Br. J. Pharmac. (1980) **69**, 565–571

α-SYMPATHOMIMETIC AMINES AND CALCIUM-MEDIATED ACTION POTENTIALS IN GUINEA-PIG VENTRICULAR MUSCLE

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1 The ability of amines, having α- or α- and β- adrenoceptor stimulating activity, to restore excitability and contractility in heart preparations partially depolarized by potassium, was investigated in guinea-pig ventricular muscle in order to elucidate the mechanism of the positive inotropic effect mediated via α-adrenoceptors.

2 In preparations in which fast sodium channels were inactivated by K^+-rich medium (22 mM) slow electrical responses as well as contractions were consistently induced by high concentrations of phenylephrine (10^{-4} to 3×10^{-4} M) and synephrine (3×10^{-4} M).

3 The restorative effects of both phenylephrine and synephrine were unaffected by phentolamine (10^{-5} M) but were readily abolished by practolol (10^{-5} M) or sotalol (10^{-5} M).

4 Methoxamine induced a dose-dependent positive inotropic effect in ventricular strips paced at 0.5 Hz in normal Tyrode solution; the maximum increase in contractile tension was obtained with methoxamine 10^{-4} M. However, at the same concentration, the amine did not induce slow electrical responses in potassium-depolarized preparations.

5 It is concluded that the induction of slow responses by phenylephrine and synephrine is due to β-adrenoceptor stimulation, and that the increase in cardiac contractility caused by α-adrenoceptor stimulation does not involve an increase in slow inward calcium current.

Introduction

Both α- and β-adrenoceptor stimulating agents increase cardiac contractility. However, there are reasons to believe that the mechanism underlying their effect is different. The positive inotropic effect mediated by β-adrenoceptors is associated with an accumulation of cellular cyclic adenosine 3',5'-monophosphate (cyclic AMP) (Kukovetz, Pöch & Wurm, 1973; Watanabe & Besh, 1974; Schümann, Endoh & Brodde, 1975; Osnes & Øye, 1975) and with an increase in the 'slow inward calcium current' (Reuter, 1967; 1974; Vassort, Rougier, Garnier, Sauviat, Coraboeuf & Gargouil, 1969). The increase in slow inward current is probably due to an increase in the number of functional Ca^{2+} channels of the cardiac cell membrane (Reuter & Scholz, 1977; for a review see Tsien, 1977).

The characteristics of the α-adrenoceptor-mediated positive inotropic effect and the differences compared with the β-adrenoceptor-mediated inotropic effect have been extensively described in myocardial preparations of man, cat, rabbit, guinea-pig and rat (Ledda, Marchetti & Mugelli, 1975; Endoh & Schümann, 1975; Endoh, Wagner & Schümann, 1975; Mugelli, Ledda & Mantelli, 1976; Brückner, Hackbarth, Meinertz, Schmlzle & Scholz, 1978; Mary-Rabine, Hordof, Bowman, Malm & Rosen, 1978; Osnes, Refsum, Skomedal & Øye, 1978; Schümann, Wagner, Knorr, Reidemeister, Sadony & Schrann, 1978).

On this basis it has been suggested that the biochemical and electrophysiological mechanisms by which the two kinds of adrenoceptors induce a positive inotropic effect are different. A dissociation between cyclic AMP levels and cardiac contractile activity has been observed in rat and rabbit heart when phenylephrine (an α- and β-adrenoceptor agonist) was administered together with a β-adrenoceptor blocking drug (Osnes & Øye, 1975; Brodde, Motomura, Endoh & Schümann, 1978). A similar cyclic AMP-independent inotropic effect has been described in rabbit and cat papillary muscle for methoxamine (Rabinowitz, Chuck, Kligerman & Parmley, 1975; Schümann *et al.*, 1975). In the rabbit papillary muscle the positive inotropic effect of phenylephrine, during blockade of β-adrenoceptors, is depressed by D600 (a 'calcium antagonistic' compound, Fleckenstein, 1971), suggesting that the effect of α-adrenoceptor stimulation may be caused mainly by a change of calcium influx through the myocardial cell membrane (Endoh *et al.*, 1975). However, in guinea-pig ventricular muscle, phenylephrine (unlike isoprenaline) does not counteract the negative inotropic action of D600, suggesting that the amine is a very weak promoter of

