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DEPOLARIZATION-INDUCED INFLUX OF SODIUM IN RESPONSE TO PHENYLEPHRINE IN RAT ATRIAL HEART MUSCLE

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SUMMARY

1. The effects of α_1-adrenoceptor stimulation on transmembrane potential, currents and ion fluxes were investigated in multicellular preparations and/or single cells obtained from the left atrium of rat hearts.

2. In multicellular preparations, phenylephrine caused a concentration-dependent positive inotropic effect, an increase in action potential duration, and a decrease in resting potential; the effects were antagonized by phentolamine.

3. In the presence of phenylephrine (100 μmol/l), two levels of resting potential were observed when the preparations were, alternately, electrically stimulated or kept at rest (-74 ± 1 mV during activity and -62 ± 4 mV at rest; means ± S.E.M.; $n = 9$).

4. In resting preparations, the depolarization in response to phenylephrine was eliminated in low-Na^+ solution (12 mmol/l) and antagonized by tetrodotoxin (10 μmol/l).

5. The phenylephrine-induced depolarization was also seen in nominally Ca^{2+}-free solution and in the presence of (−)-devapamil (1 μmol/l).

6. The alkylating agent *N*-ethyl-maleimide (30 μmol/l) abolished the depolarizing effect of phenylephrine.

7. Phorbol 12,13-dibutyrate (10 μmol/l) also abolished the depolarizing effect of phenylephrine.

8. Phenylephrine caused a significant increase of $^{22}Na^+$ uptake in resting preparations and of $^{45}Ca^{2+}$ uptake in beating preparations.

9. The depolarizing effect of phenylephrine was also observed in single atrial myocytes. Steady-state membrane currents in response to 500 ms depolarizing and hyperpolarizing voltage clamp steps were decreased. The cross-over of *I–V* curves under control and test conditions was at about −70 mV. The effects of phenylephrine were antagonized in the presence of phentolamine.

10. After suppression of potassium currents by substitution of CsCl for internal and external KCl ($[KCl]_i$ and $[KCl]_o$), phenylephrine had no effect on membrane currents.

11. In conclusion, we presume the following sequence of events in response to phenylephrine in rat atrial heart muscle. First, the stimulation of α_1-adrenoceptors

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decreases the K^+ conductance thereby producing a depolarization in the presence of an inward current. Second, the change of the membrane potential in the depolarizing direction induces a TTX-sensitive Na^+ window current which further propels the depolarization. Third, the increase in Na^+ influx may increase Ca^{2+} influx by activating the Na^+–Ca^{2+} exchange mechanism. The greater influx of Ca^{2+} may contribute to the positive inotropic effect in response to phenylephrine.

INTRODUCTION

The positive inotropic effect of catecholamines in the heart depends on the stimulation of mainly β-adrenoceptors (see Arnold, 1980). However, in several species including rat, rabbit, cat and dog, the stimulation of α_1-adrenoceptors may help to mediate the positive inotropic response to catecholamines (see Wagner & Brodde, 1978; Endoh, 1982). The increase in force of contraction mediated by β-adrenoceptor stimulation, has been ascribed to an increase of the cyclic AMP content (see Drummond & Severson, 1979) and an increase of the Ca^{2+} current, I_{Ca} (Reuter, 1974*a*). Injection of the catalytic subunit of protein kinase A mimics the effects of isoprenaline or intracellularly applied cyclic AMP on I_{Ca} (Brum, Flockerzi, Hofmann, Osterrieder & Trautwein, 1983). The subcellular mechanism of the α_1-adrenoceptor-mediated positive inotropic effect is still a matter of debate. Attempts to explain the inotropism refer to a phosphorylation of membrane proteins (Lindemann, 1986), an increase of I_{Ca} (Miura, Inui & Imamura, 1978; Brückner & Scholz, 1984), and a modification of myofibrillar responsiveness to Ca^{2+} (Endoh & Blinks, 1988).

An increasing number of observations indicates that α-adrenoceptor stimulation does not affect I_{Ca} in the heart of several species (rabbit: Hescheler, Nawrath, Tang & Trautwein, 1988; rat: Apkon & Nerbonne, 1988; cat: Hartmann, Mazzocca, Kleiman & Houser, 1988), but rather decreases K^+ currents (dog: Shah, Cohen & Rosen, 1988; rat: Apkon & Nerbonne, 1988; Ravens, Wang & Wettwer, 1989; rabbit: Fedida, Shimoni & Giles, 1989, 1990).

α_1-Adrenoceptors activate, via a GTP-binding protein (Cockcroft, 1987), the membrane-bound phospholipase C which provides, from the break-down of phosphatidyl inositol bisphosphate (PIP_2), two putative messenger molecules, diacylglycerol (DAG) and inositol trisphosphate (IP_3) (Berridge & Irvine, 1984, 1989). In the heart, α_1-adrenoceptor stimulation increases the levels of several metabolites of phosphatidyl inositol turnover (Poggioli, Sulpice & Vassort, 1986; Otani, Otani & Das, 1988; Scholz, Schaefer, Schmitz, Scholz, Steinfath, Lohse, Schwabe & Puurunen, 1988; Okumura, Kawai, Hashimoto, Ito, Ogawa & Satake, 1988). However, the precise role of DAG and/or IP_3 for the positive inotropic effect of α_1-adrenoceptor stimulation has remained unclear.

Our results confirm that the stimulation of α_1-adrenoceptors in the heart decreases K^+ currents producing a prolongation of the action potential. In addition, we show that the resting potential is decreased. Secondary to these changes, Na^+ and Ca^{2+} influx are increased. A tentative explanation for the sequence of events leading to the positive inotropic effect is provided.

