Mechanisms for the Positive Inotropic Effect of α₁-Adrenoceptor Stimulation in Rat Cardiac Myocytes

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α₁-Adrenoceptor activation can enhance myocardial contractility, and two possible inotropic mechanisms are an increase in myofilament Ca²⁺ sensitivity and action potential prolongation, which can increase net Ca²⁺ entry into cells. In adult rat ventricular myocytes (bath Ca²⁺, 1 mM; stimulated at 0.2–0.5 Hz), the drug 4-aminopyridine and the whole-cell voltage clamp have been used to control Ca²⁺ entry and differentiate between the two mechanisms. At 22–23°C the specific α₁-adrenoceptor agonist methoxamine (100 μM) prolonged action potential duration at 50% repolarization from 55±2 to 81±5 msec, delayed time to peak contraction, and increased shortening amplitude from 5.3±0.6 to 7.8±1 μm (n=18). Reduction of the transient outward current and other K⁺ currents by methoxamine was the major cause of action potential prolongation in rat myocytes with little change in the L-type calcium current. Block of the transient outward current with 2 mM 4-aminopyridine prolonged action potential duration from 52±6 to 98±12 msec and increased unloaded cell shortening from 2.9±0.4 to 6.6±0.6 μm (n=4). Subsequently, methoxamine no longer increased cell shortening, although significant potentiation of twitch amplitude was still seen after a brief rest interval. In voltage-clamp experiments, with 70–500-msec pulses, although membrane currents were reduced, methoxamine had no positive inotropic effect and reduced cell shortening from 5.3±0.7 to 4.97±0.8 μm at pulse potentials positive to −40 mV. Similar α₁-adrenoceptor responses were observed at 35°C during action potential and voltage-clamp experiments, which could be blocked by 10 μM prazosin. In myocytes loaded with the Ca²⁺ indicator indo-1, α₁-adrenoceptor stimulation or 4-aminopyridine both increased cell contraction and intracellular Ca²⁺ transients by similar amounts. As in unloaded cells, prior exposure to 4-aminopyridine prevented any inotropic effect of methoxamine without changing the systolic intracellular Ca²⁺ transient. The results indicated that under our experimental conditions positive inotropy in rat cardiomyocytes on exposure to α₁-adrenoceptor agonists was strongly correlated with the action potential prolongation that accompanied K⁺ current reduction. In addition, modulation of K⁺ channels could occur independent of changes in contractility and/or [Ca²⁺]i. (Circulation Research 1992;71:673–688)

KEY WORDS • heart • α₁-adrenoceptors • calcium transients • 4-aminopyridine

The mechanisms by which α₁-adrenoceptors increase the force of contraction in the heart are controversial and have been the subject of many recent articles. The two most attractive hypotheses used to explain the effects are 1) an increase in the Ca²⁺ sensitivity of the myofilaments and 2) agonist-dependent alterations in intracellular Ca²⁺ homeostasis caused by changes in transsarcolemmal Ca²⁺ fluxes. In rabbit papillary muscle, Endoh and Blinks were the first to demonstrate that increases in the intracellular Ca²⁺ transient were brought about by α₁-adrenoceptor stimulation but that these were proportionally less than those brought about by increases in bath Ca²⁺ for equivalent increases in contractility. Thus, it seemed likely that α₁-adrenoceptor agonists could directly increase the Ca²⁺ sensitivity of the myofilaments in mammalian ventricular muscle, and it has recently been shown that pretreatment of isolated rat ventricular cells with phenylephrine before chemical skiving results in a leftward shift in the pCa-tension curve of approximately 0.13 pCa units. In addition, though, action potential prolongation has long been known to be associated with activation of α₁-adrenoceptors and positive inotropy, and this has been attributed to an increase in the L-type calcium current or modulation of K⁺ channels. Furthermore, the Na⁺-K⁺ pump and effects on intracellular pH secondary to α₁-adrenoceptor activation all provide pathways by which changes in inotropy may be mediated. For example, stimulation of Na⁺-H⁺ exchange by α₁-adrenoceptors could raise intracellular pH and indirectly increase the sensitivity of the contractile apparatus to Ca²⁺ ions by enhancing the affinity of troponin C for Ca²⁺. Finally, inositol phospholipid hydrolysis is known to be increased on activation of α₁-adrenoceptors, and the end result is that the
intracellular mediators inositol-1,4,5-trisphosphate and protein kinase C could modulate both ion channel effects and inotropic changes.\textsuperscript{13,30,31} At present, it is unknown just how important each mechanism is in the overall inotropic effect of \(\alpha_1\)-adrenoceptors. Apart from some indirect experiments using nifedipine or \(\text{Mn}^{2+}\),\textsuperscript{29,32} few studies have clearly separated \(\text{Ca}^{2+}\) entry–dependent mechanisms from those arising from changes in the \(\text{Ca}^{2+}\) sensitivity of the myofibrils in intact contracting cardiac cells. In rat papillary muscles, Tohse et al\textsuperscript{33} showed that preexposure to 4-aminopyridine (4-AP) to block the transient outward current could convert the positive inotropic action of phenylephrine into a sustained negative response. Additionally, it is unclear whether ion channel modulation precedes the inotropic effect and is obligatory in the production of inotropy. In the present study, we report the results of experiments in which \(\alpha_1\)-adrenoceptor stimulation was carried out under conditions in which the duration of the stimulating voltage event was controlled by a combination of the whole-cell voltage-clamp technique and pharmacological inhibition of selected membrane currents during the action potential. We find that much of the inotropic effect is due to increased \(\text{Ca}^{2+}\) entry during the duty cycle without a modulatory effect on the \(L\)-type calcium current per se. In addition, \(K^+\) channel modulation can occur independent of an inotropic effect, precedes the rise of inotropy in action potential experiments, and consequently may not be related to accompanying changes of [\(\text{Ca}^{2+}\)].

**Materials and Methods**

Single myocytes were enzymatically dissociated from rat heart using methods described previously for rabbit myocytes.\textsuperscript{12,34} Whole-cell currents and membrane potentials were measured using the whole-cell variation of the patch-clamp technique.\textsuperscript{35} Electrodes were filled with internal solution containing (mM) potassium aspartate 120, KC1 30, Na\textsubscript{2}ATP 4.0, HEPES 5.0, and MgCl\textsubscript{2} 1.0, pH 7.2 with KOH. The external solution contained (mM) NaCl 121, KCl 5.0, sodium acetate 2.8, MgCl\textsubscript{2} 1.0, Na\textsubscript{2}HPO\textsubscript{4} 1.0, NaHCO\textsubscript{3} 24, glucose 5.49, and CaCl\textsubscript{2} 1.0, pH 7.4. The bath temperature was 22–23°C for all data described in this study except those in Figure 7, which were obtained at 35±1°C. Chemicals were from Sigma Chemical Co., St. Louis, Mo. All data have been corrected for a junction potential of ~10 mV. 4-AP was dissolved in distilled water to form a 0.2-M stock, and pH was corrected to 7.4 using 2N HCl. Prazosin was dissolved in 95% ethanol to a concentration of 5 mM and further diluted in external solution to give a maximum bath concentration of ethanol of 0.19%. Measurements of unloaded cell shortening were made on-line with a video edge detection device\textsuperscript{36} and stored for later analysis. Because of small changes in the radial position of the cell on the recording pipette over time, small changes of resting cell length during the course of the experiment could not be measured accurately and are not reported in this article. Propranolol (10–6 M) was present in all experiments to prevent activation of \(\beta\)-adrenoceptors.

