

## Effects of $\alpha_1$ -Adrenergic Receptor Stimulation on Intracellular $\text{Na}^+$ Activity and Twitch Force in Guinea-Pig Ventricular Muscles

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### = ABSTRACT =

The effects of  $\alpha_1$ -adrenergic receptor stimulation on membrane potential, intracellular  $\text{Na}^+$  activity, and twitch force were investigated in ventricular muscles from guinea-pig hearts. Action potentials, intracellular  $\text{Na}^+$  activity, and twitch force of ventricular papillary muscles were measured simultaneously under various experimental conditions. Stimulation of the  $\alpha_1$ -adrenergic receptor by phenylephrine produced variable changes in action potential duration, a slight hyperpolarization of the diastolic membrane potential, a decrease in intracellular  $\text{Na}^+$  activity, and a biphasic inotropic response in which a transient negative inotropic response was followed by a sustained positive inotropic response. These changes were blocked by prazosin, an antagonist of the  $\alpha_1$ -adrenergic receptor, but not by atenolol, an antagonist of the  $\beta$ -adrenergic receptor. This indicates that the changes in membrane potential, intracellular  $\text{Na}^+$  activity, and twitch force are mediated by stimulation of the  $\alpha_1$ -adrenergic receptor, but not by stimulation of  $\beta$ -adrenergic receptor. The decrease in intracellular  $\text{Na}^+$  activity was not observed in quiescent muscles, depending on the rate of the action potentials in beating muscles. The intracellular  $\text{Na}^+$  activity decrease was substantially inhibited by tetrodotoxin. However, the decrease in intracellular  $\text{Na}^+$  activity was not affected by an inhibition of the  $\text{Na}^+$ - $\text{K}^+$  pump. Therefore, the decrease in intracellular  $\text{Na}^+$  activity mediated by the  $\alpha_1$ -adrenergic receptor appears to be due to a reduction of  $\text{Na}^+$  influx during the action potential, perhaps through tetrodotoxin sensitive  $\text{Na}^+$  channels. Our study also revealed that the decrease in intracellular  $\text{Na}^+$  activity might be related to the transient negative inotropic response. The intracellular  $\text{Na}^+$  activity decrease could lower intracellular  $\text{Ca}^{2+}$  through the  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger and thereby produce a decline in twitch force.

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**Key Words:** Guinea-pig ventricle,  $\alpha_1$ -adrenergic receptor, Intracellular  $\text{Na}^+$  activity, Membrane potential, Twitch force, Biphasic inotropic response, Phenylephrine, Prazosin

### INTRODUCTION

Stimulation of  $\alpha_1$ -adrenergic receptors in cardiac

muscle elicits interesting inotropic responses. In guinea pig ventricular muscle, stimulation of the  $\alpha_1$ -adrenergic receptor by phenylephrine produces a biphasic response in twitch force: a transient decline followed by a sustained increase (Terzic et al, 1993).

In rat ventricular muscle,  $\alpha_1$ -adrenergic receptor stimulation causes a triphasic inotropic response: a small increase in twitch force followed by a transient decline and then by a sustained positive inotropic response (Otani et al, 1988; Skomedal et al, 1988; Toshe et al, 1987). Thus, stimulation of  $\alpha_1$ -adrenergic receptors produces both negative and positive inotropic responses. The biphasic and triphasic time courses of twitch force change may be caused by the combination of a negative inotropic response and a positive inotropic response each produced by a different mechanism. However, the contribution of  $\alpha$ -adrenoceptors to adrenoceptor-mediated changes of force of contraction was reported to be minimal in the guinea-pig ventricle (Hescheler et al, 1988).

Mechanisms of the inotropic responses to  $\alpha_1$ -adrenergic receptor stimulation have been studied by a number of investigators (Fedia & Bouchard, 1992; Gambassi et al, 1992; Scholz et al, 1986; Shibata et al, 1980; Skomedal et al, 1983; Terzic & Vogel, 1991). In guinea-pig and rabbit ventricular muscles, the positive inotropic responses produced by  $\alpha_1$ -adrenergic receptor stimulation were unrelated to a change in the slow inward calcium current (Hescheler et al, 1988). On the other hand, in rat ventricular muscle stimulation of the  $\alpha_1$ -adrenergic receptor prolonged the duration of the action potential and the calcium channel blocker nifedipine abolished the positive inotropic response (Otani et al, 1988; Toshe et al, 1987). This result suggests that the positive inotropic response to  $\alpha_1$ -adrenergic receptor stimulation is related to an increase of slow inward calcium current. The different results may be due to the difference between action potential shapes of guinea-pig and rat ventricular muscles. Changes in intracellular  $\text{Ca}^{2+}$  transients by  $\alpha_1$ -adrenergic receptor stimulation were recently measured in cardiac myocytes with the fluorescent  $\text{Ca}^{2+}$  indicators (O'Rourke et al, 1992; Terzic et al, 1992). It was reported that stimulation of the  $\alpha_1$ -adrenergic receptor clearly increased the  $\text{Ca}^{2+}$  transient and twitch

force in single myocytes isolated from the rat heart (O'Rourke et al, 1992). On the other hand, a similar study showed no change in the intracellular  $\text{Ca}^{2+}$  transient (Terzic et al, 1992).

As pointed out, the biphasic and triphasic time courses of twitch force changes may be caused by the combination of a negative inotropic response and a positive inotropic response. These negative and positive inotropic responses may be caused by different mechanisms with different time courses. Our study is concerned with the mechanisms of the negative inotropic response and the decrease in intracellular  $\text{Na}^+$ . The present study shows that in guinea-pig ventricular muscle, stimulation of the  $\alpha_1$ -adrenergic receptor decreases intracellular  $\text{Na}^+$  activity, which might cause a negative inotropic response. A decline in intracellular  $\text{Na}^+$  activity should decrease twitch force via  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange (Lee, 1985; Pecker et al, 1986). In guinea-pig ventricular muscle, the decrease in intracellular  $\text{Na}^+$  activity produced by  $\alpha_1$ -adrenergic receptor stimulation might be due to a reduction in  $\text{Na}^+$  influx during action potentials. In cardiac Purkinje fibers, stimulation of the  $\alpha_1$ -adrenergic receptor by phenylephrine also decreased intracellular  $\text{Na}^+$  (Zaza et al, 1990). However, in cardiac Purkinje fibers the decrease in intracellular  $\text{Na}^+$  might be due to a stimulation of the  $\text{Na}^+$ - $\text{K}^+$  pump (Williamson et al, 1993). In rat ventricular myocytes, stimulation of  $\alpha_1$ -adrenergic receptor increased  $\text{Na}^+$ - $\text{K}^+$  pump current (Williamson et al, 1999). It was suggested that the increase in  $\text{Na}^+$ - $\text{K}^+$  pump current was mediated by the  $\alpha_{1b}$ -subtype of the receptor (Williamson et al, 1993).