METHODS

Measurement of force of contraction and transmembrane potentials in multicellular preparations

Male Sprague–Dawley rats (250–400 g) were anaesthetized with ether, decapitated and bled from the carotid arteries. The hearts were removed and immersed into warmed and oxygenated Tyrode solution. Left atria were dissected and opened to prepare fine trabeculae (1–2 mm in length and 0·15–0·25 mm in diameter), which were mounted in a 2 ml organ bath and superfused with Tyrode solution containing (mmol/l): NaCl, 136·9; KCl, 5·4; $CaCl_2$, 1·8; $MgCl_2$, 1·05; NaH_2PO_4, 0·42; $NaHCO_3$, 11·9; glucose, 5·6; EDTA, 0·05; and equilibrated with 95% O_2 and 5% CO_2 at 37 °C (pH 7·4). Low-Na^+ (12 mmol/l) and nominally Ca^{2+}-free solutions were prepared as described above, but modified by substitution of choline chloride (137 mmol/l) for NaCl and omission of $CaCl_2$, respectively. The preparations were electrically driven at 1 Hz (Grass S44 and isolation unit SIU5) (square pulses $\leq$ 0·2 ms, voltage 10–15% above threshold). After a stabilization period of at least 30 min, the effects of phenylephrine were investigated by exposure to either single or cumulatively increasing concentrations, after the establishment of a stable response (about 10 min). All test solutions contained atenolol (10 μmol/l) to avoid any interference with β-adrenoceptors. For investigating the effects on resting potential, the electrical stimulation was switched off for 2 min before adding phenylephrine.

Transmembrane potential was detected intracellularly by using conventional microelectrodes filled with 3 mol/l KCl (resistances 10–20 MΩ). A voltage follower with input capacitance compensation was used to record transmembrane potential. Upstroke velocity (dV/dt) was obtained by analog differentiation, and force of contraction was recorded by means of an inductive force displacement transducer whose output was fed to a Hellige carrier frequency preamplifier. All signals were displayed on an oscilloscope (Nicolet 310) and stored digitally on videotape (VCR Panasonic NV-H75) in conjunction with an Instrutech VR 100 14-bit AD converter. For evaluation, data were plotted (Hewlett Packard, 7475A) or transcribed to an X–Y recorder (BBC Goerz Metrawatt SE 790).

Measurements of ion fluxes

The experiments were carried out both in resting and in electrically driven (1 Hz) left atria from rats. For uptake measurements, preparations were first equilibrated under control conditions or in the presence of phenylephrine (100 μmol/l) for 30 min. Thereafter, tissues were exposed for 5 min to corresponding solutions containing $^{22}Na^+$ (100 kBq) or $^{45}Ca^{2+}$ (350 kBq). After the exposure to $^{22}Na^+$, tissues were washed three times for 1 min in ice-cold solution containing (mmol/l): sucrose, 303·8; glucose, 5·6; and $CaCl_2$, 1·8. Finally, tissues were weighed, and radioactivity was determined by γ-radiation counting (Auto-Gamma Scintillation Spectrometer 5260, Packard Instruments). After the exposure to $^{45}Ca^{2+}$, tissues were washed three times for 5 min in ice-cold physiological salt solution(Leijten & van Breemen, 1984) containing (mmol/l): NaCl, 140; KCl, 4·6; EGTA, 2·0; $MgCl_2$, 1·0; glucose, 10·0; HEPES, 5·0. The pH was adjusted at 0 °C to 7·4 using 0·1 mmol/l NaOH. Finally, tissues were weighed and solubilized by the addition of 1 ml TS-1 (Zinsser, Franfurt) and keeping them at 65 °C for about 3 h. Ten millilitres of scintillation liquid (Quickszint 402, Zinsser, Frankfurt) were added to each sample and radioactivity was determined by liquid scintillation counting (Tricarb 3380, Packard Instruments).

Measurement of membrane potentials and currents in single atrial myocytes

For isolation of atrial myocytes from rats, the hearts were perfused in a Langendorff apparatus. Single cells were obtained by enzymatic dissociation, using collagenase (45 mg) and protease (6 mg), freshly dissolved in 50 ml nominally Ca^{2+}-free solution. After a perfusion period of 25 min, enzymes were washed out and cells were stored in a modified Tyrode solution (180 μmol/l Ca^{2+}). For the electrophysiological experiments, single cells were transferred to a small test chamber and superfused with Tyrode solution at 37 °C containing (mmol/l): NaCl, 137·6; KCl, 5·4; $MgCl_2$, 0·5; $CaCl_2$, 1·8; HEPES, 11·6; glucose, 5·0; pH was adjusted to 7·4 by NaOH. The whole-cell recording configuration of the single-electrode patch-clamp technique was used (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The solution in the recording pipette contained (mmol/l): KCl, 120; $MgCl_2$, 6; $CaCl_2$, 0·15; Na_2ATP, 5; HEPES, 10; EGTA, 5; pH was adjusted to 7·2 with NaOH. To

block potassium currents, test and pipette solutions were modified by substitution of CsCl ($[Cs^+]_o$ 20 mmol/l; $[Cs^+]_i$ 125 mmol/l) for KCl; pH was adjusted with CsOH.

To inactivate the fast sodium current, holding potential was set to −50 mV. Membrane currents were measured in response to depolarizing and hyperpolarizing voltage-clamp steps in the range between −110 to +40 mV. Test pulses of 500 ms were applied in 10 mV increments at 0·1 Hz. Transmembrane potentials and currents were measured with an L/M-EPC-7 amplifier (List-Medical, Darmstadt) which received rectangular command pulses from a PC equipped with a Labmaster interface (Scientific Solution, Solon). Current signals were filtered at 3 kHz prior to digitization. Data acquisition and analysis were performed with PClamp (Axon, Burlington).

Phenylephrine was added to the main Tyrode solution reservoir, always containing atenolol (10 μmol/l). Given a superfusion rate of about 3 ml/min, a stable response was generally received within 2 min.

Chemicals

The following drugs were used (abbreviations and sources in parentheses): adenosine-5′-triphosphate (Na_2ATP); collagenase (Boehringer, Mannheim); atenolol, *N*-ethyl-maleimide (NEM), phorbol 12,13-dibutyrate (PDBu), protease, tetrodotoxin (TTX) (Sigma, München); caesium hydroxide (Aldrich, Steinheim); phenylephrine hydrochloride, (PE) (Boehringer, Ingelheim); phentolamine methanesulphonate (Ciba-Geigy, Wehr); (−)-devapamil (Knoll, Ludwigshafen); $^{45}CaCl$, $^{22}NaCl$ (NEN, Dreieich). Tissue solubilizer TS-1, scintillation liquid Quickszint 402 (Zinsser, Frankfurt). All other chemicals were obtained from Merck, Darmstadt.