0007-1188/80/080565-07 $01.00

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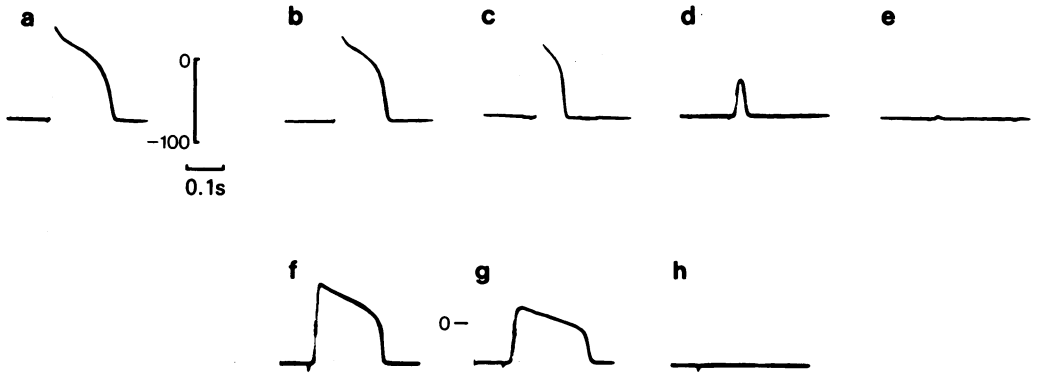

Figure 1 (a–h) Influence of EDTA on the slow electrical responses induced by isoprenaline in a potassium-depolarized preparation. Upper row: stimulation rate 2 Hz; (a) transmembrane action potential recorded from a ventricular strip of guinea-pig heart; (b–e) 1, 2, 3 and 4 min respectively after exposure to K^+-rich medium. The zero of the voltage calibration is the zero potential. Lower row: stimulation rate 0.5 Hz; (f) calcium-mediated action potential induced by isoprenaline 3×10^{-7} M in K^+-rich medium; (g–h) 10 and 30 min respectively after treatment with EDTA 10^{-5} M in the presence of isoprenaline. Zero potential between first and second panel. Temperature: 30°C.

calcium transmembrane influx, especially at concentrations at which it acts as a pure α-adrenoceptor agonist (Ledda *et al.*, 1975; Figure 3).

To clarify these points, we have used the slow responses as a test of the ability of α- and β-adrenoceptor stimulating agents to increase the calcium transmembrane influx.

A preliminary report of these studies has appeared in abstract form (Mugelli, Mantelli & Ledda, 1977).

Methods

The experiments were carried out according to the method described by Pappano (1970) with minor modifications. Right ventricle strips of guinea-pig heart were placed in a 15 ml chamber containing Tyrode solution of the following composition (mM): NaCl 115, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 10. The solution was aerated with a gas mixture of 97% O_2 and 3% CO_2; the pH of the bathing solution was 7.4 and the temperature was maintained at 30°C. The preparations were electrically stimulated at a constant rate (2 Hz) by square wave pulses delivered by a wave form generator (Tektronix 162) and a pulse generator (Tektronix 161) connected to an isolation unit. The stimulus duration was 0.3 ms, and the intensity twice the threshold voltage. Transmembrane action potentials were recorded from a single cell by microelectrodes filled with 3 M KCl (resistance about 10 MΩ).