## METHOD

### Tissue preparation and experimental solutions

Male Hartley guinea pigs weighing 250~350 g were killed by cervical dislocation. The hearts were rapidly excised and transferred to a dissection bath filled with Tyrode solution oxygenated with 97%  $\text{O}_2$

- 3%  $\text{CO}_2$ . The papillary muscle was carefully dissected free from the right ventricular wall, mounted horizontally on a narrow channel of a tissue chamber, and continuously superfused with oxygenated Tyrode solution at  $36\sim 37^\circ\text{C}$  (Lee & Dagostino, 1982). The dissected papillary muscle ranged from  $\sim 0.5$  to 1 mm at its widest diameter and from 2 to 4 mm in length. The mural end of the muscle was fixed with an insect pin to the Sylgard floor of the narrow channel. The muscle next to the insect pin was pressed against the floor by stimulating electrodes, which were used to elicit action potentials and contraction. Another L-shaped insect pin was placed  $\sim 1$  mm away from the stimulating electrodes to lightly press the muscle on the Sylgard floor so that the papillary muscle could be maintained in a stable position, with minimal movement, throughout the experiment. The distal end of the muscle was connected to a force transducer by a  $25\ \mu\text{m}$  silver wire attached to one of the chordae.

The guinea pig papillary muscle was superfused with a Tyrode solution at a constant rate. The Tyrode solution contained (in mM) 137 NaCl, 5.4 KCl, 1.05  $\text{MgCl}_2$ , 0.45  $\text{NaH}_2\text{PO}_4$ , 11.9  $\text{NaCO}_3$ , 1.8  $\text{CaCl}_2$ , and 5 dextrose. The solution was gassed with 97%  $\text{O}_2$ -3%  $\text{CO}_2$  and had a pH of 7.3~7.4.

A stock solution of phenylephrine ( $10^{-2}\text{M}$ ) (Sigma Chemical Co., St. Louis, MO) was prepared in deionized water and stored in a freezer. It was diluted with Tyrode solution just prior to use. Stock solutions of  $10^{-2}\text{M}$  strophanthidin,  $10^{-2}\text{M}$  atenolol and  $10^{-3}\text{M}$  prazosin were prepared in deionized water, stored in the refrigerator, and prior to use, diluted with Tyrode solution.

#### Measurements of intracellular Na activity and twitch force

Intracellular sodium activity ( $a_{\text{Na}}^i$ ) of the guinea pig papillary muscle was measured with  $\text{Na}^+$ -selective microelectrodes made with the ETH 227 neutral (Steiner et al, 1991). The construction and

calibration of  $\text{Na}^+$ -selective microelectrodes has been described in detail (Lee & Dagostino, 1982). The  $\text{Na}^+$ -selective microelectrodes were calibrated before and after each experiment. The papillary muscle cells stimulated at a constant rate (1 Hz) were impaled with both conventional and  $\text{Na}^+$ -selective microelectrodes. The distance between impalements with the two microelectrodes was  $<1$  mm. The  $a_{\text{Na}}^i$  of the muscle stimulated at a constant rate was measured as described previously (Lee & Dagostino, 1982). We described that a change of intracellular  $\text{Ca}^{2+}$  concentration should not affect measurements of  $a_{\text{Na}}^i$  (Vassalle & Lee, 1984). The action potential and twitch force were measured as described in our previous study (Lee & Vassalle, 1983).

All results are expressed as mean  $\pm$  standard deviation. Changes in  $a_{\text{Na}}^i$  within each intervention were analyzed by a paired t-test.

## RESULTS

#### Effects of phenylephrine on membrane potential, intracellular sodium activity, and twitch force

Fig. 1. shows the effects of phenylephrine on diastolic membrane potential, intracellular sodium activity ( $a_{\text{Na}}^i$ ), and twitch force in guinea-pig papillary muscle bathed in Tyrode solution. Traces A, B and C represent diastolic membrane potential ( $V_m$ ), intracellular sodium activity ( $a_{\text{Na}}^i$ ) and twitch force (T) respectively. Exposure of the muscle to  $10^{-5}\text{M}$  phenylephrine produced a slight hyperpolarization in  $V_m$  and a decrease in  $a_{\text{Na}}^i$  (traces A and B). Phenylephrine caused a biphasic change in twitch force: a transient decrease and then a gradual increase to a sustained level (trace C). The  $a_{\text{Na}}^i$  and twitch force started to decline almost at the same time. These results suggest that the transient decline in twitch force might be related to the decrease in  $a_{\text{Na}}^i$ . In 15 tests with 12 preparations  $a_{\text{Na}}^i$  decreased from  $5.7 \pm 1.1$  to  $5.2 \pm 1.0$  mM ( $p < 0.01$ ). Re-exposure to normal Tyrode solution caused both twitch force and  $a_{\text{Na}}^i$  to return to control levels. An under-

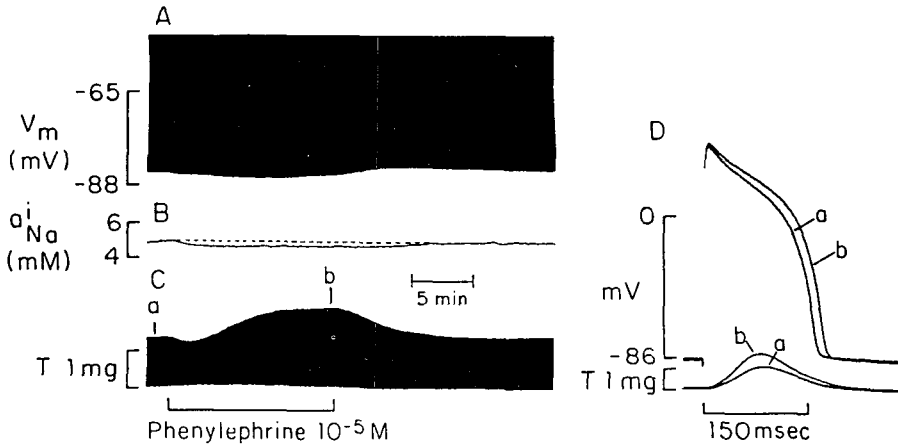


Fig. 1. Traces, A, B, and C show effects of phenylephrine on diastolic membrane potential,  $V_m$ , intracellular Na activity,  $a_{Na}^i$ , and twitch force,  $T$ , respectively in guinea-pig papillary muscle driven at a rate of 1 Hz. Panel D shows superimposed action potentials and twitch forces taken in the absence and presence of phenylephrine.

