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Effects of α-adrenoceptor stimulation with phenylephrine in the presence of propranolol on force of contraction, slow inward current and cyclic AMP content in the bovine heart

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1 The mechanism of the cyclic AMP-independent positive inotropic effect of cardiac α-adrenoceptor stimulation was studied by analyzing the effects of phenylephrine on force of contraction, calcium-dependent slow action potentials and the slow inward current (I_{si}) in bovine ventricular trabeculae. The preparations were electrically driven at 0.3 Hz in the presence of propranolol 1 $\mu mol\,l^{-1}$.

2 Phenylephrine increased the force of contraction in a concentration-dependent manner (maximum about 200% of control at 30 $\mu mol\,l^{-1}$). The effect was surmountably antagonized by phentolamine.

3 The positive inotropic effect of phenylephrine was accompanied by a concentration-dependent increase in time to peak force and occurred without any detectable increase in cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels.

4 The positive inotropic effect of phenylephrine was accompanied by an increase in action potential duration both at 20% and 90% repolarization.

5 Calcium-dependent slow action potentials were also prolonged by phenylephrine and there was a distinct increase in the maximal rate of depolarization (dV/dt_{max}) of these slow potentials. These effects were also completely reversible on washing and surmountably blocked by phentolamine. However, the increase in dV/dt_{max} was smaller than that of isoprenaline in concentrations producing similar inotropic effects.

6 Voltage-clamp experiments with the single sucrose-gap method showed that the phenylephrine-induced increase in force of contraction was associated not only with an increase in peak slow calcium inward current, $I_{si\ max}$, but also with a delay in the inactivation of I_{si}. Outward currents were not detectably altered by phenylephrine.

7 It is concluded that the α-adrenoceptor mediated, cyclic AMP-independent positive inotropic effects of phenylephrine in bovine cardiac muscle are associated with an increase in slow inward current. Additionally, the amount of calcium influx during excitation is probably increased by a delay in the inactivation of I_{si}. Both effects can explain the phenylephrine-produced prolongation of the action potential, and probably contribute to the positive inotropic effect of α-adrenoceptor stimulation. However, as the effect on dV/dt_{max} is smaller than that of isoprenaline, other (still unknown) mechanisms may also be involved.

Introduction

There is no doubt that positive inotropic effects of catecholamines in mammalian cardiac muscle are mainly due to stimulation of β-adrenoceptors (Tsien, 1977; Scholz, 1980). However, there is increasing evidence that α-adrenoceptors also exist in the myocardium and can mediate positive inotropic effects (Benfey, 1980; Schümann, 1980; Scholz, 1980; Endoh, 1982). The increase in force of con-

traction mediated by β-adrenoceptors is related to an increase in slow inward current I_{si} (Reuter & Scholz, 1977; Reuter, 1983), probably due to an elevation of intracellular cyclic AMP levels (Tsien, 1977; Osnes *et al.*, 1980; Katz, 1983). However, the mechanism(s) by which α-adrenoceptor agonists increase the force of contraction is not known. In contrast to β-adrenoceptor stimulation, α-adrenoceptor stimulation does not lead to increased myocardial cyclic AMP levels (Osnes & Øye, 1975; Endoh, *et al.*, 1976; Brodde *et al.*, 1978; Brückner *et al.*, 1978). Therefore, it appeared reasonable to investigate whether positive inotropic effects mediated through α-adrenoceptors are in any way related to changes in the slow inward current.

Previous investigations in this field have yielded rather conflicting results. Miura *et al.*, (1978) found that phenylephrine restored calcium-dependent slow action potentials in rabbit papillary muscle and attributed this effect to an enhancement of the slow inward current. In contrast, Ledda *et al.*, (1980) and Sanchez-Chapula (1981) reported that similar phenylephrine-induced effects on slow action potentials in guinea-pig papillary muscle were completely abolished by β-adrenoceptor blocking agents. Finally, Handa *et al.*, (1982) found an α-adrenoceptor-mediated increase in the maximal rate of depolarization of calcium-dependent slow action potentials, again in rabbit papillary muscle; this effect was attributed to a decrease in the time-dependent potassium outward current rather than to an increase in the slow inward current.

The aim of the present investigation was to study the effects of α-adrenoceptor stimulation on force of contraction and on the slow inward current by means of calcium-dependent slow action potentials and voltage-clamp experiments with the single sucrose-gap method. All experiments were performed with phenylephrine in the presence of propranolol 1 $\mu mol\,l^{-1}$ in order to avoid interference from β-adrenoceptor stimulation. Right ventricular trabeculae from bovine hearts were chosen for these experiments because the time-dependent potassium outward currents, which may interfere with the inactivation of the slow inward current, are small in this preparation compared with other species (McGuigan, 1974; McDonald & Trautwein, 1978; Trautwein & McDonald, 1978). The effects of phenylephrine on cyclic AMP levels and normal action potentials were also determined.

Some of the present results were presented at the 21st Spring Meeting of the Deutsche Pharmakologische Gesellschaft (Brückner & Scholz, 1980), at the 8th International Congress of Pharmacology (Scholz & Brückner, 1981a) and at the 4th European Meeting of the International Society for Heart Research (Scholz & Brückner, 1981b).