Intracellular \(\text{Ca}^{2+}\) was measured in single myocytes using the acetoxymethyl (AM) ester of indo-1 (Molecular Probes, Inc., Eugene, Ore.) and a loading procedure similar to that of duBell and Houser.\textsuperscript{37} Cells were loaded for 5–7 minutes at 22°C using 11.7 \(\mu\text{M}\) indo-1 AM, and [\(\text{Ca}^{2+}\)] was measured after excitation at 350 or 365 nm through the ratio of emission signals at 410 and 500 nm (±5 nM). An in vitro calibration was used on the experimental rig in which 2-\(\mu\)l aliquots contained 10 \(\mu\text{M}\) indo-1–free acid plus (mM) K\textsuperscript{+} 140, Mg\textsuperscript{2+} 5.5, Na\textsuperscript{+} 40, Cl\textsuperscript{−} 111, ATP 5, creatine phosphate 15, and EGTA 0.1 (all from Sigma Chemical Co.); HEPES 25 (Calbiochem Corp., La Jolla, Calif.); and K\textsubscript{2}·2·diaminohex-\(\text{ane-N}, \text{N}', \text{N}'', \text{N}''\text{-tetracetic acid} 9.9 \) (Aldrich Chemical Co., Milwaukee, Wis.). The calcium concentration was increased by adding \(\text{CaCl}_2\) in the range of 1.2 nM to 5.4 \(\mu\text{M}\) free \(\text{Ca}^{2+}\). The \(\text{Ca}^{2+}\) contamination was measured at ±10 \(\mu\text{M}\), yielding a lowest free [\(\text{Ca}^{2+}\)] of 67 nM. Methods for calculating free [\(\text{Ca}^{2+}\)], ionic strengths, ion-binding constants, and EGTA purity are described in detail elsewhere.\textsuperscript{38} The calibration bars on the intracellular \(\text{Ca}^{2+}\) transients correspond to a change of 675 nM in Figure 10C and 680 nM in Figure 11C. Because of potential problems with compartmentalization of indo-1 within the cell (e.g., see References 39 and 40) or binding to nonmobile sites within the cytosol,\textsuperscript{39} we have not included calibrations on the figures. The majority of experiments using indo-1 were performed at room temperature (22–23°C), because a more rapid loss of the intracellular \(\text{Ca}^{2+}\) indicator from the cell has been described at 37°C.\textsuperscript{40} During experiments with indo-1, an electronic shutter was used intermittently to shield the cell from continuous exposure to the ultraviolet illumination and reduce photobleaching of the intracellular \(\text{Ca}^{2+}\) indicator. The shutter was periodically opened to allow intracellular \(\text{Ca}^{2+}\) transients to be recorded. This allowed long-duration whole-cell experiments such as those described in Figures 10 and 11 to be carried out with minimal rundown of intracellular \(\text{Ca}^{2+}\) transients.

Previously, we have used rabbit myocytes to investigate the electrophysiological actions of \(\alpha_1\)-adrenoceptor agonists, but in this study we have chosen to use rat ventricular myocytes. In rabbit papillary muscle, the inotropic response is characterized by a long-lasting monophasic positive response, and it is known that the time course of the response follows changes in action potential duration.\textsuperscript{10,13} In rat papillary muscles (in which most studies of the inotropic action of \(\alpha_1\)-adrenoceptor agonists have been carried out) by contrast,\textsuperscript{32} and in single cells,\textsuperscript{41} a biphasic response to \(\alpha_1\)-adrenoceptor stimulation is seen: a negative inotropic effect of phenylephrine precedes a longer-lasting sustained positive inotropic effect. Rat ventricular myocytes also have a twofold to threefold higher density of \(\alpha_1\)-adrenoceptors than do rabbit or dog myocytes.\textsuperscript{13,42} Thus, our purpose was to investigate the effects of \(\alpha_1\)-adrenoceptor stimulation in a preparation distinct from rabbit cardiac myocytes and compare our results with those of others.

**Results**

An important association of the positive inotropic action of \(\alpha_1\)-adrenoceptors in the heart is that action potential duration increases.\textsuperscript{10,11,15,43,44} Presently, it is thought that this is the result of a decrease in outward repolarizing potassium currents rather than an increase of inward calcium or calcium-activated currents. These important results are shown and reconfirmed in Figure 1. Data are from rat ventricular myocytes, which show
robust cardiac responses to α₁-adrenoceptor activation in the heart, perhaps related to a relatively high density of α₁-adrenoceptors,13,42,46 Cells were isolated and voltage-clamped using methods we have described previously for rabbit myocytes;13 the α₁-adrenoceptor agonist methoxamine was consistently used because of its lack of β-adrenoceptor effects and demonstrated effects on the activation of pertussis toxin-insensitive G proteins in rabbit atrial myocytes.34,47 Rapid exposure of the cell to methoxamine resulted in a marked action potential prolongation (Figure 1A) that was readily reversible when the drug was removed (see also Figures 2 and 10). In 18 cells tested, action potential duration at 50% (APD₅₀) and 90% repolarization (APD₉₀) was increased from control values of 55±2 and 97±4 msec to 81±5 and 152±13 msec, respectively (Table 1). At this dose, methoxamine had no significant effect on the resting membrane potential of −81.2±0.4 mV. However, at a higher concentration (250 µM), methoxamine depolarized the membrane by 2−4 mV (n=5, data not shown), consistent with our earlier observation of a decrease of the inward rectifier K⁺ current in rabbit myocytes (Reference 48 and Table 2) and a depolarizing effect of phenylephrine in rat atrial myocytes.49

The membrane currents underlying this prolongation are shown in Figures 1B−1D. The L-type calcium current was unaffected by methoxamine during step voltage-clamp pulses from −40 to 0 mV (Figure 1B) or from −80 mV (data not shown). In contrast, the outward currents were markedly reduced by methoxamine in rat heart cells (Figure 1C). Over the whole potential range studied, the peak and steady-state currents were both reduced, and this reflects reduction of two components of outward current in rat ventricular myocytes.50 A summary of the actions of α₁-adrenoceptor agonists on the total membrane current−voltage relation is shown in Figure 1D. The inward current responsible for the maintenance of the action potential plateau (Ca²⁺
current) was unaffected by \( \alpha \)-adrenoceptor activation\(^{12,43,51} \), whereas the repolarizing currents (solid inverted triangles in Figure 1D) were reduced. These results are entirely in agreement with the observations of Apkon and Nerbome\(^{16} \) and Ravens et al\(^{17} \) in rat ventricular cells using the \( \alpha \)-adrenoceptor agonist phenylephrine and our own observations on rabbit atrial and ventricular myocytes using the \( \alpha \)-adrenoceptor agonists noradrenaline and methoxamine.\(^{11,12} \) Thus, the prolongation of the action potential that is known to occur in many species seems likely to result mainly from reduction of the repolarizing transient outward current in rat myocytes and other background K\(^+\) conductances in Purkinje fibers\(^{18} \) and rabbit ventricle.\(^{48} \) We and others\(^{17,33} \) have suggested that the K\(^+\) current reduction may thus lead to the positive inotropic effect of \( \alpha \)-adrenoceptors.\(^{12} \) In rabbit atrium\(^{10} \) and papillary muscle,\(^{13} \) the sustained phase of inotropy has been correlated with action potential prolongation, although previous studies in rats have suggested that the initial multiphasic inotropic responses to \( \alpha \)-adrenoceptor stimulation may be separated from the monophasic increase in action potential duration.\(^{13,32} \)

