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ADRENOCEPTOR-MEDIATED CHANGES OF EXCITATION AND CONTRACTION IN VENTRICULAR HEART MUSCLE FROM GUINEA-PIGS AND RABBITS

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SUMMARY

1. The influence of α-adrenoceptor stimulation on mechanical and electrophysiological parameters was investigated in ventricular preparations from guinea-pigs and rabbits. Action potential and force of contraction were measured in papillary muscles and ionic currents were measured in isolated myocytes.

2. The effects of α-adrenoceptor stimulation were compared with those of β-adrenoceptor stimulation.

3. In the guinea-pig the stimulation of α-adrenoceptors caused a small increase in the force of contraction (less than 10% of the response to β-adrenoceptor stimulation) which was not accompanied by any increase of the slow calcium inward current. β-Adrenoceptor stimulation produced large increases in both force of contraction and slow inward calcium current. The noradrenaline-induced increase in the slow inward calcium current was not affected by phentolamine.

4. In the rabbit, α-adrenoceptor stimulation produced large increases in the force of contraction (about two thirds of those seen in response to β-adrenoceptor stimulation). Whereas β-adrenoceptor stimulation also produced large increases in both maximal upstroke velocity of slow-response action potentials and slow inward calcium current, there was almost no change of both parameters in response to α-adrenoceptor stimulation.

5. We conclude that, first, the contribution of α-adrenoceptors to adrenoceptor-mediated changes of force of contraction is minimal in the guinea-pig ventricle, and second, the pronounced changes of force of contraction in the rabbit ventricle in response to α-adrenoceptor stimulation are unrelated to changes in the slow inward calcium current.

INTRODUCTION

The positive inotropic effect of catecholamines in the heart is thought to be mediated by a sequence of events that, at first, involves the release of noradrenaline from the adrenergic nerve terminals and the stimulation of β-receptors located at the postsynaptic cardiac cell membrane. More recent findings, however, suggest that

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α-adrenoceptors also may help mediate the intropic response to catecholamines (for review see Brückner, Mügge & Scholz, 1985). Typically, the stimulation of β-adrenoceptors activates the adenylate cyclase system with subsequent increases in cyclic AMP content and slow inward calcium current, I_{Ca} (Reuter & Scholz, 1977; Kameyama, Hofmann & Trautwein, 1985). The onset of the positive inotropic effect mediated by β-adrenoceptors occurs within seconds and is associated with a faster rate of relaxation (Morad & Rolett, 1972). The stimulation of the myocardium through the activation of α-adrenoceptors seems to differ both qualitatively and quantitatively. The magnitude of the positive inotropic response to α-adrenoceptor stimulants varies considerably with the species and the tissue investigated (Wagner & Brodde, 1978). The effect, albeit small, develops more slowly and is accompanied by a prolongation of the relaxation time of the twitch (Benfey, 1977). The magnitude of the α-response was described as being more pronounced in hypothyroidism (Nakashima, Maeda, Sekiya & Hagino, 1971), at low frequencies of beating (Endoh & Schümann, 1975), or in hypothermia (Kunos & Nickerson, 1976). The mechanism of the α-adrenoceptor-mediated positive inotropic effect is not known. There is a general consensus that no significant changes of cyclic AMP levels can be detected (Brodde, Motomura, Endoh & Schümann, 1978). Cholinergic stimulation antagonizes the positive inotropic effect mediated by β-adrenoceptors but not those mediated by α-adrenoceptors (Endoh and Motomura, 1979). The same dissociation was also found with adenosine (Endoh & Yamashita, 1980). Proposals for explaining the inotropic response to α-adrenoceptor agonists include increase in I_{Ca} (Miura, Inui & Imamura, 1978; Brückner and Scholz, 1984), phosphorylation of membrane proteins (Lindemann, 1986) and modification of myofibrillar responsiveness to Ca^{2+} (Blinks & Endoh, 1986). Changes of phosphatidyl inositol turnover as described for other organ systems (Berridge, 1984; Berridge & Irvine, 1984) may also be involved.

In the present study, we have investigated the effects of phenylephrine, isoprenaline, adrenaline and noradrenaline on action potential and force of contraction in papillary muscles and on I_{Ca} in single cells of ventricular heart muscle from guinea-pigs and rabbits. It will be shown that the contribution of α-adrenoceptors to adrenoceptor-mediated changes in the force of contraction is minimal in the guinea-pig ventricle. The pronounced changes in the force of contraction in response to the α-adrenoceptor agonist, phenylephrine, in the rabbit ventricle are probably unrelated to changes in I_{Ca}. A preliminary account of this work has been presented (Nawrath & Tang, 1987).