Evaluation of results

Results are either expressed as means ± S.E.M. or shown as original records. Peak levels of phasic contractions (F_C) are given as a percentage of control values. Action potentials were analysed for resting potential (RP) and time to 90% of repolarization (APD_{90}). Changes of RP in the depolarizing direction are described as a decrease in RP. The uptake of $^{45}Ca^{2+}$ and $^{22}Na^+$ was evaluated as c.p.m./100 mg wet weight. Membrane currents were measured 500 ms after onset of the pulse with reference to zero current. Where appropriate, statistically significant differences were assessed by Student's *t* test for paired or unpaired data. Statistically significant differences are marked by one ($P < 0.05$) or two asterisks ($P < 0.01$).

RESULTS

Phenylephrine (100 μmol/l) exerted a marked and persistent increase in F_C and a prominent increase in APD in rat atrial heart muscle; RP was always diminished, combined with a decrease in dV/dt_{max} (Fig. 1). Figure 2 shows the concentration–response relationships of phenylephrine including the effects on F_C, APD_{90} and RP. The effects of phenylephrine were shifted to about ten times higher concentrations of the agonist in the presence of phentolamine (1 μmol/l).

In resting preparations, phenylephrine (100 μmol/l) caused a decline in membrane potential. There was either a rapid depolarization, sometimes accelerated by one or several spontaneous action potentials at threshold potential (left panel in Fig. 3). Alternatively, the depolarization developed more slowly and continuously (right panel in Fig. 3). In either event, RP was reduced by phenylephrine from −78 ± 1 to −55 ± 1 mV (means ± S.E.M.; $n = 29$) in the steady state.

In sixteen preparations, however, virtually no response was obtained under these conditions. In these experiments, RP amounted to −80 ± 1 mV without and to −78 ± 1 mV after the addition of phenylephrine. A bimodal distribution of the resting potential, in the presence of phenylephrine, is suggested by the histogram of all values ($n = 45$) in Fig. 4. The preparations were, therefore, separated into two groups, responders ($n = 29$) and non-responders ($n = 16$). In the group of responders,

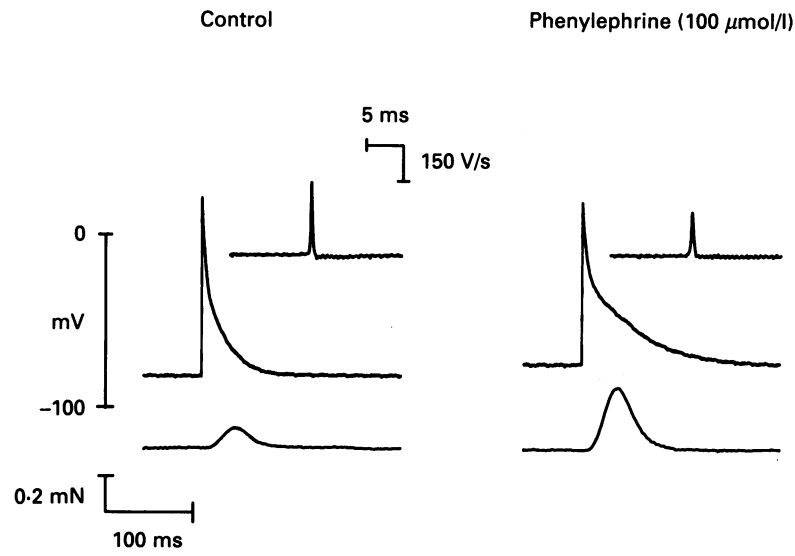

Fig. 1. Influence of phenylephrine on force of contraction (F_C; lower trace), action potential (middle trace) and upstroke velocity (dV/dt; upper trace) in the presence of atenolol (10 μmol/l). Original records before (control) and 10 min after the addition of phenylphrine (100 μmol/l). Note the increase of F_C, the prolongation of the action potential, and the decrease of the maximum diastolic potential.

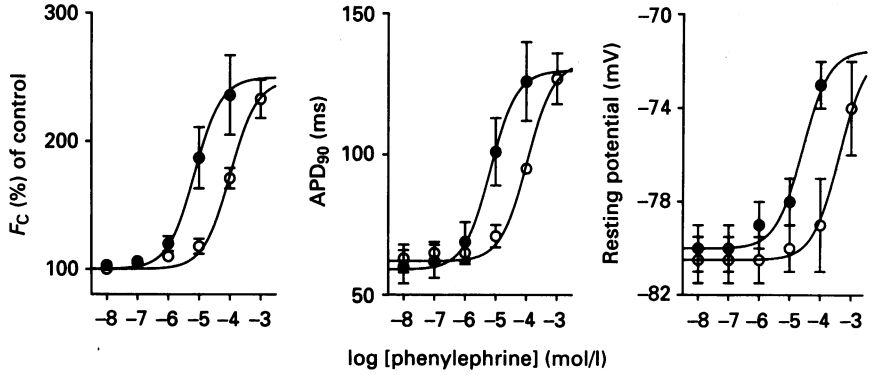

Fig. 2. Influence of phenylephrine on force of contraction (F_C), action potential duration at 90% of repolarization (APD_{90}) and maximum diastolic potential (RP). Concentration–response relationship without (●) and in the presence of phentolamine (1 μmol/l; ○). Means ± S.E.M. of two groups of ten (without phentolamine) and five (phentolamine) preparations which were exposed to cumulatively increasing concentrations of phenylephrine. Note that in the presence of phentolamine the concentration–response relationships of phenylephrine were shifted to the right by about one decade.

two levels of RP were observed after the addition of phenylephrine, a lower level in resting and a higher level in beating preparations. Figure 5 shows an example where RP amounted to −74 mV during activity (1 Hz) and to −58 mV at rest (right side of the figure); without phenylephrine RP amounted to −81 mV in both

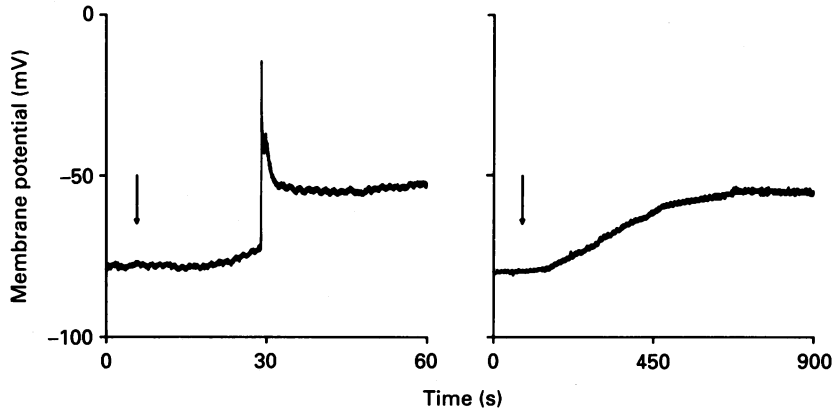

Fig. 3. Influence of phenylephrine (100 μmol/l) on the membrane potential in quiescent preparations. After the addition of phenylephrine (indicated by the arrows), the membrane potential was markedly reduced. Note the two different time courses of depolarization in the left and in the right panel. In either case, RP was finally diminished to virtually identical values.