Methods

Preparations

The experiments were performed on trabeculae excised from the right ventricles; bovine hearts were obtained from the local slaughterhouse. The diameter of the preparations varied between 0.4 and 0.9 mm, the length between 4 and 6 mm. The preparations were excised not later than 10–15 min after killing and transported to the laboratory in bathing solution (composition see below) at 4°C. The trabeculae were undamaged except at their cut ends and had no side branches over their entire length. The normal bathing solution contained ($mmol\,l^{-1}$): NaCl 136.9, KCl 5.4, CaCl 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, Na_2EDTA 0.05, ascorbic acid 0.28, glucose 5.5. The preparations were kept at 35°C and continuously aerated with 95% O_2 and 5% CO_2; the pH was 7.4.

Isometric contraction experiments

For recording isometric contractions the trabeculae were attached to bipolar platinum stimulating electrodes and mounted individually in glass tissue chambers (for further details see Meinertz *et al.*, 1976). Force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, FRG) attached to a Hellige Helco Scriptor recorder. Each muscle was stretched to the length at which force of contraction was maximal. The resting force (approximately 4 mN) was kept constant throughout the experiment. The preparations were electrically driven at 0.3 Hz with rectangular pulses of 5 ms duration (Grass Stimulator SD 9); the voltage was about 20% greater than threshold. All preparations were allowed to equilibrate for at least 60 min in drug-free bathing solution until complete mechanical stabilization was achieved. The concentration-response curves were obtained cumulatively. The rate of force development was measured with an electronic differentiator.

Electrophysiological measurements

Transmembrane potentials and membrane currents were measured by the single sucrose-gap voltage-clamp method described in detail by Reuter & Scholz (1977). Briefly, the preparations were pulled through tightly fitting holes in two rubber membranes bounding a gap of 2 mm width. The muscle length in the recording compartment was less than 1 mm. Action potentials were measured between an intracellular microelectrode filled with KCl 3 $mol\,l^{-1}$ and an extracellular Ag-AgCl electrode placed near the surface of the muscle end in the recording compartment. The maximal rate of depolarization of the

action potential was measured with an electronic differentiator (W. Fleck, Mainz). Force of contraction was monitored simultaneously from the muscle part in the recording compartment with a force displacement transducer (W. Fleck, Mainz). After the equilibration period propranolol $1\,\mu mol\,l^{-1}$ was added for 30 min before addition of the α-adrenoceptor agonist in order to avoid interference from β-adrenoceptors. Propranolol was present throughout the experiments. When fast action potentials were recorded, all chambers of the sucrose gap system were perfused with bathing solution. For recording slow action potentials the potassium concentration was increased to $22\,mmol\,l^{-1}$ without isotonic compensation in order to inactivate the fast sodium channels. For the voltage clamp experiments, the sucrose-gap was perfused with isotonic sucrose solution containing Ca^{2+} $0.01\,mmol\,l^{-1}$. The current injection chamber was perfused with bathing solution in which Na^+ was replaced by K^+ in order to reduce the input resistance of the preparation. This had no effects on resting and action potential in the recording compartment.

Cyclic AMP assay

The preparations were mounted individually in the glass tissue chambers as described above. For measuring the cyclic AMP content the preparations were quickly frozen in liquid nitrogen (for details see Dönges *et al.*, 1977). Cyclic AMP was measured by radioimmunoassay using the method of Harper & Brooker (1975) as modified by Struck *et al.*, (1977). Recoveries, run with each experiment, amounted to $95.1 \pm 4.88\%$ ($n = 6$) and were not altered by the substances under investigation. All assayable material was destroyed by treatment with phosphodiesterase.

Materials

The drugs used were (−)-phenylephrine HCl (Boehringer Ingelheim), (±)-isoprenaline HCl (Boehringer Ingelheim), (±)-propranolol HCl (Rheinpharma, Heidelberg) and phentolamine HCl (Ciba, Basel). De-ionized and twice distilled water was used throughout; the substances were freshly dissolved in bathing solution.

Propranolol, $1\,\mu mol\,l^{-1}$, by itself had a small negative inotropic effect (about 10%). At this concentration propranolol completely blocked the positive inotropic effect of a maximally effective concentration of isoprenaline ($0.3\,\mu mol\,l^{-1}$); β-adrenoceptor blockade was thus considered adequate (see also Brückner *et al.*, 1978).

Statistics

Values presented are means ± s.e.mean. Statistical significance was estimated using Student's *t* test for paired and unpaired observations. A *P* value of less than 0.05 was considered significant.

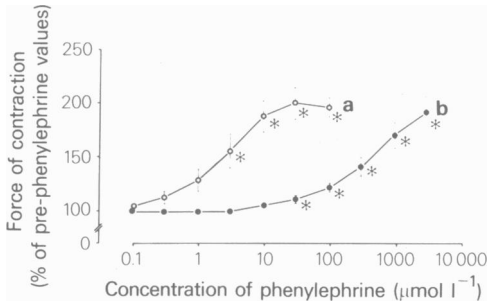

Figure 1 Cumulative concentration-response curves for the effects of phenylephrine, in the presence of propranolol ($1\,\mu mol\,l^{-1}$) (a) and in the presence of propranolol plus phentolamine ($5\,\mu mol\,l^{-1}$) (b), on force of contraction in electrically driven bovine trabeculae. The stimulation frequency was 0.3 Hz. Ordinate scale: force of contraction as % of pre-phenylephrine value. Abscissa scale: concentration of phenylephrine in $\mu mol\,l^{-1}$. The time of exposure to each concentration was 15 min. Significant differences from the corresponding pre-phenylephrine values are marked with asterisks. The pre-phenylephrine values were 3.08 ± 0.67 mN (a; $n = 7$) and 2.71 ± 0.97 mN (b; $n = 6$).