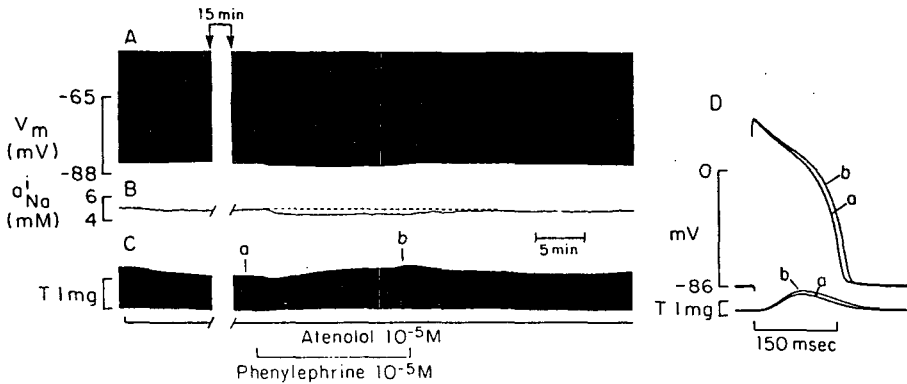


Fig. 2. Traces A, B, and C show effects of phenylephrine on  $V_m$ ,  $a_{Na}^i$ , and  $T$  respectively in the presence of atenolol. Panel D shows superimposed action potentials and twitch forces taken in the presence of atenolol alone (a) and in the presence of both atenolol and phenylephrine (b).

shoot in twitch force during recovery was not observed in our preparations although such an undershoot has been observed in cardiac Purkinje fibers (Pecker et al, 1986; Zaza et al, 1990). This finding implies that the mechanism by which phenylephrine in the papillary muscle of the guinea-pig brings about a decrease in  $a_{Na}^i$  may differ from the mechanism by which norepinephrine in the Purkinje fibers brings about a similar decrease in  $a_{Na}^i$ .

Exposure to  $10^{-5}$  M phenylephrine caused a slight hyperpolarization of the diastolic membrane potential and after washing out it recovered to control level (trace A). Panel D shows superimposed action potentials and twitch force taken at the points a and b over trace C. Exposure to  $10^{-5}$  M phenylephrine caused a small prolongation of the action potential. However, in some cases phenylephrine caused virtually no change in the duration of the action

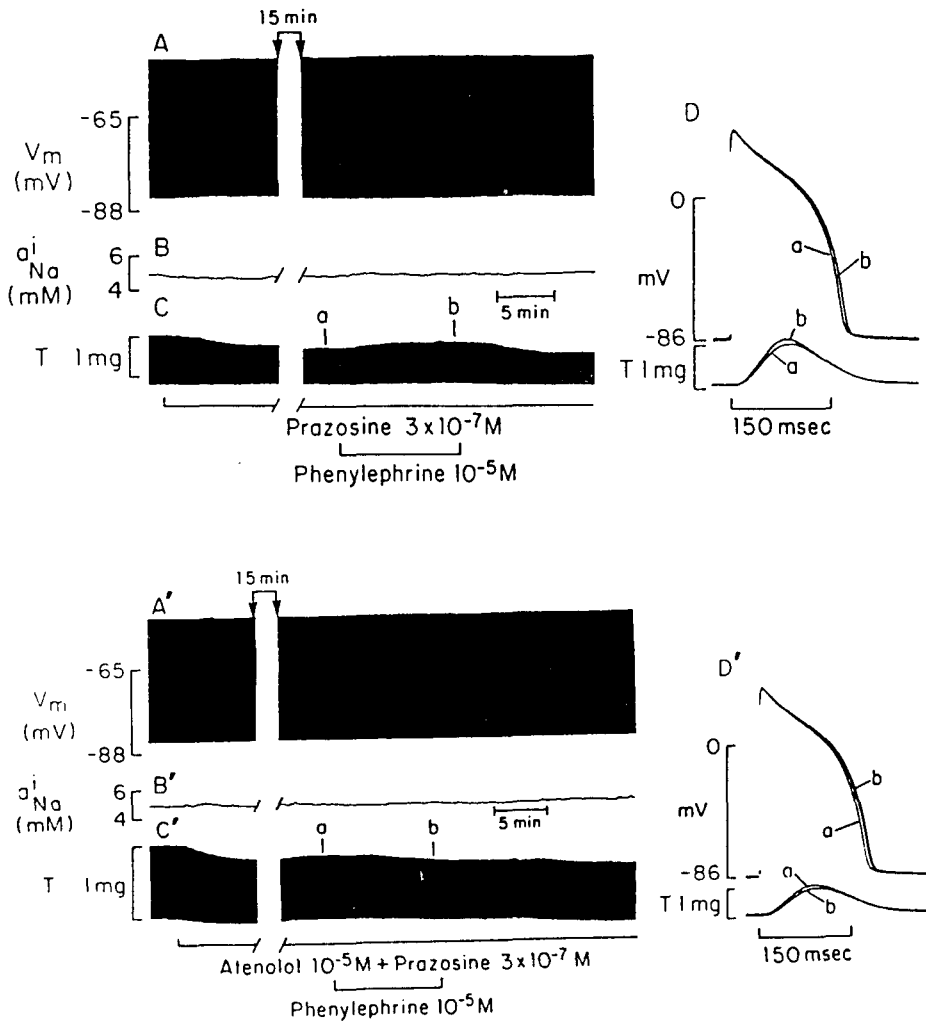


Fig. 3. Traces A, B, and C show effects of phenylephrine on  $V_m$ ,  $a^i_{Na}$ , and T respectively in the presence of prazosin. Panel D shows superimposed action potentials and twitch forces taken in the presence of prazosin alone (a) and in the presence of both prazosin and phenylephrine (b). Traces A', B', and C' show effects of phenylephrine on  $V_m$ ,  $a^i_{Na}$ , and T respectively in the presence of both atenolol and prazosin. Panel D' shows superimposed action potentials and twitch forces taken in the presence of atenolol and prazosin (a), and in the presence of atenolol, prazosin and phenylephrine (b).

potential, and in some other cases shortened the duration of the action potential. In 12 tests with 12 preparations, 7 tests showed a small prolongation in action potential duration; 2 tests showed no change; and other 3 tests showed a small reduction. This indicates that the effects of phenylephrine on the duration of the action potential in guinea-pig ventri-

cular muscle are variable.