We have attempted to control action potential duration in two different ways to attempt to dissect the role of action potential prolongation in the inotropic action of \( \alpha \)-adrenoceptors. In Figures 2–4, we have used 4-AP to block the transient outward current and thus preempt the major mechanism by which \( \alpha \)-adrenoceptors prolong the action potential. In Figures 5–8, we have used

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**TABLE 1. Changes in Electrical and Mechanical Parameters in Rat Ventricular Myocytes Exposed to 100 \( \mu\)M Methoxamine**

<table>
<thead>
<tr>
<th></th>
<th>( E_m ) (mV)</th>
<th>( \text{APD}_{90} ) (msec)</th>
<th>( \text{APD}_{90} ) (msec)</th>
<th>TPS (msec)</th>
<th>PS (( \mu)m)</th>
<th>RT(_{50}) (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-81.2±0.4</td>
<td>54.7±1.9</td>
<td>97.4±3.8</td>
<td>188±7</td>
<td>5.28±0.6</td>
<td>36±1.6</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>-81.4±0.4</td>
<td>80.8±5.2*</td>
<td>152±13*</td>
<td>209±8*</td>
<td>7.84±1.0*</td>
<td>28±1.3</td>
</tr>
<tr>
<td>Washout</td>
<td>-81.4±0.4</td>
<td>54.9±1.9</td>
<td>103±4.5</td>
<td>192±7</td>
<td>5.16±0.56</td>
<td>25±1.1</td>
</tr>
</tbody>
</table>

\( E_m \): membrane potential; \( \text{APD}_{90} \) and \( \text{APD}_{90} \) durations of action potential at 50% and 90% repolarization; respectively; TPS, time to peak shortening; PS, amplitude of peak shortening (contraction); RT\(_{50}\), time for 50% relaxation from peak shortening. Values are mean±SEM from 18 cells.

\(^*p<0.05\) vs. control by Student’s t test for unpaired data.
the voltage clamp to directly control the duration of the activating event in the myocyte and prevent any effect of α₁-adrenoceptor agonists on the length of the voltage event. A comparison of the effects of methoxamine alone and in combination with the K⁺ channel blocker 4-AP on the action potential and inotropic response to methoxamine is shown in Figure 2. Rapid exposure of the cell to methoxamine resulted in a marked action potential prolongation (Figure 2A) that was readily

![Graph](http://circres.ahajournals.org/)

**FIGURE 3.** Time courses showing changes in cell length and action potential duration in response to methoxamine (MOX) alone (panel A) and after previous exposure to 4-aminopyridine (4-AP, panel B). Panel A: Cell exposed to 100 μM MOX as indicated by the black bar during steady-state stimulation at 0.2 Hz. Note increase in duration (○) preceded increase of cell shortening (●) by a few beats. Panel B: Effect of 4-AP (2 mM) and subsequent exposure to MOX as indicated by black bars on cell shortening (●) and action potential duration (○) in another myocyte (stimulation rate, 0.25 Hz). Responses are shown as percent change from control duration at 50% repolarization or control cell shortening before exposure to MOX or 4-AP. Note that the upper portion of the left scale in panel A or panel B refers to duration change and increases upward. The lower portion of the scale refers to unloaded cell shortening and depicts an increase in cell contraction downward.

![Graph](http://circres.ahajournals.org/)

**FIGURE 4.** Recordings showing that postrest stimulation still increases cell contraction during 4-aminopyridine exposure and α₁-adrenoceptor stimulation. V_m, membrane voltage. The top of each panel shows action potentials in the steady state during stimulation at 0.25 Hz (●) and after a 14-second rest (○). The bottom of each panel shows cell shortening tracings during steady stimulation (●) and during rested beats (○) corresponding to action potentials above. Panel A: Control action potentials and contractions in the steady state. Panel B: During exposure to 4-aminopyridine alone (2 mM). Panel C: During exposure to 4-aminopyridine (2 mM) plus methoxamine (100 μM). Panel D: After washout. Note that in all four panels, postrest contractions are significantly increased over controls. Similar results were seen in three other cells. Propranolol (10⁻⁶ M) was continuously present. Simultaneous recordings of membrane potential, current, and unloaded cell shortening obtained in the steady state were usually 1–3 minutes after exposure to or washout of drugs or rate changes.

**TABLE 2.** Changes in Membrane Current and Cell Shortening Parameters During Exposure to 100 μM Methoxamine Under Voltage-Clamp Conditions

<table>
<thead>
<tr>
<th></th>
<th>I_{hold} (pA)</th>
<th>I_{peak} (pA)</th>
<th>TPS (msec)</th>
<th>PS (μM)</th>
<th>RT_{50} (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-33.1±7</td>
<td>1,302±110</td>
<td>275±13</td>
<td>5.3±0.65</td>
<td>96±4.7</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>-23.5±5*</td>
<td>923±117*</td>
<td>273±17</td>
<td>4.97±0.77</td>
<td>94±6.1</td>
</tr>
<tr>
<td>Washout</td>
<td>-33.2±7</td>
<td>1,271±140</td>
<td>272±15</td>
<td>5.2±0.68</td>
<td>95±5.5</td>
</tr>
</tbody>
</table>

I_{hold}, holding current at holding potential of -80 mV; I_{peak}, maximum outward current during voltage-clamp pulses at +20 mV; TPS, time to peak shortening; PS, amplitude of peak shortening; RT_{50}, time for 50% relaxation from peak shortening. Values are mean±SEM from 10 cells.

Pulse duration was 70–90 msec. Statistical analysis was carried out on unpaired data. Note that under voltage clamp unloaded cell shortening parameters in methoxamine do not differ significantly from control values.

*p<0.05 vs. control.
reversible when the drug was removed. Cell shortening recordings (Figure 2B) show, in single myocytes, that the longer action potential was accompanied, in the steady-state (solid circle in Figure 2B), by a more pronounced and longer-lasting cell contraction, which followed the time course of the change in action potential configuration with a delay of a few beats (Figure 3A). After methoxamine was introduced to the bathing solution, there was a rapid onefold to twofold increase in the APD₉₀. This was followed by an increase in the cell shortening response after a further delay of five beats. The positive inotropic effect of α₁-adrenoceptors in multicellular preparations is defined by an increase in peak shortening and a slight delay in time to peak shortening, but little change in the half-time of relaxation; our data in single cells confirm this. Peak shortening was increased significantly \((p<0.01)\) from 5.28±0.6 to 7.84±1 \(\mu\)m during exposure to methoxamine. Similar results were obtained for time to peak shortening, which was prolonged from 188±7 to 209±8 msec, whereas time to 50% relaxation remained unaltered from the control value of 30 msec (Table 1). As shown by recordings (open circle) in Figure 2B and the time course graph in Figure 3A, these effects were completely reversible after removal of the drug from the perfusate, with the inotropic effect taking significantly longer to dissipate than the preceding electrophysiological changes. When myocytes were exposed to the K⁺ channel antagonist 4-AP, there was marked prolongation of the action potential (open circle in Figure 2C), an expected result from transient outward current blockade. APD₉₀ and APD₉₉ were increased from 52±6 and 77±8 msec in control cells \(n=4\) to 98±12 and 133±12 msec in treated cells, and no effect was observed on the resting membrane potential of −80±0.6 mV. Accompanying this shape change, peak contraction was increased from 2.9±0.4 to 6.64±0.6 \(\mu\)m, and time to peak shortening was prolonged from 208±15 msec in control cells to 247±19 msec in treated cells (open circle in Figure 2D). Figure 3B illustrates that, as was the case for α₁-adrenoceptor stimulation, the inotropic response to 4-AP developed with a delay of three to five beats after the start of action potential prolongation. Subsequent addition of 100 \(\mu\)M methoxamine to the perfusate at this point had little effect on either the action potential shape (filled circle in Figure 2C; Figure 3B) or the extent of cell shortening (filled circle in Figure 2D; Figure 3B). Figure 2D illustrates that despite the slight prolongation of the action potential, activation of α₁-adrenoceptors under these conditions resulted in a slight negative inotropic effect rather than a positive inotropic response (see Tohse et al\(^{13}\)). There was a slight slowing of terminal repolarization of the action potential \((\text{APD}_{90} \text{ increased from } 133 \text{ to } 159 \text{ msec})\), and this effect may reflect an inhibition of the inward rectifier K⁺ current by α₁-adrenoceptors.\(^{46}\) Thus, 4-AP seems able to preemt the action potential and inotropic actions of methoxamine and prevent the α₁-adreno-
Figure 6. Graph showing the effect of α₁-adrenoceptor stimulation on peak outward current and unloaded cell shortening under varying conditions of cell Ca²⁺ loading. Methoxamine (MOX, 100 μM) was included in the perfusate at the time indicated by the horizontal bars. Initially, the cell was stimulated using 500-msec voltage-clamp pulses from −80 to +20 mV given at 0.5 Hz. Current is depicted as open circles and reversibly decreases during exposure to methoxamine. Shortening is shown as filled circles and remains relatively unchanged (see text). After the initial exposure and washout of the drug, stimulation frequency and pulse duration were reduced to 0.25 Hz and 70 msec, respectively, at the time indicated by the black bar on the right of the graph. Note the dramatic decrease in unloaded cell shortening that occurred when the pulse duration and frequency were reduced. The scale at the left axis indicates decreased shortening or membrane current as a negative percentage change from the control levels. Membrane current is still reduced by MOX (○), but there was little inotropic effect.