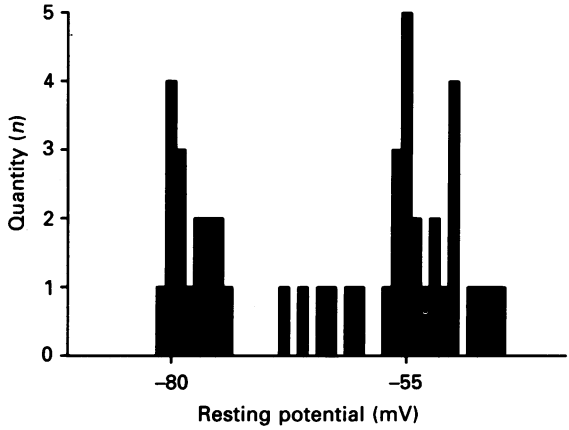

Fig. 4. Histogram of forty-five values of RP obtained 15 min after the addition of phenylephrine. The control value of all preparations amounted to -78 ± 1 mV before the addition of phenylephrine (means $\pm$ S.E.M.; $n = 45$). In view of the bimodal distribution of values, the preparations were divided into two groups, non-responders ($n = 16$) and responders ($n = 29$).

conditions (left side of the figure). Those preparations which did not react to phenylephrine at rest showed the same changes of RP and APD when electrically stimulated.

To further analyse these observations, the depolarizing effect of phenylephrine was studied during various pharmacological interventions and saline changes (Table 1). Phentolamine (3 μmol/l) antagonized the depolarization in response to phenylephrine. The depolarization was completely eliminated in low-Na^+ solution (8% of normal) and antagonized by tetrodotoxin (10 μmol/l). In contrast, the effects of phenylephrine were also seen in the presence of (−)-devapamil (1 μmol/l). Ca^{2+}-free Tyrode solution caused a depolarization by 23 ± 3 mV. Under this condition, the application of phenylephrine caused a further depolarization by 17 ± 3 mV.

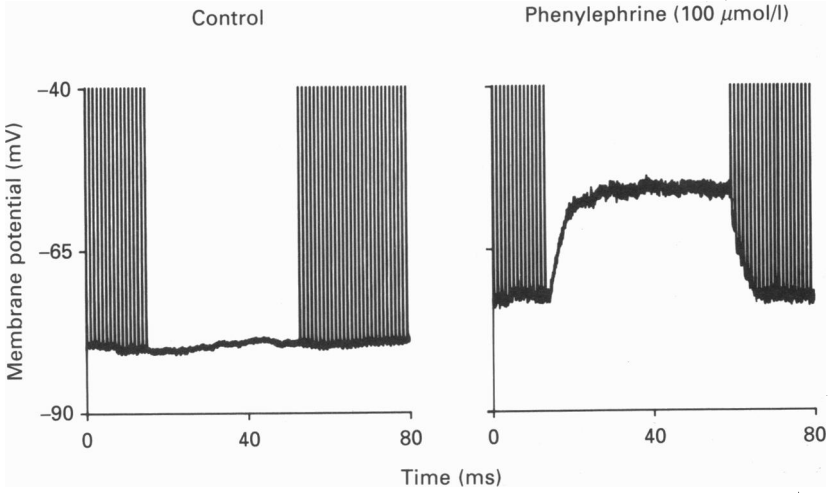

Fig. 5. Original records of the membrane potential under control conditions and in the presence of phenylephrine (100 μmol/l). The preparation was alternately electrically driven at 1 Hz or kept at rest. Control and test recordings were obtained in the same preparation (stable impalement throughout the experiment). Note the two levels of RP in the presence of phenylephrine (right side of the figure). Action potentials are cut off at −40 mV.

TABLE 1. Resting potential (mV) before (ϕ) and after (PE) the addition of phenylephrine (100 μmol/l) under control and test conditions

	Control condition		Test condition		
Test	ϕ	PE	ϕ	PE	n
Phentolamine (3 μmol/l)	−82±2	−54±1	−78±2	−73±2	4
Tetrodotoxin (10 μmol/l)	−79±2	−55±2	−78±2	−73±2	5
Low Na^+ (12 mmol/l)	−77±1	−56±2	−78±1	−77±1	4
Ca^{2+} free	−78±1	−55±5	−58±3	−41±4	6
(−)-Devapamil (1 μmol/l)	−78±1	−49±2	−81±2	−49±6	4

Values represent means±S.E.M. of preparations, which were first exposed to phenylephrine (control condition).

After wash-out (60 min), the addition of phenylephrine was repeated under the respective test condition.

To elucidate the role of the protein kinase C, the depolarizing effect of α_1-adrenoceptor stimulation was also investigated in the presence of phorbol 12,13-dibutyrate. Phorbol esters are known to induce a sustained activation of the protein kinase C (Castagna, Takai, Kaibuchi, Sano, Kikkawa & Nishizuka, 1982). The addition of PDBu (10 μmol/l) completely eliminated the response to phenylephrine within 15 min (Fig. 6*A*). Means±S.E.M. ($n = 5$) of the membrane potential amounted to −75±2 mV before the addition of phenylephrine, to −53±3 mV in the presence of phenylephrine, and to −74±2 mV after the addition of PDBu. PDBu alone had no effect on RP but also abolished the effects of phenylephrine when given previously.

The sulphhydryl-alkylating agent *N*-ethyl-maleimide (NEM; 30 μmol/l) (Harden, Scheer & Smith, 1982) also abolished the effect of phenylephrine within 30 min (Fig.

