Effect of Caffeine on Calcium Flux across the Sarcolemma of Guinea Pig Atrial Trabeculae during Contracture

Chang Kook Suh, Taick Sang Nam, and Bok Soon Kang

The changes in extracellular calcium activities during contractures of guinea pig atrial trabecular muscles were measured with Ca\(^{2+}\)-selective electrodes. When the tissue was superfused with Na\(^{+}\)-free Tyrode solution, extracellular Ca\(^{2+}\) activities were decreased and contractures were induced with some delay. When the contracture was relaxed with Na\(^{+}\)-containing Tyrode solution, extracellular Ca\(^{2+}\) activities were increased transiently and recovered in a Na\(^{+}\)-dependent manner. The magnitude of extracellular Ca\(^{2+}\) activity decreased was proportional to the maximum magnitude of contracture induced by Na\(^{+}\)-free solution. Addition of caffeine (10 mM) to Na\(^{+}\)-free solution induced transient contracture following slow development of contracture and an increase in extracellular Ca\(^{2+}\) activity. Removal of caffeine from Na\(^{+}\)-free solution caused a slow relaxation of contracture and a decrease in extracellular Ca\(^{2+}\) activity. These results confirm that caffeine blocks Ca\(^{2+}\) uptake by the sarcoplasmic reticulum (SR) resulting in an increase in sarcoplasmic Ca\(^{2+}\) activity. Ca\(^{2+}\) activity in the extracellular space, the amount of Ca\(^{2+}\) transported into the cell (Ca\(^{2+}\) depletion in the extracellular space), and the magnitude of contracture are well correlated. Present experiments suggest that extracellular use of Ca\(^{2+}\)-selective electrodes provides continuous and quantitative monitoring of Na\(^{+}\)-dependent Ca\(^{2+}\) flux across the cardiac cell membrane.

**Key Words**: Contracture, Na\(^{+}\)-Ca\(^{2+}\) exchange transport, caffeine, Ca\(^{2+}\)-selective electrodes, guinea pig atrial trabeculae

It has been known that Na\(^{+}\) has an important role in cardiac muscle contractility (Sulakhe & St. Louis 1980; Mullins 1981; Sheu & Blaustein 1986). Ca\(^{2+}\) is moved across the membrane coupled with a Na\(^{+}\) influx, in an electrogenic fashion (normally 3 Na\(^{+}\) versus 1 Ca\(^{2+}\)), depending on the prevailing Na\(^{+}\) electrochemical gradient which is maintained through the active transport of Na\(^{+}\) (Na\(^{+}\)-Ca\(^{2+}\) exchange transport). The current generated by electrogenic Na\(^{+}\)-Ca\(^{2+}\) exchange has been recorded from single myocytes (Kimura et al. 1986; Mechmann & Pott 1986, Kimura et al. 1987).

The Na\(^{+}\)-Ca\(^{2+}\) exchange mechanism has been im-

---

Received July 5, 1988
Accepted October 4, 1988
Department of Physiology, Yonsei University College of Medicine, Seoul, Korea
This work was supported by the Departmental Research Project Fund (1984-1986) from Yonsei University College of Medicine, and by a Research Grant 881-0412-018-2 from Korea Science and Engineering Foundation.
Address reprint requests to Dr. C. K. Suh, Department of Physiology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea

Number 4
and/or by inhibition of Ca" influx (Bodem & Sonnenblick 1975; Chapman & Leoty 1976). In addition to the effects on the SR and mitochondria (Blayney et al. 1978), caffeine has direct or indirect effects on membrane currents. Ca" influx through the cell membrane seems to be potentiated (Kimoto 1972; Niedergerke & Page 1981) or inhibited (Eisner et al. 1979) or unchanged (Ohba 1973) by caffeine, probably depending on the preparation. Caffeine modulation of the Ca" sensitivity of a contractile system has also been suggested by results obtained from skinned or hyperpermeable preparations (Fabiato & Fabiato 1973; Endo & Kitazawa 1978; McClellan & Winegrad 1978; Wendt & Stephenson 1983; Eisner & Valdeolmillos 1985).