ceptor agonist from having any further electrical or mechanical effects. In fact, when no change in the duration of the action potential occurred (Figures 2C, 4, 11A), activation of α₁-adrenoceptors consistently had a slight negative inotropic effect; i.e., it reduced peak cell shortening. When 4-AP was removed from the bathing medium along with methoxamine, there was a relatively rapid recovery of first APD₂ and then contraction to precontrol levels (Figure 3B).

It has been shown that at high levels of bathing calcium (~5 mM) α₁-adrenoceptor stimulation caused spontaneous calcium oscillations and symptoms of Ca²⁺ overload in rat ventricular myocytes. This prevented any inotropic effect of α₁-adrenoceptor agonists, and although we never observed oscillations of contraction or membrane potential symptomatic of Ca²⁺ overload in our cells (bath Ca²⁺, 1 mM; see also Reference 41), we were nevertheless concerned that the failure of methoxamine to produce a positive inotropic effect in the presence of 4-AP was due to saturation of the myofilaments with Ca²⁺, which was secondary to maximal action potential prolongation. To address this issue, we took advantage of the phenomenon of “postrest potentiation,” whereby resumption of rhythmic stimulation after a brief period of electrical quiescence results in a potentiation of contraction in species with well-developed sarcoplasmic reticulum (SR) networks; this potentiation is due to enhanced release of Ca²⁺ from intracellular stores. The results of one such experiment are shown in Figure 4. In the control situation, resump-

tion of stimulation after a rest interval of 14 seconds increased peak shortening by 48±12% (n=4) and modestly prolonged the duration of contraction (Figure 4A). As before (Figures 2C and 2D), sequential addition of 4-AP markedly enhanced and then methoxamine slightly reduced peak shortening in response to rhythmic stimulation at 0.25 Hz. However, postrest contractions elicited at each stage in the protocol were nearly identical, as shown in Figures 4B and 4C, and these effects were completely reversible (Figure 4D). This result suggests that under the present experimental conditions the cells retain the ability to respond inotropically to an increase in the amount of Ca²⁺ release from the SR and that the failure of α₁-adrenoceptor stimulation to elicit a further inotropic response in the presence of 4-AP was not due to saturation of the contractile apparatus with Ca²⁺. Like others who have used nifedipine and Mn²⁺ our indirect experiments to control action potential duration have demonstrated a clear dependence of the positive inotropic effect on action potential prolongation. Thus, the working hypothesis was that changes in duration of the voltage event are required to cause changes in calcium entry necessary to change the inotropic state of the cells. We have attempted to test this hypothesis directly by controlling the duration of the activating voltage event using the voltage-clamp technique.

In Figure 5, we show that more precise control over the duration of the voltage event using the voltage clamp prevents the positive inotropic effect of α₁-adrenoceptor stimulation and that this occurs despite the coexistence of modulatory effects on membrane currents. Initially, long-duration voltage-clamp pulses were used to elicit membrane currents and cell shortening at a high level of cell Ca²⁺ loading. The data in Figure 5A show that exposure of the cell to 100 μM methoxamine caused a reversible decrease of the outward K⁺ currents, as was shown in Figure 1 for cells in which the Ca²⁺ current was inhibited by addition of Cd²⁺ to the perfusate. Despite the clear reduction of membrane current, no appreciable increase in the extent or duration of cell shortening was noted (Figure 5A, bottom panel; Figure 6; and Table 2). After removal of the drug from the perfusate and return of the current waveform to the control level, the level of cell Ca²⁺ loading was reduced by slowing the stimulation rate from 0.5 to 0.25 Hz and decreasing the pulse duration from 500 to 70 msec. This was deliberately done to ensure that cells were contracting adequately for measurement but could respond in a sensitive way to any effect of α₁-adrenoceptor stimulation. Thus, the short (70-msec) voltage-clamp pulse was chosen to be about one half of the total duration of the action potential in this cell (not shown, but see Figure 1) and to reach +20 mV, which is a potential where the peak calcium current should be decreased as it falls on the ascending limb of its current–voltage relation. As illustrated in Figures 5B and 6, this had a marked effect on twitch strength, which was reduced by 65% within nine beats. However, despite the large inotropic reserve predicted under these conditions (compare with Figure 4), exposure of the cell to 100 μM methoxamine had effects on membrane currents and contractions almost identical to those depicted in Figure 5A; i.e., outward K⁺ current was reversibly decreased, but there was still no positive
FIGURE 7. Recordings showing effect of temperature and prazosin on action potentials, contractions, and membrane currents during $\alpha_1$-adrenoceptor stimulation. $V_m$, membrane voltage. Data in this figure are from three different cells studied at $35\pm1^\circ$C. Panel A: Action potential (top) and unloaded cell shortening (bottom) data from the same cell. Tracings were taken in the steady state before exposure (△), during exposure to 100 $\mu$M methoxamine (●), and after washout (○). Note that action potentials at $35^\circ$C are shorter than those at $23^\circ$C but that methoxamine causes a large relative increase in duration at 50% and 90% repolarization. Panel B: Same experiment as in panel A except that the tracing marked with the open circle was recorded in the presence of 100 $\mu$M methoxamine plus 10 $\mu$M prazosin. Prazosin completely antagonizes both the action potential prolongation and the contractility changes brought about by methoxamine. Similar action potential results were observed in five other cells. Panel C: Continuous voltage-clamp experiment at $35^\circ$C. Membrane currents (top) and cell contraction (bottom) before exposure (△), during exposure to 100 $\mu$M methoxamine (●), and after washout (○). Note that in contrast to action potential experiments in panels A and B, no increase in steady-state peak contraction occurred under voltage clamp. The cell shortening calibration at the bottom denotes 1.6 μm in panel A, 2.53 μm in panel B, and 2.43 μm in panel C. Propranolol (10 $^{-6}$ M) was continuously present. Simultaneous recordings of membrane potential, current, and unloaded cell shortening obtained in the steady state were usually 1–3 minutes after exposure to or washout of drugs or rate changes.

