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Adrenoceptive receptors in the duodenum, aorta and atria of the rabbit

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pA_{10} values for the α-receptor blocking drug phentolamine against adrenaline, noradrenaline and phenylephrine were determined in rabbit aorta and duodenum, and for the β-receptor blocking drug propranolol, against adrenaline, noradrenaline and isoprenaline in rabbit atria and duodenum. The values for phentolamine against different amines were found to be similar, varying by less than half a log unit, between or within the tissues, as were the values for propranolol. These data provide quantitative evidence that α-receptors in the aorta and duodenum are similar, as are the β-receptors in the atria and duodenum, even though both types of receptor serve divergent functions in different tissues.

ACCORDING to current concepts, α-adrenoceptive receptors subserve stimulation in most tissues but inhibition in the intestines, while β-receptors subserve inhibition in most tissues but excitation in the heart. Such divergence in function of either type of adrenoceptive receptors in different tissues calls for quantitative evidence to support the view that a particular type of receptor is the same even though it serves opposite functions in different tissues.

Drug receptors are best identified by specific antagonists. Schild (1949) introduced 'pA' as an index for measuring specificity of antagonism and Arunlakshana & Schild (1959) used this method to show that the acetylcholine receptors in skeletal muscle were different from those at the muscarinic sites while they were the same in a variety of muscarinic sites. In the present study we have determined pA_{10} values for an α- and a β-adrenergic blocking agent (phentolamine and propranolol respectively) against selected sympathomimetic amines in different tissues of the rabbit, to obtain quantitative data about the interaction between active drugs and antagonists at the α- and β-receptors.

Experimental

METHODS

Three isolated tissue preparations obtained from albino rabbits weighing between 1·5 and 3 kg were used: spirally cut aortic strips for the excitatory effect through α-receptors, excised atria for the excitatory effect through β-receptors, and duodenal segments for the inhibitory effect through both α- and β-receptors. The required tissue was removed quickly and placed directly into chilled McEwen solution (McEwen, 1956). The preparations were cleared of extraneous tissue and suspended in baths containing McEwen solution (10 ml at 37° for aortic strips and duodenal segments, and 30 ml at 29° for the atria) bubbled with a mixture of oxygen 95% and carbon dioxide 5%.

Isotonic contractions of duodenal segments and aortic strips against 4 g tension and magnified ninefold were recorded by a frontal writing lever on a smoked drum. Isometric contractions of excised atria were

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recorded on a polygraph by means of a force displacement transducer (Grass FT–03). Before making any tests, aortic strips were suspended for 90 min and duodenal segments and atria were allowed to contract for 60 min. The bathing fluid was changed every 15 min during this waiting period.

pA_{10} DETERMINATION

The dose of each agonist producing approximately 50% of the maximal effect was determined in preliminary experiments on aortic strips, and then used as the unit dose in the determination of pA_{10} values in all tissues. The effects of these doses of agonists varied widely in different tissues, but were always submaximal. The effect of this selected dose was tested repeatedly in each experiment until steady responses were obtained. The effect of a tenfold unit dose (10x) was then tested in the presence of a certain concentration of the antagonist to which the tissues had been exposed for 3 min. After allowing time for recovery of the tissues from the effect of the antagonist, the effect of the 10x dose of the agonist was measured again in the presence of a higher concentration of the antagonist. In this way the concentration of the antagonist was increased stepwise by approximately half a log unit at a time, until that concentration was reached which reduced the effect of the 10x dose of the agonist to less than the effect of the unit dose in the absence of the antagonist.

DRUGS AND SOLUTIONS

Four sympathomimetic amines were used: (—)-noradrenaline bitartrate monohydrate and phenylephrine hydrochloride as predominantly α-agonists, isoprenaline bitartrate dihydrate as a predominantly β-agonist, and (—)-adrenaline bitartrate as a potent agonist on both receptor systems. Propranolol hydrochloride, phentolamine hydrochloride and 2-bromolysergic acid diethylamide were the antagonists used. Solutions were made in 0·9% saline solution and stored in the frozen state.

Phenoxybenzamine was used in a few experiments on the duodenum. The stock solution of phenoxybenzamine hydrochloride was made in acidified propylene glycol and stored at 4°. Dilutions when required were made in 0·9% saline solution.

