

Adrenoceptors of the chick rectum

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Summary

1. The aim of the investigation was to determine whether the adrenoceptors of the chick rectum preparation complied with Ahlquist's classification; phentolamine (0.1 $\mu\text{g/ml}$) and propranolol (0.05 $\mu\text{g/ml}$) were used as specific antagonists of α - and β -adrenoceptors, respectively.
2. Propranolol antagonized phenylephrine, isoprenaline and noradrenaline in the chick rectum preparation, although according to Ahlquist's classification phenylephrine stimulates α -adrenoceptors selectively.
3. In the presence of propranolol, phenylephrine and noradrenaline produced small residual relaxations of the chick rectum preparation which were blocked by phentolamine. In the absence of propranolol, however, phentolamine did not antagonize relaxations to phenylephrine, isoprenaline or noradrenaline.
4. The adrenoceptors of the guinea-pig colon preparation complied with Ahlquist's classification in that they were like those generally present in intestine.

Introduction

The chick rectum preparation is highly sensitive to adrenaline and is used for the assay of adrenaline in mixtures of adrenaline and noradrenaline (Vane, 1969). In the present experiments α - and β -adrenoceptor antagonists have been used to compare the adrenoceptors of the chick rectum and guinea-pig colon preparations.

Methods

Isolated organs

Chicks (Brown Leghorn) aged 1 to 3 weeks, guinea-pigs and large male rats were decapitated. The lumen of the chick isolated rectum was washed through with Krebs solution (composition in g/l.: NaCl, 6.90; KCl, 0.35; CaCl₂, 0.28; KH₂PO₄, 0.15; MgSO₄.7H₂O, 0.29; NaHCO₃, 2.10; glucose, 2.00), and the preparation left open at both ends. The guinea-pig ascending colon was opened and divided into two longitudinal strips. The glandular tissue was removed from the curved blind end of the rat seminal vesicle which emptied its contents in a Petri dish of Krebs solution. The preparations were mounted in 40 ml organ baths containing Krebs solution at 35° C and gassed with 5% carbon dioxide in oxygen. The responses of the longitudinal muscle were recorded isotonicly with a tension of 1 to 2 g. In experiments where sympathomimetic amines were tested on the chick rectum preparation the bathing solution contained acetyl- β -methylcholine (2 $\mu\text{g/ml}$) to raise the tone. Preparations were rested for 30 min before testing drugs.

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Measurement of drug antagonism

Agonists were left in contact with the preparations for 45 or 60 s at intervals of 5 or occasionally 10 min, the solution in the organ bath being changed twice after each addition of the agonist. An antagonist was added to the bathing solution after responses to agonists had become constant. Antagonism was measured in terms of the dose ratio (Gaddum, Hameed, Hathway & Stephens, 1955), which is the ratio of equiactive concentrations of agonist in the presence and absence of the antagonist.

Choice of adrenoceptor blocking compounds

Adrenaline antagonists were tested on the rat seminal vesicle preparation contracting to noradrenaline and the chick rectum preparation relaxing to isoprenaline or contracting to acetylcholine and histamine. Antagonists which specifically blocked α - and β -adrenoceptors were required. Phentolamine 0.1 $\mu\text{g/ml}$ antagonized noradrenaline (dose ratio, 6.0 ± 1.0 , 4 experiments) but not isoprenaline, histamine or acetylcholine, whereas tolazoline 10 $\mu\text{g/ml}$ was approximately equipotent as an antagonist of noradrenaline but also antagonized histamine. Propranolol 0.05 $\mu\text{g/ml}$ antagonized isoprenaline (dose ratio, $1,390 \pm 626$, 4 experiments) but not noradrenaline, histamine or acetylcholine, whereas MJ-1999 50 $\mu\text{g/ml}$ was approximately equipotent as an antagonist of isoprenaline but also antagonized acetylcholine and histamine. Phentolamine (0.1 $\mu\text{g/ml}$) and propranolol (0.05 $\mu\text{g/ml}$) were therefore chosen as satisfactory antagonists of α - and β -adrenoceptors, respectively.