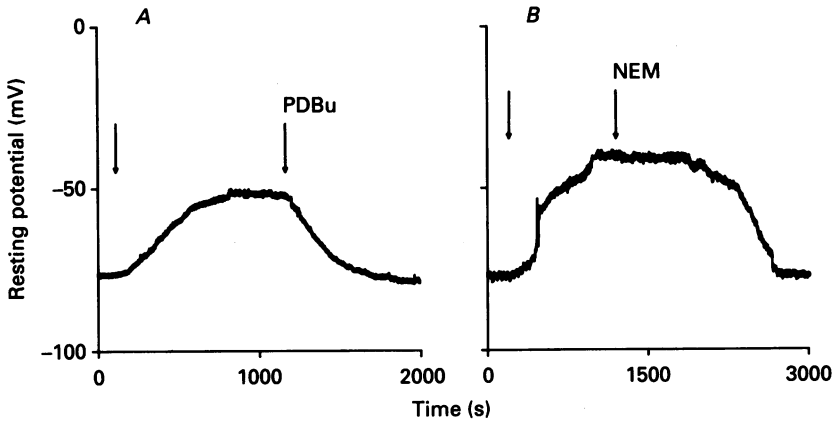

Fig. 6. Influence of phorbol 12,13-dibutyrate (10 μmol/l; *A*) or *N*-ethyl-maleimide (30 μmol/l; *B*) on the depolarizing effect of phenylephrine (100 μmol/l). Original records of the resting potential of two different preparations. The addition of phenylephrine is indicated by the first arrows on both sides of the figure. Further addition of either phorbol 12,13-dibutyrate (PDBu) or *N*-ethyl-maleimide (NEM) completely abolished the effect of phenylephrine.

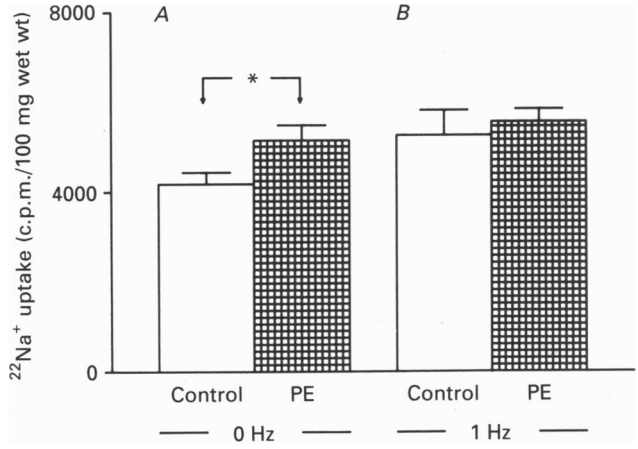

Fig. 7. $^{22}Na^+$ uptake under control conditions and in the presence of phenylephrine (PE; 100 μmol/l) in resting (*A*) and in beating preparations (*B*). Columns represent means $\pm$ S.E.M. of six preparations each. Note that the $^{22}Na^+$ uptake was significantly enhanced in resting preparations. $^*P < 0{\cdot}05$.

6*B*). Means $\pm$ S.E.M. ($n = 5$) of membrane potential amounted to -79 ± 2 mV before the addition of phenylephrine, to -52 ± 5 mV in the presence of phenylephrine, and to -78 ± 1 mV after the addition of NEM. This points to the possibility that the α-adrenoceptor may be coupled to a phospholipase C via a GTP-binding protein.

In contrast, time course and amount of depolarization produced by phenylephrine were not affected either by lithium (10 mmol/l) or 2,3-diphosphoglyceric acid (0·1 mmol/l) (data not shown), although both agents have been described as inhibiting the break-down of phosphoinositides (Rana, Sekar, Hokin & MacDonald, 1986; Drummond, 1987).

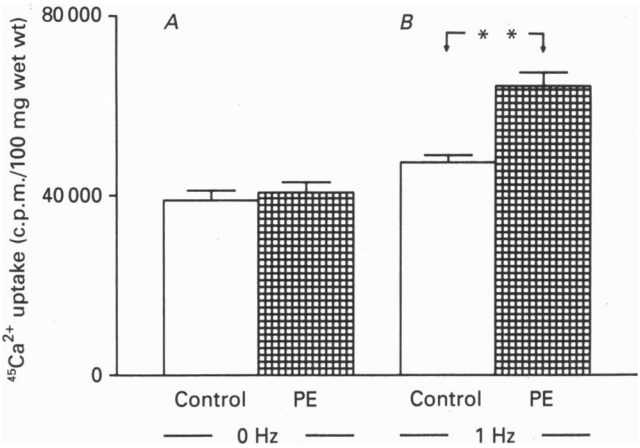

Fig. 8. $^{45}Ca^{2+}$ uptake under control conditions and in the presence of phenylephrine (PE; 100 μmol/l) in resting (*A*) and in beating preparations (*B*). Columns represent means ± S.E.M. of six preparations each. Note that $^{45}Ca^{2+}$ uptake was significantly enhanced in beating preparations. $^{**}P < 0{\cdot}01$.

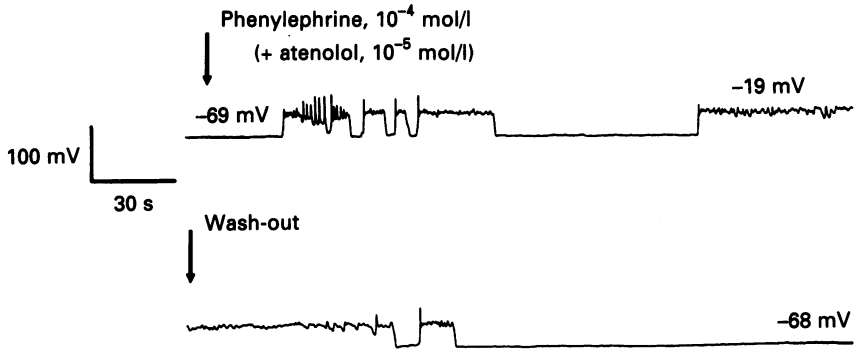

Fig. 9. Influence of phenylephrine (100 μmol/l) on the membrane potential in a single atrial myocyte. After the addition of phenylephrine (indicated by the first arrow) the membrane potential was reduced from −69 to −19 mV. Two minutes after the onset of the wash-out the membrane potential returned to the initial value.

