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INTERACTIONS OF SYMPATHOMIMETIC DRUGS, PROPRANOLOL AND PHENTOLAMINE, ON ATRIAL REFRACTORY PERIOD AND CONTRACTILITY

BY

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Govier, Mosal, Whittington & Broom (1966) reported that 5 μM of adrenaline increases atrial refractory period. As in the presence of the α-receptor antagonist phenoxybenzamine adrenaline reduced atrial refractory period, the authors concluded that the increase in atrial refractory period is an action on α-receptors. Shortening of atrial refractory period by adrenaline was assumed to be an effect on β-receptors, as the β-receptor antagonist pronethalol inhibited it.

As we had found that α-receptor antagonists such as phenoxybenzamine and phentolamine can potentiate the effect of noradrenaline on atrial contractility (Benfey & Greeff, 1961), we wondered whether the effect of phenoxybenzamine observed by Govier *et al.* (1966) was due to a potentiation of adrenaline effects, as large concentrations of adrenaline shorten refractory period. Therefore we decided to study a wide range of concentrations of various sympathomimetic drugs.

We found that isoprenaline shortens refractory period in all concentrations used and that phenylephrine prolongs it. Adrenaline had a biphasic action: it prolonged refractory period in small and shortened it in large concentrations. Noradrenaline was similar to adrenaline but less potent in prolonging the refractory period. A small concentration of phentolamine inhibited prolongation of refractory period by adrenaline, noradrenaline and phenlyephrine without increasing the effect of these drugs on contractility. In contrast, cocaine potentiated effects of noradrenaline and adrenaline on atrial contractility without inhibiting prolongation of refractory period by adrenaline. It thus appears that the prolongation of atrial refractory period by phenylephrine and small concentrations of adrenaline and noradrenaline is an effect on α- receptors.

It was observed incidentally that concentrations of the β-receptor antagonist propranolol, which were quite effective against isoprenaline, had little or no effect on the actions of adrenaline, noradrenaline and phenylephrine on atrial contractility, although these concentrations of propranolol had effects on the β-receptors responsible for shortening of the refractory period.

METHODS

Rabbits were killed by a blow on the head, the hearts rapidly excised and strips from the left atrial appendage suspended in an organ bath aerated with 95% O_2 and 5% CO_2 and containing 100 ml. of a solution of the following composition (mM): NaCl, 154; KCl, 4.0; $NaHCO_3$, 2.4; $CaCl_2$, 2.3; glucose, 5.6. Ascorbic acid (0.4 mg/ml.) was added to prevent oxidation of the sympathomimetic drugs. The bath temperature was 31°C. The atrial strips, approximately 8 mm long, 4 mm wide and 1 mm thick, were attached to a Grass force-displacement transducer and held at a resting tension of 1 g. Contractions were recorded on a Sanborn recorder. Two platinum wires, attached to the holder and approximately 10 mm long, touched the strips cross-wise. The strips were stimulated at a frequency of 1 shock/sec by square-wave pulses of 2 msec duration and a voltage 50% above threshold, delivered by a Tektronix 161 pulse generator. Refractory period was measured by delivering a second stimulus, identical to the first, at increasing intervals following the driving stimulus until a response occurred to each stimulus. The interval between stimuli at this point was taken as a measure of the functional refractory period (Govier, 1965). The atrial strips were not used until a constant refractory period was observed. The refractory period was considered to be stable when identical values were obtained in three separate determinations at 5-min intervals, which generally required 35–45 min.

Initially, various concentrations of sympathomimetic drugs were used at random in one preparation and the refractory period determined 5, 10, 15 and 20 min later. The bath was then changed and the refractory period allowed to recover, which usually required 30–90 min. The strips could often be used for several hours.

The initial results were confirmed by cumulative dose-response curves. Sympathomimetic drugs were added in increasing concentrations until the maximum effect on contractility was observed. The next higher dose was not added until the full effect of the previous concentration on contractility had developed, which required 5–15 min. Propranolol was added 45 min and phentolamine and cocaine 30 min before the sympathomimetic drugs.

Control refractory period and contractility are shown in Table 1. They varied considerably in different atrial strips. It appeared that the concentrations of propranolol, phentolamine and cocaine used were such that they did not by themselves produce a significant change in contractility or refractory period.