METHODS

Measurement of force of contraction and transmembrane potentials

Guinea-pigs or rabbits were killed by a blow to the head and bled from the carotid arteries. Suitable right ventricular papillary muscles or trabeculae, mean dimensions (±S.D.; $n = 58$) 6·9±2·7 mm length × 0·52±0·29 mm diameter, were isolated by ligating both ends with a fine silk suture and dissected from the heart. The preparations were transferred to a test chamber and electrically stimulated at 1 Hz by rectangular pulses of 0·1–1 ms duration at about 10% above threshold intensity. The force of contraction was measured isometrically after a stabilization period of at least 30 min in Tyrode solution containing (in mmol/l): NaCl, 136·9; KCl, 5·4; $MgCl_2$, 1·05; NaH_2PO_4, 0·42; $NaHCO_3$, 11·9; $CaCl_2$, 1·8; glucose, 5·6. To avoid oxidative degradation of catecholamines, ascorbic acid (50 mg/l) and EDTA (18·6 mg/l) were added. The solution was

prepared in distilled water from stock solutions and equilibrated with 95% O_2 and 5% CO_2 at 37 °C (pH 7·4). High-potassium depolarizing solution was made by replacing 21·6 mmol NaCl with equimolar amounts of KCl and addition of $BaCl_2$, 0·1 mmol/l; other ions the same. The effects of drugs were investigated by exposure to either single or cumulatively increasing concentrations, achieved by adding drugs to the main Tyrode reservoir, and increasing the concentration after the establishment of a stable response. The transmembrane potential was detected intracellularly by the use of conventional microelectrodes. Both transmembrane potential and tension were displayed on a cathode ray oscilloscope and recorded on magnetic tape for later evaluation. Maximal upstroke velocity, dV/dt_{max}, was obtained by analogue differentiation. During the course of the experiments, all signals could be observed on a Nicolet digital oscilloscope and transcribed using an X–Y recorder. Further details of the experimental procedure have been described previously (Eckel, Gristwood, Nawrath, Owen & Satter, 1982).

Measurement of membrane currents

Guinea-pigs and rabbits were anaesthetized with sodium pentobarbitone (30 mg/kg, I.P.) and the aorta was cannulated *in situ* under artificial respiration. The heart was then quickly removed and perfused in a Langendorff apparatus. Single ventricular myocytes were obtained by enzymatic dissociation as described previously (Isenberg & Klöckner, 1982; Hescheler, Kameyama & Trautwein, 1986). For the electrophysiological experiments, the single cells were transferred to a small test chamber and superfused with Tyrode solution containing (in mmol/l): NaCl, 112; $NaHCO_3$, 24; KCl, 5·4; $CaCl_2$, 1·8; $MgCl_2$, 1·0; glucose, 10 and HEPES, 5. Except for $NaHCO_3$, the salts were dissolved in distilled water and pH was adjusted to 7·4 by NaOH. Then, $NaHCO_3$ was added to the solution followed by bubbling with 95% O_2 and 5% CO_2 at 37 °C. The single-electrode patch-clamp technique in whole-cell recording configuration was used (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The solution in the recording pipette contained (in mmol/l): potassium aspartate, 80; KCl, 50; KH_2PO_4, 10; $MgSO_4$, 1; HEPES, 5; Na_2ATP, 3; EGTA, 0·1; pH was adjusted to 7·3–7·4 with KOH. The cells were clamped at their resting potential of about −80 mV. Membrane currents were measured in response to depolarizing voltage clamp steps. To inactivate the fast sodium current, clamp steps to −40 mV for 200 ms preceded test pulses of 300 ms to various potentials. The double step pulses were applied at a rate of 0·2 Hz. The effects of drugs were investigated by addition of single or cumulatively increasing doses to the main Tyrode reservoir. At the superfusion rate of about 3–5 ml/min, a stable response was generally achieved within 2 min. A PDP 11/26 computer (Digital Equipment Corp.) was used on line for generating pulses and storing the experimental data for later analysis. Further details of the Methods have been described previously (Hescheler *et al.* 1986).

Chemicals

The following drugs were used (sources in parentheses): noradrenaline bitartrate (Serva, Heidelberg); adrenaline bitartrate (Hoechst, Frankfurt); phenylephrine hydrochloride (Boehringer, Ingelheim); isoprenaline sulphate dihydrate (Boehringer, Ingelheim); propranolol hydrochloride (ICI–Pharma, Plankstadt); phentolamine methanesulphonate (Ciba-Geigy, Wehr); HEPES (Serva, Heidelberg), all other chemicals (E. Merck, Darmstadt).

Evaluation of results

Results are expressed as means ± S.E.M. The calcium current (I_{Ca}) was measured as peak inward current and the outward current (I_{out}) was measured 200 ms after onset of the pulse. Both currents were measured with reference to zero current. Peak levels of phasic contractions (F_c) and current magnitudes are given as a percentage of control values. Action potential recordings were analysed for amplitude (APA), resting potential (RP), overshoot (OS), maximal upstroke velocity (dV/dt_{max}) and duration at 20% and 90% of repolarization, APD_{20} and APD_{90}, respectively. Where appropriate, statistically significant differences were assessed by analysis of variance (repeated-measurements design according to Wallenstein, Zucker & Fleiss, 1980). Statistically significant differences are marked by an asterisk ($P < 0·05$).