Drugs

Acetylcholine chloride, acetyl- β -methylcholine chloride, histamine acid phosphate, (\pm)-isoprenaline hydrochloride, 4'-(2-isopropylamino-1-hydroxyethyl) methanesulphonanilide hydrochloride (MJ-1999), (-)-noradrenaline bitartrate, phentolamine mesylate, (-)-phenylephrine hydrochloride, propranolol hydrochloride and tolazoline hydrochloride were used. Concentrations of drugs refer to the salts.

Results

Guinea-pig colon preparation

The guinea-pig colon preparation was relaxed by phenylephrine, isoprenaline and noradrenaline, but the onset and duration of the relaxations were not the same. The relaxation reached a peak after about 1 min of exposure to phenylephrine and then gradually became less, whereas it developed slowly and was sustained for 10 min during exposure to isoprenaline. After noradrenaline, the relaxation was intermediate in onset and duration. The concentration-response curves for phenylephrine, isoprenaline and noradrenaline were parallel.

TABLE 1. Antagonism of sympathomimetic amines by propranolol (0.05 $\mu\text{g/ml}$) and phentolamine (0.1 $\mu\text{g/ml}$) in chick rectum and guinea-pig colon preparations

Preparation	Antagonist	Dose ratio \pm s.e. (No. of expts)		
		Isoprenaline	Noradrenaline	Phenylephrine
Chick rectum	Phentolamine	No antagonism	No antagonism	No antagonism
Chick rectum	Propranolol	$213 \pm 72(4)$	$89 \pm 11(4)$	$90 \pm 4(4)$
Guinea-pig colon	Phentolamine	No antagonism	$2.7 \pm 0.3(5)$	$7.7 \pm 0.6(5)$
Guinea-pig colon	Propranolol	$16.5 \pm 2.8(6)$	$1.3 \pm 0.1(6)$	No antagonism
Guinea-pig colon	Phentolamine and propranolol	$12.6 \pm 2.8(5)$	$6.3 \pm 0.8(5)$	$11.0 \pm 1.9(5)$

Phentolamine ($0.1 \mu\text{g/ml}$) and propranolol ($0.05 \mu\text{g/ml}$) did not affect the slope of the curves for phenylephrine and isoprenaline. Propranolol antagonized isoprenaline and noradrenaline but not phenylephrine (Table 1). Phentolamine antagonized noradrenaline and phenylephrine but not isoprenaline. A combination of phentolamine and propranolol antagonized noradrenaline more than did either antagonist alone ($P < 0.01$) but did not antagonize isoprenaline more than did propranolol ($P > 0.1$), or phenylephrine more than did phentolamine ($P > 0.05$).

Chick rectum preparation

Phenylephrine, isoprenaline and noradrenaline produced similar relaxations of the chick rectum preparation. The concentration-response curves for isoprenaline and noradrenaline were parallel, but the phenylephrine curve seemed to have a lower slope. When the dose interval was increased from 5 to 10 min, however, the slope of the phenylephrine curve was steeper (Fig. 1). Phenylephrine seemed to produce a transient desensitization of the adrenoceptors, for when the dose interval was 5 min a high concentration of phenylephrine ($> 10 \mu\text{g/ml}$) inhibited responses to subsequent doses of phenylephrine, isoprenaline or noradrenaline.