TABLE 1

CONTROL ATRIAL REFRACTORY PERIOD AND CONTRACTILITY

Sympathomimetic drug added afterwards	Drug pretreatment (μM)	Experiments (no.)	Refractory period (msec±standard error)	Contractility (g±standard error)
Isoprenaline	None	4	157±33	1·00±0·23
Isoprenaline	Propranolol, 0·1	6	207±14	0·54±0·09
Noradrenaline	None	4	213±20	1·03±0·26
Noradrenaline	Propranolol, 0·3	4	202±25	0·87±0·09
Noradrenaline	Cocaine, 0·3	4	206±33	0·74±0·10
Adrenaline	None	7	174±22	0·87±0·17
Adrenaline	Propranolol, 0·3	6	179±14	0·91±0·18
Adrenaline	Propranolol, 1	6	241±20	0·67±0·10
Adrenaline	Phentolamine, 0·3	5	183±10	1·07±0·13
Adrenaline	Cocaine, 0·3	4	194±16	1·04±0·26
Phenylephrine	None	4	183±18	1·05±0·21
Phenylephrine	Propranolol, 0·3	4	179±18	1·10±0·32
Phenylephrine	Phentolamine, 0·3	5	197±19	0·82±0·14

Figures 1, 3, 4 and 5 are cumulative dose-response graphs showing the effect of sympathomimetic drugs on refractory period and contractility as percent change from the control values. For the concentration-effect curve (Fig. 2) the effects were taken as a percentage of the maximum effect obtained. The dose-ratio is the concentration of a sympathomimetic drug causing 50% of the maximal effect in the presence of an antagonist divided by the concentration causing the same effect in the absence of the antagonist.

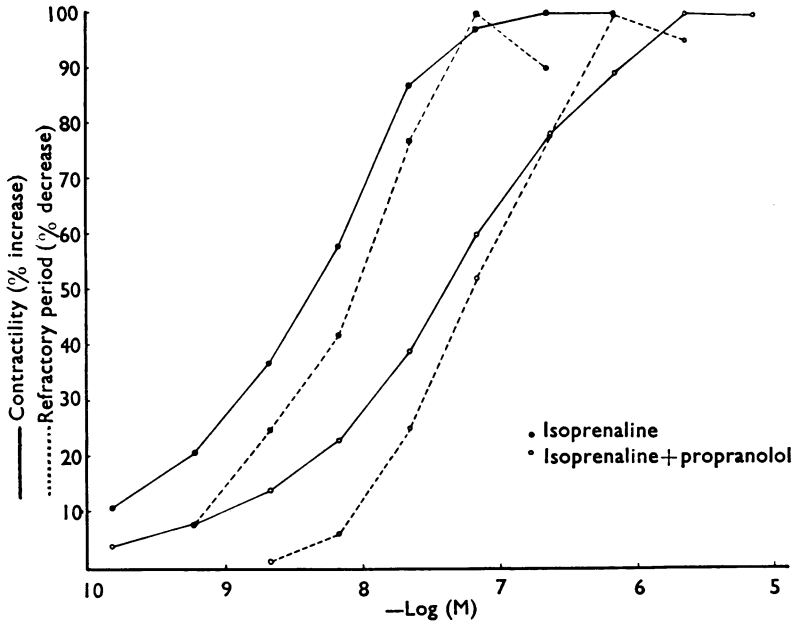

Fig. 1. Effects of isoprenaline on atrial refractory period and contractility in the absence and presence of propranolol (0.1 μM). The data represent percent changes of the controls (Table 1). The concentration of isoprenaline was increased in a cumulative fashion.

