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STUDIES ON THE POSITIVE INOTROPIC EFFECT OF PHENYLEPHRINE: A COMPARISON WITH ISOPRENALINE

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1 The effects of phenylephrine and isoprenaline on the isometric contraction of guinea-pig ventricle were compared over the whole range of their respective dose-response curves.

2 In preparations driven at 2.5 Hz the increase in contractile force induced by either isoprenaline or phenylephrine was linearly correlated to an increase in maximum velocity of force development. The relaxation time was shortened by isoprenaline but not by phenylephrine.

3 The negative inotropic effect induced by δ[N-(3,4-dimethoxyphenethyl)-*N*-methyl-amino]-α-(3,4,5-trimethoxyphenyl)α-isopropylvaleronitrile hydrochloride (D_{600}) was reversed by isoprenaline, but little influenced by phenylephrine.

4 The study of the interval-force relationship shows that the increase in contractile force induced by phenylephrine (3×10^{-5} M) was relatively greater at low frequencies of stimulation, and that the maximum effect was reached at the frequency of 1 Hz.

5 The positive inotropic effect of phenylephrine (10^{-4} M) was significantly higher at a frequency of 1 Hz than at 2.5 Hz; the effect of isoprenaline (3×10^{-8} M) was not significantly different at the two driving frequencies.

6 In preparations driven at 1 Hz the inotropic effect of the lower concentrations of phenylephrine was due to an increase in the time to peak tension without any change of the maximum velocity of force development; however an increase of this parameter became evident only after higher concentrations of the amine (10^{-5} M or more), associated with a progressive shortening of the time to peak.

7 A correlation between mechanical and electrophysiological effects of phenylephrine is attempted; the suggestion is advanced that the prolongation of the action potential and of the active state duration may be an important factor in the inotropic effect of phenylephrine.

Introduction

It is now widely accepted that the positive inotropic effect of phenylephrine is mediated through an activation of myocardial α-adrenoceptors (Benfey, 1973). In the rat ventricle (Wenzel & Su, 1966), rabbit atria (Benfey & Varma, 1967; Parr & Urquilla, 1972) and guinea-pig atria (Govier, 1967, 1968) the increase in contractility induced by the amine is inhibited by α-adrenoceptor blocking drugs.

The positive inotropic effect of phenylephrine is independent of effects on adenylcyclase and cyclic adenosine 3,5′-monophosphate (cyclic AMP) concentration (Benfey, 1971; Benfey & Carolin, 1971); moreover theophylline, in a concentration sufficient to inhibit phosphodiesterase activity and to potentiate the effect of isoprenaline, does not influence the inotropic action of the α-receptor stimulating amine (Hamakawa, Shimizu & Toda, 1973).

It has been demonstrated that the activation of myocardial α-adrenoceptors is associated with a prolongation of the action potential duration (Pappano, 1971; Giotti, Ledda & Mannaioni, 1973). An increase of the cardiac action potential duration mainly due to a lengthening of the plateau phase is induced by phenylephrine in a concentration-dependent manner (Ledda, Marchetti & Manni, 1971; Ledda & Marchetti, 1971). These observations led us to advance the hypothesis that the positive inotropic effect of phenylephrine could be due, at least in part, to a prolongation of the duration of the active state. Therefore in this study the effects of phenylephrine on the isometric contraction of guinea-pig