After a 60 min period of equilibration, the preparations were exposed to a high potassium Tyrode solution, containing 22 mM KCl (with an equimolar reduction of NaCl), which depolarized them and made them inexcitable within a few minutes. The stimulation rate was then reduced to 0.5 or 0.4 Hz and the stimulus intensity and duration were doubled. Increasing concentrations of adrenoceptor stimulating amines were then added to the bathing solution at 10 min intervals; the threshold concentration of the amine capable of restoring electrical activity as well as contraction was determined. The isometric contractions were measured by means of an isometric transducer and a d.c. amplifier. The preparations were mounted vertically in a double walled chamber containing 20 ml of Tyrode solution having the same composition as the one used for electrophysiological experiments, except that the calcium concentration was 3.6 mM.

In each experiment one or more antagonists were used in order to identify the receptors involved in the responses. The following substances were used: (±)-isoprenaline hydrochloride (Fluka); (−)-phenylephrine hydrochloride (K. and K. Laboratories); (−)-synephrine tartrate (kindly supplied by Boehringer, Ingelheim); methoxamine hydrochloride (kindly supplied by Wellcome); (±)-practolol hydrochloride (kindly supplied by CIBA); tetrodotoxin (TTX, Sankyo); disodium edetate (EDTA, Merck); D_{600} hydrochloride (δ[N-(3,4-dimethoxyphenethyl)-N-methylamino] - α - (3,4,5 - trimethoxyphenyl)α - iso - propylvaleronitrile hydrochloride; Knoll).

Results

Figure 1 shows the progressive depolarization obtained by superfusing the preparation with a potassium-rich solution. After the preparation had

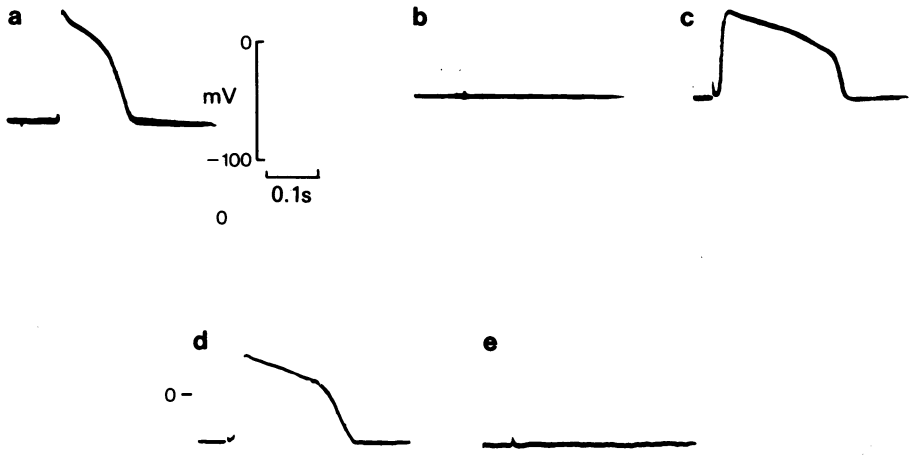

Figure 2 (a–e) Influence of tetrodotoxin, phentolamine and sotalol on the slow electrical responses induced by phenylephrine in a potassium-depolarized preparation. Upper row: (a) transmembrane action potential recorded from a ventricular strip of guinea-pig heart; (b) depolarization and inexcitability after exposure to K^+-rich medium; (c) calcium-mediated action potential induced by phenylephrine 10^{-4} M in K^+-rich medium after 5 min of exposure. Zero of the voltage calibration represents the zero potential. Lower row: (d) persistence of the phenylephrine-induced response after treatment with tetrodotoxin 10^{-5} M for 15 min and phentolamine 10^{-5} M for 10 min; (e) blockade of the phenylephrine-induced response by sotalol 10^{-5} M after 8 min. Zero potential on the left side. Records (a–b) stimulation rate 2 Hz; records (c–e) stimulation rate 0.4 Hz. Temperature 30°C.

become inexcitable, isoprenaline, a pure β-adrenoceptor agonist, was added to the bath; in four experiments slow electrical responses (calcium-mediated action potentials) as well as contractions (not shown) were consistently induced by isoprenaline (10^{-7} to 3×10^{-7} M); these responses were consistently blocked by EDTA (10^{-5} M) or by the inhibitor of calcium inward current, D600 (0.5 μg/ml; not shown).

