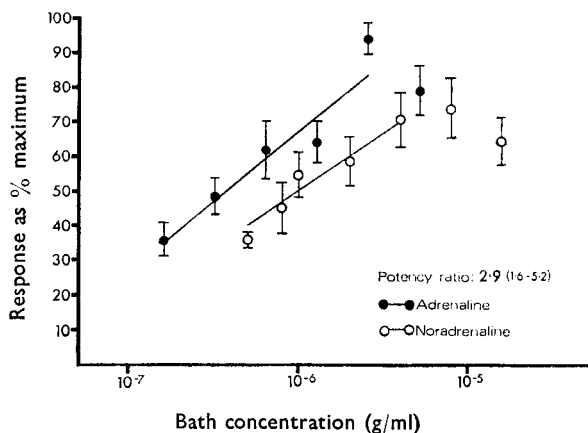

Fig. 2.   Mean dose-response lines for adrenaline (●) and noradrenaline (○) contractions of guinea-pig isolated trachea in the presence of propranolol hydrochloride 10^{-5} g/ml.   Each line represents the mean results from six experiments.   Vertical bars represent standard errors of the means.

calculated from the pooled results of each series of experiments.   The large standard errors of the means of the points plotted are due to the variation in responses within the same experiment, as shown in Fig. 1, and also to the differences in sensitivity between preparations.   Statistical analysis of the results established that both regressions were linear over the concentration range indicated.   No significant departure from parallelism was found and the lines did not coincide.   Adrenaline was 2·9 (95% limits, 1·6–5·2) times more potent than noradrenaline.   The responses to adrenaline and noradrenaline diminished at concentrations greater than those producing peak effects. If higher concentrations than those shown in Fig. 2 were used, then a relaxation occurred due to antagonism of the propranolol β-adrenergic receptor blockade.

An estimate of the size of the catecholamine contractions in relation to the maximum acetylcholine response was obtained by expressing the largest catecholamine response in each experiment as a percentage of the acetylcholine maximum.   The mean response from the six adrenaline experiments was 38·4 (s.e. $\pm$ 2·1)%.   The corresponding figure from the noradrenaline series of experiments was 32·0 (s.e. $\pm$ 3·5)%. There was no significant difference (Student's test) between the two means.

Effect of α-adrenergic blocking agents on adrenaline contractions

The effect of phenoxybenzamine and dibenamine was investigated on adrenaline responses at various points on the adrenaline dose response curve.   At concentrations

ranging from 10^{-6} to 10^{-5} g/ml, both phenoxybenzamine and dibenamine exerted partial to almost complete antagonism of the responses elicited by adrenaline in the presence of propranolol 10^{-5} g/ml. The effect of phenoxybenzamine 8×10^{-6} g/ml on the contractions due to adrenaline 2×10^{-6} g/ml is illustrated in Fig. 3. Potentiation of


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   AD            AD    PB    AD
2 × 10⁻⁶              8 × 10⁻⁶
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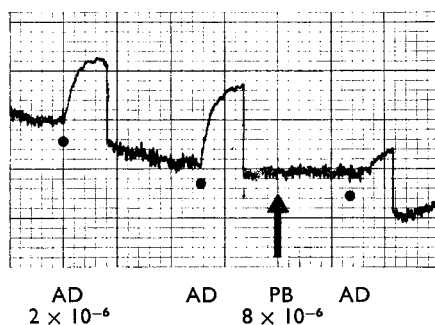
FIG. 3. Isolated guinea-pig trachea suspended in Krebs-Henseleit solution at 37° containing propranolol hydrochloride 10^{-5} g/ml. Antagonism of adrenaline (AD) contractions by phenoxy benzamine (PB). Concentrations are in g/ml. Adrenaline contact time: 4 min.

the adrenaline contractile responses by either phenoxybenzamine or dibenamine was never observed.

Effect of other blocking agents on adrenaline contractions

The contractions produced by adrenaline 2×10^{-6} g/ml in the presence of propranolol 10^{-5} g/ml, were unaffected by either atropine (5×10^{-7} g/ml) or diphenhydramine (10^{-6} g/ml). These concentrations of antagonists were shown to be sufficient to block the effects of acetylcholine and histamine respectively.

DISCUSSION

The guinea-pig isolated trachea has been shown to contract in response to adrenaline and noradrenaline, when propranolol is present in sufficient concentrations to block the β-adrenergic effects of these catecholamines. The size of the responses was shown to be related to inherent tone of the tissue. The largest responses elicited by either adrenaline or noradrenaline occurred when the tissue had minimal tone. Adrenaline was more potent than noradrenaline; however, isoprenaline exhibited no activity under the same conditions. This finding substantiates the observation by Widdicombe (1963) concerning the lack of reports of airway constriction with isoprenaline. The contractions elicited by adrenaline in the presence of propranolol were antagonized by the α-adrenergic blocking agents, phenoxybenzamine and dibenamine, but were unaffected by atropine or diphenhydramine. Thus, the antagonism exhibited would seem to be unrelated to the reported anticholinergic and antihistamine activities of phenoxybenzamine and dibenamine (Goodman & Gilman, 1965).

The order of potency of the three catecholamines in the experiments described coincides with the order of α-adrenergic activity found by Ahlquist (1966) in a wide range of biological actions. This, together with the antagonism exhibited by phenoxybenzamine and dibenamine, satisfies the adrenergic receptor characterization criteria of Ahlquist, and leads to the conclusion that the catecholamine contractions of the guinea-pig isolated trachea are mediated by α-adrenergic receptors.

The results reported here are in agreement with the findings of Takagi, Osada & others (1967), but at variance with those of Foster (1966). Foster never observed contractions to noradrenaline in the presence of propranolol in the concentrations as high as 2×10^{-5} g/ml and concluded that the adrenergic receptors of the guinea-pig trachea were β-receptors. The potentiation of the catecholamine relaxing responses by α-adrenergic blocking agents was explained as being solely due to inhibition of catecholamine uptake (Foster, 1966). Blockade of the α-adrenergic receptors may contribute to this phenomenon.

The previously reported existence of α-adrenergic receptors in dog bronchioles (Castro de la Mata & others, 1962) and now in the guinea-pig trachea, raises the possibility of the existence of similar receptors in human bronchial tissue. This would seem especially pertinent in view of the similar drug sensitivities of guinea-pig tracheal and human bronchial preparations (Hawkins & Schild, 1951), and the similar behaviour of guinea-pig bronchial and human asthmatic bronchial tissues (Herxheimer, 1967). If such receptors were shown to exist, the question should then be raised as to the role of these receptors in the regulation of bronchial tone in both the normal and asthmatic states.

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