The recordings in Fig. 2. are a continuation of the recordings in Fig. 1. In the presence of  $10^{-5}M$  atenolol (a  $\beta$ -adrenergic antagonist), phenylephrine still caused a decrease in  $a^i_{Na}$ , a transient decrease in twitch force, and hyperpolarization of the diastolic membrane potential. This result suggests that

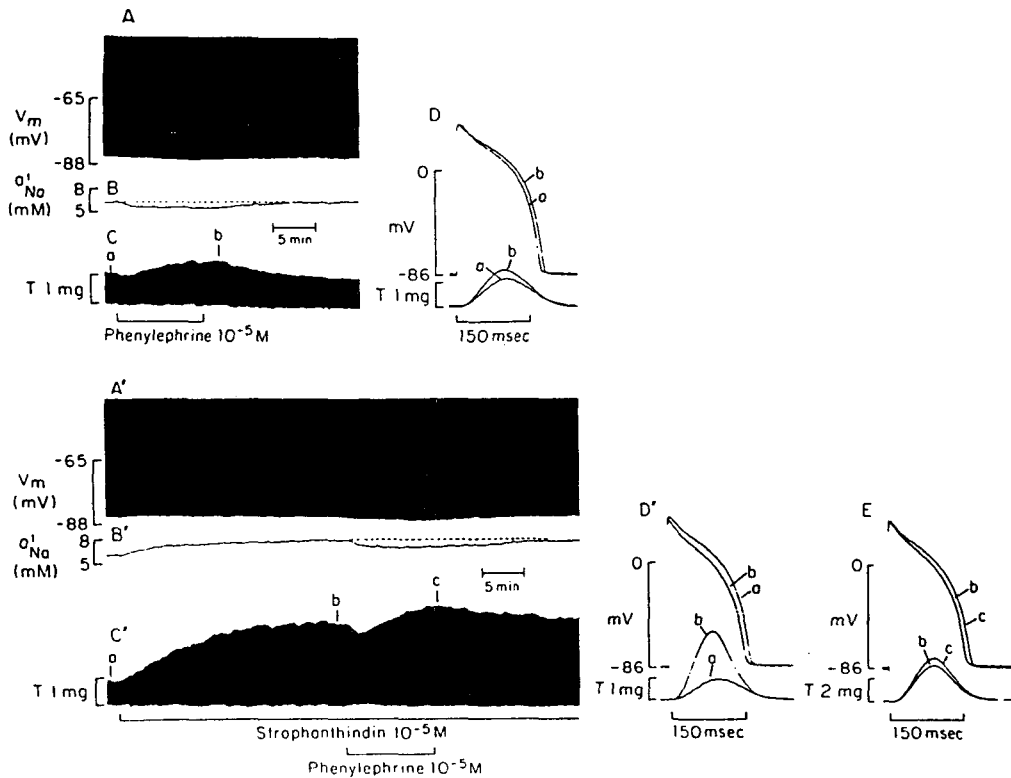
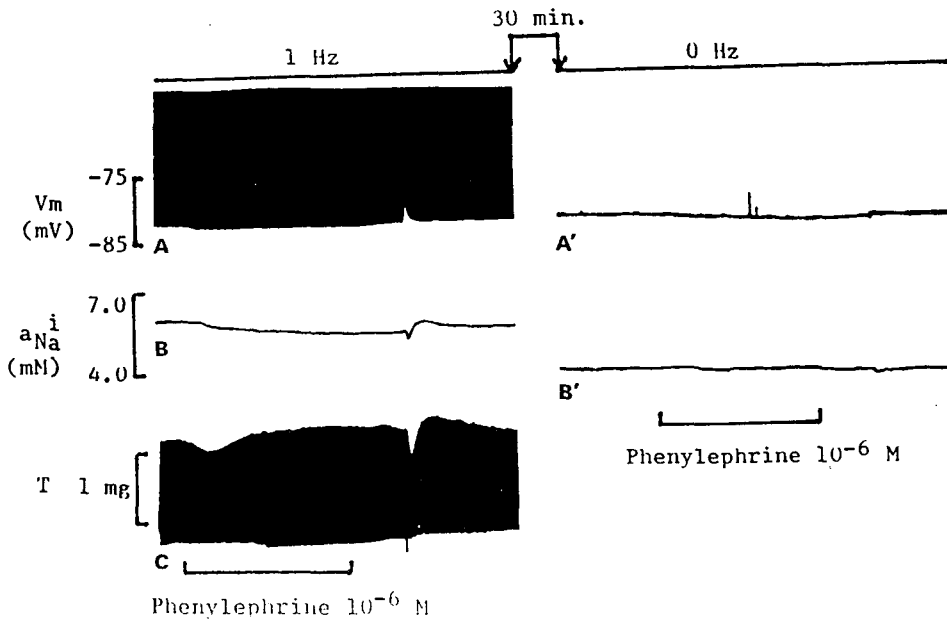


Fig. 4. Traces A, B, and C show effects of phenylephrine on  $V_m$ ,  $a_{Na}^i$ , and  $T$  respectively. Panel D shows superimposed action potentials and twitch forces in the absence (a) and presence (b) of phenylephrine. Traces A', B', and C' show effects of phenylephrine on  $V_m$ ,  $a_{Na}^i$ , and  $T$  respectively in the presence of strophanthidin. Panel D' shows superimposed action potentials and twitch forces in the absence (a) and presence (b) of strophanthidin. Panel E shows superimposed action potentials and twitch forces in the presence of strophanthidin alone (b) and in the presence of both strophanthidin and phenylephrine (c). The experiments were done in the presence of atenolol ( $10^5M$ ).

these effects of phenylephrine are due to stimulation of the  $\alpha_1$ -adrenergic receptor, not the  $\beta$ -adrenergic receptor. In 8 tests with 7 preparations phenylephrine decreased  $a_{Na}^i$  from  $6.0 \pm 1.2$  to  $5.6 \pm 1.1$  mM ( $p < 0.01$ ). In the presence of atenolol, the positive inotropic response caused by phenylephrine was smaller than that observed in the absence of the  $\beta$ -adrenergic antagonist (Fig. 1). This indicates that phenylephrine might increase twitch force partially through stimulating  $\beta$ -adrenergic receptors. This means that phenylephrine produces its positive inotropic effects through both  $\alpha_1$ - and  $\beta$ -adrenergic receptors.