The inotropic effect of the $\alpha_1$-adrenoceptor agonist, and again a slight decrease in cell shortening occurred (filled circle in Figure 5B) that was clearly reversible on washout of the drug (open circle in Figure 5B). The time course of the effects of methoxamine on contraction and membrane current during continuous voltage clamp are shown in Figure 6. The effect of exposure to 100 $\mu$M methoxamine was a clear repeatable decrease in the membrane current response that was reversible on washout of the $\alpha_1$-adrenoceptor agonist. However, whether cells are exposed to methoxamine at a high level of Ca$^{2+}$ loading during 500-msec voltage-clamp pulses or at a low level during 70-msec voltage-clamp pulses, there was only a small reversible decrease in cell contraction. The results of these experiments (Table 2) were thus quite similar to those obtained in current-clamp conditions with previous exposure of the cell to 4-AP, in which peak shortening was slightly depressed, rather than enhanced, during $\alpha_1$-adrenoceptor stimulation.

Confirmation that these results were representative of the physiological situation at $35^\circ$C is shown in Figure 7. Action potentials were shorter than those at $23^\circ$C but still showed a large relative prolongation on exposure to 100 $\mu$M methoxamine. This was accompanied by increases in contractility in the steady state (Figure 7A) that were reversible on washout. Both the increase in action potential duration and in contractility could also be reversed by exposure to 10 $\mu$M prazosin, a specific $\alpha_1$-adrenoceptor antagonist (Figure 7B). Rapid superfusion with prazosin resulted in a complete reversal of the effects of the $\alpha_1$-adrenoceptor agonist, and no effect of prazosin alone was observed during rapid exposures. At $35^\circ$C in seven cells during action potential stimulation, 100 $\mu$M methoxamine increased mean contractility by 139±24% (mean±SEM). In contrast, under continuous voltage clamp, methoxamine failed to increase cell contractility in the steady state (Figure 7C). Despite a large reduction in membrane outward current brought about by methoxamine (Figure 7C), there was little change in cell contractility in the steady state, although, as in Figures 7A and 7B and in the results obtained with action potentials at $22^\circ$–$23^\circ$C (Figure 2), the $\alpha_1$-adrenoceptor agonist delayed time to peak contraction slightly. Under voltage clamp at $35^\circ$C, methoxamine reduced peak steady-state contraction to 96.3±3.8% of that in control conditions (n=9).

One concern in the voltage-clamp experiments was that some change in the voltage dependence of cell contraction was brought about by the $\alpha_1$-adrenoceptor agonist so that by pulsing to one fixed clamp pulse potential we were missing an increase in contractility at
Another pulse potential. The experiment illustrated in Figure 8 was aimed at examining this point. As shown in Figure 8A, the depressant effect of 100 μM methoxamine on membrane currents and the slight decrease of contraction were fully reversible. The protocol for determination of the voltage dependence of contraction is shown in Figure 8B. Control currents and contractions for 80-msec clamp pulses from −80 mV to potentials from threshold (for the transient outward current and contraction) of approximately −40 mV to +40 mV are shown. As expected, there are progressive increases in peak outward current and contraction as the step potential is made more positive. In four cells studied with this protocol, similar to the response observed with a 100-mV step from −80 to +20 mV (Figure 5 and Table 2), we found that peak shortening was slightly depressed at all potentials tested positive to −40 mV. This effect on twitch amplitude contrasts with the significant decrease of membrane current in the same potential range, which amounted to approximately 30% in this experiment (Figure 8C). Similar results were observed in three other cells. Thus, we conclude that under constant voltage-clamp conditions, stimulation of α1-adrenoceptors did not cause a shift in the shortening-voltage relation and was unable to exert a positive inotropic effect at any potential studied.

The quantitative relation between the electrophysiological and corresponding mechanical alterations after α1-adrenoceptor stimulation is summarized in Figure 9. Figure 9A shows the results of a regression analysis in which the increase in APD50 in response to 100 μM methoxamine and 1 μM propranolol has been plotted against the equivalent inotropic response. The correlation coefficient for cells exposed to methoxamine alone was r=−0.918 (n=18), which reveals a significant relation (p<0.001) between the degree of action potential pro-

**Figure 8.** Effect of α1-adrenoceptor stimulation on voltage dependence of membrane current (Im) and contraction. Vm, membrane voltage; MOX, methoxamine. Panel A: Recordings showing Im (middle tracing) and contractions (bottom tracing) for single voltage-clamp pulses from −80 to +20 mV (protocol is top tracing). Measurements were obtained before exposure (left tracings), during exposure to 100 μM MOX (middle tracings), and after washout (right tracings). Panel B: Recordings showing voltage dependence of Im (middle tracing) and unloaded cell shortening (bottom tracing) under control (CTL) conditions. Vm was stepped from a holding potential of −80 mV to test potentials ranging from −40 to +40 mV for 80 msec. Panel C: Graph showing current–voltage relation for peak outward current (circles) and shortening–voltage relation (inverted triangles) before (open symbols) and after (filled symbols) exposure to 100 μM MOX. Note that stimulation of α1-adrenoceptors did not shift the current–voltage relation or shortening–voltage relation along the voltage axis. An increase in peak shortening is shown downward. Propranolol (10−6 M) was continuously present. Simultaneous recordings of membrane potential, current, and unloaded cell shortening obtained in the steady state were usually 1–3 minutes after exposure to or washout of drugs or rate changes.
longation and the corresponding inotropic response. For comparative purposes, data have also been included from four cells in which APD_90 was increased with 2 mM 4-AP in the absence of α₁-adrenoceptor stimulation. This did not significantly change the result (r=0.906, n=22, p<0.001) and serves to illustrate the similarities between the electrophysiological and inotropic changes obtained with α₁-adrenoceptor stimulation and those interventions thought to increase contractility primarily through an increase in Ca^{2+} uptake and release from the SR. 56–58 Conversely, Figure 9B depicts the lack of a significant relation between the reduction of peak outward current and peak unloaded cell shortening in response to α₁-adrenoceptor stimulation under continuous voltage clamp (r=0.158, n=10). Unlike the large positive inotropic effect observed under current clamp (Figure 9A and Table 1), stimulation of α₁-adrenoceptors under voltage clamp had a slight depressant effect on peak shortening of approximately 6% (Table 2), which was independent of pulse duration (Figures 5 and 6) or the accompanying reduction of outward K⁺ currents (Figures 1 and 5).

As discussed above, several lines of evidence suggest that activation of α₁-adrenoceptors may result from an increase in the sensitivity of the myofilaments to Ca^{2+}. 6,6,22 Although we have not directly measured changes in the Ca^{2+} sensitivity of the myofilaments, our indirect experiments on rat ventricular myocytes in Figures 1–5 suggest that, under voltage clamp at least, any increase in the Ca^{2+} sensitivity of the myofilbrs does not result in a net positive inotropic effect of α₁-adrenoceptor stimulation under the present experimental conditions. To further investigate this issue, we have measured intracellular Ca^{2+} with the fluorescent indicator indo-1. In Figure 10 are data showing action potentials, cell shortening, and intracellular Ca^{2+} transients recorded simultaneously from a single rat ventricular myocyte. Similar to results obtained in field-stimulated rat ventricular cells loaded with indo-1 AM 49 or fura-2 AM, 59 we found that loading of cells with indo-1 AM for 5–10 minutes (total indo-1, 11.7 μM) had a significant effect on the mechanics of cell shortening in response to injection of a brief current pulse. Time to peak shortening was prolonged slightly from 188±7 msec in control to 256±13 msec after loading of the indicator. The majority of twitch lengthening occurred during the relaxation phase of contraction, and the time to 50% relaxation was increased from 35±1.6 msec in the control group to 141.7±13 msec in cells loaded with indo-1 (n=8). Although the twitch was generally prolonged, peak contraction (mean, 5.45 μm) was not statistically different from non-indo-loaded cells (Table 1).

