Effects of Phenylephrine on Intracellular Na^+ and H^+ Activities in Guinea-Pig Cardiac Ventricular Papillary Muscles

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Abstract: Effects of phenylephrine (PE) on the contractility, intracellular Na^+ activity (a_{Na}^i), intracellular H^+ activity (pH_i), and membrane potential in guinea-pig cardiac ventricular papillary muscles were studied. In beating fibers, PE significantly increased the twitch tension, pH_i and the action potential duration but decreased a_{Na}^i . These effects could be inhibited by phentolamine, but not by propranolol. In quiescent fibers, PE also increased the resting tension and pH_i , and decreased a_{Na}^i . Pretreatment with phentolamine completely abolished the effect of PE. Methylisobutyl amiloride (MIA), a potent inhibitor of Na^+ - H^+ exchanger, significantly decreased membrane potential, a_{Na}^i , and pH_i without changing the resting tension. PE in the presence of MIA increased the resting tension and further de-

creased a_{Na}^i without significant changes in membrane potential and pH_i . Ouabain significantly decreased membrane potential and pH_i , and increased the resting tension and a_{Na}^i . In the ouabain-treated fiber, PE further increased the resting tension without decreasing a_{Na}^i or increasing pH_i . From these results, it appears that PE activates the Na^+ - K^+ pump and Na^+ - H^+ exchange through α -adrenoceptors. Activation of the Na^+ - K^+ pump contributes, at least in part, to the enhanced Na^+ - H^+ exchange. The increase in contractile force associated with a decrease in a_{Na}^i is suggested to result from the intracellular alkalization and increased sensitivity of contractile protein to Ca^{2+} . [Japanese Journal of Physiology, 46, 137–144, 1996]

Key words: guinea-pig cardiac ventricular papillary muscle, phenylephrine, intracellular Na^+ activity, pH_i , Na^+ - H^+ exchange.

Cardiac tissues are known to possess α_1 - but not α_2 -adrenoceptors [1]. Activation of α -adrenoceptors leads to a breakdown of membrane phosphoinositides, producing intermediates, diacylglycerol (DAG) and inositol trisphosphates (IP_3). These intermediates in turn induce alterations of electrical and mechanical events, e.g., increases in the generation of contractile force, Na^+ - K^+ pump activity, Na^+ - H^+ exchange, Na^+ - Ca^{2+} exchange, intracellular Na^+ activity (a_{Na}^i), intracellular pH (pH_i), and membrane depolarization [2–6].

In guinea-pig ventricular papillary muscles, phenylephrine, an α -adrenoceptor agonist, has been shown to cause positive inotropy through activation of α -

adrenoceptors in the myocardial membrane [7]. Stimulation of α -adrenoceptors enhances the Na^+ - K^+ pump activity, resulting in a decrease of a_{Na}^i [8]. Other evidence suggests that the Na^+ - H^+ exchange and Na^+ - HCO_3^- symport are oppositely regulated by α_1 -adrenoceptor activation [9]. However, the precise relationship between the activities of the Na^+ - H^+ exchange and Na^+ - K^+ pump and their roles in electrical and mechanical events remain ambiguous.

In the present study, we explored the effects of phenylephrine on guinea-pig papillary muscles by simultaneously measuring the contractile force, a_{Na}^i or pH_i , and membrane potential. Ouabain and methylisobutyl amiloride (MIA), respective inhibitors of the

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$\text{Na}^+\text{-K}^+$ pump and $\text{Na}^+\text{-H}^+$ exchanger [10, 11], were employed to test the involvement of the $\text{Na}^+\text{-K}^+$ pump and $\text{Na}^+\text{-H}^+$ exchanger.

