Sodium and Potassium Sensitivity of Calcium Uptake and Calcium Binding by Dog Cardiac Microsomes

By Arnold M. Katz, M.D., and Doris I. Repke, C.T.

ABSTRACT

Concentrations of KCl and NaCl below 1.0 M increased Ca uptake (in the presence of oxalate) by dog cardiac microsomes purified on sucrose density gradients; higher concentrations of these salts decreased Ca uptake. At lower microsome concentrations, where calcium uptake was maximal, replacement of KCl by equimolar amounts of NaCl decreased both the rate and extent of Ca uptake. The uptake of Ca in mixtures of NaCl and KCl was that expected from the presence of each cation alone, and bore no obvious relationship to the (Na+ + K+)-activated ATPase associated with sodium transport. Ca binding (in the absence of oxalate) was increased by concentrations of NaCl and KCl up to approximately 0.12 M when the reaction mixture contained 5 mM ATP; higher concentrations of the alkali-metal salts reduced Ca binding. A similar dual action of Na+ and K+ on Ca binding was seen in the absence of ATP, but Ca binding was maximal at approximately 0.01 M KCl. The reduced Ca binding at high concentrations of KCl in the presence of ATP showed some characteristics suggesting competition between K+ and Ca2+ for a single binding site, but double reciprocal plots of the data did not meet the strict criteria for a classical competitive reaction. Thus, while these microsomal membranes show some of the properties of an ion exchanger, these interactions are more complex, in that, at low ionic strengths, Ca binding is facilitated by addition of alkali-metal salts. Ca binding was not altered by replacing K+ with Na+, and specific competition between Na+ and Ca2+ was not seen in deoxycholate-treated or sonicated microsomes. Thus, the site for the proposed Na+-Ca2+ competition that controls myocardial contractility has not been found.

ADDITIONAL KEY WORDS excitation-contraction coupling sarcoplasmic reticulum cardiac muscle myocardial contractility

Cardiac performance is regulated primarily by variations in end-diastolic fiber length (the Frank-Starling relationship) and by changes in myocardial contractility which alter the capacity of heart muscle to do work (1). The functionally important variations in myocardial contractility most likely represent modulations in the activity of the contractile proteins of the heart (2, 3), and can be brought about by a number of different physiological, hormonal and pharmacological procedures. Several investigators have presented evidence that suggests that variations in the amount of intracellular calcium ion released by the sarcoplasmic reticulum during excitation-contraction coupling may be the mechanism responsible for the regulation of myocardial contractility (4-6).

Many of these inotropic changes are associated with a loss of intracellular potassium ion (1, 7, 8), which appears to be exchanged for sodium ion that enters from the extra-
cellular fluid (9, 10). We have, therefore, examined the effects of Na\(^+\) and K\(^+\) on the calcium binding and calcium uptake\(^1\) of cardiac microsomal vesicles, which presumably are derived largely from the sarcoplasmic reticulum.

**Methods**

The dog cardiac microsomes were isolated by differential ultracentrifugation, and purified on sucrose density gradients by methods described previously (11). The uptake of \(^{45}\text{Ca}\) by purified microsomes\(^2\) (0.01 to 0.05 mg/ml) was measured in the presence of 2.5 mM Tris oxalate. Calcium binding was determined at higher concentrations of microsomal protein in the absence of oxalate. The \(^{45}\text{Ca}\) remaining in microsome-free filtrates, prepared by filtration through 13 mm HA (0.45 μl) Millipore filters, was determined in a liquid scintillation spectrometer (11). In some experiments, microsomes were collected by centrifugation at 145,000 \(\times \) g for 30 min. The microsomal pellet was washed twice with a small volume of buffered 0.3 M sucrose, the microsomes being collected by ultracentrifugation (12). The microsomal protein was precipitated with trichloroacetic acid and the radioactivity of the supernatant fraction counted in Bray's solution \(^3\) in the liquid scintillation spectrometer. This method was used instead of the Millipore filtration method when microsomal protein concentrations exceeded 0.2 mg/ml.

Deoxycholate-treated microsomes were prepared by adding 2% deoxycholate to the sucrose-histidine solution immediately before homogenization of the ventricles. In some experiments, microsomes were sonicated in a Branson Sonifier, model S75, operated with a current of 7 amp. In the studies on deoxycholate-treated and sonicated microsomes, the purification on the sucrose density gradient was omitted.

All chemicals used were reagent grade. Tris\(^3\) ATP was prepared by passage of sodium ATP (Sigma Chemical Co.) through Dowex 50 in the H\(^+\)-form followed by neutralization with Tris. