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Characterization of α-adrenoceptors in the myocardium

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Summary

1. In the driven rabbit left atrium α-adrenoceptors mediate the inotropic effect of phenylephrine and the prolongation of functional refractory period by sympathomimetic amines.

2. These receptors are highly sensitive to blockade by phentolamine and phenoxybenzamine.

3. Prolongation of functional refractory period is greater with the secondary amines, phenylephrine, adrenaline and epinine, than with the primary amines, norphenylephrine, noradrenaline and dopamine.

Introduction

The effects of phenylephrine on the isolated heart, involving both α- and β-adrenoceptors, remain a matter of controversy. For example, McNeill, Davis & Muschek (1972) wrote: 'Phenylephrine is only 24% as effective as norepinephrine on guinea-pig atria and 56% as effective as norepinephrine in the experiments with rabbit atria. These results are in contrast to those obtained by Benfey & Carolin (1971) who found that phenylephrine was as effective as epinephrine with regard to the positive inotropic effect of the amines.' Disregarding Koch-Weser & Blinks' (1963) warning that changes in the strength of contraction of spontaneously beating preparations cannot be relied on because the strength of contraction is greatly influenced by the frequency of contraction, McNeill *et al.* (1972) recorded cardiac contractility in spontaneously beating atria whereas Benfey & Carolin (1971) used electrically driven preparations. The work described here shows that the inotropic effects of phenylephrine on the spontaneously beating isolated mammalian heart and on the driven preparation are not the same.

Methods

These were the same as those used by Benfey & Varma (1967). Atrial strips were driven at a rate of 1 Hz and the 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. Cumulative concentration-effect curves were obtained by increasing the concentration of the agonists stepwise until the maximal effect was reached.

In the present studies, control contractions were approximately 0·8 g tension and the refractory period was approximately 170 milliseconds.

Drugs in addition to those formerly used (Benfey & Varma, 1967) included norphenylephrine hydrochloride, epinine hydrochloride and dopamine hydrochloride (K & K Labs.), methoxamine hydrochloride (Burroughs Wellcome), phenoxybenz-

amine hydrochloride (Smith, Kline & French) and reserpine (Ciba ; 5 mg/kg was injected i.p. a day before the experiment).

Results

Direct effects of phenylephrine

Phenylephrine prolonged the refractory period and increased the force of contractions of the rate-controlled rabbit left atrium (Figure 1). These effects were not inhibited by reserpine pretreatment or by cocaine (Figure 1). This was taken as evidence that phenylephrine acts directly on the receptors.