It is known that phenylephrine activates both α- and β-adrenoceptors (Schümann, Endoh & Wagner, 1974; Wagner, Endoh & Reinhardt, 1974; Ledda *et al.*, 1975). In six experiments, phenylephrine (10^{-4} to 3×10^{-4} M) restored excitability in K^+-depolarized preparations in less than 10 min (Figure 2). The calcium-mediated action potentials induced by the amine were not blocked by 10^{-5} M tetrodotoxin (2 experiments) or by 10^{-5} M phentolamine (Figure 2), but were abolished within a few minutes by β-blocking drugs (10^{-5} M sotalol in 3 experiments, 10^{-5} M practolol in 3 experiments, see Figure 2). Also in 6 experiments the phenylephrine-induced restoration of mechanical activity was blocked by 10^{-5} M practolol but not by 10^{-5} M phentolamine (Figure 3).

Synephrine stimulates both α- and β-adrenoceptors (Endoh, Schümann, Krappitz & Hillen, 1976); synephrine (3×10^{-4} M) induced slow electrical responses (4 experiments) and restored contractility (3 experiments). These effects were blocked consistently by 10^{-5} M practolol but not by 10^{-5} M phentolamine (Figure 4 and Figure 5, respectively).

Methoxamine causes a frequency-dependent positive inotropic effect through α-adrenoceptor stimulation (Endoh & Schümann, 1975; Rabinowitz *et al.*, 1975) and is a weak antagonist of β-adrenoceptors (Imai, Shigei & Hashimoto, 1961). We have confirmed that in guinea-pig ventricular strips paced at 0.5 Hz, methoxamine consistently caused a dose-dependent positive inotropic effect (Figure 6); the maximum increase in contractile tension was obtained with methoxamine 10^{-4} M (+22.3% ± 6.0: mean values ± s.e. of 5 experiments). However, at the same concentration methoxamine did not induce slow electrical responses even after a long period of exposure (3 experiments). In this group of experiments electrical activity as well as contractility were restored by the application of drugs which activate the calcium inward current (histamine 10^{-5} M, 1 experiment; isoprenaline 3×10^{-7} M, 2 experiments).

Discussion

It has been demonstrated repeatedly that catecholamines cause the appearance of slow electrical responses in potassium-depolarized cardiac preparations through β-adrenoceptor stimulation (Carmeliet & Vereecke, 1969; Pappano, 1970).

Cardiac tissues, partially depolarized by potassium, are considered as suitable preparations to investigate drug effects on the slow inward current by many authors (Pappano, 1970; Shigenobu & Sperelakis, 1972; Tritthart, Volkmann, Weiss &