In contrast to caffeine-induced transient contracts (Chapman & Leoty 1976), other studies have found that caffeine does not produce a contracture when applied under normal conditions (Blinks et al. 1972; Chapman & Miller 1974; Jundt et al. 1975; Busselen & van Kerkhove 1978). However, if external Na" is removed or the membrane is depolarized, the subsequent addition of caffeine produces a contracture (Chapman & Miller 1974; Jundt et al. 1975; Busselen & van Kerkhove 1978). This result has been explained by suggesting that, under normal conditions, any Ca" released from the SR by caffeine can be transported out of the cell via a sarcolemmal Na"-Ca" exchange. If the preparation is depolarized or exposed to a Na"-free solution, this Ca" efflux is decreased and the Ca" released by caffeine likely activates contracture. Similarly, when the ability of the Na"-Ca" exchange to extrude Ca" is reduced by an increase in the intracellular Na" concentration, depolarization increases the resting or tonic force (Eisner & Lederer 1979). The addition of caffeine markedly increases this component of force (Eisner & Lederer 1982; Karagueuzian & Katzung 1982).

In view of the foregoing ideas, direct measurements of Ca" fluxes across the sarcolemma via Na"-Ca" exchange and examination of the contribution of Ca" flux to contracture are worthwhile. The development of Ca"-selective electrodes has made it possible to continuously measure the Ca" flux across the sarcolemma (Suh & Sleator 1982; Bers & MacLeod 1986; Suh et al. 1987).

In this study, extracellular Ca" activities of guinea pig atrial trabeculae were measured with Ca"-selective electrodes and the changes in extracellular Ca" were correlated with contractures induced by Na"-deficient solutions. The effects of caffeine on contractile strength along with Ca" movements across the sarcolemma during the contracture was investigated.

**METHODS**

Guinea pigs of either sex ranging in size from 200 to 500 grams were sacrificed with a blow to the head. The heart was rapidly excised and placed in oxygenated (100% O") Tyrode solution. To reduce diffusion delay, which seems to be essential to observe caffeine contracture in cardiac preparation (Chapman & Leoty 1976), thin trabecular muscles of the left atrium were dissected under a microscope and glued with surgical silk threads (7/0 Mersilk, Ethicon) and then transferred to a recording chamber which was continuously perfused with the oxygenated Tyrode solution. Tension was monitored by a Model 400A Force Transducer System (Cambridge Technology, Inc.) and displayed on a Philips PM 3305 digital storage oscilloscope and on a Gould Brush 220 recorder along with the signal from the differential electrometer for the Ca"-selective electrode (E")]. The temperature in the recording chamber was maintained at 27.0±0.2°C. The Tyrode solution had the following composition in millimoles per liter (mM): NaCl 133.5; KCl 4.0; KH₂PO₄ 0.3; MgSO₄ 7H₂O 1.2; CaCl₂ 1.8; Tris 10; dextrose 10. For Na"-deficient solutions, NaCl was isotonically replaced with tetramethylammonium (TMA) chloride since the ability of the cardiac tissue to withstand multiple exposure to Na"-deficient solutions was greatest among Na" substitutes (Chapman et al. 1983). The flow rate of superfusion solutions was kept constant (about 5 ml/minute) in such a way that the solution levels of the solution containers were maintained constant with spring-aided small supports.

Ca"-selective electrodes and reference electrodes were manufactured as described previously (Suh, et al. 1987) with some modifications for extracellular recordings. The tip of the microelectrode, which was beveled with a Narishige EG-3 microgrinder and a Narishige MF-83 microforge, had an opening of 2 to 5 μm in diameter. The resin used for the Ca"-selective electrodes was provided by Professor W. Simon. The mixture was that of Oehme et al. (Oehme et al. 1976). Polyvinylchloride (PVC), approximately 10% by weight, was added to the mixture for better stability of the Ca"-selective electrodes.

The e.m.f. from the Ca"-selective electrode was measured with an electrometer (Analog Devices 311 J) or AD515 operational amplifier, Norwood, MA U.S.A., and the membrane potential measured by the reference electrode was electronically subtracted from the e.m.f. measured with the Ca"-selective electrode.
Each Ca²⁺-selective electrode was tested for response before and after the experiments, and was equilibrated in the Tyrode solution for at least one hour before being used. The Ca²⁺ activity coefficient of 0.32 was used to calculate Ca²⁺ activity in the solution (Lee 1981).