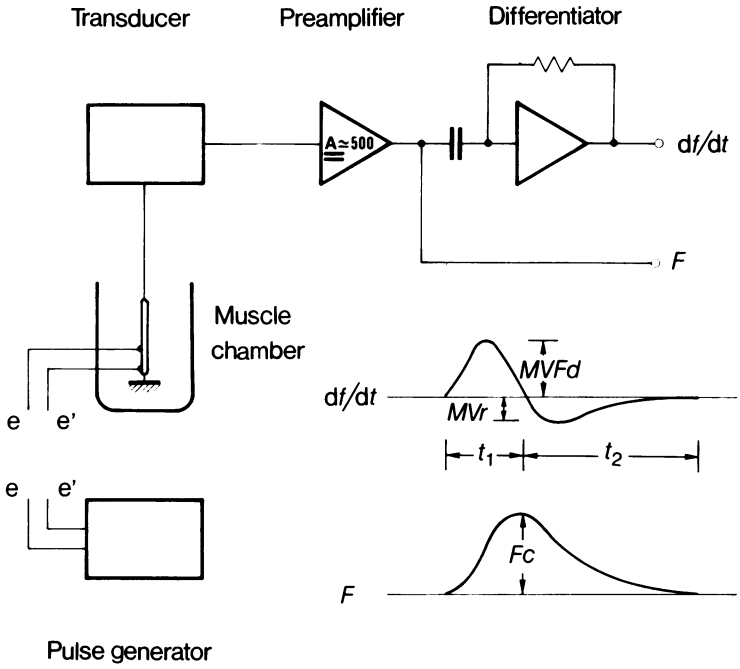

Figure 1 Block diagram of the experimental set-up, showing the isometric contraction curve, the first derivative, and the measurements taken.

ventricle were observed over the whole range of the dose-response curve. In addition the effect of the amine was compared with that of isoprenaline to obtain some information about the possible differences between the mechanical responses elicited in cardiac muscle by α- and β-adrenoceptor agonists.

Methods

Isolated ventricle strips (about 2 mm wide and 10 mm long) obtained from guinea-pig hearts were mounted vertically in a double walled chamber containing 20 ml of perfusion fluid with the following composition (mM): NaCl 115; KCl 4.7; $CaCl_2$ 3.6; $MgSO_4$ 1.2; KH_2PO_4 1.2; NaH_2CO_3 25; glucose 10. The solution was constantly gassed with a mixture of 97% O_2 and 3% CO_2, and kept at 30° C.

The preparations were stimulated electrically at constant rates (1 and 2.5 Hz) by square wave pulses delivered by a pulse generator.

The length of the muscle was adjusted by stretch to produce a resting force of 0.8 g; the resting force was then maintained constant during the whole experiment.

The isometric contraction curves were obtained by an isometric transducer and a d.c. preamplifier; the first derivative of tension was obtained by a differentiator (operational amplifier) with a linear output between 0 and 100 V/second. The two variables were displayed on a dual beam oscilloscope, and were photographed at high sweep speed (100 ms/cm) by means of a Grass C4-K camera.

The following measurements were taken (Figure 1): Fc (force of contraction); t_1 (time to peak tension); t_2 (relaxation time); $MVfd$ (maximum velocity of force development); MVr (maximum velocity of relaxation); ΔFc (inotropic effect: change in peak force of contraction).

The experiment started after a 30-60 min period of equilibration. Cumulative dose-response curves were obtained according to the technique described by Van Rossum (1963), with the 1/2 $\log_{10}$ procedure.

Tension-frequency curves were obtained by raising stepwise the driving frequency from 0.1 to 4.0 Hz; constant frequencies of stimulation were maintained until a steady state contractile tension was attained.

The following substances were used: (±)-isoprenaline hydrochloride (Fluka), (−)-phenylephrine hydrochloride (K and K Lab.), δ[N-(3,4-dimethoxyphenethyl)-*N*-methylamino]-α-(3,4,5-