RESULTS

Guinea-pig ventricular preparations

Phenylephrine, which is thought to activate principally α-adrenoceptors and only to a minor extent β-adrenoceptors (Starke, Endo & Taube, 1975), exerted a concentration-dependent positive inotropic effect. When added in a cumulative

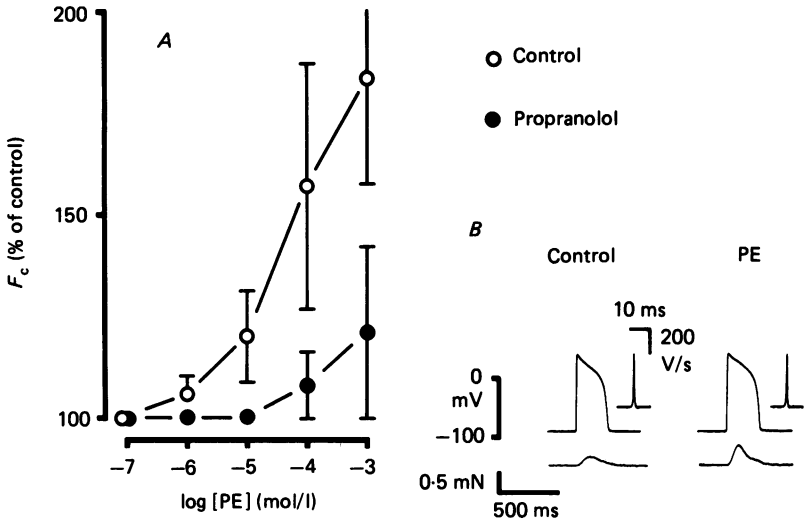

Fig. 1. Influence of phenylephrine (PE) on force of contraction (F_c) and action potential parameters in guinea-pig papillary muscles. *A*, cumulative concentration–response relationships (F_c) without (○) and in the presence of propranolol (0·3 μmol/l; ●). Means ±S.E.M. of two groups of seven preparations each. Note that in the presence of propranolol the concentration–response relationships of phenylephrine were shifted to the right by about two decades indicating a virtually pure β-effect. *B*, records of action potential, dV/dt and F_c under control conditions (left) and 15 min after the addition of the maximally effective concentration of phenylephrine (1 mmol/l; right). Note the slight prolongation of the action potential.

manner, the threshold concentration for the effects was around 0·1 μmol/l and maximal effects were seen at 1000 μmol/l where the force of contraction (F_c) was about doubled. Half-maximal effects occurred at 30 μmol/l (EC_{50}). The Hill coefficient was 0·9. Blockade of β-receptors with propranolol (0·3 μmol/l) greatly depressed the inotropic response. For example, the same effect as was obtained with phenylephrine (10 μmol/l) without propranolol was seen only at a concentration 100 times higher when propranolol was present (Fig. 1*A*). The original records in Fig. 1*B* show a marked increase by phenylephrine of the force of contraction and slight changes of the configuration of the action potential. The mean values ±S.E.M. of all action potential parameters are summarized in Table 1. At maximally effective concentrations of phenylephrine, resting potential, overshoot and APD_{20} were slightly increased. The small changes of the action potential were abolished when propranolol was present as described earlier for the changes in F_c.

Figure 2 compares the effects of α- and β-adrenoceptor stimulation when the papillary muscles were exposed immediately to submaximally effective concen-

TABLE 1. Influence of phenylephrine on action potential parameters in guinea-pig papillary muscles (means ±S.E.M.; $n = 7$)

	Phenylephrine concentration (mol/l)				
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
APA (mV)	122±2	122±2	123±2	124±2	125±2
RP	−88±1	−88±1	−88±1	−89±1	−89±1
OS	34±1	34±1	34±1	35±2	36±1
APD_{20} (ms)	101±10	105±11	107±11	113±11	117±11
APD_{90}	198±16	196±15	198±16	198±17	199±17
dV/dt_{max} (V/s)	268±28	269±28	268±29	258±31	247±28

For abbreviations see text.

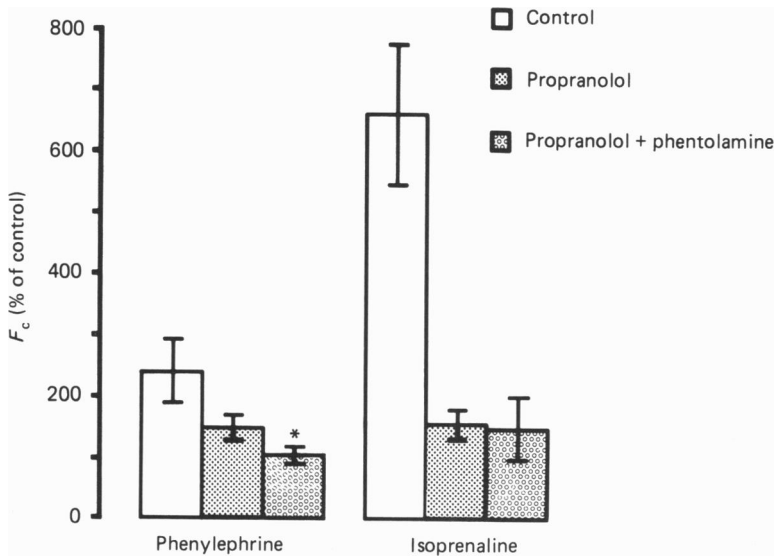

Fig. 2. Influence of phenylephrine (100 μmol/l; left group of columns) and isoprenaline (1 μmol/l; right group of columns), on F_c under control conditions (open columns), in the presence of propranolol (1 μmol/l; shaded columns) and in the presence of both propranolol (1 μmol/l) and phentolamine (1 μmol/l; dotted columns) in guinea-pig papillary muscles. Means ±S.E.M. of two groups of seven preparations each which were treated first with the agonist, then with propranolol and finally with phentolamine. The drugs were added cumulatively after a steady state of effects had been reached (between 10 and 30 min). Note that a small portion of the effect of phenylephrine can be attributed to α-adrenoceptors. The asterisk denotes a significant difference between the effects of phenylephrine in the presence of propranolol + phentolamine and the effects of both phenylephrine alone or phenylephrine + propranolol.