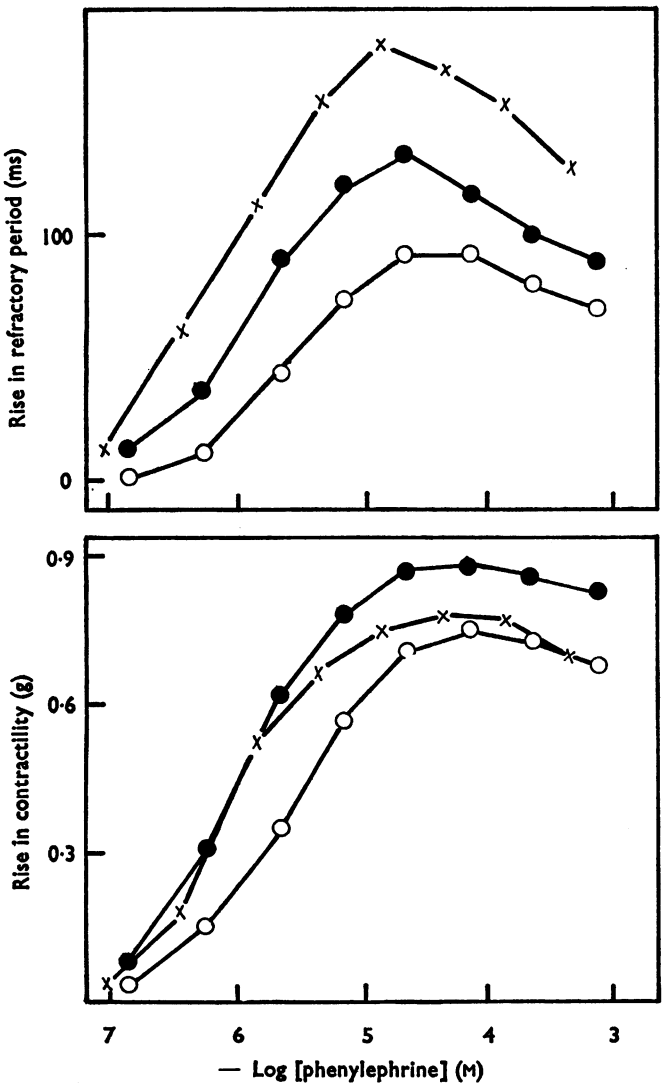

FIG. 1. Effects of phenylephrine on refractory period and contractility of the driven rabbit left atrium, alone (○), following reserpine pretreatment (×) and in the presence of 2·5 μM cocaine (●). Means of 5–7 experiments.

Inhibition of phenylephrine effects

In concentrations as low as 0·03 and 0·1 μM phentolamine, the α-adrenoceptor blocking drug inhibited the effects of phenylephrine on refractory period and on contractility (Figure 2). From concentration-effect curves of phenylephrine in the presence of 0·03–10 μM phentolamine the apparent dissociation constant, K_B (Furchgott,

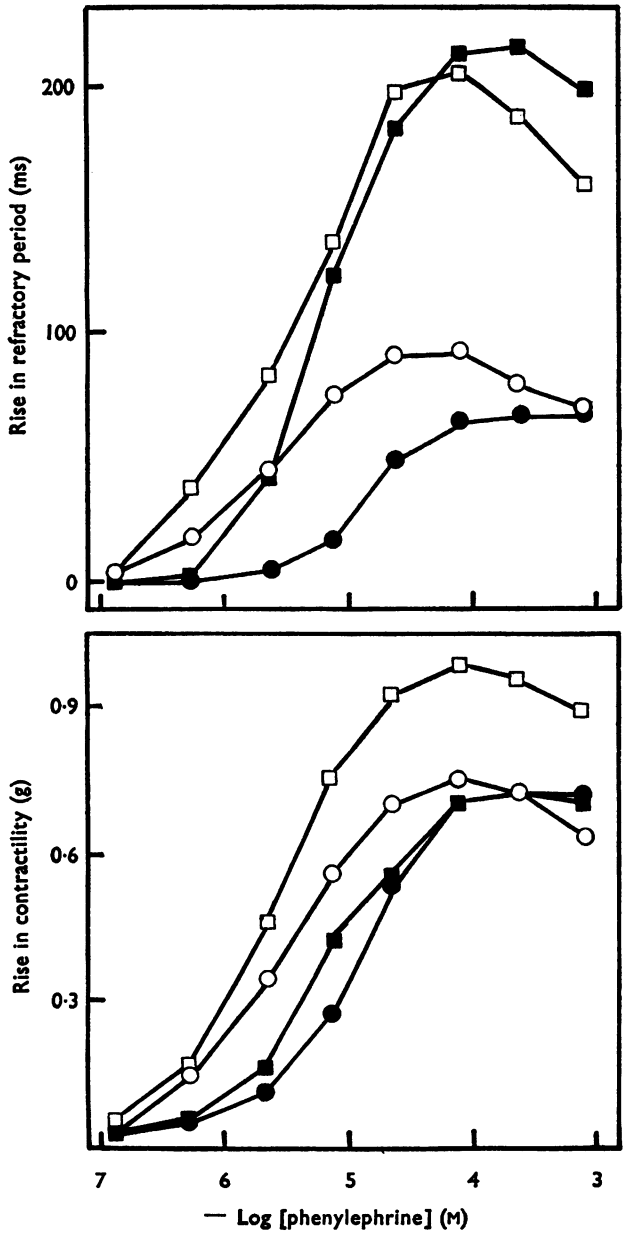

FIG. 2. Effects of phenylephrine on refractory period and contractility of the driven rabbit left atrium in the absence (○) and the presence of 0·1 μM phentolamine (●), 0·3 μM propranolol (□), and 0·3 μM propranolol plus 0·03 μM phentolamine (■). Means of 5–7 experiments.