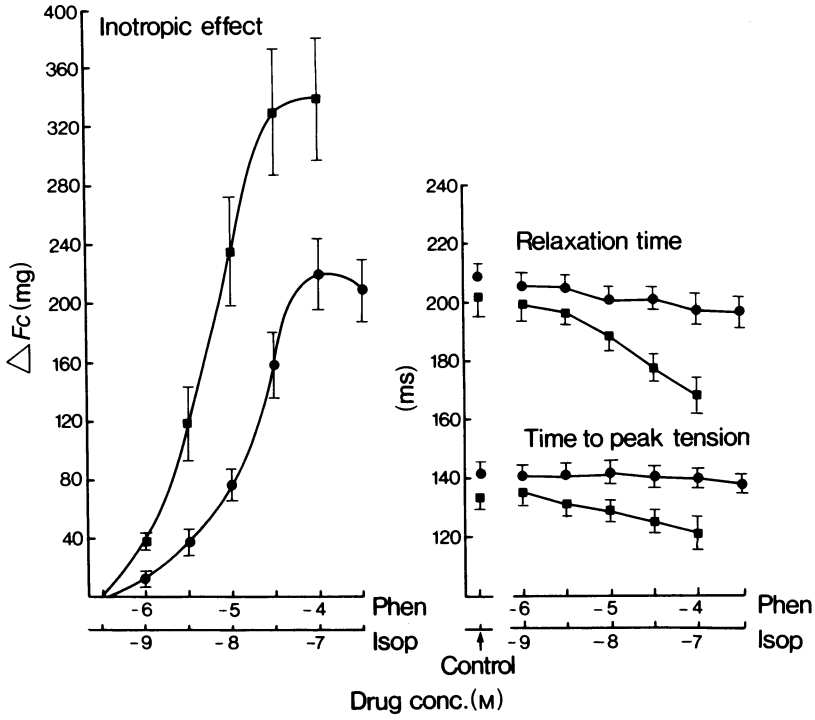

Figure 2 Inotropic effect, relaxation time and time to peak tension as influenced by phenylephrine (mean values of 12 experiments: (●)) and isoprenaline (mean values of 11 experiments: (■)) in cumulative concentrations. Vertical bars show s.e.mean. Stimulation frequency: 2.5 Hz. Temperature: 30° C.

trimethoxyphenyl)α-isopropylvaleronitrile hydrochloride (D_{600} hydrochloride, kindly supplied by Knoll).

Results

Inotropic effects in preparations driven at 2.5 Hz

Cumulative dose-inotropic response curves for isoprenaline and phenylephrine are shown in Figure 2. A positive inotropic effect was induced by isoprenaline in concentrations ranging from 10^{-9} to 10^{-7} M; the phenylephrine effect was obtained with concentrations ranging from 10^{-6} to 10^{-4} M. The endpoint of the concentration-effect curve for isoprenaline was considerably higher than that for phenylephrine: the maximum increase of contractile force (ΔFc) induced by the β-adrenoceptor agonist (10^{-7} M) was 337.1 ± 53.6 mg, while that induced by the α-adrenoceptor agonist (10^{-4} M) was 220.0 ± 23.5 (mean values of 7 and 12 experiments respectively).

Besides this quantitative difference in the inotropic effect, the two amines also had different effects upon the shape of the isometric contraction curves. Isoprenaline shortened the relaxation time (t_2) whereas this parameter was not affected by phenylephrine.

Time to peak tension (t_1) was only slightly and not significantly affected by both substances (Figure 2).

The increase in contractile force (ΔFc) induced by either isoprenaline or phenylephrine was linearly correlated to the increase in maximum velocity of force development (*MVfd*): the correlation coefficients (r) are 0.908 and 0.803, and the regression coefficients (b) are 0.020 and 0.023 respectively.

A straight correlation was also found between ΔFc and *MVr* (r = 0.927 and b = 0.018 for isoprenaline; r = 0.889 and b = 0.020 for phenylephrine).

Reversal of D_{600}-induced negative inotropic effect

D_{600}, a methoxyderivative of verapamil, is a negative-inotropic agent which can inhibit excitation-contraction coupling (Fleckenstein, 1971), by