RESULTS

Prior to testing the effect of caffeine, the competence of the experimental technique to detect the changes in extracellular Ca²⁺ by Ca²⁺-selective electrodes was determined by measuring extracellular Ca²⁺ activities (αCa) and contractile strength of the left atrial trabecular muscle stimulated at rate of 2 contractions per second (Fig. 1). When the rest intervals were given, extracellular Ca²⁺ activities started to increase and reached a maximum level in approximately 30 seconds (about 1.7 mV, which is equivalent to 330 μM Ca²⁺ activity change). As the rest interval lasted longer, Ca²⁺ activity slowly decreased to the basal level. When the tissue was stimulated again, the contractile strength increased to that of the steady-state contractions, while extracellular Ca²⁺ activities decreased. These measurements were done before the main (contracture) experiments, which assured the position of the Ca²⁺-selective electrodes in the extracellular space (Suh et al. 1987).

The effects of Na⁺ concentration in the superfusion solutions on muscle contractility and extracellular Ca²⁺ activity were measured. Fig. 2 shows a series of recordings of extracellular Ca²⁺ activities and contractility induced by different concentrations of Na⁺ in the superfusion solution, which also contained 10 mM caffeine to induce strong contraction. When the tissue was superfused with Na⁺-free, caffeine (10mM) containing Tyrode solution, Ca²⁺ activity in the extracellular space started to decline and with some delay the contracture was induced. As the superfusion of Na⁺-free solution continued, αCa remained decreased (3.9mV; 690μM) and the contracture reached the maximum contractility (2.6mN) and spontaneously relaxed on occasion. When the tissue was superfused with 100% Na⁺ solution, the contracture was relaxed accompanying an increase in αCa (4.1 mV; 760 μM) which was decreased to the basal level with about 1 minute’s delay, as the superfusion of 100% Na⁺ solution continued. When 50% Na⁺-solution was superfused following the contracture induced with Na⁺-free solution, αCa did not increase as much as when superfused with 100% Na⁺ (2.4 mV; 420 μM), although the contracture was relaxed. When an additional superfusion of 100% Na⁺ solution was followed, αCa was increased back to the level (0.9 mV; 220 μM) which was reached with 100% Na⁺-solution alone.

On the other hand, when the tissue was superfused with 50% Na⁺-solution following the relaxation with 100% Na⁺-solution, αCa was decreased (2.5 mV; 250 μM) but not as much as when superfused with Na⁺-free solution and no visible contracture was recorded. However, when the tissue was again superfused with

![Fig. 1. Changes in extracellular Ca²⁺ activity during rest period.](image)

Horizontal scale = 10 seconds. Vertical scale = 5 mV for $E_{Ca}$ (upper trace); 10 mN for tension (lower trace). The lower bar of the horizontal scale also represents the level of Ca²⁺ activity in the superfusion solution.
Fig. 2. Changes in extracellular Ca\(^{2+}\) activity during contractures induced with different concentrations of Na\(^{+}\). Horizontal scale = 2 minutes. Vertical scale = 5 mV for \(E_{Ca}^{0}\) (upper trace); 5 mN for tension (lower trace). The horizontal bars (between upper and lower traces in both A and B) represent the level of Na\(^{+}\) activity (0, 50, 100 from the top) in the superfusion solutions.

Fig. 3. Ca\(^{2+}\)-dependence of changes in extracellular Ca\(^{2+}\) activity during contracture. Horizontal scale = 2 minutes. Vertical scale = 5 mV for \(E_{Ca}^{0}\) (upper trace); 5 mN for tension (lower trace). The horizontal bars (between upper and lower traces) represent the periods when Na\(^{+}\)-free solutions with different concentrations of Ca\(^{2+}\) (A: 1.0 mM; B: 2.5 mM; C: 5.0 mM Ca\(^{2+}\)) were superfused. The superfusion with 1.0 mM Ca\(^{2+}\) solution induced greater \(E_{Ca}^{0}\) changes than with higher concentrations of Ca\(^{2+}\).
Table 1. Extracellular Ca\(^{2+}\) depletion and magnitude of contracture