1967), of phentolamine in the absence and presence of 0·3 μM propranolol was calculated for the inotropic effect as 76 and 28 nM respectively and for the effect on refractory period as 63 and 37 nM, respectively.

The α-adrenoceptor blocking drug, phenoxybenzamine, depressed the maximum of the effects of phenylephrine in a concentration as low as 1 nM and reversed the effect of phenylephrine on refractory period in a concentration of 10 nM (Figure 3).

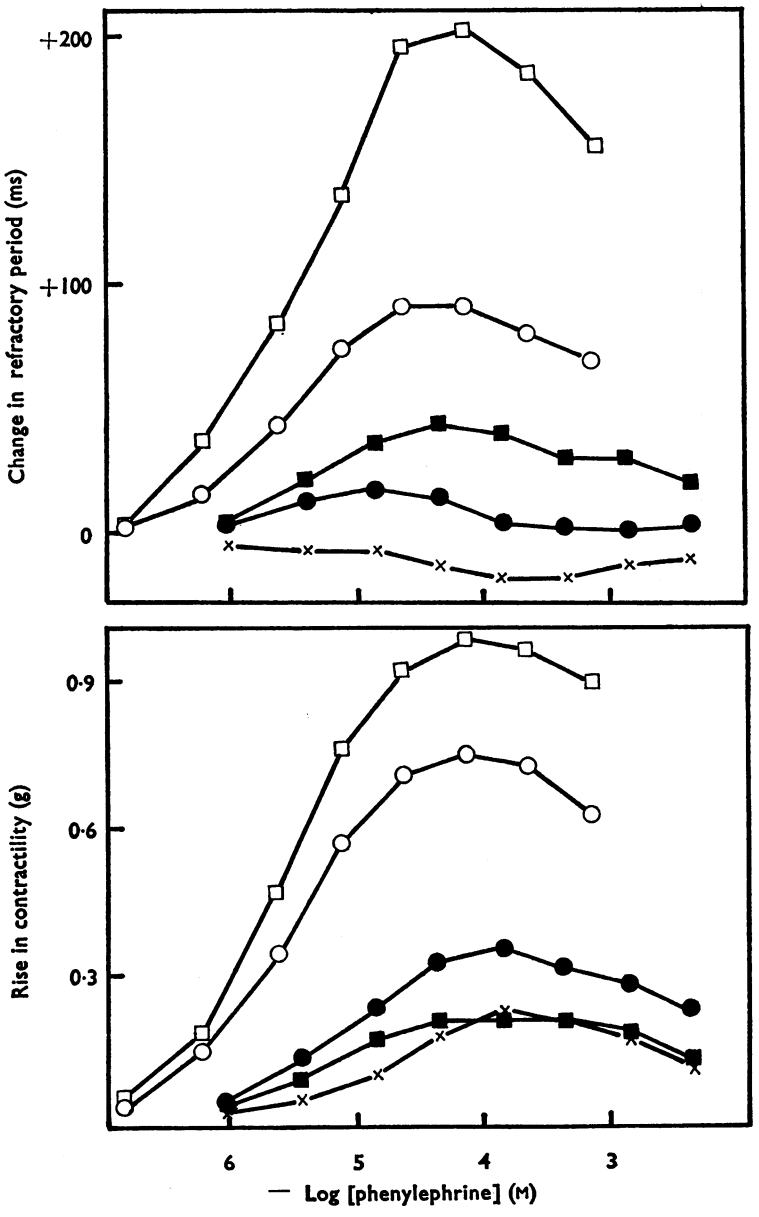

FIG. 3. Effects of phenylephrine on refractory period and contractility of the driven rabbit left atrium in the absence (○) and presence of 1 nM phenoxybenzamine (●), 10 nM phenoxybenzamine (×), 0·3 μM propranolol (□) and 0·3 μM propranolol plus 1 nM phenoxybenzamine (■). Means of 4–7 experiments.