trations of the respective agonist. Under these conditions, there was a somewhat larger effect of phenylephrine on F_c than with cumulatively increasing concentrations (230% of control instead of 160% of control at 100 μmol/l). There was a much greater response to isoprenaline, where F_c was increased to about 680% of control values. Both the effects of phenylephrine and isoprenaline were effectively antagonized when propranolol (1 μmol/l) was present, i.e. F_c returned to 150% of control values. In control experiments, propranolol (1 μmol/l) did not

significantly affect F_c. The addition of phentolamine (1 μmol/l) further decreased the inotropic response to phenylephrine to 125% of control. This antagonizing effect of phentolamine did not occur in the case of isoprenaline (compare the dotted columns in Fig. 2).

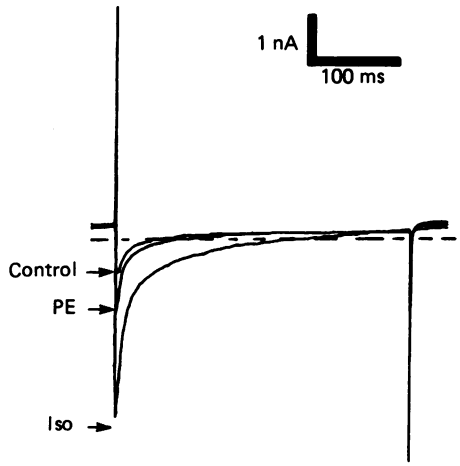

Fig. 3. Influence of phenylephrine (100 μmol/l) and isoprenaline (1 μmol/l) on I_{Ca} in a guinea-pig ventricular myocyte. The preparation was first treated with phenylephrine, then washed and finally treated with isoprenaline (exposure times, 5 min each). Current traces in response to depolarizing voltage clamp steps from −40 to 0 mV for 300 ms. The dashed line refers to zero current at the holding potential of −80 mV. Records under control and test conditions were superimposed. Note that the increase by phenylephrine of I_{Ca} amounts to about half of that in response to isoprenaline.

Since it has been shown that the positive inotropism of β-adrenoceptor stimulation is causally related to an increase in I_{Ca} (Reuter & Scholz, 1977), we tested the effects of α- and β-adrenoreceptor stimulation on I_{Ca} in isolated myocytes. Figure 3 demonstrates a moderate increase in I_{Ca} in response to phenylephrine (100 μmol/l) from 0·81 to 1·6 nA, whereas isoprenaline (1 μmol/l) increased I_{Ca} to 3·6 nA. The mean increases (means ±S.E.M.) of I_{Ca} amounted to 181±31% of control for phenylephrine (100 μmol/l; $n = 6$) and to 354±94% of control for isoprenaline (1 μmol/l; $n = 3$). Again, for separation of α- and β-adrenergic effects, we superfused the cells with propranolol (1 μmol/l) for 5 min which itself had no significant effect on I_{Ca}. When, under this condition, either phenylephrine (0·1–1000 μmol/l) or isoprenaline (1 μmol/l) were applied, no changes in I_{Ca} were detected (not shown).

So far, evidence has been presented that the inotropic and electrophysiological effects of phenylephrine and isoprenaline on the heart are obviously mainly due to the stimulation of β-adrenoceptors. Only a small fraction of the inotropic response of phenylephrine could be attributed to the stimulation of α-adrenoceptors. These results do not necessarily exclude a somewhat greater participation of α-adrenoceptors in the effects of endogenous noradrenaline or adrenaline in the heart. Both transmitters have been shown to be more potent than phenylephrine at α-adrenoceptors (Starke *et al.* 1975). To test this possibility, we investigated the effects of noradrenaline on I_{Ca} in the presence and absence of the α-adrenoceptor blocking

agent, phentolamine. It is shown in Fig. 4 that the two concentration–response relationships were superimposable, suggesting that α-receptors do not participate in the response to noradrenaline. The same results were also obtained with adrenaline (not shown).