As described earlier, for non-indo-loaded cells (Figures 1 and 2 and Table 1), stimulation of α₁-adrenoceptors resulted in a marked prolongation of action potential duration (filled circle in Figure 10A), which was fully reversible after removal of the drug from the perfusate (open circle in Figure 10A) and could be repeated several times in the same cell without desensitization of the response. The cell shown in Figure 10 was chosen for study because of its small contraction in response to 0.25-Hz stimulation in the control situation, which was probably associated with the rather short action potential duration recorded under those conditions (APD_90, 45 msec). APD_90 was increased in this cell from 45 to 243 msec in the presence of methoxamine and was reduced to 54 msec after washout. Peak shortening in response to α₁-adrenoceptor stimulation was
markedly enhanced (filled circle in Figure 10B), the extent of which was related to the large inotropic reserve under these conditions (compare with Figure 6). Peak shortening was increased from 2.5 \( \mu \)m in control to 16.1 \( \mu \)m after rapid exposure to methoxamine. As observed in non-indo-loaded cells (Table 1), the rate of relaxation was generally enhanced, with the exception of terminal relaxation, which was prolonged. In this cell, the peak of the intracellular \( Ca^{2+} \) transient was increased by 92\%, and little change in the time to peak \( [Ca^{2+}]_c \) was noted. The rate of decline of the intracellular \( Ca^{2+} \) transient was enhanced, but the total duration of the transient was little changed, consistent with the effects of methoxamine observed on the time to 50\% relaxation in control cells (Table 1) and cells loaded with indo-1. After removal of the drug from the perfusate, both peak shortening and the intracellular \( Ca^{2+} \) transient returned to their respective control values. Similar results were obtained in two other cells.

The results of this experiment are consistent with those obtained from fura-2-loaded rabbit papillary muscles\(^5\) and in field-stimulated rat ventricular myocytes,\(^6\) in which \( \alpha_1 \)-adrenoceptor stimulation has been reported to cause significantly larger increases in peak twitch strength than peak \( [Ca^{2+}]_c \). To address this issue, the inotropic response obtained in the presence of methoxamine alone was compared with that elicited by exposure of the cell to 4-AP in the absence and presence of \( \alpha_1 \)-adrenoceptor stimulation. The result of this experiment is shown in Figure 11, which illustrates that the effect of 2 mM 4-AP on action potentials, unloaded cell shortening, and the underlying intracellular \( Ca^{2+} \) transient is quite similar to that described in Figure 10 for the \( \alpha_1 \)-adrenoceptor agonist. Addition of 4-AP to the perfusate increased APD\(_{90} \) from 49 to 188 msec. Peak shortening was increased from 2.35 to 16.4 \( \mu \)m, and the peak amplitude of the corresponding intracellular \( Ca^{2+} \) transient was increased by 85\%. Subsequent addition of methoxamine to the bath further prolonged APD\(_{90} \) from 188 to 215 msec and, as in non-indo-1–loaded cells under current clamp (Figure 2D) or voltage clamp (Figure 8), elicited a small negative inotropic effect. In addition, we observed no measurable effect on the peak or kinetics of the intracellular \( Ca^{2+} \) transient in this or two other cells tested with this protocol. Thus, the effects of 4-AP on action potential duration, cell shortening, and the intracellular \( Ca^{2+} \) transient parallel those obtained with \( \alpha_1 \)-adrenoceptor stimulation and are similar to effects reported in earlier studies in isolated rat ventricular myocytes loaded with either indo-1 of fura-2. These studies reported that unloaded shortening had a greater sensitivity than did intracellular \( Ca^{2+} \) transients to changes of bath \( Ca^{2+} \), stimulation frequency,\(^5\) or pulse duration.\(^5\)

**Discussion**

**Mechanisms of Action Potential Prolongation**

Action potential prolongation has long been known to occur in those species and tissues in which significant
inotropic responses to α1-adrenoceptor stimulation are observed. In the absence of changes in the L-type calcium current during this prolongation in rat, cat, and rabbit, attention has focused on the role of a reduction of outward currents and especially the transient outward current in this action potential prolongation. Under current-clamp conditions, action potential prolongation was invariably observed with the α1-adrenoceptor agonist. In voltage-clamp experiments, we failed to observe changes in the Ca++ current (Figure 1), but there were always large reductions of outward currents at plateau potentials in rat ventricular myocytes (Figures 1, 5, 7, and 10), action potential prolongation was unchanged from the control value (Table 1), but a depolarizing effect of 2–4 mV was noted at higher concentrations. This may reflect a significant reduction of the inward rectifier current by α1-agonists. The reduction of transient outward current in rabbit atrium was found to be independent of intracellular Ca++ concentration, because external CdCl2 (see also Figure 1) or 10 mM EGTA in the pipette filling solution had no effect on the current reduction. Similarly, release of intracellular Ca++ from stores as a result of inositol phosphate hydrolysis did not seem important, because inclusion of inositol-1,4,5-trisphosphate or inositol-1,3,4,5-tetrakisphosphate in the pipette filling solution did not affect the transient outward current or the reduction of the current on activation of α1-adrenoceptors. The net decrease of outward current is capable of causing a respectable action potential prolongation as illustrated by the use of 4-AP, a relatively selective blocker of the transient current (Figure 2). This prolongation could cause Ca++ current to flow for longer periods of time during the prolonged action potential plateau to increase the entry of Ca++ into cells, which accumulates during successive cardiac cycles. Further, Ca++ extrusion via Na+-Ca++ exchange will be reduced during the prolonged plateau phase because of its inherent stoichiometry and voltage dependence, which result in an unfavorable driving force for Ca++ extrusion at positive potentials. Hence, the amount of Ca++ released from the SR in response to an action potential is increased. In support of a “calcium entry” mechanism, Miura and Inui showed large changes in action potential duration and increases in contractility in rabbit atrium that were simultaneous in time course (see also Reference 13). Also, the Ca++ entry blockers, Ni++, Mn++, and nifedipine re-
duced or abolished the sustained positive inotropic response to α1-adrenoceptor stimulation. Some care must be taken with the interpretation of some of these earlier results as total abolition of all contractions can result from block of the L-type calcium current (see Reference 63 for an example). Despite this, these observations are all strong evidence that reductions of transmembrane Ca2+ flux can directly modulate the inotropic effect.