Results

Effects on force of contraction

Figure 1 shows concentration-response curves for the effects of phenylephrine on the force of contraction in the presence of either propranolol or propranolol plus phentolamine. In the presence of propranolol the positive inotropic effect of phenylephrine started at a concentration of $0.1\,\mu mol\,l^{-1}$ and reached its maximum ($200.3 \pm 9.4\%$; $n = 7$) at $30\,\mu mol\,l^{-1}$. Phentolamine, $5\,\mu mol\,l^{-1}$, shifted the concentration-response curve of phenylephrine to the right by about two log units.

Figure 2 shows the influence of phenylephrine (in the presence of propranolol) on the individual parameters of the isometric contraction curve. The positive inotropic effect of phenylephrine was accompanied by an increased rate of force development and, in contrast to β-adrenoceptor stimulation, an increase in time to peak force. The increase in rate of force development was less pronounced than the increase in peak force, indicating that the increase in time to peak force contributes to the development of the positive inotropic effect.

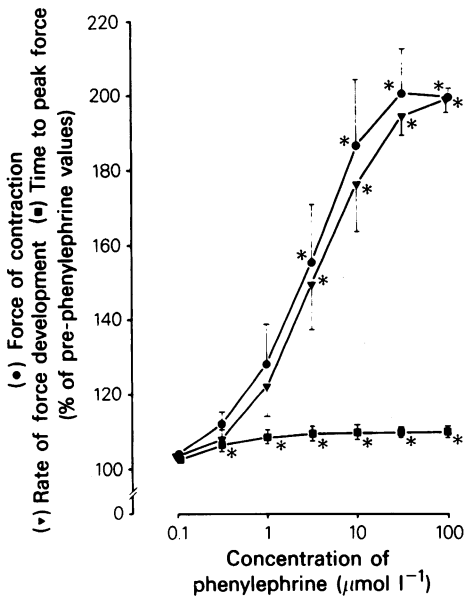

Figure 2 Cumulative concentration-response curves for the effects of phenylephrine in the presence of propranolol 1 μmol l^{-1} on force of contraction (●), time to peak force (■) and maximal rate of force development (▼) in electrically driven bovine trabeculae stimulated at a frequency of 0.3 Hz. Ordinate scale: force of contraction, rate of force development and time to peak force in % of pre-phenylephrine values. Abscissa scale: concentration of phenylephrine in μmol l^{-1}. The time of exposure to each concentration was 15 min; $n = 6$. Significant differences from the corresponding pre-phenylephrine values are marked with asterisks. The pre-phenylephrine values were 3.08 ± 0.67 mN (●); 143.3 ± 5.4 ms (■) and 36.8 ± 9.02 mN s^{-1} (▼).

Effects on cyclic AMP content

These experiments were performed with phenylephrine 10 μmol l^{-1}, i.e. a nearly maximally effective concentration as far as the positive inotropic effect is concerned. After pre-incubation with propranolol 1 μmol l^{-1} the cyclic AMP content was measured before and 0.5, 2 and 15 min after the addition of phenylephrine. The results are summarized in Table 1. It is evident that the increase in force of contraction was not accompanied by a significant increase in cyclic AMP levels at any time studied.

Table 1 Effects of phenylephrine (10 μmol l^{-1}) in the presence of propranolol (1 μmol l^{-1}) on force of contraction and cyclic AMP content in bovine trabeculae

Incubation time (min)	*Force of contraction* (mN)	*Cyclic AMP-content* (pmol mg^{-1} ww)
0	2.33 ± 1.23 (4)	0.60 ± 0.06 (4)
0.5	1.91 ± 0.61 (3)	0.67 ± 0.07 (3)
2	2.45 ± 0.61 (4)	0.75 ± 0.08 (4)
15	5.49 ± 0.44* (4)	0.56 ± 0.07 (4)

The incubation time for phenylephrine was 0 min (control), 0.5 min, 2 min, and 15 min. The numbers in parentheses give the number of experiments.
* $P < 0.05$ vs. control

Effects on fast action potentials

In an initial series of experiments the effects of phenylephrine on normal action potentials were investigated. Phenylephrine was studied at a concentration 10 μmol l^{-1}, which produced a nearly maximal increase in force of contraction and which could be blocked by phentolamine. Figure 3 shows that the positive inotropic effect of phenylephrine was accompanied by a marked increase in action potential duration (Figure 3b). Propranolol, which itself marginally shortened the action potential duration (about 10%, not shown) was regarded as control. At 20% repolarization (APD_{20}), phenylephrine increased the duration of the action potential from 261.9 ± 22.9 to 352.7 ± 23.3 ms; at 90% repolariza-

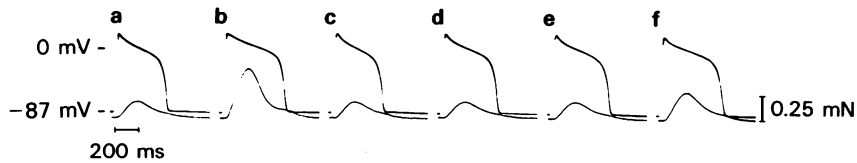

Figure 3 Effects of phenylephrine, in the presence of propranolol (1 μmol l^{-1}) (b) and propranolol plus phentolamine (10 μmol l^{-1} for 15 min) (e, f), on transmembrane action potentials (upper trace) and force of contraction (lower trace) in an electrically driven (0.3 Hz) bovine trabecula. Propranolol 1 μmol l^{-1} (a) is designated as control I (blockade of β-adrenoceptors) and propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} (d) as control II (blockade of β- and α-adrenoceptors). (a) Propranolol 1 μmol l^{-1} (30 min, control I); (b) propranolol 1 μmol l^{-1} + phenylephrine 10 μmol l^{-1} (15 min); (c) propranolol 1 μmol l^{-1} (30 min, back control); (d) propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} (30 min, control II); (e) propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} + phenylephrine 10 μmol l^{-1} (15 min); (f) propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} + phenylephrine 3000 μmol l^{-1} (15 min).