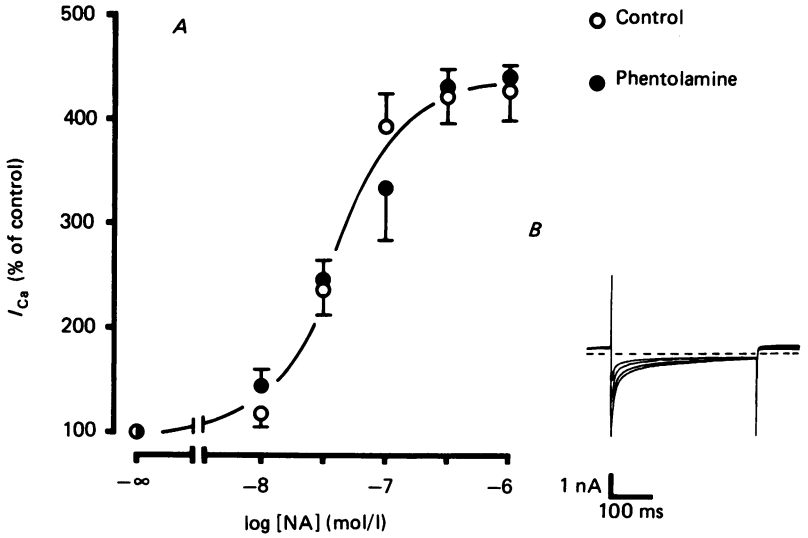

Fig. 4. Influence of noradrenaline (NA) on I_{Ca} in guinea-pig ventricular myocytes. *A*, concentration–response relationships without (○) and in the presence of phentolamine (1 μmol/l; ●). Means ±S.E.M. of two groups of four (control) and three (phentolamine) preparations which were exposed to cumulatively increasing concentrations of NA. Note that the concentration–response relationships of NA were unchanged by phentolamine. *B*, current traces in response to depolarizing voltage clamp steps from −40 to 0 mV for 300 ms. The dashed line refers to zero current at the holding potential of −80 mV. Records obtained under control conditions and with cumulatively increasing concentrations of NA (0·01, 0·1 and 1·0 μmol/l) were superimposed.

Rabbit ventricular preparations

In contrast to the relatively weak effects of α-adrenoceptor stimulation in the guinea-pig ventricle, marked positive inotropic effects can be demonstrated in rabbit ventricular preparations. Figure 5 shows typical traces of twitches in response to α- and β-adrenoceptor stimulation, achieved by the addition of different concentrations of phenylephrine and of isoprenaline, respectively. Since phenylephrine at high concentrations stimulates not only α- but also β-adrenoceptors, all experiments with phenylephrine in rabbit ventricular preparations were carried out in the presence of propranolol (1 μmol/l) which by itself did not significantly change the control values. Phenylephrine increased F_c from 2·1 to 5·8 mN and slightly prolonged both time to peak tension and relaxation time. Isoprenaline increased F_c from 2·3 to 8·4 mN and significantly diminished time to peak tension and relaxation time. The concentration–response relationships of phenylephrine and isoprenaline (Fig. 6) demonstrate that the maximal effects of phenylephrine amount to about 2/3 of those seen in response to isoprenaline, even though reached with about hundred times higher concentrations. The threshold and the maximal concentrations ranged from

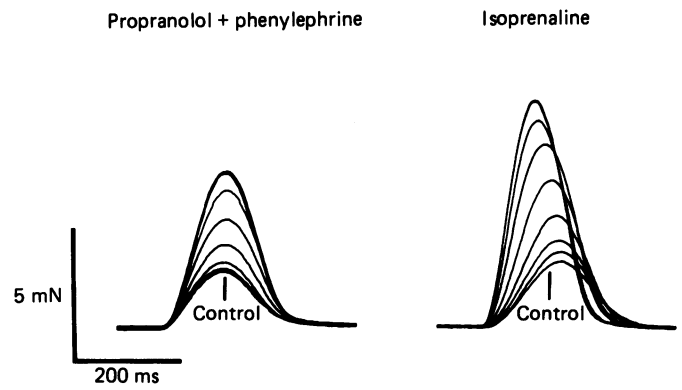

Fig. 5. Influence of α-adrenoceptor stimulation (phenylephrine + propranolol, 1 μmol/l) and β-adrenoceptor stimulation (isoprenaline) on force of contraction (F_c) in rabbit papillary muscles. Original traces of two preparations which were exposed to cumulatively increasing concentrations of either phenylephrine (0·03, 0·1, 0·3, 1·0, 3·0, 10·0, 30·0, and 100 μmol/l) or isoprenaline (0·001, 0·003, 0·01, 0·03, 0·1, 0·3, and 1 μmol/l). The records under control and test conditions were superimposed. Note that, first, the maximal increase by phenylephrine of F_c amounted to about two thirds of that seen in response to isoprenaline, and second, the contraction time was slightly prolonged by phenylephrine but not shortened, as with isoprenaline.

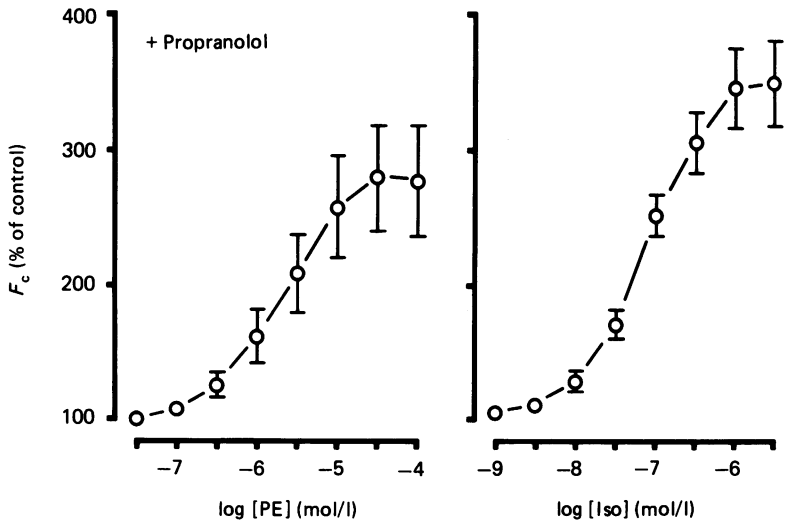

Fig. 6. Influence of α-adrenoceptor stimulation (phenylphrine + propranolol 1 μmol/l; left) and β-adrenoceptor stimulation (isoprenaline; right) on force of contraction (F_c) in rabbit papillary muscles. Concentration–response relationships (means ± S.E.M.). Two groups of ten preparations each were treated by cumulatively increasing concentrations of either phenylephrine (PE) or isoprenaline (Iso). Note that the maximal effects obtained with phenylephrine amounted to about two thirds of those seen in response to isoprenaline.