<table>
<thead>
<tr>
<th>Na(^{-})-free solutions</th>
<th>(E_{\text{Ca}}) (mV)</th>
<th>Ca(^{2+}) depletion ((\mu\text{M}))</th>
<th>Contracture (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Ca(^{2+})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mM</td>
<td>4.3±0.1</td>
<td>345±2</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>2.8±0.2</td>
<td>465±34</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>5.0 mM</td>
<td>1.9±0.1</td>
<td>593±32</td>
<td>2.6±0.1</td>
</tr>
<tr>
<td>B. Caffeine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mM</td>
<td>7.8±0.6</td>
<td>464±42</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>10 mM</td>
<td>8.9±0.1</td>
<td>446±37</td>
<td>2.2±0.2</td>
</tr>
</tbody>
</table>

* Due to valiant and deteriorating conditions of the tissue during the contracture-relaxation cycles, the number of data collected from the single preparation was limited to n=4. Numbers represent mean±S.D.

Na\(^{-}\)-free solution, \(a_{\text{Ca}}\) was further decreased (1.1 mV; 170 \(\mu\text{M}\)) and the contracture (2.6 mN) was induced. The magnitude of contracture induced with Na\(^{-}\)-free solution following 50% Na\(^+\) solution was the same as that induced with Na\(^{-}\)-free solution alone and the rising phase of contracture was faster with the pretreatment of 50% Na\(^+\) solution.

Na\(^{-}\)-dependent changes in \(a_{\text{Ca}}\) were further studied by changing the Ca\(^{2+}\) concentration of the superfusion solutions (Fig. 3 and Table 1). When the tissue was superfused with Na\(^{-}\)-free, 2.5 mM Ca\(^{2+}\) solution, the magnitude of decrease in \(E_{\text{Ca}}\) was 2.8±0.2 mV, which was equivalent to about 465±34 \(\mu\text{M}\), and the magnitude of contracture induced was 2.5±0.3 mN. When the Ca\(^{2+}\) concentration in the superfusion solutions was raised to 5.0 mM, the change in \(E_{\text{Ca}}\) was only 1.9±0.1 mV, which is much smaller than that with 2.5 mM Ca\(^{2+}\). However, this recorded electrical signal was equivalent to a change in \(a_{\text{Ca}}\) of 593.0±32 \(\mu\text{M}\), and the magnitude of contracture was 2.6±0.1 mN. When superfused with Na\(^{-}\)-free solutions containing 1.0 mM Ca\(^{2+}\), the change in \(E_{\text{Ca}}\) was 4.3±0.1 mV, which is equivalent to 345±2 \(\mu\text{M}\) and the magnitude of induced contracture was 1.8±0.1 mN.

The maximal strengths of contractures from different preparations, which were induced by Na\(^{-}\)-free solution with various Ca\(^{2+}\) concentrations, were plotted against the accompanying changes in \(a_{\text{Ca}}\) (Fig. 4). The contractile strengths were increased as the decrease in \(a_{\text{Ca}}\) got larger. As the more extracellular Ca\(^{2+}\) was depleted, the strength of the corresponding contracture got stronger in a proportionate manner.

In order to look into the effect of caffeine on extracellular Ca\(^{2+}\) depletion and tension, the contracture and accompanying extracellular Ca\(^{2+}\) depletion induced by Na\(^{-}\)-free solution alone were compared with those induced with caffeine-containing Na\(^{-}\)-free solution (Table 1). The magnitude of contracture induced without caffeine was about 50% of that induced with caffeine (1.1±0.1 mN vs. 2.2±0.2 mN) and the extracellular Ca\(^{2+}\) depletion was larger without caffeine (464±42 \(\mu\text{M}\) vs. 446±37 \(\mu\text{M}\)), but not significantly. The effect of caffeine during contracture was further measured with successive superfusion of Na\(^{-}\)-free solution and caffeine-containing solution (Fig. 5). When superfused with Na\(^{-}\)-free caffeine (10 mM) solution, a strong contracture was induced as normally seen. While the contracture was sustained, an additional superfusion of Na\(^{-}\)-free solution without caffeine induced slow relaxation of contracture along with an additional depletion of \(a_{\text{Ca}}\) (Fig. 5-A). When the tissue was superfused with Na\(^{-}\)-free solution without caffeine, a much weaker contracture was slowly developed and depletion of \(a_{\text{Ca}}\) was sustained for longer than 5 min, compared to about 1 min with caffeine containing solution (Fig. 5-B). When caffeine (10 mM) was added into the Na\(^{-}\)-free solution, fast and strong contracture was developed in 1 min and relaxed spontaneously. The addition of caffeine also caused a slow but significant increase in \(a_{\text{Ca}}\) until the contracture already started to relax (Fig. 5-B).
**DISCUSSION**

There is a universal agreement that the excitation-contraction coupling of cardiac muscle is completed by the release of Ca$^{2+}$ into the sarcoplasm of cardiac cells, and that Ca$^{2+}$ reacts with the contractile proteins to produce contraction.