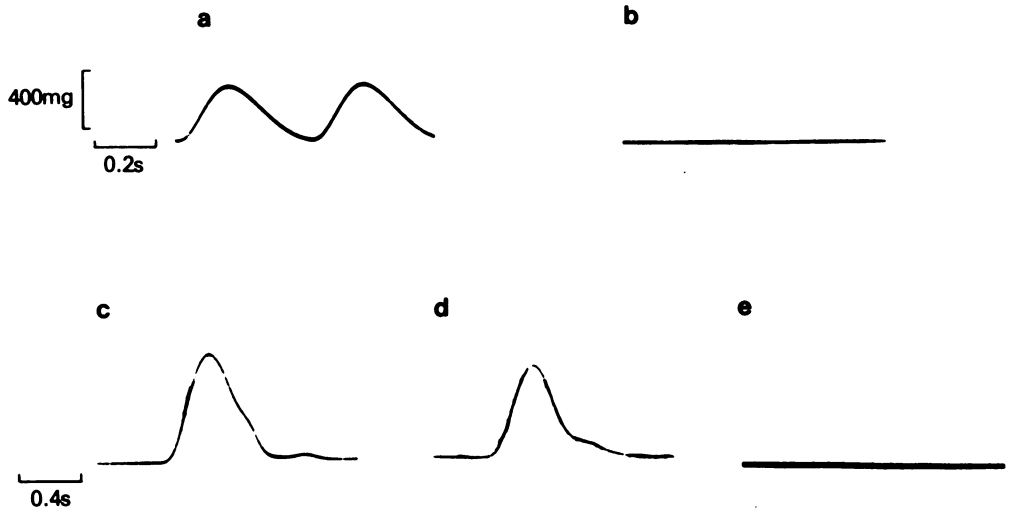

Figure 3 (a–e) Influence of phentolamine and practolol on the phenylephrine-induced contractions in a potassium-depolarized preparation. Upper row: stimulation rate 2.5 Hz; (a) isometric contraction recorded from a ventricular strip of guinea-pig heart; (b) inexcitability after exposure to K^+-rich medium. Lower row: stimulation rate 0.4 Hz; (c) restoration of mechanical activity induced by phenylephrine 3×10^{-4} M in K^+-rich medium after 10 min of exposure; (d) persistence of mechanical activity 15 min after phentolamine 10^{-5} M; (e) blockade of the phenylephrine-induced response after 14 min treatment with practolol 10^{-5} M, in the presence of phentolamine. Temperature 30°C.

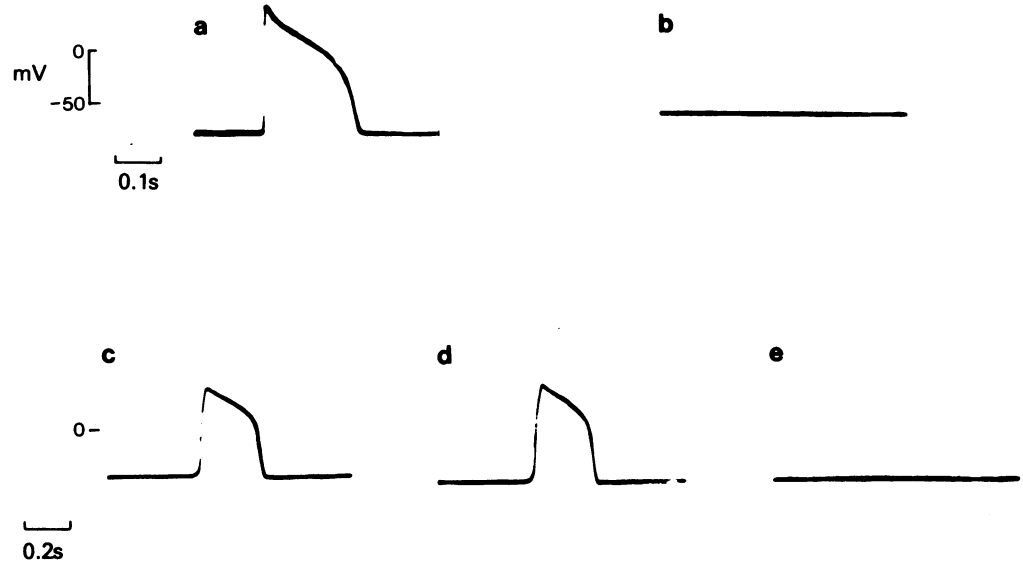

Figure 4 (a–e) Influence of phentolamine and practolol on the slow electrical responses induced by synephrine in a potassium-depolarized preparation. Upper row: stimulation rate 2 Hz; (a) transmembrane action potential recorded from a ventricular strip of guinea-pig heart; (b) depolarization and inexcitability during exposure to K^+-rich medium. The zero of the voltage calibration is the zero potential. Lower row: stimulation rate 0.5 Hz; (c) calcium-mediated action potential induced by synephrine 3×10^{-4} M in K^+-rich medium after 6 min of exposure; (d) persistence of the synephrine-induced response after 10 min treatment with phentolamine 10^{-5} M; (e) blockade by practolol 10^{-5} M of the synephrine-induced response after 10 min. Zero potential on the left side of first panel. Temperature: 30°C.