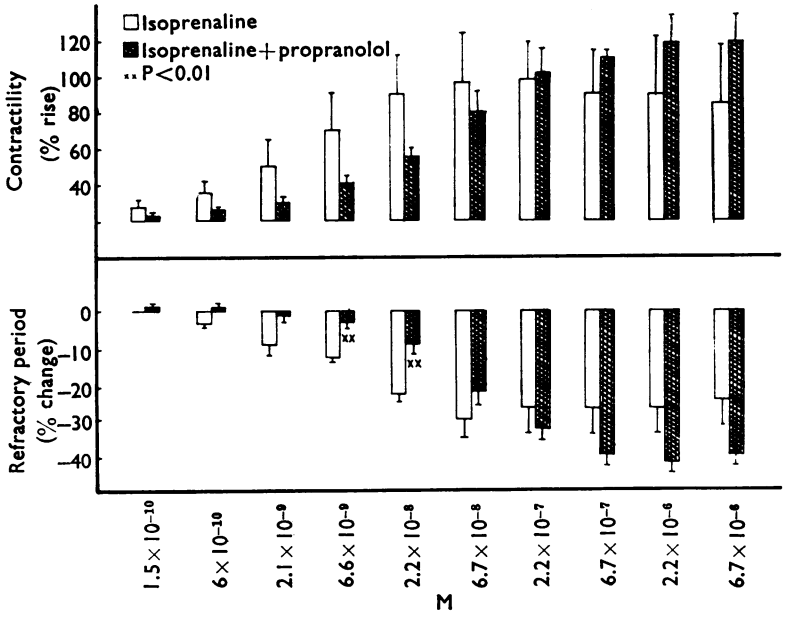

Fig. 2. Effects of isoprenaline on atrial refractory period and contractility in the absence and presence of propranolol (0.1 μM). The data represent percent changes of the maximal effect. The concentration of isoprenaline was increased in a cumulative fashion.

The drugs used were isoprenaline (1-isoproterenol d-bitartrate dihydrate, Winthrop), noradrenaline (levarterenol bitartrate, Winthrop), adrenaline (1-epinephrine bitartrate, Winthrop), 1-phenylephrine HCl (K. & K. Laboratories), propranolol HCl (AY-64043, Ayerst, McKenna & Harrison), phentolamine methanesulphonate (Ciba) and cocaine HCl. Dilutions of the sympathomimetic drugs were made in 0.9% NaCl containing 10 mg/100 ml. ascorbic acid. The statistical calculations were made according to conventional procedures (Mainland, 1952).

RESULTS

Isoprenaline increased atrial contractility and reduced refractory period in all concentrations used ; these effects were inhibited by propranolol (0.1 μM, Fig. 1). Figure 2 shows that propranolol shifted the dose-response curve to the right ; the dose ratio for contractility was 9.1 and that for shortening of the refractory period 6.9.

Noradrenaline slightly prolonged atrial refractory period in small concentrations and shortened it in larger concentrations (Fig. 3). In the presence of propranolol (0.3 μM) prolongation of refractory period became pronounced and the effect of noradrenaline on contractility was slightly reduced (dose-ratio 2.7). Cocaine (0.3 μM) potentiated the

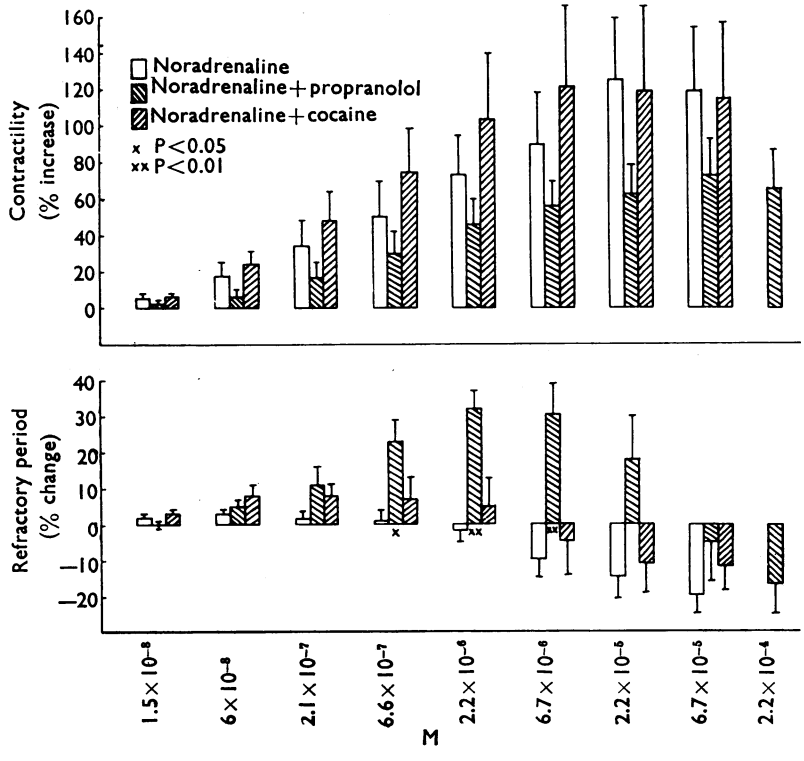

Fig. 3. Effects of noradrenaline on atrial refractory period and contractility in the absence and presence of propranolol (0.3 μM) and cocaine (0.3 μM). The data represent percent changes of the controls (Table 1). The concentration of noradrenaline was increased in a cumulative fashion.

effect of noradrenaline on contractility by a factor of 2.0 and slightly increased the prolongation of refractory period.