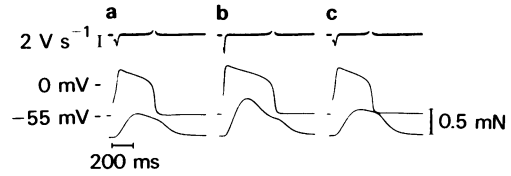

Figure 4 Effects of phenylephrine, in the presence of propranolol, on slow action potentials and force of contraction in a potassium-depolarized electrically driven bovine trabecula. Stimulation frequency, 0.3 Hz; Ca^{2+} 1.8 mmol l^{-1}, K^+ 22 mmol l^{-1}. Upper trace: maximal rate of depolarization in V s^{-1}; middle trace: membrane potential in mV; lower trace: force of contraction in mN. (a) Propranolol 1 μmol l^{-1} (30 min, control); (b) propranolol 1 μmol l^{-1} + phenylephrine 10 μmol l^{-1} (15 min); (c) propranolol 1 μmol l^{-1} (30 min, back control).

tion (APD_{90}) the increase was from 467.7 ± 15.5 to 576.0 ± 24.8 ms ($n = 11$). Thus, the increase in the two parameters of the action potential duration were nearly the same (about 100 ms) and showed a roughly parallel shift of the repolarization phase. The resting potential and the amplitude of the action potential remained unchanged. Mechanical and electrophysiological effects followed the same time course and were maximal after 10–15 min. They were completely reversible after washing with propranolol 1 μmol l^{-1} for 30 min (Figure 3c). After this washing period some of the preparations ($n = 4$) were additionally incubated with phentolamine 5 μmol l^{-1} for 30 min. Phentolamine which by itself exerted a small increase in APD_{90} (about 10%; Figure 3d), completely blocked the mechanical and electrophysiological effects of phenylephrine 10 μmol l^{-1} (Figure 3e). As illustrated in Figure 3f the antagonistic effect of phentolamine could be surmounted by a very high concentration of phenylephrine (3000 μmol l^{-1}; two experiments). Although the mechanical and electrophysiological effects again show the changes characteristic of α-adrenoceptor stimulation, involvement of β-adrenoceptors of course cannot be excluded at this high phenylephrine concentration.

Effects on slow action potentials

The effects of phenylephrine on slow action potentials were studied in preparations depolarized to about −45 mV by raising the potassium concentration in the bathing solution from 5.4 to 22 mmol l^{-1}. It is generally accepted that phase 0 of such action potentials is mainly carried by calcium ions and that changes in their maximal rate of depolarization (dV/dt_{max}) reflect changes in slow inward current (cf. Carmeliet, 1980).

Figure 4 shows that the duration of slow action potentials was also prolonged by phenylephrine in the presence of propranolol. Furthermore, the positive inotropic effect was accompanied by an increase in dV/dt_{max} (Figure 4b). The phenylephrine-induced increase in force of contraction and dV/dt_{max} was approximately 100% and 50% respectively (Table 2). The effects were completely reversible within

Table 2 Effects of phenylephrine, in the presence of propranolol (A–C) and propranolol plus phentolamine (D–F), on maximal rate of depolarization of slow action potentials and on force of contraction in bovine trabeculae

	(A) *Propranolol 1 μmol l^{-1} (control)*	**(B)** *Propranolol 1 μmol l^{-1} + phenylephrine 10 μmol l^{-1}*	**(C)** *Propranolol 1 μmol l^{-1} (return to control)*
Force of contraction (mN)	0.501 ± 0.09 (11)	1.032 ± 0.20* (11)	0.534 ± 0.11 (11)
dV/dt_{max} (V s^{-1})	2.08 ± 0.14 (11)	3.28 ± 0.27* (11)	2.25 ± 0.17 (11)
	(D) *Propranolol 1 μmol l^{-1}*	**(E)** *Propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1}*	**(F)** *Propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} + phenylephrine 10 μmol l^{-1}*
Force of contraction (mN)	0.391 ± 0.11 (5)	0.376 ± 0.11 (5)	0.373 ± 0.10 (5)
dV/dt_{max} (V s^{-1})	2.28 ± 0.26 (5)	2.20 ± 0.27 (5)	2.26 ± 0.30 (5)

The numbers in parentheses give the number of experiments.
* $P < 0.05$ vs. (A)

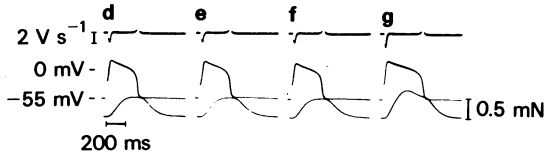

Figure 5 Effects of phenylephrine, in the presence of propranolol and phentolamine, on slow action potentials and force of contraction in a potassium-depolarized electrically driven bovine trabecula. Stimulation frequency, 0.3 Hz; Ca^{2+} 1.8 mmol l^{-1}; K^+ 22 mol l^{-1}. Upper trace: maximal rate of depolarization in V s^{-1}; middle trace: membrane potential in mV; lower trace: force of contraction in mN. (d) Propranolol 1 μmol l^{-1} (30 min); (e) propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} (30 min, control); (f) propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} + phenylephrine 10 μmol l^{-1} (15 min); (g) propranolol 1 μmol l^{-1} + phentolamine 5 μmol l^{-1} + phenylephrine 3000 μmol l^{-1} (15 min).