In the chick rectum, all three amines were antagonized by propranolol but not by phentolamine, the effect of the antagonists on the responses to phenylephrine being shown in Fig. 2. Propranolol ($0.05 \mu\text{g/ml}$) and phentolamine ($0.1 \mu\text{g/ml}$) did not affect the slopes of the concentration-response curves of the amines. The dose

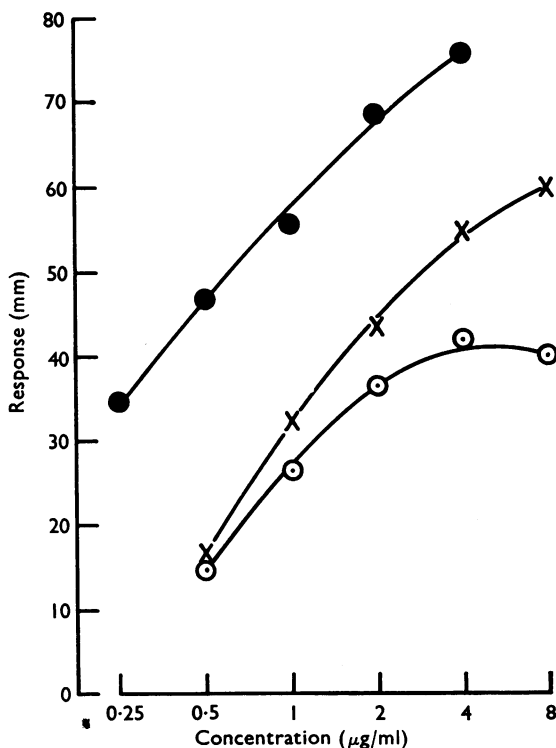


FIG. 1. Chick rectum preparation, tone raised by acetyl- β -methylcholine ($2 \mu\text{g/ml}$). Ordinate, relaxations ($\times 13$); abscissa, concentration of amines ($\mu\text{g/ml}$). Concentration-response curves for noradrenaline (●—●) and phenylephrine (○—○) tested at intervals of 5 min and phenylephrine (×—×) tested at intervals of 10 min.

ratios for isoprenaline and noradrenaline (Table 1) were obtained in experiments in which these amines were tested at intervals of 5 min, but dose ratios for phenylephrine were obtained with preparations exposed only every 10 min to the amine. In earlier experiments (Bartlet & Hassan, 1969) phenylephrine may have desensitized the adrenoceptors as it was added to the bath fluid at intervals of 5 min and this produced dose ratios greater than those reported now.

In the presence of propranolol (0.05 $\mu\text{g}/\text{ml}$), phenylephrine and noradrenaline usually produced small residual relaxations which were abolished by phentolamine