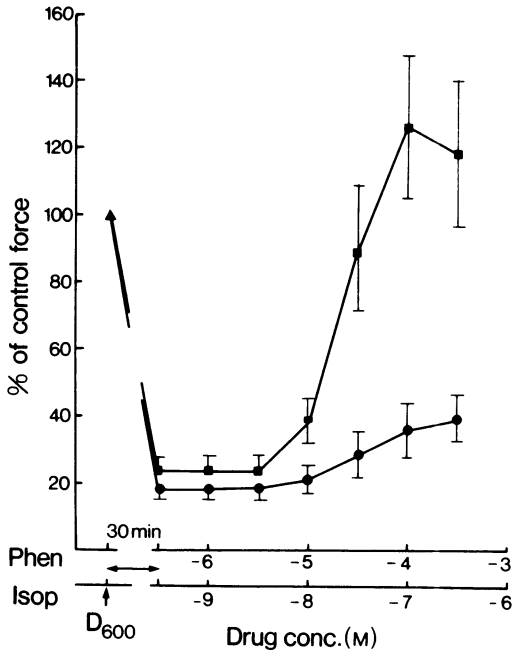

Figure 3 Reversal of the D_{600} (0.25 μg/ml)-induced negative inotropic effect by phenylephrine (mean values of 5 experiments: (●)) and isoprenaline (mean values of 5 experiments: (■)) in cumulative concentrations. Vertical bars show s.e.mean. Stimulation frequency: 1.5 Hz. Temperature 30° C.

selectively blocking the calcium transmembrane influx into the excited myocardial fibres (Kohlhardt, Bauer, Krause & Fleckenstein, 1972). The contractile tension of preparations stimulated at 2.5 Hz was reduced by about 80% after treatment for 30 min with 0.25 μg/ml of the drug.

Isoprenaline added in a cumulative way reversed the D_{600}-induced negative inotropic effect: the contractile tension, which decreased to 23.3 ± 4.3% of control values, returned to 126.7 ± 21.8% after isoprenaline 10^{-7} M.

Phenylephrine, even at the highest concentration (3×10^{-4} M), scarcely influenced the effect of the negative inotropic agent: the contractile tension, decreased to 18.08 ± 4.3% by D_{600}, was increased only to 40.2 ± 7.4% after phenylephrine 3×10^{-4} M (Figure 3).

Frequency-dependence of inotropic effects

Tension frequency curves obtained from 6 preparations before and 5 min after treatment with phenylephrine at a concentration able to induce a submaximal inotropic effect (3×10^{-5} M) showed an upward shift induced by the amine

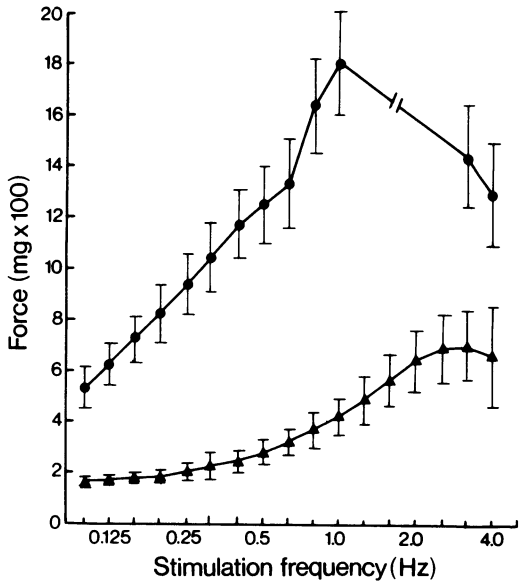

Figure 4 Tension-frequency curves obtained in 6 preparations before (▲) and 5 min after phenylephrine 3×10^{-5} M (●); mean values are given. Vertical bars show s.e.mean. Temperature 30° C.

(Figure 4). Moreover the interval-force relationship was altered in some respects: the increase in contractile force induced by phenylephrine was relatively higher at low frequencies of stimulation, and a maximum was reached at the frequency of 1 Hz; as the interval between beats was further shortened, the decrease in contractility was earlier in the preparation treated with phenylephrine than in controls. The positive inotropic effect of phenylephrine is either quantitatively or qualitatively dependent on stimulation rate.

Table 1 shows that the net increase in contractile force induced by phenylephrine 10^{-4} M was significantly higher ($P < 0.01$) in preparations driven at 1 Hz than in those driven at 2.5 Hz.