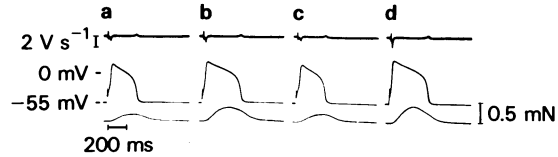

Figure 6 Comparison of the effects phenylephrine in the presence of propranolol (α-adrenoceptor stimulation) and of isoprenaline (β-adrenoceptor stimulation) on slow potentials and on force of contraction in a potassium-depolarized electrically driven bovine trabecula. Stimulation frequency 0.3 Hz; Ca^{2+} 1.8 mmol l^{-1}; K^+ 22 mol l^{-1}. Upper trace: maximal rate of depolarization in V s^{-1}; middle trace: membrane potential in mV; lower trace: force of contraction in mN. As the effects of propranolol are only incompletely reversible and in order to allow a direct comparison in the very same preparation, β-adrenoceptor stimulation with isoprenaline was also performed in the presence of propranolol. The concentration of isoprenaline was selected such to produce a positive inotropic effect similar to that of phenylephrine. (a) Propranolol 1 μmol l^{-1} (30 min, control); (b) propranolol 1 μmol l^{-1} + phenylephrine 10 μmol l^{-1} (15 min); (c) propranolol 1 μmol l^{-1} (30 min, back control); (d) propranolol 1 μmol l^{-1} + isoprenaline 30 μmol l^{-1} (15 min).

30 min of washing (return to control, Figure 4c). The effect of phenylephrine on force of contraction as well as on dV/dt_{max} remained unchanged when the concentration of propranolol was increased from 1 to 5 μmol l^{-1} (not shown).

Figure 5 illustrates that phentolamine 5 μmol l^{-1}, which itself did not affect phase 0 of the slow action potential (Figure 5), completely blocked the increase in dV/dt_{max} as well as the positive inotropic effect of phenylephrine 10 μmol l^{-1} (Figure 5f and Table 2, D–F). The blocking effect of phentolamine on the phenylephrine-produced increase in dV/dt_{max} was surmounted by phenylephrine 3000 μmol l^{-1} (Figure 5g compare also Figure 3f). Thus, the positive inotropic effect of phenylephrine in the presence of propranolol was accompanied by an increase in the dV/dt_{max} of calcium-mediated slow action potentials indicating an increase in calcium inward current. The effects were reversible on washing and blocked by phentolamine.

In order to quantify further the electrophysiological effects of phenylephrine, we compared the effects of phenylephrine 10 μmol l^{-1} in the presence of propranolol (α-adrenoceptor stimulation) on slow action potentials in a second series with those of β-adrenoceptor stimulation (with isoprenaline 30 μmol l^{-1} in the presence of propranolol) in the same preparations. Figure 6 shows a typical example and all the results are summarized in Table 3. Stimu-

Table 3 Comparison of the effects of α- and β-adrenoceptor stimulation on maximal rate of depolarization of slow action potentials and force of contraction

	Force of contraction (mN)	(% of control)	*Maximal rate of depolarization* (V s^{-1})	(% of control)
α-Adrenoceptor stimulation				
Propranolol 1 μmol l^{-1} (control)	0.51 ± 0.09 (11)	100 (11)	2.08 ± 0.14 (11)	100 (11)
Propranolol 1 μmol l^{-1} + phenylephrine 10 μmol l^{-1}	1.03 ± 0.20 (11)	219.0 ± 23.8 (11)	3.28 ± 0.27 (11)	157.5 ± 8.6 (11)
β-Adrenoceptor stimulation				
Propranolol 1 μmol l^{-1} (control)	0.49 ± 0.16 (7)	100 (7)	2.31 ± 0.34 (7)	100 (7)
Propranolol 1 μmol l^{-1} + isoprenaline 30 μmol l^{-1}	1.10 ± 0.44 (7)	202.0 ± 17.7 (7)	5.20 ± 0.69 (7)	239.0 ± 24.8 (7)

The numbers in parentheses give the number of experiments.

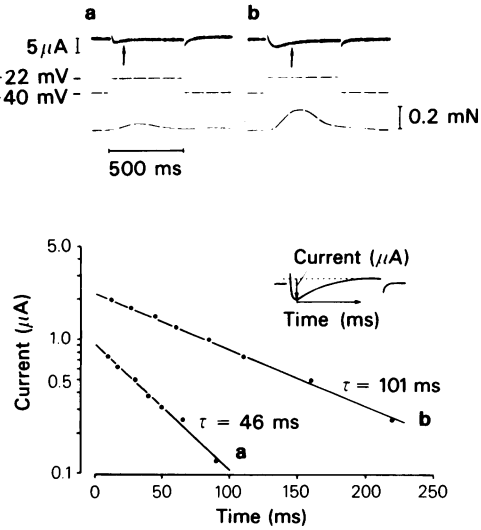

Figure 7 Effect of phenylephrine in the presence of propranolol on the slow inward current in an electrically driven bovine trabecula. Stimulation frequency 0.3 Hz; Ca^{2+} 1.8 mmol l^{-1}. Upper panel: original recordings of a voltage clamp experiment. Upper trace: membrane current in μA; the arrows indicate the time constant of inactivation of the slow inward current, I_{si}, as determined below. Middle trace: membrane potential in mV; clamp step from a holding potential of −40 mV to −22 mV for 500 ms. Lower trace: force of contraction in mN. Lower panel: semilogarithmic plot of the inactivation of the slow inward current, I_{si}, in the same experiment. Ordinate scale: membrane current in μA. Abscissa scale: time after peak current in ms (see inset). The time constant of inactivation was 46 ms before and 101 ms after addition of phenylephrine. (a) Propranolol 1 μmol l^{-1} (30 min, control); (b) propranolol 1 μmol l^{-1} + phenylephrine 10 μmol l^{-1} (10 min).