METHODS

Male guinea-pigs (390 ± 15 g) were sacrificed by cervical dislocation. A strand of fine guinea-pig cardiac papillary muscle, from the right ventricle roughly 0.5 mm in diameter and 3 mm in length, was carefully dissected and then mounted in a narrow perfusing chamber. The superfusing Tyrode solution was oxygenated with a gas mixture of 97% O_2 and 3% CO_2 , to give a pH of 7.4, and was maintained at 37°C . The composition of the solution was (in mM): NaCl 135, KCl 5.4, CaCl_2 1.8, NaHCO_3 12, MgCl_2 1.1, NaH_2PO_4 0.5, glucose 5.0. One end of the fiber was fixed and driven with a Grass stimulator. The other end was tied to a force displacement transducer (Cambridge, 403A) to measure tension. The sensitivity of the transducer was adjusted at 30 mV/mg. One or two L-shaped insect-pins were used to fix the fiber near the driven end that left a segment to be impaled without vigorous movement. The free end of the fiber left for twitching was nearly 0.5 mm in length and 0.3 mm in diameter that was used to detect the twitch tension. In stimulation studies, the preparation was driven at a rate of 60 beats/min and stretched until the contractions reached about 60% of the maximum. In non-stimulation studies, the experiment was conducted after the preparation was equilibrated for an hour and mounted with a preload of about 3 mg.

The constructions of Na^+ -selective, H^+ -selective and conventional microelectrodes have been described previously [12]. Briefly, the conventional microelectrode was made from borosilicate micropipettes that had a tip resistance in the range of 10–40 M Ω when backfilled with 3 M KCl solution. The action potential was displayed on an oscilloscope (Gould model 1600). The maximum rate of the rise of the action potential ($\dot{V}_{\text{max}}$) was recorded after the signal of the action potential passed through a differential amplifier. The Na^+ -selective microelectrode, made from thick-wall pipettes (1.8 mm o.d., 1.1 mm i.d.), was beveled and backfilled with 100 mM NaCl after silanization with a tiny amount of *n*-tributylchlorosilane. A 100–300 μm column of Na^+ -selective liquid sensor (Fluka) was drawn into the tip of microelectrodes. The sodium electrode was calibrated by the method using single-electrolyte solutions, containing 100, 10, and 1 mM NaCl, 100 mM KCl, and 1 mM CaCl_2 , individually, before and after each experiment [13]. The potential response of Na^+ -electrodes was 58.6 ± 0.5 mV ($n=42$)

per tenfold sodium activity change at 37°C . The selectivity coefficients for K^+ (k_{NaK}) and Ca^{2+} (k_{NaCa}) were less than 0.02 and 2, respectively. Since the selectivity coefficient for calcium was rather high, the Na^+ -selective microelectrode was also calibrated in solutions containing 10^{-5} , 10^{-6} , 10^{-7} , and 10^{-8} M [Ca^{2+}]. This was done by using an apparent stability constant of $3.2 \times 10^6 \text{ M}^{-1}$ for Ca-EGTA. All test solutions contained 140 mM KCl, 10 mM NaCl, and 1 mM MgCl_2 , with similar ionic strength and buffered by imidazole at pH 7.0. The Na^+ -selective microelectrodes did not typically respond to calcium changes below 10^{-6} M when the value of the selectivity coefficient was below 2 measured by the method using single-electrolytes solution (1 mM CaCl_2). Signals from the conventional and Na^+ -selective microelectrodes passed through two identical low-pass filters with a fixed frequency of 0.2 Hz (A.P. Circuit Corp). The electrical potentials of the sodium electrode (E_{Na}^i) and conventional electrode ($\bar{V}_m$) and their difference (a_{Na}^i) were recorded on a chart recorder. The intracellular Na^+ activity of muscle fibers was calculated according to a modified Nicolsky equation [13]:

$$E_{\text{Na}}^i - \bar{V}_m = E_o + S \log[a_{\text{Na}}^i + k_{\text{NaK}} a_{\text{K}}^i + k_{\text{NaCa}} (a_{\text{Ca}}^i)^{1/2}],$$

where E_{Na}^i and $\bar{V}_m$ are the respective potentials of the sodium and conventional electrodes in cells. E_o is the constant potential of the electrometric system. S is the slope of the potential response in calibration solutions for each electrode. a_{K}^i and a_{Ca}^i are the intracellular K^+ and Ca^{2+} activities, respectively, and the reported values of 100 mM and 87 nM in cardiac ventricular muscles were used for calculation [14, 15]. Possible small changes of a_{K}^i and a_{Ca}^i were assumed to have insignificant interference with the sodium electrode [16, 17], and were neglected in this study.