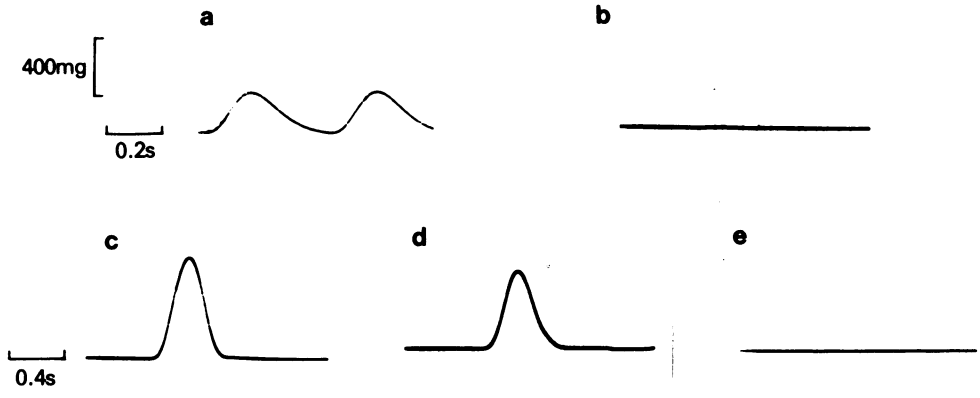

Figure 5 (a–e) Influence of phentolamine and practolol on the synephrine-induced contractions in a potassium-depolarized preparation. Upper row: stimulation rate 2.5 Hz; (a) isometric contraction recorded from a ventricular strip of guinea-pig heart; (b) inexcitability during exposure to K^+-rich medium. Lower row: stimulation rate 0.4 Hz; (c) restoration of mechanical activity induced by synephrine 3×10^{-4} M in K^+-rich medium; (d) persistence of mechanical activity 10 min after phentolamine 10^{-5} M; (e) blockade of the synephrine-induced response by treatment with practolol 10^{-5} M for 3 min. Temperature: 30°C.

Eibach, 1976; Inui & Imamura, 1976); the restoration of excitability and contractility in these preparations can be taken to indicate that the drugs increase calcium influx.

In the present study it was observed that both phenylephrine and synephrine induced slow responses. Evidence has been previously obtained to show that the positive inotropic effect of phenylephrine is mediated by α-adrenoceptors at concentrations of up to 3×10^{-6} M and by β-adrenoceptors at higher concentrations both in the rabbit papillary muscle (Schümann *et al.*, 1974) and in guinea-pig ventricular strip (Ledda *et al.*, 1975).

Synephrine shares with phenylephrine the ability to stimulate both α- and β-adrenoceptors (Endoh *et al.*, 1976). The induction of slow responses by phenylephrine and synephrine observed in the present study can be ascribed to β-adrenoceptor stimulation; in fact such restorative effect was induced only by high concentrations of the amines, and was consistently blocked by β-adrenoceptor blocking drugs but not by phentolamine. However, our observations are not in