Effects of other sympathomimetic amines

Sympathomimetic amines have a biphasic effect on refractory period, prolonging

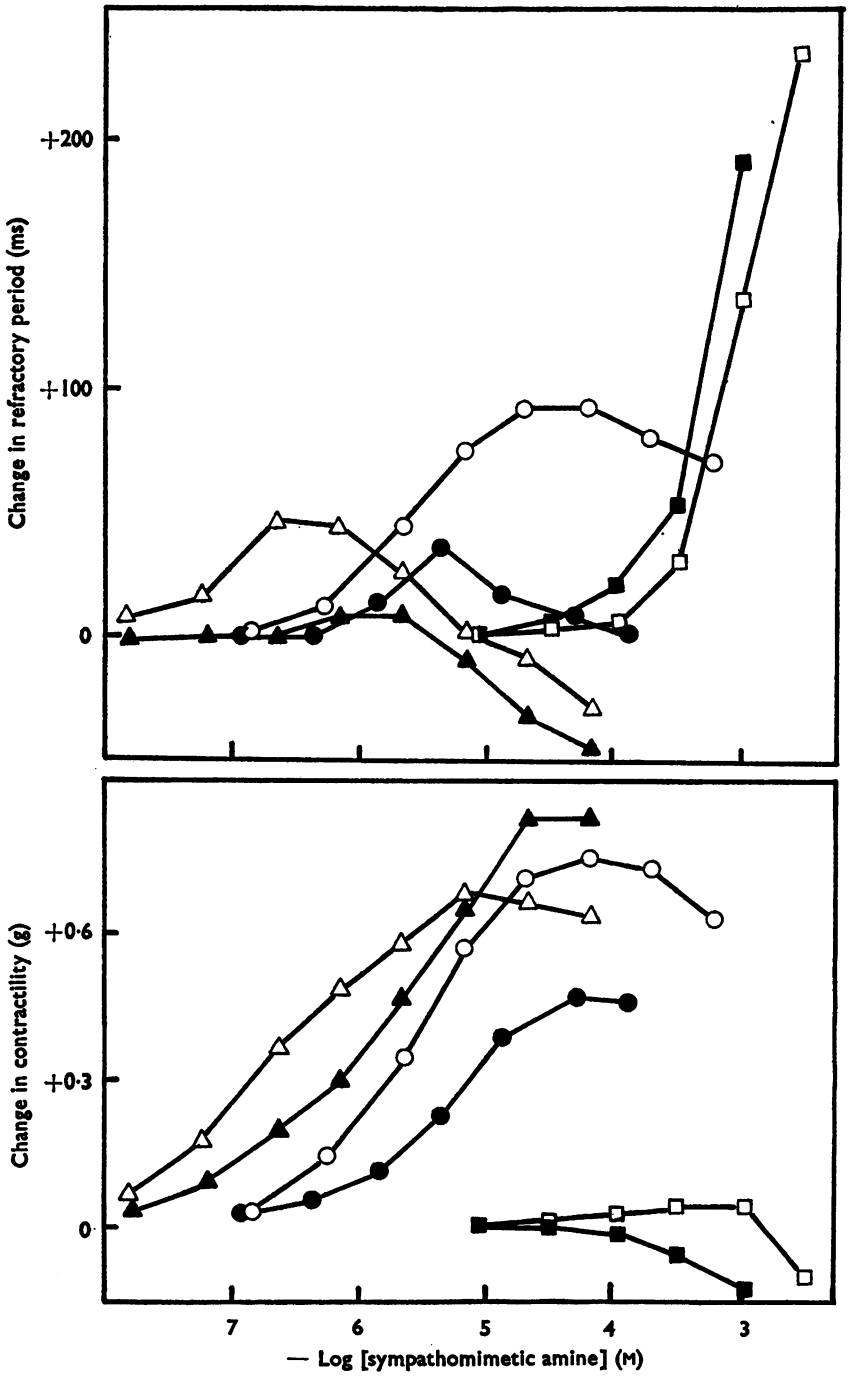

FIG. 4. Effects of phenylephrine (○), norphenylephrine (●), adrenaline (△), noradrenaline (▲), methoxamine (□), and methoxamine plus 10 μM phentolamine (■) on refractory period and contractility of the driven rabbit left atrium. Means of 4–8 experiments.

it through α-adrenoceptor stimulation and shortening it through β-adrenoceptor stimulation (Govier, Mosal, Whittington & Broom, 1966; Benfey & Varma, 1967). In a comparison of secondary and primary amines, phenylephrine and adrenaline prolonged refractory period more than did norphenylephrine and noradrenaline (Figure 4). Epinine and dopamine showed the same behaviour; quantitatively their effects on refractory period were intermediate between those of the ethanolamines.