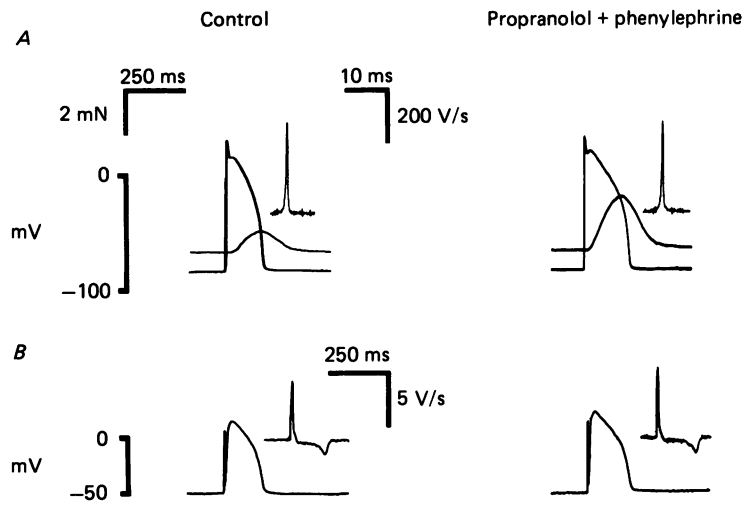

Fig. 7. Influence of α-adrenoceptor stimulation (phenylephrine, 100 μmol/l+propranolol, 1 μmol/l) on force of contraction (F_c) and action potential parameters in a rabbit papillary muscle. The preparation was first exposed to phenylephrine in normal Tyrode solution, then washed and finally exposed to phenylephrine again in Tyrode solution containing KCl (21·6 mmol/l) and $BaCl_2$ (0·1 mmol/l). Propranolol (1 μmol/l) was present in all solutions. Stable microelectrode impalement existed throughout the experiment. *A*, recordings of action potential, dV/dt and F_c under control conditions (left) and after α-adrenoceptor stimulation (right). Note that F_c was increased and the action potential duration slightly prolonged under test conditions. *B*, recordings of slow-response action potentials and dV/dt under control conditions (left) and after α-adrenoceptor stimulation (right). Note that overshoot, duration and dV/dt_{max} were slightly increased under test conditions.

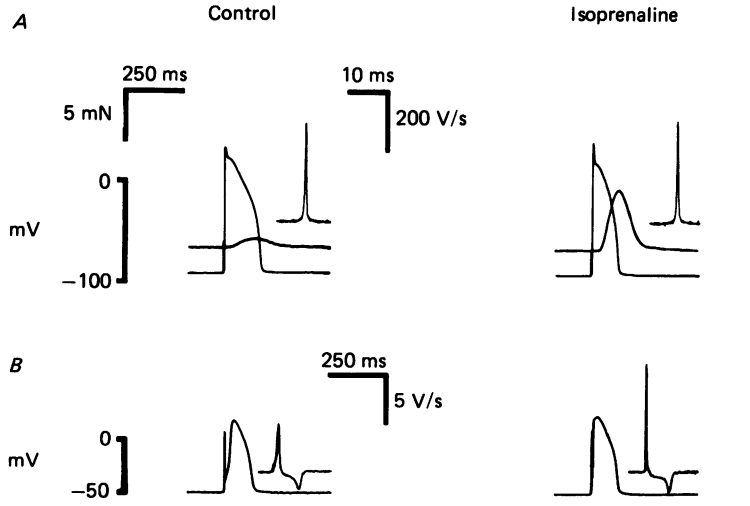

Fig. 8. Influence of β-adrenoceptor-stimulation (isoprenaline, 1 μmol/l) on force of contraction (F_c and action potential parameters in a rabbit papillary muscle. The experimental protocol and the graphic presentation of records are the same as described in the legend to Fig. 7 except that isoprenaline was used instead of phenylephrine and propranolol was absent. Note that in *A*, F_c was increased and the action potential shortened and in *B*, overshoot and dV/dt_{max} of the slow-response action potential were largely increased by isoprenaline.