lation with isoprenaline was performed in the presence of propranolol because the effects of propranolol are only incompletely reversible. The concentrations of phenylephrine and isoprenaline were chosen such that they were equieffective (about 200%) with respect to their inotropic effects. However, as far as dV/dt_{max} is concerned α-adrenoceptor stimulation produced an increase to 157% of control, whereas the effect of β-adrenoceptor stimulation was much more pronounced (239%).

Voltage clamp experiments

Theoretically, the increase in the rate of depolarization of slow action potentials described above may not only be due to an increase in slow inward current but also to a decrease in outward currents (Carmeliet, 1980). In an attempt to provide direct information on whether or not an increase in I_{si} is involved in the α-adrenoceptor mediated response, voltage-clamp experiments were performed with the single sucrose gap technique (Fozzard & Beeler, 1975; Coraboeuf, 1980). The upper panel of Figure 7 shows the effect of phenylephrine in the presence of propranolol on the slow inward current in such an experiment. The holding potential was kept at −40 mV in order to inactivate the fast sodium channels (Reuter, 1973; Trautwein, 1973). It is evident that phenylephrine exerted not only an increase in peak current, $I_{si\,max}$, but also a marked delay of the current's decay together with the positive inotropic effect. The outward currents at the end of the clamp step remained unchanged. The semilogarithmic plot of the decay of the slow inward current (Figure 7, lower panel) shows that the time constant of the inactivation increased from 46 ms to 101 ms. Similar results were obtained in three other experiments.

Discussion

Positive inotropic effects of phenylephrine, after adequate β-adrenoceptor blockade, have been demonstrated in various cardiac preparations (cf. Benfey, 1980; Scholz, 1980). These effects were qualitatively different from those elicited by stimulation of β-adrenoceptors and were probably mediated by α-adrenoceptors (Ledda *et al.*, 1975; Endoh *et al.*, 1976; Skomedal *et al.*, 1982).

In the present paper a positive inotropic effect of phenylephrine in the presence of propranolol was demonstrated in bovine ventricular trabeculae. This effect of phenylephrine was surmountably blocked by the α-adrenoceptor blocking agent phentolamine and was accompanied both by an increase in rate of force development and by a concentration-dependent increase in time to peak force. There was no increase in myocardial cyclic AMP content. Thus the positive inotropic effect of phenylephrine in the bovine heart revealed the known characteristics for α-adrenoceptor-mediated cardiac effects. Therefore it is concluded that α-adrenoceptors exist in the bovine ventricle mediating cyclic AMP-independent positive inotropic effects.

The positive inotropic effect of phenylephrine in the presence of propranolol was associated with a phentolamine-sensitive increase in action potential duration. Stimulation of β-adrenoceptors mostly results in a prolongation of the plateau (phase 2) whereas the phase of final repolarization (phase 3) is often shortened (Nathan & Beeler, 1975). However, phenylephrine prolonged the action potential duration both at 20% and 90% repolarization to the same amount. These results are in accord with those of Ledda *et al.* (1971) who found a prolongation of the action potential duration and the effective refractory

period in sheep Purkinje fibres with phenylephrine. This supports the hypothesis, that the α-adrenoceptor-mediated, cyclic AMP-independent effects of phenylephrine in the bovine heart are produced by mechanisms different from those of β-adrenoceptor stimulation.

The duration of calcium-dependent slow action potentials was also prolonged by phenylephrine. Additionally, a distinct increase in the maximal rate of depolarization occurred. These effects were also completely reversible on washing, were surmountably blocked by phentolamine and were not altered by increasing the concentration of propranolol from 1 μmol l^{-1} to 5 μmol l^{-1}. Moreover, mechanical and electrophysiological effects followed the same time course. These results are in accord with those of Miura *et al.* (1978) who found that phenylephrine in the presence of the β-adrenoceptor blocking agent bufetolol, induced phentolamine-sensitive slow action potentials in rabbit papillary muscles. These and the present results suggest that the positive inotropic effect of phenylephrine in the presence of propranolol, at least in rabbit and cattle, is mediated by α-adrenoceptors and that it is probably related to an increase in the slow calcium inward current. However, contradictory results were reported by Ledda *et al.* (1980) and Sanchez-Chapula (1981) who found that the effects of phenylephrine on slow action potentials in guinea-pig papillary muscles were completely abolished by β-blocking agents. These discrepancies do not necessarily contradict the conclusion drawn above; they are more likely to be due to species differencies. Obviously, α-adrenoceptors are of minor importance or only poorly developed in guinea-pig ventricular tissue, as has been pointed out by Shibata *et al.* (1980).