To reveal the ionic nature of observations, flux experiments with $^{22}Na^{+}$ and $^{45}Ca^{2+}$ were performed. Phenylephrine increased the uptake of $^{22}Na^{+}$ in resting preparations by about 25%; in beating preparations, no significant differences between control and test values were found (Fig. 7). The uptake of $^{45}Ca^{2+}$ was increased by 35% in beating but not in resting preparations (Fig. 8).

In the next series of experiments, we investigated the effect of phenylephrine on membrane potential and steady-state currents of single atrial myocytes. In the experiment shown in Fig. 9, the membrane potential was decreased in the presence of phenylephrine (100 μmol/l) by 50 mV; the effect was reversible after wash-out. Membrane currents were evaluated during hyper- and depolarizing clamp pulses of 500 ms duration (V_H −50 mV). The original records depicted in Fig. 10 show a decrease of both outwardly and inwardly directed currents at the end of the step. The

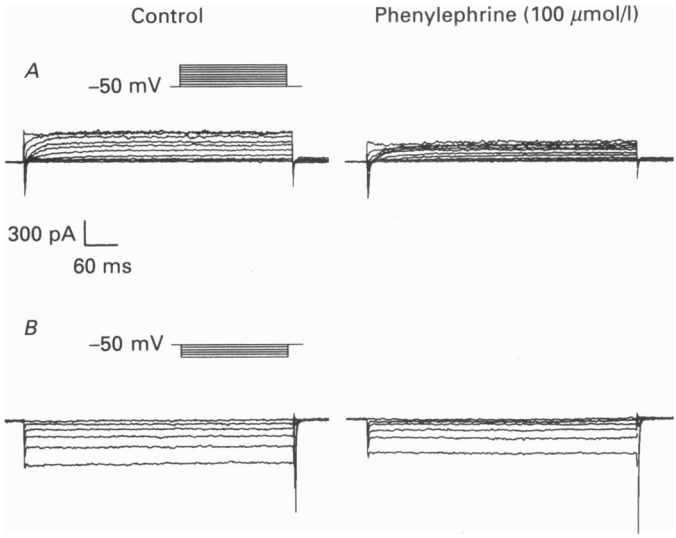

Fig. 10. Influence of phenylephrine (100 μmol/l) on membrane currents in rat atrial cell. Current traces in response to depolarizing voltage-clamp steps from -50 to $+40$ mV (*A*) and hyperpolarizing steps from -50 to -100 mV (*B*). Test pulses of 500 ms were applied in 10 mV increments at 0·1 Hz. Records under control condition (left panels) and in the presence of phenylephrine (right panels). In response to phenylephrine, a decrease in steady-state inwardly and outwardly directed currents was obtained. Note that I_{Ca} was not affected in the presence of phenylephrine.

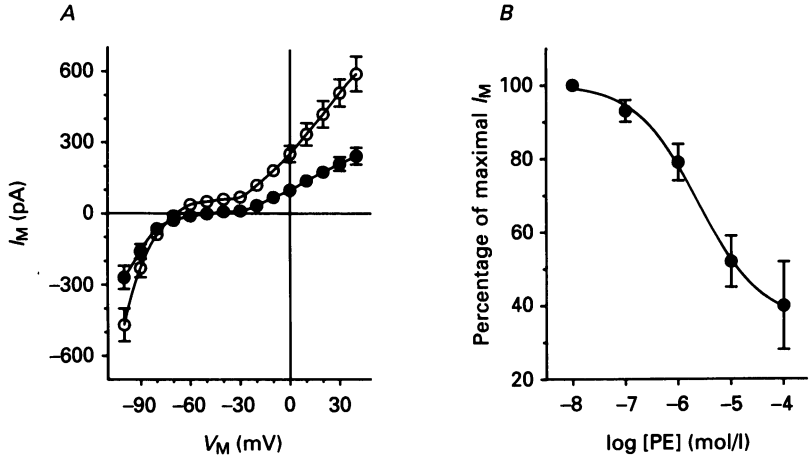

Fig. 11. Influence of phenylephrine on steady-state membrane current in rat atrial cells. *A*, current–voltage relationships without (○) and in the presence (●) of phenylephrine (PE; 100 μmol/l). Values are means ± S.E.M. of six cells. Note the decrease of both outwardly and inwardly directed membrane currents and the lack of a pronounced anomalous rectification. *B*, concentration–response relationship. Means ± S.E.M. of six cells which were exposed to cumulatively increasing concentrations of phenylephrine. Membrane currents were obtained in response to depolarizing voltage-clamp steps from -50 to $+10$ mV for 500 ms.

change in current was virtually the same when measured at the end or at the beginning of the voltage-clamp step indicating that no time-dependent currents are affected by phenylephrine. The current–voltage relationships of all cells without and with phenylephrine are shown in Fig. 11*A*. In contrast to the significant changes of

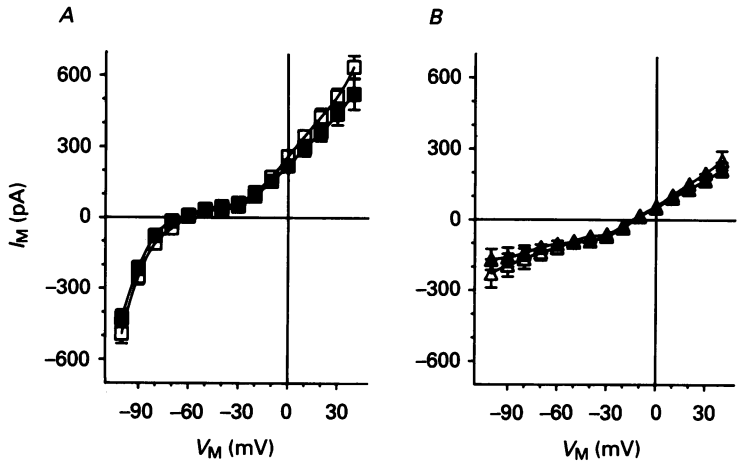

Fig. 12. Influence of phentolamine and caesium on phenylephrine-induced changes of steady-state membrane currents in rat atrial cells. *A*, current–voltage relationship before (□) and after the addition of phenylephrine (100 μmol/l; ■) in the presence of phentolamine (2 μmol/l). Values are means ± S.E.M. of six cells. Phentolamine antagonizes the phenylephrine-induced decrease of membrane currents. *B*, current–voltage relationship before (△) and after the addition of phenylephrine (100 μmol/l; ▲) in the presence of caesium. Values are means ± S.E.M. of five cells. $[Cs^+]_o$ (20 mmol/l) and $[Cs^+]_i$ (125 mmol/l) was substituted for KCl. Under this condition, membrane potential depolarized to about −10 mV and the phenylephrine-induced changes in membrane currents were abolished.