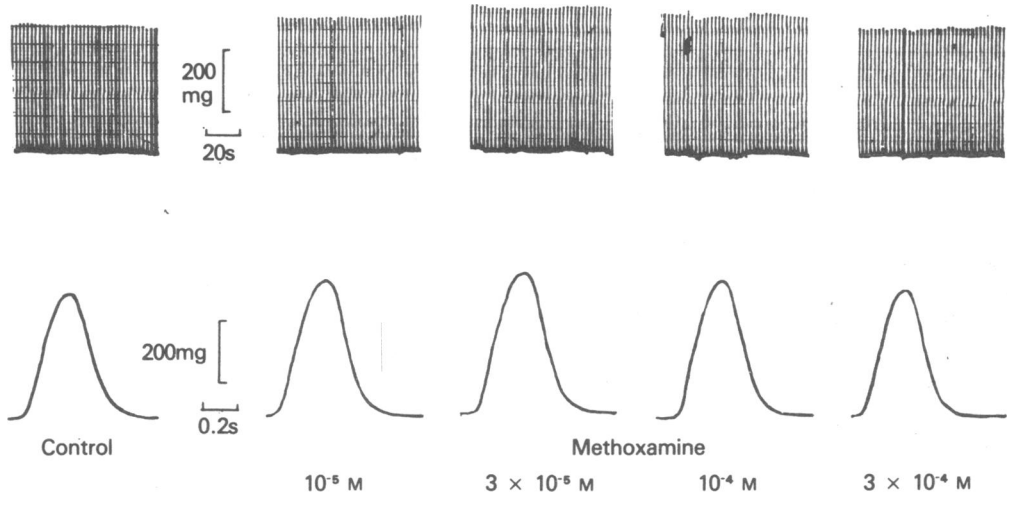

Figure 6 Effect of methoxamine on contractile tension in guinea-pig ventricular muscle. Chart recorded traces. The effect of methoxamine on contractile tension is shown at low (above) and fast (below) chart speed. Stimulation rate 0.5 Hz. Temperature: 30°C.

agreement with those obtained by Miura, Inui & Imamura (1978), who found that phenylephrine (10^{-6} to 10^{-4} M) induced slow responses in partially depolarized rabbit papillary muscle in the presence of a β-adrenoceptor blocking drug (bufetolol 10^{-6} M); this effect was suppressed by phentolamine 10^{-6} M in 4 out of 7 experiments.

Possible explanations of this discrepancy can be found either in the species difference or in the differences in experimental techniques used: namely, the use of field stimulation with a higher stimulus duration and of a lower concentration of β-adrenoceptor blocking drug. In fact, in our experimental conditions we never observed slow responses with phenylephrine concentrations up to 10^{-4} M in the presence of practolol (10^{-6} to 10^{-5} M).

As phenylephrine and synephrine stimulate both α- and β-adrenoceptors, we considered it useful to study the effect of methoxamine, which is able to induce a positive inotropic effect through α-adrenoceptor stimulation and is devoid of any β-adrenoceptor stimulating activity (Endoh & Schümann, 1975; Rabinowitz *et al.*, 1975).

Our results showed that, despite its positive inotropic effect, methoxamine failed to restore the excitability and contractility in potassium depolarized preparations. It could be suggested that methoxamine increased calcium influx but failed to induce an action potential, because simultaneously it increased the potassium efflux. This interpretation is made unlikely by the findings of Hauswirth, Wehner & Ziskoven (1976), who demonstrated that methoxamine actually suppresses the background potassium current (i_{k1}). However, the effect on outward current seems to be of very little significance in the induction of slow responses by phenylephrine. Such a suppression of i_{k1} would cause a depolarization, that has not been observed with phenylephrine (Ledda, Marchetti & Manni, 1971).

From our results it appears that the stimulation of α-adrenoceptors does not induce slow responses, and that the α-adrenoceptor blocking drug, phentolamine, is not able to antagonize the restorative effect of either phenylephrine or synephrine.

These results indicate that the α-adrenoceptor-mediated positive inotropic effect is elicited through a mechanism other than that induced by stimulation of β-adrenoceptors. They support the hypothesis that this mechanism is not associated with an increase in slow inward calcium current and confirm our previous observation that α-adrenoceptor stimulation does not antagonize the negative inotropic effect of D600 (Ledda *et al.*, 1975).

We wish to thank Professor Mario Vassalle for reading the manuscript and offering helpful suggestions. This work was partly supported by a grant from the Italian Research Council (CT 77-01386).

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(Received May 15, 1979
Revised August 7, 1979.)