In mammalian cardiac muscle, a small but significant amount of Ca$^{2+}$ enters the myocardial cells from the extracellular space via a slow inward current and outward (reverse) mode of Na$^+$-Ca$^{2+}$ exchange during the plateau phase of the action potential (Reuter 1979; Noble 1984; Fedida et al. 1987). This Ca$^{2+}$ is not immediately available for contraction, but can replenish or augment the amount of Ca$^{2+}$ in intracellular stores (Winegrad 1979). As the depolarization occurs, mammalian cardiac muscle is activated primarily by Ca$^{2+}$ released from the SR (Chapman 1983; Winegrad 1979). The mechanism by which depolarization activates Ca$^{2+}$ release from the SR is not well known, but there is evidence that a Ca$^{2+}$-induced Ca$^{2+}$ release mechanism may be involved in cardiac muscle.

---

**Fig. 5.** Effect of caffeine on extracellular Ca$^{2+}$ activity and contracture.

Horizontal scale = 2 minutes. Vertical scale = 5 mV for $E_{Ca}$ (upper trace); 5 mN for tension (lower trace). The horizontal bars (between upper and lower traces) represent the level of caffeine (10 mM, upper; 0 mM, lower) concentration in the superfusion solutions. A: When superfused with Na$^+$-free caffeine (10 mM) solution, strong contracture was induced as normally seen. While the contracture was sustained, an additional superfusion of Na$^+$-free solution induced slow relaxation of contracture along with an additional depletion of $a_{Ca}$. B: When the tissue was superfused with Na$^+$-free solution without caffeine, a much weaker contracture was slowly developed and depletion of $a_{Ca}$ was sustained for longer than 5 min, compared to about 1 min with the caffeine containing solution. When caffeine (10 mM) was added into the Na$^+$-free solution, fast and strong contracture was developed in 1 min and relaxed spontaneously. Addition of caffeine also caused a slow but significant increase in $a_{Ca}$ until the contracture already started to relax.
Na*-dependent Ca** Flux across the Cardiac Cell Membrane

(Fabiato 1983; Fabiato 1985ab).

Since Ca** enters the cardiac cell during each action potential, to attain a steady-state there must be a mechanism to get Ca** out of the cell. Although a Ca**-activated ATPase in SR vesicles has been shown to be associated with the Ca** pump, this has not been clearly identified in cardiac sarcolemma. Thus, the Ca** efflux across the sarcolemma is believed to be accomplished by means of a Na*-Ca** exchange transport (Mullins 1981; Sheu & Blaustein 1986).

The contribution of the Na*-Ca** exchange transport to the maintenance of a low Ca** concentration in the sarcoplasm (and a relaxed state of the contractile system) was shown by measuring a net release of radioisotope 43Ca** and resting tension from isolated guinea pig auricles under conditions known to change the intracellular free Ca** activity (Jundt et al. 1975). The rate of net efflux of Ca** from the cell, in the presence of caffeine, depends upon the Na* concentration of the bathing medium. Recent development of Ca**-selective electrodes has made it possible to continuously measure the Ca** flux across the sarcolemma (Suh & Sleator 1982; Bers & MacLeod 1986; Suh et al. 1987). Ca** sensitive dyes were also used to measure Ca** changes in extracellular space of mammalian cardiac muscle (Hilgemann et al. 1983; Hilgemann 1986). Both of these approaches have indicated that the cumulative Ca** depletions (and accumulations) observed can be accounted for by Ca** uptake (and release) by the SR. After a long rest period the SR is depleted of Ca** and resumption of stimulation will increase Ca** influx and progressively refill the SR. This will require net movement of Ca** from the extracellular space into the cell and will decrease extracellular Ca** activity (Ca** depletion) as shown in Fig. 1. When Ca** continuously leaks from the SR during the resting state (Kitazawa 1984; Bridge 1986), or the SR releases all of its Ca** to the sarcoplasm and cannot resequester this Ca** by pharmacological agents (Fabiato & Fabiato 1973), some of that Ca** will be extruded from the cell and increase extracellular Ca** activity (Ca** accumulation), in a Na*-dependent fashion. This is clearly shown in Fig. 1 and 2.