phenylephrine on steady-state currents, no effects on I_{Ca} were observed. Fig. 11*B* shows that the decrease in outward current by phenylephrine was concentration dependent. Phentolamine (2 μmol/l) antagonized the changes of membrane currents by phenylephrine (Fig. 12*A*). The voltage-clamp experiments were also performed in the presence of Cs^+ ($[Cs^+]_o$ 20 mmol/l; $[Cs^+]_i$ 125 mmol/l). Under these conditions, total membrane currents were decreased and the effect of phenylephrine was abolished (Fig. 12*B*).

DISCUSSION

Phenylephrine produced a marked depolarization in left atrial heart muscle preparations which can be ascribed to a decrease in K^+ currents in the presence of a depolarizing inward current. The effects were absent in low-Na^+ solution and diminished by TTX, indicating that the inward current producing the depolarization is carried by Na^+.

Furthermore, the uptake of $^{22}Na^+$ was significantly increased by phenylephrine in resting preparations but unchanged in beating preparations. These effects may be due secondarily to the decrease in RP which is expected to produce an increased Na^+ window current but a decreased Na^+ influx during the action potential.

The effects of phenylephrine on RP were much greater in resting than in beating preparations, as is obvious from the two distinct levels of maximum diastolic potential, when the preparations were alternately electrically driven or kept at rest. It is assumed that, during the action potential, a K^+ outward current is activated which counteracts the depolarization brought about by phenylephrine. The observed changes of membrane potential may be directly related to the arrhythmogenic properties of α-adrenoceptor agonists during ischaemia and reperfusion (Corr, Shayman, Kramer & Kipnis, 1981; Hamra & Rosen, 1988).

The positive inotropic effect of α_1-adrenoceptor stimulation is possibly a consequence of the depolarization-induced opening of Na^+ channels. The influx of Na^+ increases the ratio of intracellular to extracellular Na^+ activity, a^i_{Na}/a^o_{Na}. The increase of $[Na^+]_i$ will result in a change of $[Ca^{2+}]_i$ due to its effects on Na^+–Ca^{2+} exchange (see Reuter, 1974*b*; for more recent references see Sonn & Lee, 1988).

A rise in $[Na^+]_i$ which ultimately increases $[Ca^{2+}]_i$ may be accomplished by various pharmacological means. Depolarization-induced opening of Na^+ channels by phenylephrine (this paper), inhibition of Na^+ channel inactivation by ceveratrum alkaloids (Honerjäger, 1982) or sea anemone toxin ATX II (Isenberg & Ravens, 1984), inhibition of Na^+–Ca^{2+} exchange (Kennedy, Berlin, Ng, Akera & Brody, 1986), or inhibition of Na^+–K^+-ATPase (see Akera & Brody, 1978) represent some of the major pathways which have been discussed so far. A rise in $[Na^+]_i$, due to the activation of receptor-coupled Na^+ channels, has been proposed as the mechanism of a positive inotropic response to high concentrations of carbachol in guinea-pig ventricular heart muscle (Korth & Kühlkamp, 1985). Matsumoto & Pappano (1989) have shown that carbachol induces a Na^+-dependent membrane current in single guinea-pig ventricular myocytes.

We have shown that the uptake of $^{45}Ca^{2+}$ is indeed augmented by phenylephrine in beating but not in resting preparations, although the primary membrane effects were also seen in Ca^{2+}-free solution or in the presence of the Ca^{2+} channel blocker (−)-devapamil. Moreover, I_{Ca} was unchanged by phenylephrine.

Therefore, the enhanced uptake of Ca^{2+} by phenylephrine probably does not reflect a change in Ca^{2+} conductance. This confirms earlier reports that I_{Ca} in the heart is not increased by α_1-adrenoceptor stimulation (Hartmann *et al.* 1988; Hescheler *et al.* 1988).

Endoh & Blinks (1988) have proposed that the positive inotropic response to phenylephrine may be due to an increased sensitivity of the myofibrils against Ca^{2+}. The evidence for this hypothesis was derived from experiments in rabbit papillary muscles microinjected with the bioluminescent protein aequorin. In contrast to other positive inotropic interventions, phenylephrine failed to enhance markedly the $[Ca^{2+}]_i$ transient associated with the contraction cycle. It has to be noted, however, that the light emission of aequorin is nearly independent of $[Ca^{2+}]_i$ at low concentrations of the electrolyte (Allen, Blinks & Prendergast, 1977). Therefore, small changes of steady-state values of $[Ca^{2+}]_i$ may have escaped detection. On the other hand, a positive inotropic effect must finally occur as a result of an increase in systolic $[Ca^{2+}]_i$. Aequorin is a very effective $[Ca^{2+}]_i$ indicator in this range and phenylephrine is unusual amongst inotropic agents in not increasing the systolic $[Ca^{2+}]_i$ to a major extent, as measured by aequorin in rabbit papillary muscles. At

present, no obvious explanation for the discrepancy of our and their results can be given. It seems possible, however, that the effects of phenylephrine may be mediated by completely different mechanisms, not only in different species, but also in the various parts of the heart (atrium, ventricle).

Several recent studies in isolated heart cells have shown that α_1-adrenoceptor stimulation decreases outward currents carried by K^+ (Apkon & Nerbonne, 1988; Shah, Cohen & Rosen, 1988; Fedida *et al.* 1989, 1990; Ravens *et al.* 1989).

Our voltage-clamp experiments in atrial myocytes confirm these earlier observations. Phenylephrine reduced steady-state currents in response to depolarizing and hyperpolarizing voltage-clamp steps. The *I–V* curves under control conditions and in the presence of phenylephrine intersected at about −70 mV. This suggests that phenylephrine reduces a K^+ conductance rather than increasing a Na^+ conductance. Correspondingly, the effects of phenylephrine on the steady-state *I–V* relationships were abolished when Cs^+ was substituted for K^+ both inside the pipette and the external bath solution.