Fig. 3. shows the effects of phenylephrine on diastolic membrane potential (trace A),  $a_{Na}^i$  (trace B), twitch force (trace C) and action potential (panel D) in the presence of  $3 \times 10^7$  M prazosin ( $\alpha_1$ -adrenergic antagonist). The recordings in Fig. 3 are a continuation of those in Fig. 2. Fig. 3 shows that the effects of phenylephrine on diastolic membrane potential,  $a_{Na}^i$ , and twitch force (negative inotropic response) are abolished by prazosin. Therefore, the hyperpolarization of the diastolic membrane potential, the decrease in  $a_{Na}^i$  and the negative inotropic response are mediated by  $\alpha_1$ -adrenergic receptors. However, phenylephrine produces a small increase



*Fig. 5. Traces A, B, and C show effects of phenylephrine on  $V_m$ ,  $a_{\text{Na}}^i$ , and twitch force respectively in the papillary muscle driven at a rate of 1 Hz. Traces A' and B' show effects of phenylephrine on membrane potential and  $a_{\text{Na}}^i$  respectively in quiescent papillary muscle. Traces A' and B' are a continuation of traces A and B respectively. The experiments were done in the presence of atenolol ( $10^5\text{M}$ ).*

in twitch force and action potential duration in the presence of prazosin (trace C and panel D). In 5 tests from 5 preparations, similar results were obtained.

Recordings of A', B', C' and D' shows the effects of phenylephrine on diastolic membrane potential,  $a_{\text{Na}}^i$ , twitch force and action potential in the presence of both atenolol and prazosin. The recordings are a continuation of recordings of A, B and C. This experiment demonstrates that atenolol and prazosin together completely abolish the effects of phenylephrine on diastolic membrane potential,  $a_{\text{Na}}^i$ , and twitch force (negative and positive inotropic responses). In 4 tests from 4 preparations, similar results were obtained.

**Effects of phenylephrine on membrane potential,  $a_{\text{Na}}^i$ , and twitch force in the presence of either strophanthidin or tetrodotoxin**

In cardiac muscle cells, intracellular  $\text{Na}^+$  is regu-

lated by  $\text{Na}^+$  influx through  $\text{Na}^+$  channels,  $\text{Na}^+\text{-Cl}^-$  cotransport,  $\text{Na}^+\text{-H}^+$  exchanger and  $\text{Na}^+$  efflux via the  $\text{Na}^+\text{-K}^+$  pump. Thus, the change in  $a_{\text{Na}}^i$  caused by phenylephrine might be due to an alteration in  $\text{Na}^+$  influx or the  $\text{Na}^+\text{-K}^+$  pump. To elucidate the mechanism by which phenylephrine decreases  $a_{\text{Na}}^i$ , we tested the effects of phenylephrine on  $a_{\text{Na}}^i$  in the presence of either strophanthidin or tetrodotoxin (TTX).

Fig. 4. shows the effects of  $10^5\text{M}$  phenylephrine on  $V_m$ ,  $a_{\text{Na}}^i$ , twitch force, and action potentials in both the absence and presence of  $10^5\text{M}$  strophanthidin. As usual, phenylephrine caused a small hyperpolarization of the diastolic membrane potential (trace A), a decrease in  $a_{\text{Na}}^i$  (trace B), and biphasic change in twitch force (trace C). Then the muscle was exposed to  $10^5\text{M}$  strophanthidin, which substantially increased  $a_{\text{Na}}^i$  and twitch force (trace B' and C'). Traces A', B' and C' are a continuation of traces A, B and C respectively. When  $a_{\text{Na}}^i$  and

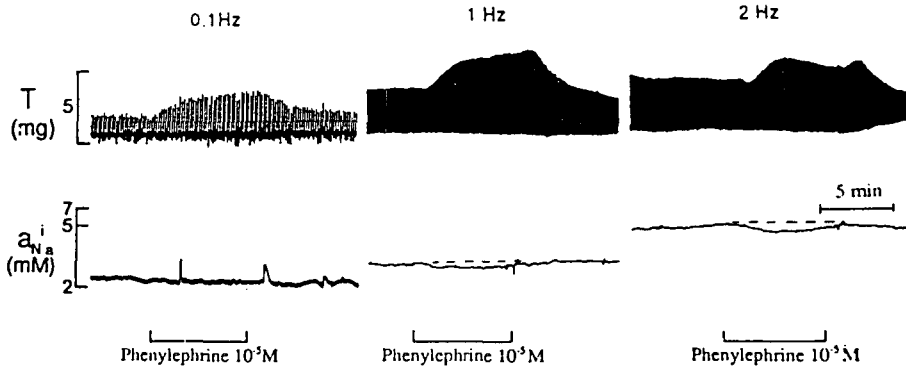


Fig. 6. Effects of phenylephrine on twitch force ( $T$ ) and  $a_{Na}^i$  in papillary muscle driven at rates of 0.1, 1 and 2 Hz. Twitch force and  $a_{Na}^i$  at the different stimulation rates were recorded in the same muscle cells. The experiments were done in the presence of atenolol ( $10^{-5}M$ ).

twitch force had stabilized, phenylephrine was added in the presence of strophanthidin. In the presence of strophanthidin, phenylephrine hyperpolarized the diastolic membrane potential slightly (trace A'), decreased  $a_{Na}^i$  (trace B'), caused a biphasic change in twitch force (trace C'), and prolonged the duration of the action potential (panel E). The decrease in  $a_{Na}^i$  by phenylephrine in the presence of strophanthidin is even greater than that seen in the absence of strophanthidin. In 4 tests with 4 preparations, similar results were obtained. This result indicates that the decrease in  $a_{Na}^i$  by phenylephrine is not due to a stimulation of the  $Na^+K^+$  pump.