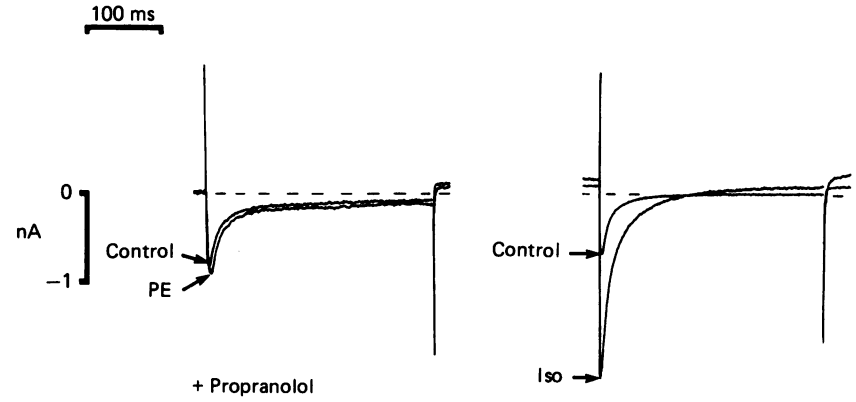

Fig. 9. Influence of α-adrenoceptor stimulation (phenylephrine (PE) 100 μmol/l + propranolol 1 μmol/l; left) and β-adrenoceptor stimulation (isoprenaline (Iso), 1 μmol/l; right) on I_{Ca} in rabbit ventricular myocytes. One preparation was exposed to PE, the other to Iso. Current traces in response to depolarizing voltage clamp steps from −40 to 0 mV for 300 ms. The dashed lines refer to zero current at the holding potential of −80 mV. Records under control (C) and test conditions (PE, Iso) were superimposed. Note the very small increase in I_{Ca} in response to PE and the large increase in I_{Ca} in response to Iso. In response to Iso, there was also a slight change of the outward current.

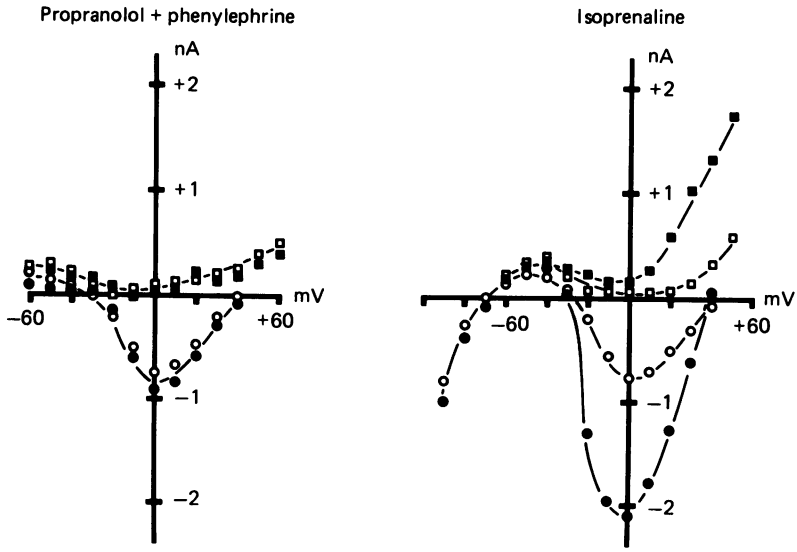

Fig. 10. Influence of α-adrenoceptor stimulation (phenylephrine, 100 μmol/l + propranolol, 1 μmol/l; left) and β-adrenoceptor stimulation (isoprenaline, 1 μmol/l; right) on inward and outward currents in rabbit ventricular myocytes. Current–voltage relationships. Note that in response to phenylephrine, the current–voltage relationships were very little affected. In response to isoprenaline, large changes of both inward and outward currents were detected at different voltage levels. The latter result explains the observations that in response to isoprenaline either a shortening of the action potential (Fig. 8) or a prolongation (Eckel *et al.* 1982) can be seen depending on the balance of both currents.

0·03 to 100 μmol/l for phenylephrine and from 0·001 to 3 μmol/l for isoprenaline. Hill coefficients amounted to 0·98 and to 1·2 for phenylephrine and isoprenaline, respectively.

Action potential recordings revealed a slight retardation of repolarization in response to phenylephrine; in Fig. 7, the maximal upstroke velocity (dV/dt_{max}) of the slow-response action potential was increased from 5·5 to 6·4 V/s. In contrast, isoprenaline shortened the action potential duration, and increased dV/dt_{max} of the slow-response action potential from 4·6 to 10·0 V/s (Fig. 8). The statistical evaluation showed that phenylephrine (100 μmol/l) increased dV/dt_{max} by $27 \pm 13\%$, whereas isoprenaline produced an increase of $153 \pm 15\%$ (means $\pm$ S.E.M.; $n = 10$). The effects of both isoprenaline and phenylephrine were observed in the same preparations (stable impalements throughout the experiments).

In the isolated myocyte, phenylephrine barely affected the magnitude of I_{Ca}, whereas isoprenaline increased I_{Ca} almost threefold (Fig. 9). In two groups of preparations, phenylephrine (100 μmol/l) and isoprenaline (1 μmol/l) increased I_{Ca} by $7·7 \pm 4·4\%$ (means $\pm$ S.E.M.; $n = 7$) and by $174 \pm 7·0\%$ (means $\pm$ S.E.M.; $n = 6$), respectively. The current–voltage relationships in Fig. 10 show that both I_{Ca} and I_{out} were increased by isoprenaline (1 μmol/l) at different voltage clamp levels. In contrast, the current–voltage relationships were barely affected by phenylephrine (100 μmol/l).