Adrenaline prolonged atrial refractory period in small concentrations and shortened it in large concentrations (Fig. 4). In the presence of propranolol (0.3 and 1 μM) prolongation of atrial refractory period was greater. While 0.3 μM propranolol did not inhibit the action of adrenaline on contractility, 1 μM led to a dose-ratio of 2.3. Phentolamine (0.3 μM) inhibited both the prolongation of atrial refractory period and the action of small concentrations of adrenaline on contractility. Cocaine (0.3 μM) potentiated the effects of small concentrations of adrenaline on contractility and refractory period.

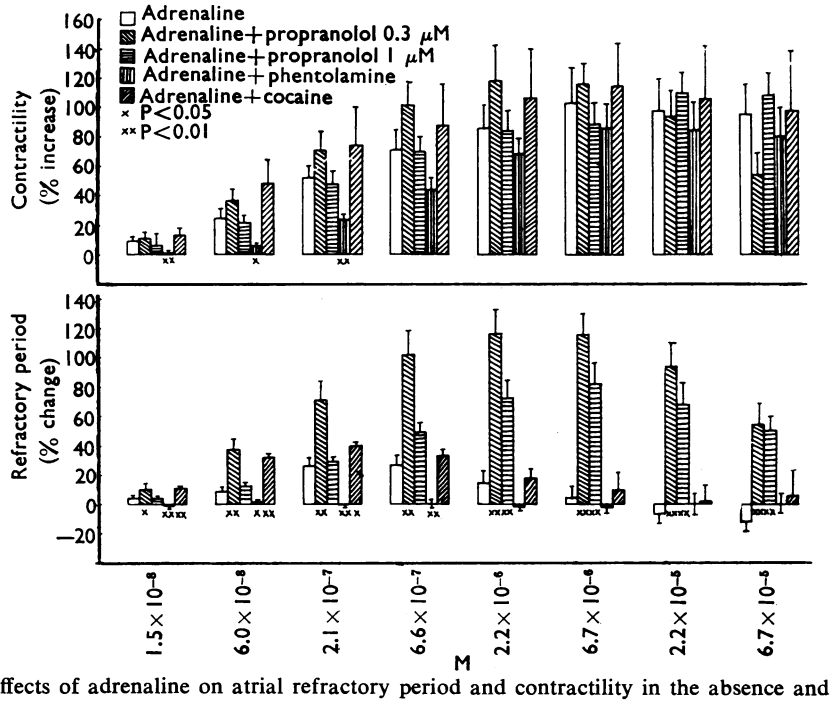

Fig. 4. Effects of adrenaline on atrial refractory period and contractility in the absence and presence of propranolol (0.3 and 1 μM), phentolamine (0.3 μM) and cocaine (0.3 μM). The data represent percent changes of the controls (Table 1). The concentration of adrenaline was increased in a cumulative fashion.

Phenylephrine increased atrial refractory period in all concentrations used (Fig. 5). Propranolol (0.3 μM) did not inhibit the action of phenylephrine on contractility and increased the prolongation of refractory period. Phentolamine (0.3 μM) inhibited the effect of phenylephrine on refractory period.

DISCUSSION

Isoprenaline reduced the refractory period and, as this was inhibited by propranolol, shortening of refractory period by isoprenaline may be regarded as an effect on β-receptors in agreement with Govier *et al.* (1966).