Effects of α1-Adrenoceptor Stimulation on Unloaded Cell Shortening

The effects of α1-adrenoceptor stimulation on twitch mechanics in the single cell are similar to those reported for multicellular cardiac tissues. Time to peak shortening (Figure 2) or peak force is delayed, whereas time to 50% relaxation is slightly reduced (Figures 2 and 4) or remains unaffected. Similarly, both peak shortening in isotonically contracting cells (Figures 2, 7, and 10 and Reference 41) and peak force in isometrically contracting muscles are enhanced significantly in the presence of β-adrenoceptor blockade. Our data on the time course of changes in action potential duration and contractility are somewhat different from those of others. We too show an increase in steady-state action potential duration and contractility (Figure 2), but time course data (Figure 3A) reveal that the inotropic effect lags behind the action potential prolongation by a few beats. We suspect that this delay reflects time taken for calcium entering the cell to appear at SR release sites before an increase in contraction becomes apparent. Similarly, when the α1-adrenoceptor agonist is washed out, time is taken for the extra calcium load to be extruded from the cell, presumably via Na2+/Ca2+ exchange. The lag observed before the inotropic effect suggests the involvement of an intermediate step in the inotropic response of cells to α1-adrenoceptor stimulation. This delay in the onset of the positive inotropic effect was not only observed for α1-adrenoceptor stimulation (Figure 3A) but also for the inotropic effects after action potential prolongation with 4-AP (Figure 3B) and a change of voltage-clamp pulse duration (Figure 6). The delay in the development of the inotropic effect can also be seen after immediate changes in the L-type calcium current, plateau height or duration, and pulse duration or after rapid changes in the duration of pulses of Ca2+ on skinned cardiac cells. Other evidence that the SR is the location for an intermediate pool of calcium during α1-adrenoceptor stimulation is that the magnitude (Figure 9) and time course (Figure 3) of the response are related to the degree of action potential prolongation and also that low concentrations of ryanodine (1–10 μM) prevent an inotropic response to α1-adrenoceptor stimulation in isolated perfused rat hearts.

Control Over the Duration of the Activating Event Prevents a Positive Inotropic Effect

Two methods have been used that revealed an important role for action potential prolongation in the inotropic action of α1-adrenoceptors under our experimental conditions. First, an understanding that the major mechanism underlying the action potential prolongation is a reduction of the transient outward current enabled us to use 4-AP to block this current. The resultant action potential prolongation resulted in an inotropic effect (Figures 2C and 2D) similar to that observed with methoxamine alone. An additional important result was that further exposure to 4-AP plus methoxamine had no positive inotropic effect above that of 4-AP alone. In our experiments, these effects were entirely reversible on washout, and failure of the cells to respond to the α1-adrenoceptor agonist was not caused by myofilament overload with Ca2+. Capogrossi et al. have shown that, in 1 mM external calcium, cells are well able to sustain such inotropic interventions. Also, postrest experiments using 4-AP and methoxamine revealed that cells were able to respond inotropically to a short rest (Figure 4), so that the failure of cells to respond to α1-adrenoceptor stimulation in the presence of 4-AP could not be attributed to saturation of the myofilaments of the SR with Ca2+. A more controlled way to prevent changes in action potential shape was to use the voltage clamp (Figures 5–8). This method completely abolished the positive inotropic action of α1-adrenoceptor agonists under our conditions. Short voltage-clamp pulses were deliberately chosen to keep cell calcium loading low and allow the cell to show a large inotropic response. This was manifested as small evoked contractions in control conditions, which allowed a sensitive detection of small changes in released Ca2+ from inside the cell. However, no positive inotropic action of α1-adrenoceptors was observed. Longer voltage-clamp pulses, which allowed enhanced calcium entry, had an immediate and large inotropic effect, but again α1-adrenoceptor agonists had no effect under voltage clamp (Figures 5–7). This is in contrast to the observation of Hartmann et al. who showed that prazosin was able to reduce contractility induced by phenylephrine during 500-msec voltage-clamp pulses. The reasons for the differences between our observations and ours are not clear. In our experiments, this lack of an inotropic response was not due to an effect of low temperature, because similar steady-state results were observed at 35°C (Figure 7), nor was it due to a shift of the voltage dependence of contraction by α1-adrenoceptor agonists as shown by the full current– and contraction–voltage relations in Figure 8. It is possible that species-dependent differences are responsible for the contrasting observations between our data and the data of Hartmann et al. One very important aspect of our experiments is that membrane current was measured and outward current clearly decreased during exposure to methoxamine (Figures 5–8). This provides proof that cells responded to α1-adrenoceptor stimulation with a decrease in outward current, but this could clearly be separated from the inotropic effect by controlling the duration of the voltage event. Many of these data are summarized in the regression plots shown in Figure 9. Under current-clamp conditions (Figure 9A), the magnitude of the inotropic response to α1-adrenoceptor stimulation was well correlated with the change in action potential duration whether this was brought about by the α1-agonist or the drug 4-AP. When the duration of the activating voltage event was closely controlled by the voltage clamp, a small negative inotropic effect resulted from α1-adrenoceptor stimulation, and no correlation was seen between the inotropic changes and the K+ current reduction that still occurred.
A number of authors have noted a biphasic or even triphasic time course to the inotropic response in rat myocardium, usually when using phenylephrine to stimulate \( \alpha_1 \)-adrenoceptors. An initial decrease in contractility is followed by a sustained increase in contraction. This apparently can dissociate the inotropic response from monotonic increases in action potential duration, and it has been suggested that this response results from activation of protein kinase C. In our experiments, when methoxamine was used and rapid solution changes in 1 mM external \( Ca^{2+} \) were made, action potential prolongation occurred very rapidly and was followed soon after by an increase in cell shortening (Figure 3A). Perhaps because of our choice of \( \alpha_1 \)-adrenoceptor agonist or the use of rapid solution changes, we did not observe a negative inotropic phase often described in rat ventricular tissue until the action potential prolonging effect of methoxamine was preempted by 4-AP (Figures 2C, 2D, and 3B) or by using the voltage clamp (Figures 5 and 6). It seems likely that at least two processes are at work. A calcium entry-dependent positive inotropic effect that requires action potential prolongation supercedes with time an early negative inotropic effect that relates to enhanced cellular inositol phospholipid metabolism or increased protein kinase C activity. When the duration of the voltage event was controlled (e.g., see Figures 5–8 and 11) in our experiments, it is possible that the small negative inotropic effect that we observed during voltage-clamp experiments reflects this increased enzyme activity.

**\( \alpha_1 \)-Adrenoceptor Actions on the Calcium Transient**

Two distinct mechanisms for inotropy due to \( \alpha_1 \)-adrenoceptors have arisen from the study of intracellular \( Ca^{2+} \) transients in mammalian cardiac muscle. In beating rabbit papillary muscles, Endoh and Blinks concluded that \( \alpha_1 \)-adrenoceptor stimulation produced a modest increase in the amplitude of the intracellular \( Ca^{2+} \) transient but that the major inotropic response resulted from an increase in the responsiveness of the myocardial contractile apparatus to \( Ca^{2+} \). In single skinned rat cardiac cells studied at 20–22°C, Puccetti et al. have shown that 10 \( \mu M \) phenylephrine can shift the pCa-tension relation to the left by approximately 0.1–0.2 pCa units. Thus, in skinned cells an increase in the \( Ca^{2+} \) sensitivity of the myofilaments can be brought about by \( \alpha_1 \)-adrenoceptor stimulation. However, \( \alpha_1 \)-adrenoceptor activation also changes the size of the intracellular \( Ca^{2+} \) transient. Increases in the systolic transient have been reported using acquirin or indo-1 in rat ventricular myocytes and whole rat hearts. Increases in diastolic \( [Ca^{2+}]_i \) have been recorded in resting cell preparations and indo-1-loaded whole hearts stimulated at 220 beats per minute.