The hydrogen electrode was prepared similarly to the sodium electrode except that the sensor was H^+ -selective. The back-filled solution was Tris-buffered at pH 7 containing 140 mM KCl and 10 mM NaCl. Calibration solutions of pH 6, 7, 8 were prepared by 10 mM Tris-HCl buffer containing 140 mM KCl and 10 mM NaCl. After both H^+ -selective and conventional microelectrodes were impaled in cells, the membrane potential was subtracted from the hydrogen electrode potential. The difference obtained in voltage was used to interpolate the unknown pH value using the calibration curve of the particular microelectrode.

L-Phenylephrine hydrochloride, propranolol hydrochloride and ouabain octahydrate (Sigma) were dissolved in distilled water to make stock solutions of 1 mM. Stock solutions of methylisobutyl amiloride (10 mM; Research Biochemicals International) and

phentolamine base (1 mM; Sigma) were dissolved in dimethylsulfoxide and ethanol, respectively. The experimental data were pooled for analysis. Only those experiments in which the recovery reached above a level of 80% of the control during washing out were included. A Newman-Krue test or Student's *t*-test was employed to analyze the experimental data. The difference was considered significant when the *p* value was less than 0.05.

RESULTS

Effects of phenylephrine and phentolamine

Figure 1 shows that phenylephrine induced a concentration-dependent positive inotropic effect on guinea-pig ventricular papillary muscles driven at 1 Hz. The increase in contraction was abolished by pretreatment with phentolamine. In addition to the increase in twitch tension, there were also a slight increase in the action potential duration at 90% repolarization (APD_{90}) and a decrease of a_{Na}^i without change of $\dot{V}_{\text{max}}$ after 3 μM phenylephrine, as illustrated in an

Fig. 1. Concentration-response curves for the positive inotropic effect of phenylephrine in the absence (□) and presence (◇) of 3 μM phentolamine in guinea-pig ventricular muscles. ○, normalized from the data obtained in the presence of 3 μM phentolamine by taking the twitch tension at 0 μM phenylephrine as 0% in ordinate. The ordinate indicates increments of the twitch tension induced by phenylephrine (cumulative administration, $n=9$), expressed as percent change from the control value. * $p < 0.05$ in comparison with the value at 0 μM phenylephrine.

Fig. 2. Effects of phentolamine (Pht) and propranolol (PPL) on the effects of phenylephrine (PE) in beating guinea-pig ventricular muscles. **A:** PE alone, **B:** Pht and PE, **C:** PPL and PE. *T*, twitch tension; a_{Na}^i , intracellular Na^+ activity; $\bar{V}_m$, filtered membrane potential. Fast recordings of the action potential and the maximum rate of rise of the upstrokes were recorded at points a, b, and c indicated in the left panels.

example shown in Fig. 2A. These effects were abolished in the presence of 3 μM phentolamine (Fig. 2B), but not of 3 μM propranolol (Fig. 2C). In nine fibers tested for the action of 3 μM phenylephrine, the twitch tension was increased by $57.9 \pm 6.6\%$, APD_{90} prolonged from 220.7 ± 11.4 to 228.5 ± 11.1 ms, and a_{Na}^i decreased from 4.5 ± 0.1 to 3.9 ± 0.1 mM (-13.3%), significantly. The pH_i , measured in six fibers, was significantly increased from 7.14 ± 0.01 to 7.19 ± 0.01 .