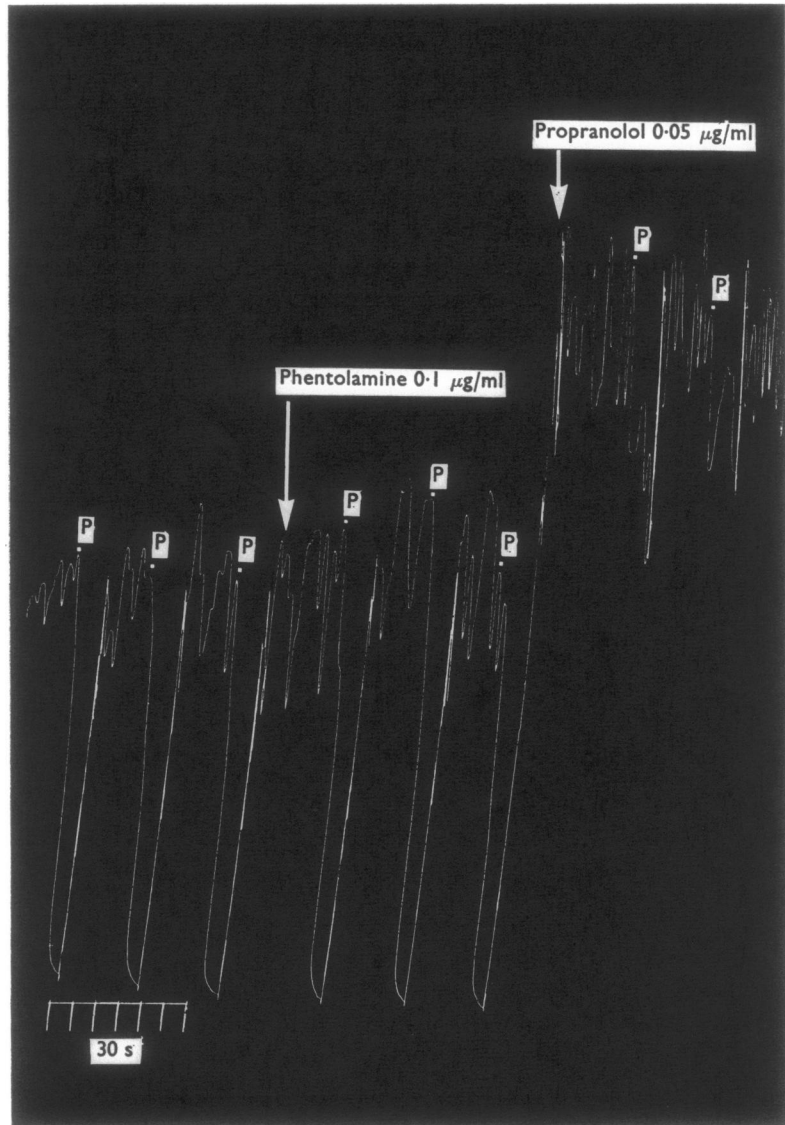


FIG. 2. Chick rectum preparation, tone raised by acetyl- β -methylcholine (2 $\mu\text{g}/\text{ml}$). The dots mark exposure to phenylephrine (1 $\mu\text{g}/\text{ml}$) for 45 s every 10 min. At the first arrow phentolamine (0.1 $\mu\text{g}/\text{ml}$) was added to the bathing solution, and at the second arrow phentolamine was replaced by propranolol (0.05 $\mu\text{g}/\text{ml}$). Time, 30 s.

(0.1 $\mu\text{g/ml}$) but not by propranolol at a higher concentration (1 $\mu\text{g/ml}$). When the concentrations of phenylephrine and noradrenaline were increased to surmount the propranolol antagonism, additional exposure to phentolamine (0.1 $\mu\text{g/ml}$) produced no further measurable antagonism.

Discussion

Phentolamine and propranolol did not alter the slope of the concentration-response curves obtained for isoprenaline, noradrenaline and phenylephrine, an observation which suggests that the antagonists act competitively.

Phenylephrine produced a prompt relaxation of the guinea-pig colon which was blocked by phentolamine, and isoprenaline produced a slow relaxation which was blocked by propranolol; thus, α - and β -adrenoceptors are present in the preparation. The onset and duration of the response to noradrenaline were intermediate to those produced by phenylephrine and isoprenaline and since noradrenaline was antagonized by phentolamine or propranolol it would seem to stimulate both α - and β -adrenoceptors. Thus, the adrenoceptors of the guinea-pig colon preparation are similar to those generally present in the intestine (Levy & Ahlquist, 1967).

In the chick rectum preparation, however, phenylephrine, isoprenaline and noradrenaline produced relaxations which were similar in onset and duration. Moreover, propranolol, but not phentolamine, antagonized phenylephrine, isoprenaline and noradrenaline. It may therefore be concluded that the chick rectum preparation contains adrenoceptors which are readily stimulated by phenylephrine and blocked by propranolol: these receptors do not comply with Ahlquist's classification (Ahlquist, 1948; Levy & Ahlquist, 1967) which designates phenylephrine a selective stimulant of α -adrenoceptors and propranolol an antagonist of β -adrenoceptors only. The predominance of adrenoceptors which combine readily with propranolol and phenylephrine may account for the preparation's ability to discriminate between adrenaline and noradrenaline. Propranolol did not completely antagonize the effects of noradrenaline and phenylephrine in the chick rectum preparation, however, and the small residual relaxations were blocked by phentolamine, suggesting the presence of α -adrenoceptors. When the concentrations of phenylephrine and noradrenaline were increased to surmount the effect of propranolol, additional exposure to phentolamine produced no further demonstrable antagonism, indicating that α -adrenoceptors are a small component of the receptors in the chick rectum preparation.

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