The sum of evidence therefore suggests that the primary membrane effect of phenylephrine, also in rat atrial heart muscle, is to reduce a K^+ conductance. The resulting depolarization may then induce secondary changes which can be summarized as follows: a decrease in K^+ conductance will cause the membrane to depolarize in the presence of an inward current. Such depolarization will result in a greater Na^+ influx through the Na^+ window causing an enhancement of $[Na^+]_i$. The increase in $[Na^+]_i$ will cause a rise of $[Ca^{2+}]_i$ via the Na^+–Ca^{2+} exchange. Alternatively, $[Ca^{2+}]_i$ may be increased due to the prolongation of APD and a greater influx of Ca^{2+} through a Ca^{2+} window. The latter possibility has been discussed in detail by Fedida *et al.* (1989).

The shallow *I–V* relationship in rat atrial myocytes in the range between −70 and −30 mV permits relatively large changes of membrane potential at minor changes of membrane currents. The rather critical balance of inwardly and outwardly directed current, determining the membrane potential in this region, may explain the bimodal distribution of the effects of phenylephrine on RP.

Figure 11*A* shows that the inward rectifier is decreased by phenylephrine. At positive potentials, the outward current is also significantly decreased by phenylephrine. This decrease can explain the prolongation of APD. This effect is not due to a change in time-dependent current, but we do not know the exact nature of the channel which is affected by phenylephrine in the plateau range. Fedida *et al.* (1989, 1990) and Apkon & Nerbonne (1988) have shown that a transient outward current (I_{to}) is significantly reduced in rabbit atrial and rat ventricular heart muscle, respectively. Alternatively, a time-independent current in the plateau level called I_{Kp} by Yue & Marban (1988) could also be affected by phenylephrine. A single-channel analysis should reveal the nature, the kinetics and the pharmacology of the affected channels. Given the fact that the channel closes at the resting potential in response to phenylephrine, without the need of a voltage change, it may be classified as a receptor- or ligand-operated channel (see Reuter, 1987).

It also remains unclear how the signal transfer between α_1-adrenoceptor activation and the electrophysiological changes is accomplished. Activation of protein kinase C by a phorbol ester abolished the effects of phenylephrine on APD and F_C in the rabbit

papillary muscle (Kushida, Hiramoto, Satoh & Endoh, 1988; Nawrath & Rombusch, 1988) and on RP in rat atrial heart muscle (this paper). This may be explained by phosphorylation and uncoupling of α_1-adrenoceptors from the phosphatidyl inositol turnover (Leeb-Lundberg, Cotecchia, Lomasney, DeBernadis, Lefkowitz & Caron, 1985), by the activation of IP_3 5-phosphomonoesterase promoting the break-down of IP_3 to IP_2 (Majerus, Connolly, Deckmyn, Ross, Bross, Ishii, Bansal & Wilson, 1986), or by the translocation of protein kinase C from cytosolic to membrane compartments (Yuan, Sunahara & Sen, 1987). Taken together, these results support the view that protein kinase C provides a negative feedback control over cell-signalling processes and may act to terminate the effects of α_1-adrenoceptor stimulation which involves the production of IP_3 (Orellana, Solski & Brown, 1987; Nishizuka, 1988).

Tohse, Kameyama & Irisawa (1987) have shown that activation of protein kinase C by a phorbol ester increases the time-dependent K^+ current I_K in guinea-pig ventricular heart muscle; this effect was mimicked by phenylephrine. Whereas an increase of a K^+ current by the phorbol ester may have antagonized the effects of phenylephrine on K^+ currents in our experiments, the effects of phenylephrine on K^+ currents are clearly conflicting when comparing the results from different species.

Diacylglycerol (Okumura *et al.* 1988) and IP_3 levels (Poggioli *et al.* 1986; Otani *et al.* 1988; Scholz *et al.* 1988) are increased in the heart in response to α_1-adrenoceptor stimulation. Direct effects of IP_3 on membrane conductances have not been shown. IP_3 releases Ca^{2+} from non-mitochondrial stores in pancreatic acinar cells (Streb, Irvine, Berridge & Schulz, 1983). In the heart, the effects of IP_3 on Ca^{2+} release from the sarcoplasmic reticulum are still controversial (Hirata, Suematsu, Hashimoto, Hamachi & Koga, 1984; Movsesian, Thomas, Selak & Williamson, 1985; Nosek, Williams, Zeigler & Godt, 1986; Erlich & Watras, 1988).

In connection with our results, it may be assumed that the stimulation of α_1-adrenoceptors in the heart activates a phospholipase C, probably involving a GTP-binding protein which was found to be sensitive to pertussis toxin in Purkinje myocytes (Shah *et al.* 1988) but insensitive in rat left auricles (Böhm, Schmitz & Scholz, 1987). *N*-Ethyl-maleimide abolished the effects of phenylephrine on RP. This indicates that a G-protein may be involved in mediating the effects of α_1-adrenoceptor stimulation. However, interference with other critical S–H groups of the hormone receptor or direct changes of ion-channel proteins may modify the effects of phenylephrine as well. The latter possibility has been discussed by Braun & Sperelakis (1988) who found that *N*-ethyl-maleimide attenuates the cholinergically induced shortening of action potential duration in guinea-pig right atrium.

The cleavage of phosphatidylinositol 4,5-bisphosphate enhances the IP_3 level which may then phosphorylate membrane channels of the myocardial cell membrane. Lindemann (1986) has described a phosphorylation of a 15 kDa protein which may be associated with this process.

Our results do not contradict the hypothesis that the effect of α_1-adrenoceptor stimulation in the heart are partially mediated through a release by IP_3 of Ca^{2+} from the sarcoplasmic reticulum. The long-lasting positive inotropic effects in response to α_1-adrenoceptor stimulation, however, seem to require a sink to refill the intracellular stores. Putney (1987) has suggested, that a fraction of the sarcoplasmic reticulum which lies closely to the plasma membrane may have some direct connection to the

extracellular space managing a Ca^{2+} entry. The present report has described an alternative pathway, via the decrease of K^+ conductance leading to a depolarization, a secondary increase of Na^+ influx and an activation of the Na^+–Ca^{2+} exchange, whereby the intracellular Ca^{2+} concentration could be sustained at a higher level.

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