Previously, the amount of Ca** transported across the sarcolemma into the cell was also estimated from the extracellular Ca** depletion caused by steady-state contractions (Bers 1983; Suh & Sleator 1983). However, the slow time response of Ca**-selective electrodes (compared to the time course of action potential) complicates the interpretation of extracellular Ca** changes during excitation. Measurements of Ca** flux across the sarcolemma during the resting state can minimize this problem and the Ca** flux during the resting state can be enhanced with greater efficiency of Na*-Ca** exchange which is dependent on the Na* gradient across the sarcolemma. In this study, all of the Na* was removed from the superfusion solution to have Na*-Ca** exchange work in the outward mode: Ca** influx coupled with Na* efflux. As Ca** is transported into the cell, extracellular Ca** is depleted and intracellular Ca** activity is raised to induce an increase in tonic tension (Na* withdrawal contracture). If some of the Ca** that enters the cell is taken up by the SR, as reported in mammalian trabeculae (Chapman et al. 1983), the blockage of SR sequestering activity with caffeine would enhance the Na* withdrawal contracture, as discussed later.

The magnitude of change in $E_{\text{Ca}}$ is a function of Ca** activity in the extracellular space:

$$E_{\text{Ca}} = s \cdot \log (1 + \Delta a_{\text{Ca}}^*/a_{\text{Ca}}^*)$$

where $s$ is a calibration constant (about 30 mV), and $a_{\text{Ca}}^*$ and $\Delta a_{\text{Ca}}^*$ are Ca** activity and its change, respectively. Assuming the changes in extracellular Ca** activity ($\Delta a_{\text{Ca}}^*$) do not vary to a large extent in different background Ca** activity ($a_{\text{Ca}}^*$), lower Ca** activity in the superfusion solution yields larger recorded $E_{\text{Ca}}$ than higher activity (Fig. 3). These results, along with the Na*-dependence of $a_{\text{Ca}}^*$ in Fig. 2 strongly support the empirical justification of this study: extracellular recordings of Ca** flux with Ca**-selective electrodes.

With Na*-Ca** exchange transport maximized, the amount of Ca** transported is determined by the amount of Ca** available in the extracellular space. The amount of Ca** transported determines the magnitude of contracture, in a proportionate way (with the physiological range), if intracellular stores do not take up any fraction of the Ca** which entered. These three parameters, namely Ca** activity in the extracellular space, the amount of Ca** transported into the cell (Ca** depletion in the extracellular space), and the magnitude of contracture are well correlated as shown in Fig. 4.

It does not appear that caffeine has a direct effect on the Ca** flux across the sarcolemma, since $a_{\text{Ca}}^*$ is increased when the addition of caffeine to Na*-free solution induces strong transient contracture (Fig. 5). The removal of Na* increases Ca** influx via Na*-Ca** exchange and consequently increases sarcoplasmic Ca** activity which leads to the induction of contracture. Meanwhile, the SR also takes up Ca** from the sarcoplasm and lessens the amount of Ca** available for tension generation. Thus, Ca** keeps being transported into the cell depleting extracellular Ca** as shown in this study and the resting tension con-
tinuously increases. The blockade of the SR Ca\textsuperscript{2+} uptake by caffeine further increases the sarcoplasmic Ca\textsuperscript{2+} activity and enhances the available amount of Ca\textsuperscript{2+} for tension generation. Since the operating mode of Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange is also dependent upon sarcoplasmic Ca\textsuperscript{2+} activity and Na\textsuperscript{+} may not be completely removed from the extracellular space, it is possible that Ca\textsuperscript{2+} is transported out by Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange under the condition that induces Na\textsuperscript{+}-free contracture. When caffeine is removed from the Na\textsuperscript{+}-free solution, extracellular Ca\textsuperscript{2+} activity transiently decreases under the condition of slow relaxation of contracture. It could happen if the SR sequesters and lowers sarcoplasmic Ca\textsuperscript{2+} activity to the level where Ca\textsuperscript{2+} is transported into the cell under Na\textsuperscript{+}-free conditions. This explanation is persuasive if Ca\textsuperscript{2+} kinetics among intracellular binding sites, e.g. the SR and myofilaments and sarcoplasm are known. Direct recordings of intracellular Ca\textsuperscript{2+} activity during contractures are needed for further information.