Fig. 5. shows the effects of phenylephrine on  $a_{Na}^i$  and membrane potential in both driven and quiescent papillary muscle. In the driven preparation, as usual phenylephrine produced a slight hyperpolarization of  $V_m$  (trace A), a decrease of  $a_{Na}^i$  (trace B), and a biphasic change in twitch force (trace C). However, in the quiescent preparation phenylephrine had no effect on membrane potential and  $a_{Na}^i$  (traces A' and B'). In 5 tests with 4 preparations, similar results were obtained. This result suggests that the effect of phenylephrine on  $a_{Na}^i$  is action potential-dependent. Therefore we investigated the effect of phenylephrine on  $a_{Na}^i$  in the muscle stimulated at different rates (Fig. 6). Fig. 6 shows the effects of

phenylephrine on twitch force ( $T$ ) and  $a_{Na}^i$  in the muscle that elicits action potentials at the rate of 0.1, 1, and 2 Hz. The results show that as the rate of action potential was increased, the decrease in  $a_{Na}^i$  caused by phenylephrine became greater. In 3 tests with 3 preparations, similar results were obtained. Therefore, these results indicate that the decrease in  $a_{Na}^i$  produced by phenylephrine is related to  $Na^+$  movement during an action potential.

Fig. 7. shows the effects of phenylephrine on diastolic membrane potential,  $a_{Na}^i$ , twitch force, and action potential in the absence and presence of TTX. In a control experiment, phenylephrine caused a hyperpolarization of the diastolic membrane potential (trace A), a decrease in  $a_{Na}^i$  (trace B), biphasic change in twitch force (trace C), and almost no change in duration of the action potential (panel D). Then, the preparation was exposed to TTX ( $10^{-5}M$ ). TTX decreased  $a_{Na}^i$  and twitch force (traces B' and C') and shortened the duration of the action potential (panel D'). Traces A', B', and C' are a continuation of traces A, B, and C respectively. When  $a_{Na}^i$  and twitch force were stabilized, phenylephrine was added. In the presence of TTX, phenylephrine produced almost no change in  $a_{Na}^i$ . Further, a clear negative inotropic response by phenylephrine was not be observed with TTX. The positive inotropic



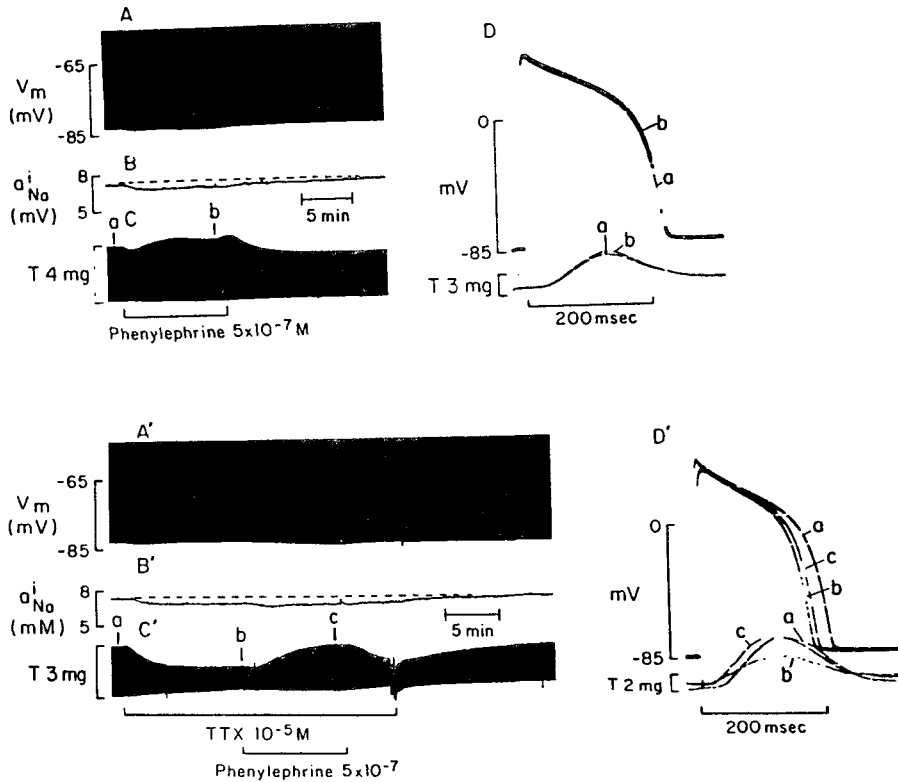


Fig. 7. Traces A, B, C (control experiment) show effects of phenylephrine on  $V_m$ ,  $a_{\text{Na}}^i$ , and twitch force ( $T$ ). Panel D shows superimposed action potentials and twitch forces in the absence (a) and presence (b) of phenylephrine. Traces A', B', C' shows effects of phenylephrine on  $V_m$ ,  $a_{\text{Na}}^i$ , and twitch forces ( $T$ ) in the presence of tetrodotoxin. Panel D' shows superimposed action potentials and twitch forces in the control condition (a), in the presence of TTX alone (b), and in the presence of both TTX and phenylephrine (c). The experiments were done in the presence of atenolol ( $10^{-5} \text{ M}$ ).

response produced by phenylephrine was relatively larger than the response that occurred in the absence of TTX. In 3 tests with 3 preparations, similar results were obtained. This result indicates that both the decrease in  $a_{\text{Na}}^i$  and the negative phase in twitch force that are caused by phenylephrine can be substantially reduced by TTX. Therefore, we suggest that the decrease in  $a_{\text{Na}}^i$  might be due to a reduction of  $\text{Na}^+$  influx through TTX-sensitive  $\text{Na}^+$  channels, and that the negative inotropic response might be related to the decrease in  $a_{\text{Na}}^i$ .