A comparison between the maximum inotropic effect of isoprenaline and phenylephrine at the lower frequency of stimulation was made impossible by the frequent development of spontaneous high frequency arrhythmias induced by isoprenaline concentrations above 3×10^{-8} M in preparations driven at 1 Hz. For this reason a concentration of 3×10^{-8} M isoprenaline was chosen for the comparison between the effects of the two amines at the two frequencies of stimulation. As shown in Table 1, the net increase of contractile tension induced by phenylephrine 10^{-4} M was significantly higher ($P < 0.01$) than that induced by isoprenaline 3×10^{-8} M at 1 Hz

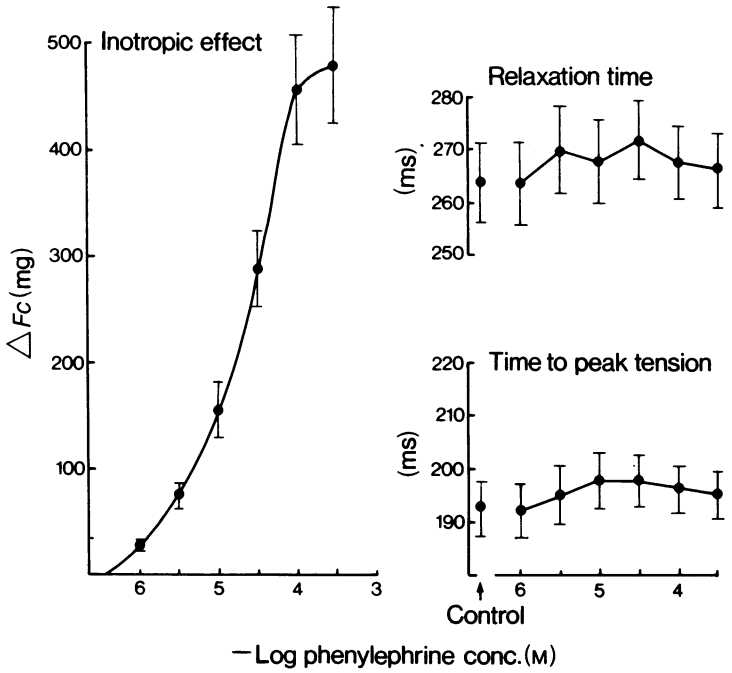

Figure 5 Inotropic effect, relaxation time and time to peak tension as influenced by phenylephrine in cumulative concentrations. Mean values of 11 experiments; vertical bars show s.e.mean. Stimulation frequency: 1 Hz. Temperature 30° C.

whereas the reverse is true at 2.5 Hz ($P = 0.05$). The effects of isoprenaline 3×10^{-8} M were not significantly different at the two driving frequencies.

With regard to qualitative differences Figure 5 shows that the inotropic effect of phenylephrine at 1 Hz was associated with a slight increase of both the time to peak tension and the time of relaxation, whereas no variations of the two parameters were detectable at 2.5 Hz (for comparison see Figure 2).

Although these changes in isometric contraction parameters do not reach the level of statistical significance, because of considerable variation among preparations, individual tracings often showed a distinct difference between the effects of

Table 1 Differences between the inotropic effects of isoprenaline (3×10^{-8} M) and phenylephrine (10^{-4} M) in preparations driven either at 1 Hz or at 2.5 Hz

Drug (molar concentration)	*Net increase of contractile tension (ΔFc) (mg)*	
	1 Hz	*2.5 Hz*
Isoprenaline (3×10^{-8} M)	A_1 285.0 ± 43.2 (7)	A_2 329.0 ± 41.7 (11)
Phenylephrine (10^{-4} M)	B_1 460.4 ± 54.1 (11)	B_2 220.0 ± 23.5 (12)

Statistical significance of differences*: A_1-A_2 = NS
B_1-B_2 = $P < 0.01$
A_1-B_1 = $P < 0.01$
A_2-B_2 = $P = 0.05$

Number of experiments in parentheses.
* Statistical analysis was performed according to the variance analysis of a factorial experiment (m x n) followed by *t* test of non orthogonal comparisons.