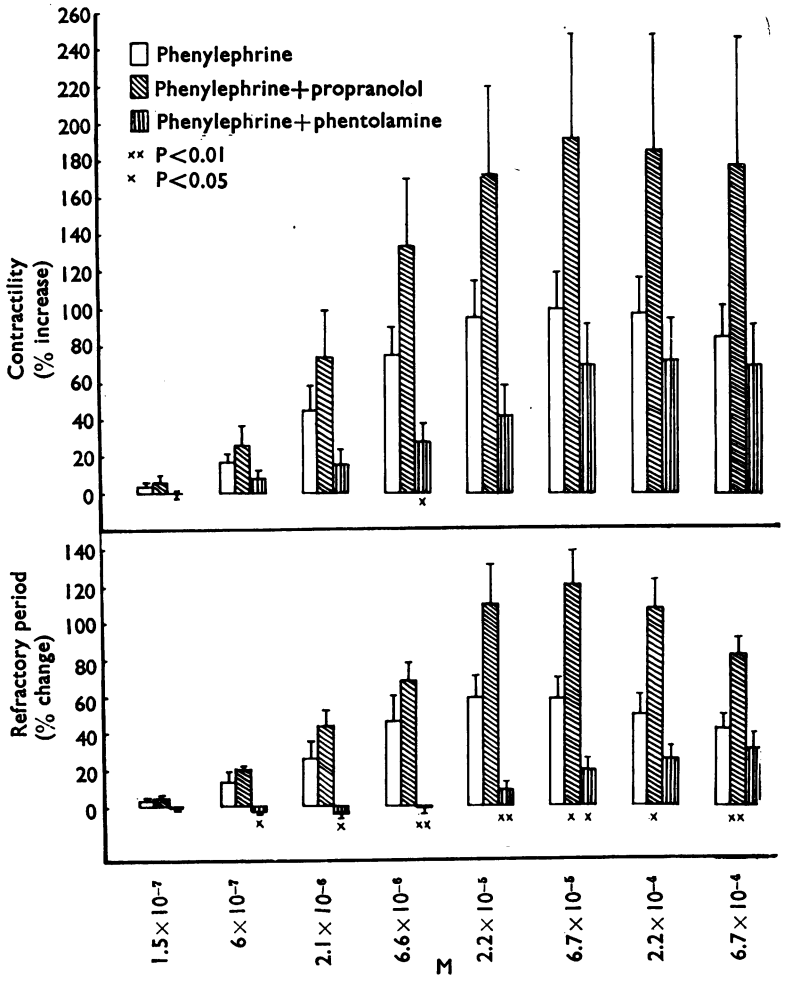

Fig. 5. Effects of phenylephrine on atrial refractory period and contractility in the absence and presence of propranolol (0.3 μM) and phentolamine (0.3 μM). The data represent percent changes of the controls (Table 1). The concentration of phenylephrine was increased in a cumulative fashion.

Adrenaline had a biphasic action on refractory period ; it increased refractory period in small concentrations (0.015–6.7 μM) and reduced it in higher concentrations (22 and 67 μM). Govier *et al.* (1966) showed that 5 μM of adrenaline increases refractory period but shortens it in the presence of 1 μM of phenoxybenzamine, and that pronethalol (0.1 μg/ml.) potentiates the prolongation of refractory period by adrenaline. The present results with phentolamine and propranolol support the conclusion of Govier *et al.* (1966) that the prolongation of atrial refractory period by adrenaline is an effect on α-receptors and the shortening an effect on β-receptors.

Noradrenaline increased refractory period slightly in concentrations of 0.015–0.66 μM and decreased it in concentrations of 2.2–67 μM. Govier *et al.* (1966) found that

noradrenaline (5 μM) reduces refractory period within 5 min and increases it after 20–30 min but had no effect in concentrations of 0.1 and 1 μM. Propranolol significantly increased the prolongation of refractory period by noradrenaline. It appears that when the β-receptors are blocked by propranolol the effect of α-receptor stimulation is stronger.

Phenylephrine, which is known to have prominent effects on α-receptors, increased refractory period in all concentrations used. Propranolol augmented this effect.

Propranolol has an antifibrillatory action and it reduced the maximal rate at which the guinea-pig isolated atrium followed an electrical stimulus (Benfey & Varma, 1966). It appears that this action is greater in the presence of sympathomimetic drugs such as noradrenaline, adrenaline and phenylephrine.