## DISCUSSION

### $\alpha_1$ -Adrenergic receptors and cardiac inotropic responses

In mammalian heart muscles, stimulation of the  $\alpha_1$ -adrenergic receptor produces multiphasic inotropic responses (Otani et al, 1988; Terzic et al, 1993; Toshe et al, 1987). In rat ventricular muscle, stimulation of the receptor by phenylephrine produces a triphasic inotropic response: a small transient positive inotropic response followed by a transient negative inotropic response, and then a sustained

positive inotropic response. Our study shows that in guinea-pig ventricular muscle, stimulation of the  $\alpha_1$ -adrenergic receptor by phenylephrine produces a transient negative inotropic response followed by a sustained positive inotropic response. Thus, in guinea-pig ventricular muscle, an early small positive inotropic response is not seen and phenylephrine causes a biphasic inotropic response. The mechanisms of the multiphasic inotropic responses are poorly understood.

The possible mechanisms underlying triphasic inotropic responses have been examined (Otani et al, 1988; Toshe et al, 1987). It was suggested that the inotropic responses might be provoked by  $IP_3$  and diacylglycerol (DG) generated through the receptor-linked degradation of  $PIP_2$  (Endoh et al, 1991; Heathers et al, 1989; Kohl et al, 1990; Scholz et al, 1988). The second messengers,  $IP_3$  and DG could activate separate intracellular pathways along different time scales, thereby producing positive inotropic responses. It is not clear what causes the transient negative inotropic response produced by phenylephrine. It was suggested that the transient negative inotropic response might be due to an  $Ca^{2+}$  overload produced by  $IP_3$  (Otani et al, 1988). However, this is unlikely because the transient positive inotropic response that precedes the negative inotropic response is very small. Furthermore, whether  $IP_3$  plays a significant role in the mobilization of  $Ca^{2+}$  from intracellular stores (sarcoplasmic reticulus) in cardiac muscles is subject to debate (Movesian et al, 1985). Therefore, further studies were required to elucidate the mechanism underlying the negative inotropic response. One possible explanation was that the transient negative inotropic response might be due to a decrease in intracellular  $Ca^{2+}$  through  $Na^+-Ca^{2+}$  exchange (see the discussion in the next section). Intracellular  $Ca^{2+}$  concentration could be decreased by  $Na^+-Ca^{2+}$  exchange when intracellular  $Na^+$  activity is lowered (January & Fozzard, 1984; Vassalle & Lee, 1984).

### $\alpha_1$ -Adrenergic receptors, negative inotropic response and $Na^+$ influx

Our study shows that in guinea-pig ventricular muscle phenylephrine produces a decrease in intracellular  $Na^+$  activity. The initial time course of the decrease in intracellular  $Na^+$  activity is similar to that of the decline in twitch force (Fig. 1). This suggests that the transient negative inotropic response might be related to the decrease in intracellular  $Na^+$  activity. The decrease in intracellular  $Na^+$  activity should decrease intracellular  $Ca^{2+}$  through  $Na^+-Ca^{2+}$  exchange (January & Fozzard, 1984).

Because phenylephrine is mainly known as an agonist of the  $\alpha_1$ -adrenergic receptor, it might be that the decrease in intracellular  $Na^+$  activity is mediated by stimulation of this receptor. If that were the case, blocking the  $\alpha_1$ -adrenergic receptor should prevent any decrease in intracellular  $Na^+$  activity. Indeed, our study shows that when the  $\alpha_1$ -adrenergic receptor is blocked by prazosin, phenylephrine does not produce a decrease in intracellular  $Na^+$  activity (Fig. 3). This is a strong indication that the decrease in intracellular  $Na^+$  activity is mediated by activation of the  $\alpha_1$ -adrenergic receptor. In the presence of prazosin, phenylephrine produced a small increase in twitch force (Fig 3). This suggests that phenylephrine might increase intracellular  $Ca^{2+}$  via stimulation of the  $\beta$ -adrenergic receptor.

Previous studies have shown that in cardiac cells stimulation of  $\beta$ -adrenergic receptors causes a decrease in intracellular  $Na^+$  activity (Desiletes & Baumgarten, 1986; Lee & Vassalle, 1983; Wasserstrom et al, 1982). Stimulation of the  $\beta$ -adrenergic receptor increases the intracellular cAMP level, which then leads to a fall in intracellular  $Na^+$  activity via stimulation of the  $Na^+-K^+$  pump (Desiletes & Baumgarten, 1986; Pecker et al, 1986; Wasserstrom et al, 1982; Zaza et al, 1990). Is the decrease in intracellular  $Na^+$  activity caused by phenylephrine related to stimulation of the  $\beta$

-adrenergic receptor and the  $\text{Na}^+\text{-K}^+$  pump? When the  $\beta$ -adrenergic receptor is blocked by atenolol, phenylephrine still produces a decrease in intracellular  $\text{Na}^+$  activity (Fig. 2). Such decreases were similar in magnitude, whether or not atenolol (a  $\beta$ -adrenergic blocker) was present. This supports the notion that the decrease in intracellular  $\text{Na}^+$  activity by phenylephrine is mediated by stimulation of the  $\alpha_1$ -adrenergic receptor, but not by stimulation of the  $\beta$ -adrenergic receptor. Moreover, in the presence of both prazosin and atenolol, phenylephrine did not produce a decrease in intracellular  $\text{Na}^+$  activity or twitch force (Fig. 3).

The decrease in intracellular  $\text{Na}^+$  activity brought about by phenylephrine could be caused by either a decrease in  $\text{Na}^+$  influx or an increase in  $\text{Na}^+$  efflux. We tested whether the decrease in intracellular  $\text{Na}^+$  activity was due to a change in  $\text{Na}^+$  influx or in  $\text{Na}^+$  efflux. In cardiac muscle cells, the major  $\text{Na}^+$  influx is by way of ion movement through  $\text{Na}^+$  channels that can be blocked by tetrodotoxin (TTX). If the decrease in intracellular  $\text{Na}^+$  activity by phenylephrine is due to a reduction in  $\text{Na}^+$  influx through  $\text{Na}^+$  channels, TTX should prevent or substantially reduce the change in intracellular  $\text{Na}^+$  activity. Our results indicate that the decrease in intracellular  $\text{Na}^+$  activity by phenylephrine is much smaller in the presence of TTX than in its absence (Fig. 7). This suggests that when  $\text{Na}^+$  channels are blocked by TTX, the transient negative inotropic response by phenylephrine is attenuated.