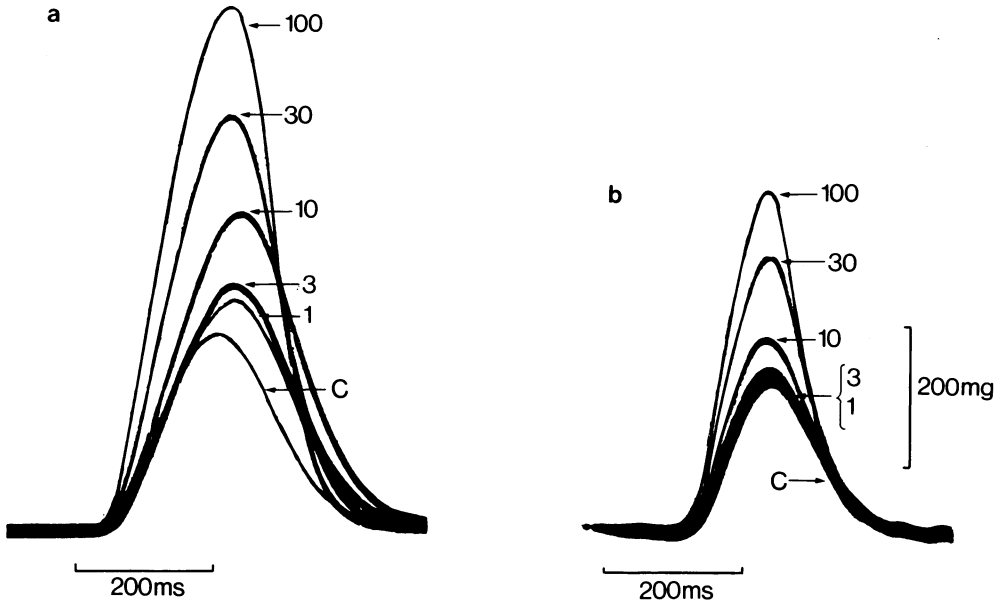

Figure 6 Superimposed concentration-inotropic effect curves obtained in the same preparation treated with phenylephrine and driven either (a) at 1 Hz or (b) at 2.5 Hz. Numbers indicate drug concentration ($\times 10^{-6}$ M); C = control. Temperature: 30° C.

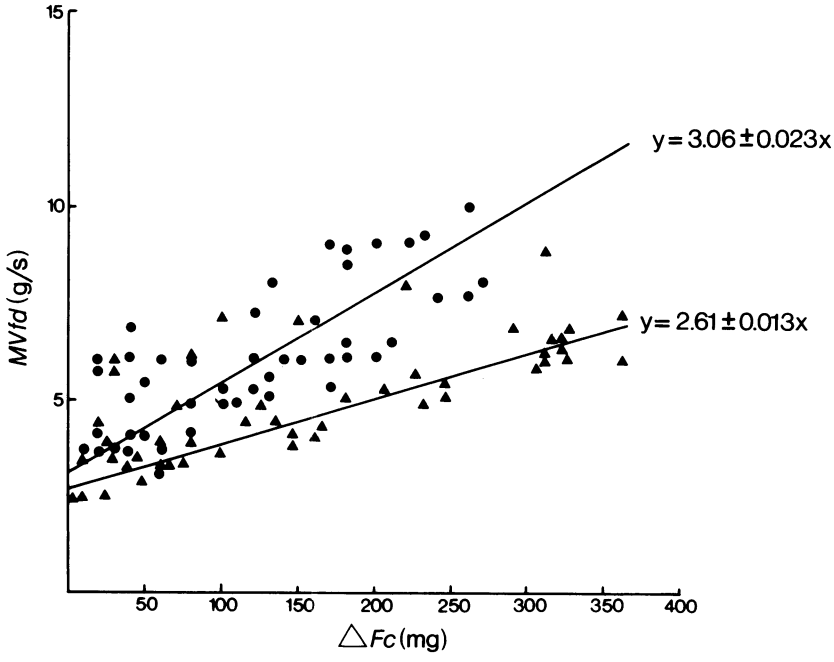

Figure 7 Correlation between inotropic effect and maximum velocity of force development in preparations treated with phenylephrine and driven either at 1 Hz (▲) or at 2.5 Hz (●). Incubation temperature: 30° C.