In another five fibers tested with 3 μM phentolamine pretreatment, the effects of 3 μM phenylephrine were completely abolished. Phentolamine at 3 μM alone significantly decreased the twitch tension by $15.2 \pm 0.9\%$, a_{Na}^i from 4.9 ± 0.1 to 4.4 ± 0.1 mM and $\dot{V}_{\text{max}}$ from 93.5 ± 1.0 to 83.1 ± 1.3 V/s, but increased APD_{90} from 230.0 ± 14.3 to 234.6 ± 13.4 ms. In five additional fibers tested, 3 μM propranolol significantly decreased the twitch tension by $21.6 \pm 3.3\%$, a_{Na}^i from 4.5 ± 0.1 to 4.1 ± 0.1 mM (-8.9%), $\dot{V}_{\text{max}}$ from 102.0 ± 4.6 to 94.1 ± 4.6 V/s, and APD_{90} from 180.6 ± 12.6 to 176.7 ± 13.5 ms. Phenylephrine (3 μM) in the presence of 3 μM propranolol significantly decreased a_{Na}^i from 4.1 ± 0.1 to 3.8 ± 0.1 mM, and increased APD_{90} from 176.7 ± 13.5 to 185 ± 11.7 ms and the twitch tension to $116.0 \pm 4.9\%$ of the control value. The normalized increment of the twitch tension by phenylephrine in propranolol-treated preparations was about 47.9% which

was 10% less than those in propranolol-nontreated preparations.

In quiescent fibers, as illustrated in examples shown in the left traces of Fig. 3A and B, 3 μM phenylephrine significantly decreased a_{Na}^i from 3.3 ± 0.1 to 3.0 ± 0.1 mM (-8.9% , $n=7$), increased the resting tension and increased pH_i from 7.15 ± 0.003 to 7.18 ± 0.002 ($n=4$) without change in the membrane potential. Phentolamine (1 μM) significantly decreased the resting tension, a_{Na}^i (from 3.4 ± 0.1 to 3.1 ± 0.1 mM), and the membrane potential (from -82.7 ± 1.0 to -80.3 ± 0.8 mV) ($n=4$) without alteration of pH_i 7.15 ± 0.001 ($n=5$). After pretreatment with phentolamine, phenylephrine had no effect on any of the above variables (Fig. 3, right traces).

Effects of methylisobutyl amiloride

Figure 4 illustrates examples of the effects of 1 μM methylisobutyl amiloride (MIA) and 3 μM phenylephrine on quiescent fibers. MIA, a potent inhibitor of the Na^+/H^+ exchanger, did not change the resting tension but significantly decreased a_{Na}^i from 3.4 ± 0.1 to 3.0 ± 0.1 mM, pH_i from 7.16 ± 0.001 to 7.10 ± 0.001 , and the membrane potential from -83.1 ± 0.7 to -81.4 ± 0.9 mV ($n=5$). Phenylephrine applied in the presence of MIA further and significantly decreased a_{Na}^i to 2.7 ± 0.1 mM (by 8.8% of control), and increased the

Fig. 3. Modification of the effects of phenylephrine (PE) by phentolamine (Pht) on resting tension (T), membrane potential ($\bar{V}_m$), and intracellular sodium activity (a_{Na}^i) or intracellular pH (pH_i) in quiescent guinea-pig ventricular muscles.

Fig. 4. Modification of phenylephrine (PE) effects by methylisobutyl amiloride (MIA) in quiescent guinea-pig ventricular muscles. Other abbreviations are the same as indicated in the legend for Fig. 3.

resting tension without changing the membrane potential or pH_i ($n=5$).

Effects of ouabain

It can be seen in Fig. 5 that $100\text{ }\mu\text{M}$ ouabain increased the resting tension and a_{Na}^i , and decreased pH_i and the membrane potential in a quiescent papillary muscle. Both changes in a_{Na}^i and pH_i occurred rather rapidly in the first 8 min; thereafter, the changes developed more slowly. The a_{Na}^i and pH_i were measured in five fibers each. Ouabain significantly increased the resting tension, increased a_{Na}^i (from 3.7 ± 0.1 to $6.6\pm0.1\text{ mM}$), decreased pH_i (from 7.16 ± 0.01 to 6.92 ± 0.01) and decreased the resting membrane potential (from -84.0 ± 0.9 to $-82.0\pm1.2\text{ mV}$), in the first 15 min. The following addition of $3\text{ }\mu\text{M}$ phenylephrine did not alter the membrane potential, but significantly increased the tension. During the application of phenylephrine, the a_{Na}^i continued to increase from 6.6 ± 0.1 to $7.1\pm0.2\text{ mM}$ (Fig. 5A) and pH_i decreased from 6.92 ± 0.01 to 6.87 ± 0.01 (Fig. 5B), without any obvious acceleration of the rate of change in a_{Na}^i and pH_i .