Results

Preliminary experiments indicated that the pA_{10} values for phentolamine against different agonists were between 7 and 6 in both tissues and the pA_{10} values for propranolol were between 6 and 5. For the final determination of the pA_{10} values, therefore, closely spaced concentrations of the antagonists within the range of 1 to 1·5 log units were used. Fig. 1 shows the protocol of a typical experiment. After washing out the antagonist and 10x dose of the agonist, the unit dose of the agonist was added every 30 min to determine when the tissue had recovered from the effect of the previous dose of the antagonist (only the first and the last of these responses are shown in Fig. 1).

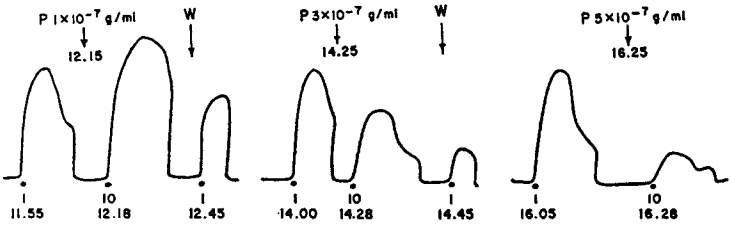

FIG. 1. pA_{10} determination of phentolamine against adrenaline on rabbit aortic strip. All contractions with adrenaline (1) 3×10^{-8} and (10) 3×10^{-7} g/ml. At P ↓ the indicated concentration of phentolamine was added 3 min before testing the effect of 10x concentration of adrenaline. At W ↓ phentolamine was washed out. The time sequence is also shown.

In the experiments on aortic strips, the effect of a 10x dose of the agonist in the presence of different concentrations of the antagonist was calculated as a percentage of the effect of the unit dose in the absence of the antagonist. With duodenal segments and atria, the effect of the unit dose as well as 10x dose of the agonist was expressed as a percentage change in the height of spontaneous contraction. The results from two experiments, one with duodenum and another with aorta, have been plotted in Fig. 2 to illustrate the method used for deriving the concentra-

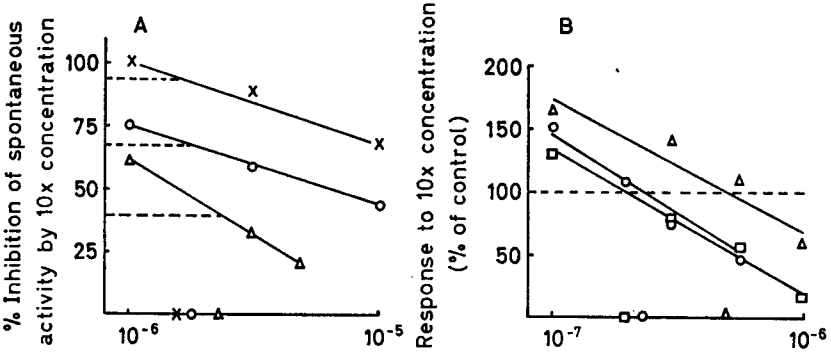

FIG. 2. Graphic derivation of the concentration equivalent to pA_{10} of (A) propranolol (g/ml) on duodenum and (B) phentolamine (g/ml) on aortic strip; ○ adrenaline, × isoprenaline, △ noradrenaline, □ phenylephrine. The broken lines are drawn through the effect of the unit dose in the absence of the antagonist. The symbols on the abscissae indicate the concentrations of either antagonist corresponding to the pA_{10} values against each agonist.

tion from which pA_{10} was calculated. Using this procedure, pA_{10} values for each agonist-antagonist pair were determined in 3–6 experiments in each tissue and the results are presented in Table 1.

pA_{10} values of phentolamine against each agonist were lower in the aorta than in the duodenum. Moreover the values for noradrenaline in either tissue were lower by 0·3–0·6 log units than the values for adrenaline or phenylephrine. For propranolol, the values for all the agonists within each tissue were nearly identical, but the values in the duodenum compared to the atria were consistently lower by about

TABLE 1. pA_{10} VALUES ± S.E. OF PHENTOLAMINE AND PROPRANOLOL AGAINST DIFFERENT SYMPATHOMIMETIC AMINES IN RABBIT AORTA, DUODENUM, AND ATRIA