DISCUSSION

In the present study, exposure of guinea-pig ventricular preparations to phenylephrine resulted in moderate increases of both F_c and I_{Ca}, the duration of the action potential was slightly prolonged. The effects of phenylephrine on I_{Ca} were comparably small with respect to isoprenaline and completely eliminated in the presence of propranolol. A small portion of the inotropic response to phenylephrine was sensitive to phentolamine. These results are consistent with the view that, in guinea-pig papillary muscles, the existence of α-adrenoceptors is of minor importance. Also, the effect of noradrenaline, which is a potent stimulator of α-adrenoceptors suggests that there are mainly β-adrenoceptors in the guinea-pig heart, since the increase in I_{Ca} was not significantly affected by the α-adrenoceptor blocking agent, phentolamine.

The rabbit ventricle was much more sensitive to α-adrenoceptor stimulation (phenylephrine + propranolol), although the effects on F_c were still smaller than with β-adrenoceptor stimulation (isoprenaline). The inotropic effects of β-adrenoceptor stimulation in the rabbit ventricle were accompanied by large increases in both dV/dt_{max} of slow-response action potentials and I_{Ca}. In contrast, α-adrenoceptor stimulation did not significantly affect either slow-response action potentials or magnitude of I_{Ca}. The very small increase in I_{Ca} caused by phenylephrine probably does not account for the positive inotropic effect of the drug and may rather reflect a secondary effect induced by a slight change of the intracellular calcium concentration (Isenberg, 1977). Earlier reports on the effects of phenylephrine in rabbit and bovine ventricle may have overestimated the importance of changes in I_{Ca} for the positive inotropic effect of phenylephrine, although the point was stressed that the effects were smaller than those in response to isoprenaline and that other

mechanisms may contribute to the positive inotropic effect (Brückner & Scholz, 1984). Our results are in contrast with the observation of the latter authors that the inactivation time constant of I_{Ca} is about doubled by phenylephrine. This effect would add to the greater peak inward current observed by the authors and further increase calcium entry during excitation. The reason for the discrepancy between our results and theirs is not entirely clear, although it seems that determinations of time constants obtained from experiments with the sucrose-gap voltage-clamp method are more likely to be prone to erroneous interpretation (see their Fig. 7).

Lindemann (1986) suggested that phosphorylation of a sarcolemmal 15 kDa protein may be involved in increases in I_{Ca} produced by stimulation of either α- or β-adrenoceptors in rat hearts. So far, it is not clear whether or not the 15 kDa protein can somehow be associated with the regulation or with the calcium channel itself. Recent studies rather suggest that the phosphorylation of a 142 kDa protein is of functional importance for calcium channels (Flockerzi, Oeken, Hofmann, Pelzer, Cavalié & Trautwein, 1986). Blinks & Endoh (1986) demonstrated that many positive inotropic interventions alter the intracellular calcium transient as assessed by the injection of aequorin. For a given increase in F_c there was, however, a much smaller increase in the height of the aequorin signal under the influence of phenylephrine than with other positive inotropic agents. The authors therefore proposed an increase in the responsiveness of the contractile myofilaments to Ca^{2+} as the underlying mechanism of α-adrenoceptor stimulation. This interpretation is in line with the lack of an effect of phenylephrine on I_{Ca}.

As yet, little information exists as to how the signal transfer between α_1-adrenoceptor and the contractile machinery could be accomplished. Whereas β_1-, β_2- and α_2-adrenoceptors are linked to the adenylate cyclase system in different organ systems (Rodbell, 1980), the activation of α_1-adrenoceptors has been described as mobilizing intracellular Ca^{2+} through the generation of second messenger molecules of the polyphosphoinositides (Berridge, 1984; Berridge & Irvine, 1984). The increase in tonic tension by activation of α_1-adrenoceptors in smooth muscle may be explained in this way (Abdel-Latif, 1986). The existence of a similar mechanism in the heart remains to be established. A possible participation of polyphosphoinositide metabolism in the excitation–contraction coupling in the heart was investigated by Poggioli, Sulpice & Vassort (1986). In the latter study, both noradrenaline and carbachol increased the basal levels of several phosphoinositides through activation of either α_1-adrenoceptors or muscarine receptors. The authors concluded that inositol triphosphate may be involved in mediating the effects of α_1-adrenoceptor stimulation.

This study and many other studies have shown that the myocardial response to α-adrenoceptor stimulation can differ between almost none and relatively large effects amounting to about two thirds of those seen after β-adrenoceptor stimulation. The differences depend on species and tissue investigated and may be related to receptor density and/or variations in coupling mechanisms between receptor activation and physiological response. The functional role of α-adrenoceptors in the heart is therefore not easy to discuss. Some authors think the α-receptors in the heart act as a reserve mechanism to stimulate the heart under various conditions such as hypothyroidism, diabetes or ischaemia (for review see Brückner *et al.* 1985). Others

have demonstrated that α-adrenoceptors are involved in regulating myocardial hypertrophy (Simpson, Bishopric, Coughlin, Karliner, Ordahl, Starksen, Tsao, White & Williams, 1980). Different life cycles of α- and β-adrenoceptors (Maisel, Motulsky & Insel, 1987) or interconversion of α- and β-adrenoceptors (Kunos & Nickerson, 1976) under various pharmacological conditions or disease settings may contribute to the variability of results.

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