DISCUSSION

In the present study, we have demonstrated that, in the

guinea-pig ventricular papillary muscles, phenylephrine induced an increase in contractile force, intracellular alkalization, and a decrease in a_{Na}^i . These actions are blocked by phentolamine, an α -adrenergic antagonist, but not by the β -adrenergic antagonist propranolol. Therefore, the effects of phenylephrine are considered to be elicited by activation of α -adrenoceptors. Indeed, the presence of α -adrenoceptors in the guinea-pig myocardial membrane has been demonstrated from radioligand binding studies [7]. However, $3\text{ }\mu\text{M}$ phenylephrine increased the twitch tension by 57.9% in fibers without pretreatment with propranolol, but only 47.9% in the propranolol-treated fibers, suggesting the existence of some stimulating effect of phenylephrine on the β -adrenoceptors as well as on the α -adrenoceptors. An increase of diastolic tension seen after the application of phenylephrine in the presence of the β -blocker (Fig. 2C), which was not seen in the control (Fig. 2A), also alludes to the involvement of some β -effect. The intracellular alkalization induced by phenylephrine can be attributed to the activation of α -adrenoceptor-mediated $\text{Na}^+\text{-H}^+$ exchange because this effect could be inhibited by MIA, a potent and selective inhibitor of the $\text{Na}^+\text{-H}^+$ exchanger [2–5, 10, 11]. The decreased a_{Na}^i in response to phenylephrine was not affected by blockade of the $\text{Na}^+\text{-H}^+$ exchange with MIA, but was com-

Fig. 5. Modification of phenylephrine (PE) effects by ouabain in quiescent guinea-pig ventricular muscles. Other abbreviations are the same as indicated in the legend for Fig. 3.

pletely abolished after inhibition of $\text{Na}^+\text{-K}^+$ pump activity by ouabain. An inhibition of fast sodium channels to explain the decrease of a_{Na}^i is unlikely since the $\dot{V}_{\text{max}}$ was not affected by phenylephrine. It thus appears that stimulation of α -adrenoceptors activates the $\text{Na}^+\text{-K}^+$ pump. Interestingly, neither blockade of the $\text{Na}^+\text{-H}^+$ exchange nor inhibition of the $\text{Na}^+\text{-K}^+$ pump had an appreciable effect on the increase in resting tension induced by phenylephrine.

Amiloride has been shown to inhibit the $\text{Na}^+\text{-Ca}^{2+}$ exchange in guinea-pig hearts and, from radioligand binding studies, to interact with the α -adrenoceptors in rat hearts [18, 19]. It might be argued that MIA, an analog of amiloride, exerts such an amiloride-like effects. In the resting steady state, the $\text{Na}^+\text{-Ca}^{2+}$ exchange is responsible for maintaining a net efflux of calcium in exchange for sodium entry [20]. Therefore, a decrease of a_{Na}^i may lead to a decrease in the cytosolic calcium concentration and the tension. In the present study, MIA decreased a_{Na}^i without changes in the resting tension. It is plausible that the inhibitory effect of MIA on the $\text{Na}^+\text{-Ca}^{2+}$ exchange prevented the level of cytosolic calcium from decreasing and the tension might have remained unchanged. Since MIA did not

block the phenylephrine-induced increase in tension and decrease in a_{Na}^i , a significant blocking effect of MIA on the adrenoceptors could not be ruled out.