	Phentolamine	
	Aorta	Duodenum
Adrenaline	6·14 ± 0·06 (6)	6·49 ± 0·02 (3)
Noradrenaline	5·74 ± 0·07 (5)	6·17 ± 0·25 (3)
Phenylephrine	6·12 ± 0·08 (6)	6·50 ± 0·23 (3)
	Propranolol	
	Atria	*Duodenum
Adrenaline	5·71 ± 0·08 (3)	5·14 ± 0·36 (3)
Noradrenaline	5·75 ± 0·41 (3)	5·10 ± 0·38 (5)
Isoprenaline	5·65 ± 0·33 (3)	5·06 ± 0·13 (5)

* Values determined on duodenal segments pre-exposed to 10^{-6} g/ml of phenoxybenzamine for 15 min. Figures in parentheses are number of experiments.

0·5 log units. The values for propranolol in the duodenum were obtained with segments pretreated for 15 min with phenoxybenzamine 10^{-6} g/ml, because in the absence of the latter drug there was wide variation from experiment to experiment in the degree of blockade produced by propranolol. Vanov (1963) has also noted inconsistent blockade by pronethalol against sympathomimetics in the rabbit duodenum. Exposure to this concentration of phenoxybenzamine did not appreciably alter the doses of agonists nor the spontaneous activity of the preparations.

Phenylephrine caused stimulation instead of inhibition in some duodenal segments after pretreatment with phentolamine (α-receptor blockade), the tone of these segments being increased while the amplitude of rhythmic contractions was reduced. The degree of stimulation caused by a constant dose of phenylephrine was related directly to the concentration of phentolamine. Furthermore this stimulatory effect was not modified by propranolol added before or during exposure to phenylephrine. However 2-bromolysergic acid diethylamide (BOL) in a concentration of 10^{-6} g/ml completely abolished this stimulatory effect of phenylephrine.

Discussion

It is generally accepted that receptors of similar configuration are concerned in situations where an antagonist gives the same pA_x against different agonists on a given tissue or where it gives the same pA_x against a given agonist in different tissues. The theoretical basis for this relationship has been discussed by Arunlakshana & Schild (1959), Ariëns (1964) and Furchgott (1964), the crucial point being that pA is strictly a measure of affinity of the antagonist. The question, however, is what degree of difference in the pA values indicates different receptors. Clark & Raventos (1937) noted a difference of more than 4 log units in the pA_{10} values of atropine–acetylcholine between the frog rectus muscle and the frog auricles. Arunlakshana & Schild (1959) reported a difference

of 3 log units between the pA_2 values of atropine against acetylcholine and histamine. Similarly Kohli & Innes (1964) reported a difference of nearly 3 log units between the pA_2 values of BOL against 5-hydroxytryptamine (5-HT) and noradrenaline. In the present study, the pA_{10} values of phentolamine against the various sympathomimetic amines varied by less than 0·5 log unit both within and between the tissues. Similarly the values for propranolol were close, both within and between the tissues. These results therefore suggest (*a*) that the sympathomimetics examined all act on the same receptor in a given tissue, and (*b*) that α-receptors in the aorta and duodenum are similar, as are the β-receptors in the atria and the duodenum.

Because few quantitative studies of this type are available, it is difficult to say what the significance of the small differences in the pA_{10} values between different tissues might be (Table 1). Furchgott (1964) has pointed out that pA values calculated on the basis of drug concentrations in the bath fluid may not be true. Therefore the differences in the pA values between the tissues may not be real but may be explained by the difference in the partition coefficients of the tissues between the aqueous phase and the biophase. Another possible explanation for this difference in pA_{10} values may be that sympathomimetic amines can interact with more than one type of receptor and the different tissues may differ in the proportion of different receptors on which these agonists can act. In this regard it is interesting that phenylephrine caused contraction instead of relaxation after previous exposure to phentolamine and that this stimulatory effect was not modified by propranolol but was abolished by BOL. This observation suggests that phenylephrine might produce this stimulatory effect, directly or indirectly, through 5-HT receptors. The ability of some sympathomimetics to interact with 5-HT receptors in other tissues has been reported previously (Vane, 1960; Innes, 1963; Kohli, 1965).

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