The substitution of Na\textsuperscript{+} with TMA might cause potential offsets of the difference signal from the Ca\textsuperscript{2+}-selective electrode and reference electrode, which originates from the different selectivity coefficients of Ca\textsuperscript{2+}-resin toward Na\textsuperscript{+} and TMA. The magnitude of potential offsets is a few mV in negative and may cause overestimation of Ca\textsuperscript{2+} depletion and underestimation of Ca\textsuperscript{2+} accumulation. To make the situation more complicated, the flow rate of superfusion solutions, which seems to be one of the important factors to induce contracture (Chapman 1983) determines the degree of Ca\textsuperscript{2+} activity changes such as Ca\textsuperscript{2+} accumulation following Ca\textsuperscript{2+} efflux. Thus the quantification of changes in Ca\textsuperscript{2+} activity needs careful measurements and interpretation of recorded data.

The present experiments do not provide direct information about the quantification of Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange transport. If the changes in $a_{Ca}$ brought about by the [Na\textsuperscript{+}] and [Ca\textsuperscript{2+}] in the superfusion solutions is to be interpreted as a movement of Ca\textsuperscript{2+} across the cell membrane, the net Ca\textsuperscript{2+} influx can be calculated from the following equation

$$\text{net Ca}^{2+} \text{ influx} = K_i \cdot [\text{Ca}^{2+}] \cdot V_o / A$$

where $K_i$ is an influx rate constant, $V_o$ is the volume of extracellular space, and $A$ is the surface area of the cells. The influx rate constant $K_i$ is the initial rate of $E_{Ca}$ change assuming there is no Ca\textsuperscript{2+} efflux during the period, and with the information on extracellular space to cell surface area, the net Ca\textsuperscript{2+} influx can be estimated. However, it does appear that Ca\textsuperscript{2+} activity in the the extracellular space, the amount of Ca\textsuperscript{2+} transported into the cell (Ca\textsuperscript{2+} depletion in the extracellular space), and the magnitude of contracture are well correlated. At this point it is reasonable to say that the measurement of extracellular Ca\textsuperscript{2+} activity with Ca\textsuperscript{2+}-selective electrodes provides continuous and direct monitoring of Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} flux across the cell membrane.

ACKNOWLEDGMENT

The authors thank Prof. D.H. Kang for reading the manuscript and for thoughtful discussion.

REFERENCES


Bridge JB: Relationships between the sarcoplasmic reticulum and sarcolemmal calcium transport revealed by rapidly cooling rabbit ventricular muscle. J Gen Physiol 88:437-473, 1986


Eisner DA, Lederer WJ: Effects of caffeine on the transient
Na⁺-dependent Ca²⁺ Flux across the Cardiac Cell Membrane

Eisner DA, Lederer WJ, Noble D: Caffeine and tetracaine abolish the slow inward current in sheep cardiac Purkinje fibres. J Physiol 293:76-77, 1979
Fabiato A: Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol 245:C1-C14, 1983
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 cell. J Gen Physiol 85:247-289, 1985a
Fabiato A: Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J Gen Physiol 85:291-320, 1985b
Hilgemann DW: Extracellular calcium transients at single excitation in rabbit atrium measured with tetramethylruthenide. J Gen Physiol 87:707-735, 1986
Sheu S.S., Blaustein MP: Sodium/calcium exchange and regulation of cell calcium and contractility in cardiac muscle, with a note about vascular smooth muscle. In Fozzard et al., eds. The Heart and Cardiovascular System, New York, Raven Press, 1986, 509-535
Wendt IR, Stephenson DC: Effects of caffeine on Ca⁺⁺-activated force production in skinned cardiac and skeletal muscle fibres of the rat. Pflugers Arch 398:210-216, 1983
Winegrad S: Electromechanical coupling in heart muscle. In Handbook of Physiology, the cardiovascular system. Am Physiol Soc 1979, 393-428