In guinea-pig heart cells, the regulation of pH_i is controlled by a dual extrusion system ($\text{Na}^+\text{-H}^+$ exchange and $\text{Na}^+\text{-HCO}_3^-$ symport) which is activated by intracellular acid load. Stimulation of α -adrenoceptors results in activation of the $\text{Na}^+\text{-H}^+$ exchange and inhibition of the $\text{Na}^+\text{-HCO}_3^-$ symport system [9]. The stoichiometry of $\text{Na}^+\text{-H}^+$ exchange is electroneutral and voltage-independent [21]. The driving force for H^+ extrusion is provided by the Na^+ gradient across the membrane. The sodium gradient depends on the activity of the $\text{Na}^+\text{-K}^+$ pump. With increased pump activity more sodium is pumped out, and the resulting increase of the driving force for Na^+ across the membrane may enhance the rate of $\text{Na}^+\text{-H}^+$ exchange with eventual increases in pH_i . Thus, changes of a_{Na}^i are the result of activation of both the $\text{Na}^+\text{-H}^+$ exchanger and the $\text{Na}^+\text{-K}^+$ pump.

We have consistently demonstrated a decrease in a_{Na}^i after phenylephrine. In the control, the beating fiber has a larger a_{Na}^i than the quiescent fiber because the former has more sodium influx through generation

of action potentials. In either case, we have found a decrease of a_{Na}^i after phenylephrine. The decrease can be attributed to an activation of the $\text{Na}^+\text{-K}^+$ pump because after blocking the pump activity with ouabain, phenylephrine no longer caused a decrease of a_{Na}^i (Fig. 5). In fact, a_{Na}^i actually increased, rather than decreased. We speculate that the decrease in a_{Na}^i caused by phenylephrine-enhanced $\text{Na}^+\text{-K}^+$ pump activity led to an enhanced extrusion of intracellular H^+ by accelerating $\text{Na}^+\text{-H}^+$ exchange (due to increased Na^+ gradient across the membrane) and increased pH_i . This idea is supported by the observation that phenylephrine decreased a_{Na}^i (via activation of the $\text{Na}^+\text{-K}^+$ pump) even in the presence of MIA (i.e., under blockade of $\text{Na}^+\text{-H}^+$ exchange with intact $\text{Na}^+\text{-K}^+$ pump) (Fig. 4A), and failed to increase pH_i , evidence of the blockade of $\text{Na}^+\text{-H}^+$ exchanger (Fig. 4B). The finding of a decrease of a_{Na}^i is contrary to the general belief that an increase of contractile force is associated with an increase in a_{Na}^i which leads to a diminished $\text{Na}^+\text{-Ca}^{2+}$ exchange with subsequent increases of intracellular Ca^{2+} and contraction. Reports on guinea-pig papillary muscles and rat cardiomyocytes have shown that a_{Na}^i was increased after the application of norepinephrine and phenylephrine, and this increase was thought to be responsible for the enhanced contractile force [22–24]. Nevertheless, our finding of a decrease in a_{Na}^i after the application of phenylephrine does not exclude the possibility that there had been an increase in sodium influx after stimulation of the α -adrenoceptor, whilst this influx was overcome by the enhanced Na^+ efflux via activation of the $\text{Na}^+\text{-K}^+$ pump.

Our experimental results do not offer a clear clue as to the mechanism of the positive inotropic action of phenylephrine. The subcellular mechanism during activation of α -adrenoceptors is known to involve increased hydrolysis of inositol phospholipids, producing IP_3 and DAG. In cardiac muscles, the IP_3 generation is transient [6] and therefore probably is not responsible for the sustained increase in contraction, although IP_3 has been reported to cause increases in the magnitude and frequency of contractile oscillations [25]. Alternatively, DAG initiates the activation of protein kinase C that increases intracellular pH and the myofibrillar sensitivity to Ca^{2+} [2–4, 26]. This increase in the sensitivity of the contractile protein to Ca^{2+} probably contributes to the increased twitch and resting tensions induced by phenylephrine, as claimed by Endoh and Blinks [27] and Fabiato and Fabiato [28].

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