The relation between a decrease in intracellular  $\text{Na}^+$  activity and TTX-sensitive  $\text{Na}^+$  channels is further supported by the observation that the decrease associated with phenylephrine is produced only in the muscle cells generating action potentials, not in quiescent muscle cells. Most  $\text{Na}^+$  channels in quiescent cells are inactivated, and  $\text{Na}^+$  influx should be small. Thus, an application of phenylephrine would not be expected to decrease intracellular  $\text{Na}^+$  activity through the  $\text{Na}^+$  channels. On the other hand, during action potentials in beating muscle cells  $\text{Na}^+$

channels are open, and  $\text{Na}^+$  influx may be large. Under this condition, inhibiting the  $\text{Na}^+$  channels could decrease  $\text{Na}^+$  influx and produce a fall in intracellular  $\text{Na}^+$  activity. Further, we found that the decrease in intracellular  $\text{Na}^+$  activity caused by phenylephrine was dependent on the rate of action potential (Fig. 5, 6).

#### $\alpha_1$ -Adrenergic receptors and $\text{Na}^+$ efflux

As mentioned above, an increase in  $\text{Na}^+$  efflux should decrease intracellular  $\text{Na}^+$  activity. In cardiac muscle cells,  $\text{Na}^+$  efflux is mainly due to the  $\text{Na}^+\text{-K}^+$  pump. Thus, stimulation of the  $\text{Na}^+\text{-K}^+$  pump should decrease intracellular  $\text{Na}^+$  activity. It was reported that phenylephrine decreased intracellular  $\text{Na}^+$  activity by stimulation of the  $\text{Na}^+\text{-K}^+$  pump in canine cardiac Purkinje fibers (Zaza et al, 1990). It was also shown that catecholamines decreased intracellular  $\text{Na}^+$  activity by stimulation of the  $\text{Na}^+\text{-K}^+$  pump in cardiac Purkinje fibers (Lee & Vassalle, 1983; Wasserstrom et al, 1982) and cardiac myocytes (Desiletes & Baumgarten, 1986). The decrease in intracellular  $\text{Na}^+$  activity by catecholamines was inhibited by propranolol or strophanthidin (Desiletes & Baumgarten, 1986; Lee & Vassalle, 1983; Wasserstrom et al, 1982). These results indicate that stimulation of  $\alpha_1$ -adrenergic or  $\beta$ -adrenergic receptors in cardiac Purkinje fibers stimulates the  $\text{Na}^+\text{-K}^+$  pump and thereby decreases intracellular  $\text{Na}^+$  activity. Hyperpolarization of the diastolic membrane potential is consistent with stimulation of the  $\text{Na}^+\text{-K}^+$  pump.

Although the decrease in intracellular  $\text{Na}^+$  activity caused by phenylephrine is consistent with stimulation of the  $\text{Na}^+\text{-K}^+$  pump in cardiac Purkinje fibers, our results, obtained from guinea-pig ventricular muscle, do not support the notion of  $\text{Na}^+\text{-K}^+$  pump stimulation. When  $a_{\text{Na}}^i$  is increased by the  $\text{Na}^+\text{-K}^+$  pump inhibition, the inward  $\text{Na}^+$  driving force should decrease and be less than it would be in the absence of strophanthidin (Fig. 4). This should decrease  $\text{Na}^+$  influx. This means that the rate of the

$\text{Na}^+\text{-K}^+$  pump should be slower to match the decreased  $\text{Na}^+$  influx. Therefore, the  $\text{Na}^+\text{-K}^+$  pump activity in the presence of strophanthidin (Fig. 4B') should be lower than that in the absence of strophanthidin (Fig. 4B). If phenylephrine stimulates the  $\text{Na}^+\text{-K}^+$  pump, the decrease in  $a_{\text{Na}}^i$  by phenylephrine with low  $\text{Na}^+\text{-K}^+$  pump activity should be less than that seen with a normal  $\text{Na}^+\text{-K}^+$  pump activity. However, our experiments show that inhibition of the  $\text{Na}^+\text{-K}^+$  pump (a low  $\text{Na}^+\text{-K}^+$  pump activity) does not reduce the decrease in intracellular  $\text{Na}^+$  activity brought about by phenylephrine. When guinea-pig papillary muscle was exposed to  $10^{-5}$  M strophanthidin and the  $\text{Na}^+\text{-K}^+$  pump was inhibited, phenylephrine still produced a decrease in intracellular  $\text{Na}^+$  activity and a transient fall in twitch force (Fig. 4) which were similar to those in the absence of strophanthidin. In some cases, the decrease in intracellular  $\text{Na}^+$  activity was even greater in the presence of strophanthidin than in its absence. Similar results were obtained when the guinea-pig papillary muscles were exposed to  $10^{-4}$  M strophanthidin. These results are in contrast to those observed in cardiac Purkinje fibers. A possible explanation for these differing results may lie in the tissue difference between ventricular muscle and Purkinje fibers. It is also possible that the pump stimulation might be due to the  $\alpha_{1b}$ -subtype of  $\alpha_1$ -adrenergic receptor. Recently it was reported that in rat ventricular myocytes the  $\text{Na}^+\text{-K}^+$  pump stimulation was caused by selective stimulation of the  $\alpha_{1b}$ -adrenergic receptor subtype (Williamson et al, 1993).

Other studies have indicated that in cardiac muscle cells agonists of  $\alpha_1$ -adrenergic receptors stimulate  $\text{Na}^+\text{-H}^+$  exchange (Astarie et al, 1991; Iwakura et al, 1990; Terzic et al, 1991; Terzic et al, 1992). If the  $\text{Na}^+\text{-H}^+$  exchange is stimulated, intracellular  $\text{Na}^+$  activity should increase. However, stimulation of the  $\alpha_1$ -adrenergic receptor by phenylephrine cause a decrease, not an increase in intracellular  $\text{Na}^+$  activity. Therefore, it is unclear whether

stimulation of the  $\alpha_1$ -adrenergic receptor affects  $\text{Na}^+\text{-H}^+$  exchange in cardiac muscles. It is possible that the decrease in intracellular  $\text{Na}^+$  activity produced by phenylephrine overshadows the smaller increase in  $\text{Na}^+$  activity caused by stimulation of  $\text{Na}^+\text{-H}^+$  exchange.

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