Although the upstroke of the slow action potential is widely accepted to reflect the slow inward current, its shape may theoretically be modulated by changes in potassium permeability in addition to changes in I_{si}. Handa *et al.* (1982), working with rabbit papillary muscles, pointed out that the prolongation of the action potential produced by phenylephrine remained unchanged if action potentials were recorded in the presence of the slow channel blocking ion, Mn^{2+}. They concluded that a decrease in the time-dependent potassium outward current is thus involved in this effect. A decrease in potassium conductance would allow more calcium to enter the cell and would thus elicit the positive inotropic response. This, however, was not the case in the present study, where the direct determination of the effect of phenylephrine on I_{si} with the voltage-clamp technique revealed that the drug increased the magnitude, $I_{si\,max}$, and decelerated the inactivation of I_{si} without any detectable change in potassium outward currents. Both the increase in peak current and the delay in inactivation result in an increased amount of calcium entering the cell through the slow channel. Thus it is not unreasonable to conclude that in this preparation the phentolamine-sensitive (and hence presumably α-adrenoceptor-mediated) effects of phenylephrine on normal and slow action potentials are mainly, if not entirely, due to an increase in I_{si}.

It is generally accepted that β-adrenoceptor stimulating agents increase force of contraction and I_{si} to about the same extent and that these effects are related to an increase in cyclic AMP (Reuter, 1983; Katz, 1983). The present study shows that the positive inotropic effect of phenylephrine in the presence of propranolol is likewise accompanied by an increase in slow calcium inward current, which, however, occurred without an increase in myocardial cyclic AMP levels. However, the phenylephrine-induced increase in I_{si}, as judged from the effect on the rate of depolarization of slow action potentials, was smaller than that of an equieffective positive inotropic concentration of isoprenaline. Thus, the present results suggest that the increase in I_{si} contributes to the positive inotropic effect of α-adrenoceptor stimulation, though it is likely that other (still unknown) mechanisms are also involved in the α-adrenoceptor increase in myocardial force of contraction.

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References

BENFEY, B.G. (1980). Cardiac alpha-adrenoceptors. *Can. J. Physiol. Pharmac.*, **58**, 1145–1157.

BRODDE, O.E., MOTOMURA, S., ENDOH, M. & SCHÜMANN, H.J. (1978). Lack of correlation between the positive inotropic effect evoked by alpha-adrenoceptor stimulation and the levels of cyclic AMP and or cyclic GMP in the isolated ventricle strip of the rabbit. *J. mol. cell. Cardiol.*, **10**, 205–211.

BRÜCKNER, R., HACKBARTH, I., MEINERTZ, T., SCHMELZLE, B., SCHOLZ, H. (1978). The positive inotropic effect of phenylephrine in the presence of propranolol. Increase in time to peak force and in relaxation time without increase in cAMP. *Naunyn Schmiedebergs Arch. Pharmac.*, **303**, 205–211.

BRÜCKNER, R. & SCHOLZ, H. (1980). Effects of phenylephrine in the presence of propranolol on slow potentials in mammalian ventricular fibres. *Naunyn Schmiedebergs Arch. Pharmac.*, **311**, R 37.

CARMELIET, E. (1980). The slow inward current: non-voltage-clamp studies. In *The Slow Inward Current and Cardiac Arrhythmias.* ed. Zipes, D.P., Bailey, J.C. & Elharrar, V. pp. 97–110. The Hague, Boston and London: Martinus Nijhoff Publishers.

CORABOEUF, E. (1980). Voltage clamp studies of the slow inward current. In *The Slow Inward Current and Cardiac Arrhythmias.* ed. Zipes, D.P., Bailey, J.C. & Elharrar, V. pp. 25–95. The Hague, Boston and London: Martinus Nijhoff Publishers.

DÖNGES, C., HEITMANN, M., JUNGBLUTH, H., MEINERTZ, T., SCHMELZLE, B. & SCHOLZ, H. (1977). Effectiveness of theophylline to increase cyclic AMP levels and force of contraction in electrically paced guinea-pig auricles. Comparison with isoprenaline, calcium and ouabain. *Naunyn Schmiedebergs Arch. Pharmac.*, **301,** 87–97.

ENDOH, M. (1982). Adrenoceptors and the myocardial inotropic response: do alpha and beta receptor sites functionally coexist? In *Trends in Autonomic Pharmacology*, Vol. 2, ed. Kalsner, S. pp. 303–322. Baltimore, Munich: Urban & Schwarzenberg.

ENDOH, M., BRODDE, O.E., SCHÜMANN, H.J. (1976). Relationship between the level of cAMP and the contractile force under stimulation of alpha- and beta-adrenoceptors by phenylephrine in the isolated rabbit papillary muscle. *Naunyn Schmiedebergs Arch. Pharmac.*, **295,** 109–115.

FOZZARD, H.A. & BEELER, G.W. (1975). The voltage clamp and cardiac electrophysiology. *Circulation. Res.*, **37,** 403–413.

HANDA, Y., WAGNER, J., INUI, J., AVERESCH, H. & SCHÜMANN, H.J. (1982). Effect of alpha- and beta-sympathomimetic agonists on calcium-dependent slow action potential and force of contraction in the rabbit papillary muscle. *Naunyn Schmiedebergs Arch. Pharmac.*, **318,** 330–335.

HARPER, G.F. & BROOKER, G. (1975). Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2′-0-acetylation by acetic anhydride in aqueous solution. *J. Cyclic Nucl. Res.*, **1,** 207–218.

KATZ, A. (1983). Cyclic adenosine monophosphate effects on the myocardium: A man who blows hot and cold with one breath. *J. Am. Coll. Cardiol.*, **2,** 143–150.