An incidental finding was that propranolol is less potent against the effect of adrenaline, noradrenaline and phenylephrine on atrial contractility than against that of isoprenaline. For example, 1 μM propranolol increased the dose-ratio of adrenaline 2.3-fold, while 0.1 μM propranolol increased the dose-ratio of isoprenaline 9.1-fold. In a concentration of 0.3 μM propranolol increased the dose-ratio of noradrenaline 2.7-fold and had no effect on the actions of adrenaline and phenylephrine on atrial contractility.

On the other hand, 0.3 μM propranolol significantly increased the prolongation of atrial refractory period by noradrenaline, adrenaline and phenylephrine. Thus propranolol seems to be more potent on the β-receptors responsible for shortening of the refractory period than on the β-receptors responsible for the increase in atrial contractility. It appears that propranolol blocks the β-receptors responsible for shortening of the refractory period, thus leading to a greater effect of adrenaline, noradrenaline and phenylephrine on the α-receptors responsible for prolongation of refractory period.

Stimulation of cardiac contractility and rate by sympathomimetic drugs is competitively inhibited by adrenaline β-receptor blocking drugs. Thus in kitten papillary muscle driven at a frequency of 12 beats/min Koch-Weser (1964) found that pronethalol shifts the inotropic concentration-effect curve of noradrenaline to the right in a parallel fashion. The antagonism of pronethalol and noradrenaline appeared to be a simple competitive one. We showed that pronethalol and propranolol competitively inhibit the effect of noradrenaline on contractility in the spontaneously beating guinea-pig atrium (Benfey & Varma, 1966).

Inhibition of sympathomimetic drug effects on cardiac contractility by adrenaline α-receptor antagonists has also been observed (Cotten, Moran & Stopp, 1957; Nickerson & Chan, 1961). Nickerson and Chan (1961) found that 10 μg/ml. phenoxybenzamine doubles the strength of contraction of the cat papillary muscle and reduces the effect of adrenaline and $CaCl_2$ and concluded that "maximum tolerated doses of phenoxybenzamine did not depress the responses of the myocardium to adrenaline, except in preparations and under conditions in which a comparable decrease in response to calcium occurred."

In the present studies 0.3 μM phentolamine (and 0.3 μM phenoxybenzamine) significantly inhibited the effect of small concentrations of adrenaline and phenylephrine on atrial contractility without significantly altering control contractility. The concentration of phenoxybenzamine (0.3 μM) was 0.113 μg/ml. Nickerson & Chan (1961) reported that exposure of the rabbit isolated aorta to 0.05 μg/ml. phenoxybenzamine for 5 min almost

completely eliminated the response to adrenaline and that significant blockade could be obtained with concentrations of 0.01 μg/ml. or less. Thus the concentration of phenoxybenzamine we used was not much greater than that required for effects on the α-receptors of the aorta. However, the mechanism of the inhibition of the effects of adrenaline and phenylephrine on atrial contractility by α-receptor antagonists requires further study. Large concentrations of phenoxybenzamine (25 μg/ml.) and phentolamine (17 μg/ml.) increased contractility and potentiated the effect of noradrenaline on contractility of the spontaneously beating guinea-pig atrium (Benfey & Greeff, 1961).

SUMMARY

1. The interactions of isoprenaline, noradrenaline, adrenaline and phenylephrine and the receptor antagonists phentolamine and propranolol on atrial refractory period and contractility were studied.

2. Isoprenaline increased contractility and shortened refractory period; these effects were competitively antagonized by the β-receptor antagonist propranolol.

3. Noradrenaline slightly increased refractory period in small concentrations and shortened it in large concentrations. In the presence of propranolol the prolongation of refractory period was greater.

4. Adrenaline increased refractory period in small concentrations and shortened it in large concentrations. In the presence of propranolol the prolongation of refractory period was greater. The α-receptor antagonist phentolamine inhibited the prolongation of refractory period.

5. Phenylephrine increased refractory period in all concentrations used which was potentiated by propranolol and inhibited by phentolamine.

6. It is concluded that prolongation of atrial refractory period by noradrenaline, adrenaline and phenylephrine is an effect on α-receptors, while shortening of refractory period by isoprenaline and large concentrations of adrenaline and noradrenaline is an effect on β-receptors.

7. Propranolol was much less effective in inhibiting the action of noradrenaline, adrenaline and phenylephrine on atrial contractility than in inhibiting that of isoprenaline. Phentolamine significantly inhibited the effect of small concentrations of adrenaline and phenylephrine on atrial contractility.

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