The experiments shown in Figures 10 and 11 attempted to assess the influence of \( Ca^{2+} \) entry on intracellular \( Ca^{2+} \) transients and the inotropic responses to the \( \alpha_1 \)-adrenoceptor agonist. We found clear increases in the intracellular \( Ca^{2+} \) transient that were very similar in magnitude whether the action potential was prolonged by methoxamine or 4-AP (Figures 10 and 11), and both interventions resulted in similar large increases in cell contraction. After the action potential had been prolonged by 4-AP, further addition of methoxamine had little effect on the action potential, and there was no change in the magnitude of the intracellular \( Ca^{2+} \) transient or cell contraction (Figures 11B and 11C). These responses were similar to those observed in non–indo-1–loaded cells (Figure 2D). It is known that \( \alpha_1 \)-adrenoceptor stimulation enhances the breakdown of membrane inositol phospholipids and that this is circumstantially related to the magnitude and time course of the inotropic effect. However, no evidence supports the direct involvement of inositol-1,4,5-trisphosphate in the inotropic effect of \( \alpha_1 \)-adrenoceptor stimulation. Indeed Nosek et al. have reported that 38 \( \mu M \) inositol-1,4,5-trisphosphate had no direct effect on skinned rabbit or rat ventricular muscle in a pCa range similar to that expected during contraction under our experimental conditions (pCa 5.2–6.6). It is possible that \( Ca^{2+} \) released by inositol-1,4,5-trisphosphate might increase either the peak systolic calcium level recorded or influence the resting level of cell \( Ca^{2+} \). Zhu and Nosek have shown that inositol-1,4,5-trisphosphate does not affect \( Ca^{2+} \)-induced \( Ca^{2+} \) release from the cardiac SR and that its role in excitation–contraction coupling is minimal. Inositol-1,4,5-trisphosphate can enhance \( Ca^{2+} \) oscillations and may have a role in arrhythmogenesis. These findings are in keeping with our observations that, in the presence of 4-AP, methoxamine did not enhance the amplitude of intracellular \( Ca^{2+} \) transients. Small changes in diastolic levels of \( [Ca^{2+}]_i \), could not be discriminated using indo-1 AM because of the likelihood of small background signals from the fluorescence of indicator within intracellular organelles (see “Materials and Methods” and Reference 39). In the absence of changes in the intracellular \( Ca^{2+} \) transient, the lack of inotropic effect shown in Figure 11B on exposure to methoxamine argues against the large \( \alpha_1 \)-adrenoceptor–induced increase in the \( Ca^{2+} \) sensitivity of the myofilaments under these experimental conditions. It should be noted that these experiments were performed at room temperature, so we are unable to comment on the importance of \( Ca^{2+} \) sensitivity changes at 37°C. The much larger increase in contraction than in the intracellular \( Ca^{2+} \) transient during exposure to methoxamine (Figure 10) or 4-AP (Figure 11) is interesting and may reflect the nonlinearity of the pCa-tension relation in cardiac muscle, especially at the foot of the curve. It is possible that in 1 mM bathing \( Ca^{2+} \) the cell is positioned at the foot of the pCa-tension relation, where a relatively small increase in \( [Ca^{2+}]_i \), can give much larger changes in contractility. Similar explanations have been used to explain the different sensitivity of cell shortening and the peak amplitude of intracellular \( Ca^{2+} \) transients in rat ventricular cells to changes of extracellular \( Ca^{2+} \) concentration or pulse duration. In a recent study the effect of phenylephrine on contraction and intracellular \( Ca^{2+} \) transients in rat myocytes, also using 1 mM external \( Ca^{2+} \), a doubling of the peak cell shortening resulted in only a 25% increase in the peak amplitude of the intracellular \( Ca^{2+} \) transient (b and c of Figure 2 in Reference 41). A more rapid rate of decay of the intracellular \( Ca^{2+} \) transient was also seen when it was increased by methoxamine or 4-AP (Figures 10 and 11). The fact that this effect on \( [Ca^{2+}]_i \) was induced by both \( \alpha_1 \)-adrenoceptor stimulation and exposure to 4-AP suggests that it may reflect a nonspecific \( [Ca^{2+}]_i \) dependence of \( Ca^{2+} \) sequestration or extrusion from the cell.
rather than an agonist-dependent modulation of the contractile apparatus. Taken together, our indo-1 data completely agree with the recordings made from unloaded cells. In the presence of 4-AP, we were unable to observe a positive inotropic effect of α₁-adrenoceptor stimulation with methoxamine, and there was little effect on the amplitude of the intracellular Ca²⁺ transient.

We conclude that there is an important requirement for action potential prolongation in the positive inotropic action of α₁-adrenoceptors under our experimental conditions. The modulation of K⁺ channels by α₁-adrenoceptors is the major causal factor preceding action potential prolongation (Figures 1–3) and may be separated from inotropy under voltage clamp. The reduction of K⁺ currents does not appear to involve global changes in [Ca²⁺], as was suggested by experiments using intracellular EGTA. Under current clamp, intracellular Ca²⁺ transients recorded with indo-1 increased in a similar manner with exposure to α₁-adrenoceptor agonists or 4-AP for equivalent increases in cell contractility. Thus, our data lead to the conclusion that it is Ca²⁺ entry secondary to action potential prolongation that accounts for a significant fraction of the positive inotropic action of α₁-adrenoceptor agonists under our experimental conditions. Our observation that blockade of the transient outward current with 4-AP or use of the voltage clamp prevented comparable effects of α₁-adrenoceptor activation on action potential duration, unloaded cell shortening, or the underlying intracellular Ca²⁺ transient appears to argue against physiologically important changes in inotropy brought about by changes in the calcium sensitivity of the myofibrils under the present experimental conditions. We feel that further experiments using the voltage-clamp technique to control Ca²⁺ entry are necessary to elucidate the importance of changes in the Ca²⁺ sensitivity of the myofilaments in the inotropic response of cardiac myocytes to α₁-adrenoceptor stimulation. If indeed the inotropic effects depend in an important way on changes of Ca²⁺ entry into cardiac cells, increases in [Ca²⁺], in the failing heart could lead to the arrhythmogenesis known to be associated with activation of α₁-adrenoceptors via activation of Ca²⁺-activated transient inward currents.

Acknowledgments

We particularly thank Dr. W. Giles for his encouragement and support in the execution of this study. We also thank Dr. C. Lamont for her help in the design and manufacture of calibrating solutions for [Ca²⁺] measurements.

References

7. Dickerson RT, Shah R-C, Williford DJ, Sheu S-S: Alpha₁-adrenoceptor stimulation produces a positive inotropic effect which occurs with a decrease in the Ca²⁺ transient and the action potential duration in guinea-pig ventricle. (abstract) Biophys J 1991;59:282a
15. Pappano AJ: Propranolol-insensitive effects of epinephrine on action potential repolarization in electrically driven atria of the guinea-pig. J Pharmacol Exp Ther 1971;177:85–95


56. Fabiato A: Simulated cardiac current can both cause calcium loading and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje fiber. J Gen Physiol 1985;85:291–320

57. Cleemann L, Morad M: Role of Ca²⁺ channel in cardiac excitation-contraction coupling in the rat: Evidence from Ca²⁺ transients and contraction. J Physiol (Lond) 1991;432:283–312


61. Fabiato A: Time and calcium dependence of activation and inactivation of calcium induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje fiber cell. J Gen Physiol 1985;85:247–289


63. Beuckelmann DJ, Wier WG: Mechanism of release of calcium from sarcoplasmic reticulum of guinea-pig cardiac cells. J Physiol (Lond) 1988;405:233–255


68. Zhu Y, Nosek TM: Inositol trisphosphate enhances Ca²⁺ oscillations but not Ca²⁺-induced Ca²⁺ release from cardiac sarcoplasmic reticulum. Pflugers Arch 1991;418:1–6


Mechanisms for the positive inotropic effect of alpha 1-adrenoceptor stimulation in rat cardiac myocytes.
D Fedida and R A Bouchard

Circ Res. 1992;71:673-688
doi: 10.1161/01.RES.71.3.673

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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