LEDDA, F., MARCHETTI, P. & MANNI, A. (1971). Influence of phenylephrine on transmembrane potentials and effective refractory period of single Purkinje fibres of sheep heart. *Pharmac. Res. Commun.*, **3,** 195–206.

LEDDA, F., MARCHETTI, P. & MUGELLI, A. (1975). Studies on the positive inotropic effect of phenylephrine: A comparison with isoprenaline. *Br. J. Pharmac.*, **54,** 83–90.

LEDDA, F., MANTELLI, L. & MUGELLI, A. (1980). Alpha-sympathomimetic amines and calcium-mediated action potentials in guinea-pig ventricular muscle. *Br. J. Pharmac.*, **69,** 565–571.

McGUIGAN, J.A.S. (1974). Some limitations of the double sucrose gap, and its use in a study of the slow outward current in mammalian ventricular muscle. *J. Physiol.*, **240,** 775–806.

McDONALD, T.F. & TRAUTWEIN, W. (1978). Membrane currents in cat myocardium: separation of inward and outward components. *J. Physiol.*, **274,** 193–216.

MEINERTZ, T., NAWRATH, H. & SCHOLZ, H. (1976). Possible role of cyclic AMP in the relaxation process of mammalian heart: effects of dibuturyl cyclic AMP and theophylline on potassium contractures in cat papillary muscles. *Naunyn Schmiedebergs Arch. Pharmac.*, **293,** 129–137.

MIURA, Y., INUI, J. & IMAMURA, H. (1978). Alpha-adrenoceptor-mediated restoration of calcium-dependent potentials in the partially depolarized papillary muscle. *Naunyn Schmiedebergs Arch. Pharmac.*, **301,** 201–205.

NATHAN, D. & BEELER, G.W. (1975). Electrophysiologic correlates of the inotropic effects of isoproterenol in canine myocardium. *J. mol. cell. Cardiol.*, **7,** 1–15.

OSNES, J.B. & ØYE, I. (1975). Relationship between cyclic AMP metabolism and inotropic response of perfused rat hearts to phenylephrine and other adrenergic amines. *Adv. Cyclic Nucleotide Res.*, **5,** 415–433.

OSNES, J.B., SKOMEDAL, T. & ØYE, I. (1980). On the role of cyclic nucleotides in the heart muscle contraction and relaxation. *Prog. Pharmac.*, **4,** 49–62.

REUTER, H. (1973). Divalent cations as charge carriers in excitable membranes. *Prog. Biophys. Mol. Biol.*, **26,** 1–43.

REUTER, H. (1983). Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature*, **301,** 569–574.

REUTER, H. & SCHOLZ, H. (1977). A study of the ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian cardiac muscle. *J. Physiol.*, **264,** 17–47.

SANCHEZ-CHAPULA, J. (1981). Multiple effects of putative alpha-adrenoceptor agonists on the electrical and mechanical activity of guinea-pig papillary muscle. *Naunyn Schmiedebergs Arch. Pharmac.*, **316,** 108–111.

SCHOLZ, H. (1980). Effects of beta- and alpha-adrenoceptor activators and adrenergic transmitter releasing agents on the mechanical activity of the heart. In *Adrenergic Activators and Inhibitors, Handb. exp. Pharmac.*, Vol. 54/I. ed. Szekeres, L. pp. 651–733. Berlin, Heidelberg and New York: Springer-Verlag.

SCHOLZ, H. & BRÜCKNER, R. (1981a). Effects of alpha-adrenoceptor stimulating agents on cardiac mechanical and electrophysiological properties. *Abstr. 8th Int. Congr. Pharmac.*, Tokyo, p. 512.

SCHOLZ, H. & BRÜCKNER, R. (1981b). Effects of beta- and alpha-adrenoceptor stimulating agents on mechanical activity, electrophysiological parameters and cyclic nucleotide levels in the heart. *J. mol. cell. Cardiol.*, **13,** (Suppl. 1), 84.

SCHÜMANN, H.J. (1980). Are there alpha-adrenoceptors in the mammalian heart? *Trends Pharmac. Sci.*, **1,** 195–197.

SHIBATA, S. SERIGUCHI, D.G., IWADARE, S., ISHIDA, Y. SHIBATA, T. (1980). The regional and species differences on the activation of myocardial alpha-adrenoceptors by phenylephrine and methoxamine. *Gen. Pharmac.*, **11,** 173–180.

SKOMEDAL, T., OSNES, J.B. & ØYE, I. (1982). Differences between alpha-adrenergic and beta-adrenergic inotropic effects in rat heart papillary muscles. *Acta Pharmac. Tox. (Copenh)*, **50,** 1–12.

STRUCK, C.J., AHNERT, G., GLOSSMANN, H. & SCHAEG, W. (1977). Solid phase radioimmunoassay for cyclic AMP using staphylococcal protein A-antibody absorbent. *Naunyn Schmiedebergs Arch. Pharmac.*, **298,** 67–73.

TRAUTWEIN, W. (1973). Membrane currents in cardiac muscle fibres. *Physiol. Rev.*, **53,** 793–835.

TRAUTWEIN, W. & McDONALD, T.F. (1978). Current voltage relations in ventricular muscle preparations from different species. *Pflügers Arch.*, **374,** 79–89.

TSIEN, R.W. (1977). Cyclic AMP and contractile activity in heart. *Adv. Cyclic Nucleotide Res.*, **8,** 363–420.

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