phenylephrine at low and high stimulation frequencies. Figure 6 shows the recordings of the concentration-inotropic effect curves obtained in the same preparation treated with phenylephrine and driven either at 1 Hz (a) or at 2.5 Hz (b). The difference in the endpoint of the curves obtained at the two frequencies is evident. Moreover at 1 Hz the inotropic effect of the lower concentrations of the amine was due to an increase in the time to peak tension without any change of *MVtd*; an increase of this parameter is evident only at the higher concentrations, associated with a progressive shortening of the time to peak. It is noteworthy that the linear correlation existing between the phenylephrine-induced ΔFc and *MVfd* ($r = 0.861$) at 1 Hz shows a regression coefficient (0.013) significantly lower ($P < 0.001$) than the one observed at 2.5 Hz (Figure 7).

Discussion

In preparations driven at 2.5 Hz both phenylephrine and isoprenaline induce an increase of contractile force by a rise of *MVfd*, which can be considered an index of the intensity of the active state (Panefsky, 1971). However, a distinct difference concerning the relaxation time can be found between the inotropic effects of the two amines. This implies that the cellular mechanisms involved in the effects of the α- and β-adrenoceptor agonists are not the same.

With regard to the cellular mechanism of the inotropic actions, the interaction of the amines with compound D_{600} is important. The increase of cardiac contractility induced by isoprenaline, as well as by other catecholamines is ascribed to an increase of calcium influx probably mediated by an increase in the level of cellular cyclic AMP (Kukovetz & Poch, 1972). Our findings confirm that isoprenaline, acting as a promoter of calcium transmembrane influx is able to counteract the negative inotropic effect of D_{600} (Fleckenstein, 1971). On the basis of its antagonistic action against D_{600}, phenylephrine seems to be a weak promoter of calcium transmembrane influx. This is in agreement with the observed lack of effect of phenylephrine on cyclic AMP levels (Benfey, 1971; Benfey & Carolin, 1971).

Moreover the inotropic effect of phenylephrine shows an evident frequency-dependence: the net increase of contractile tension induced by the same concentration of the amine is significantly higher at 1 Hz than at 2.5 Hz. Despite the increased contractility, the slope of the linear correlation between ΔFc and *MVfd* is significantly lower at 1 Hz than at 2.5 Hz; this implies that the increased intensity of the active state is of less importance at 1 Hz than at 2.5 Hz and that some other factor must play a role in the inotropic response at the lower frequency of stimulation. The suggestion can be advanced that this factor is a prolongation of the active state, because an increase of time to peak force is seen in preparations treated with phenylephrine at the lower but not at the higher stimulation rate.

In the light of these observations a correlation can be attempted between mechanical and electrophysiological effects of phenylephrine on cardiac muscle. (Ledda & Marchetti, 1971; Ledda *et al.*, 1971.) Studies on correlation between electrical and mechanical events show that the prolongation of the action potential duration tends to increase the time to peak force thus increasing developed tension, either in mammalian (Morad & Trautwein, 1968; Braveny & Sumbera, 1970) or in amphibian heart (Vassort & Rougier, 1972). If the prolongation of the plateau phase of the action potential is an important factor in the mechanism of the inotropic action of phenylephrine, it is possible to explain the smaller effect of the amine at the higher frequency of stimulation on the basis of the well known inverse relationship between action potential duration and rate.

With regard to the two different mechanisms which seem to be involved in the inotropic effect of phenylephrine at the lower frequency, namely an increase in time to peak tension at the lower concentrations and an increase in maximum velocity of force development at the higher, it is necessary to bear in mind that phenylephrine is a weak agonist of β-adrenoceptors (Furchgott, 1972). Therefore it is conceivable that the isoprenaline-like effect (i.e. increase of *MVfd*) of the higher concentrations of the amine could be due to activation of β-receptors, which seem to be dominant in the heart. This interpretation is in agreement with the observation of Govier (1968) and Parr & Urquilla (1972) that the positive inotropic effects of high doses of phenylephrine are antagonized by β-adrenoceptor antagonists.

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