Methoxamine increased the refractory period at a concentration one hundred times higher than that of phenylephrine and this effect was not inhibited by phentolamine (Figure 4). This observation in addition to the fact that methoxamine did not increase cardiac contractility makes it unlikely that the prolongation of refractory period by methoxamine is the result of α-adrenoceptor stimulation.

Discussion

The existence of myocardial α-adrenoceptors has been demonstrated previously. α-Adrenoceptor blocking drugs inhibited the increase in contractility produced by phenylephrine in the driven rat ventricle (Wenzel & Su, 1966), rabbit atrium (Benfey & Varma, 1967) and guinea-pig atrium (Govier, 1967a, 1968) and also inhibited the prolongation of refractory period caused by adrenaline and phenylephrine in the rabbit and guinea-pig atrium (Govier *et al.*, 1966; Govier, 1967b; Benfey & Varma, 1967). Relatively high concentrations of phentolamine and phenoxybenzamine were used in these experiments and no further attempt was made to characterize the myocardial α-adrenoceptors.

The present results confirm that phenylephrine is an effective stimulant of myocardial α-adrenoceptors and that it is less potent than noradrenaline (Benfey & Varma, 1967; Govier, 1968). The same result was obtained in other tissues in which phenylephrine is an α-adrenoceptor stimulant with a potency five times lower than that of noradrenaline (Furchgott, 1970). In the presence of propranolol the values of the apparent dissociation constant, K_B, of phentolamine against phenylephrine were 28 and 37 nM which compares favourably with the values ranging from 7·3 to 15·6 nM found in other tissues treated with reserpine or in the presence of cocaine (Furchgott, 1970).

The chronotropic effect of phenylephrine on the isolated rabbit atrium (Leong & Benfey, 1968) and guinea-pig atrium (Krell & Patil, 1969) is inhibited by β-adrenoceptor blocking drugs. As a β-adrenoceptor stimulant phenylephrine is not fully efficacious (Trendelenburg, Gomez Alonso de la Sierra & Muskus, 1963) and much less potent than noradrenaline (Furchgott, 1970).

β-Adrenoceptor blocking drugs have been reported to inhibit the inotropic effect of phenylephrine in the spontaneously beating rabbit isolated atrium (Lee & Yoo, 1964; Yoo & Lee, 1970) and dog isolated heart (Kabela, Jalife, Peon, Cros & Mendez, 1969), and α-adrenoceptor blocking drugs were found ineffective. Provided the inotropic effect of phenylephrine is not the result of the increase in rate (see **Introduction**), this finding would indicate that α-adrenoceptors do not respond to phenylephrine when the heart is under the control of the normal pacemaker.

β-Adrenoceptor stimulation is known to increase myocardial cyclic AMP concentration, and in the isolated perfused rat heart Drummond & Hemmings (personal communication) found a small rise in cyclic AMP following administration of a

high dose of phenylephrine. α-Adrenoceptor stimulation does not appear to be associated with a rise in cyclic AMP. In rabbit heart slices Benfey (1971) found that, although 10 μM noradrenaline increased cyclic AMP accumulation four-fold, 1 mM phenylephrine was ineffective.

Prolongation of refractory period by methoxamine did not seem to be mediated by α-adrenoceptors as it was not inhibited by phentolamine. As an α-adrenoceptor stimulant methoxamine is 33 times less potent than phenylephrine (Furchgott, 1970). In the isolated heart methoxamine is known to block β-adrenoceptors and depress contractility (Blinks, 1964). The cardiac depressant effect of methoxamine appears to dominate the present results.

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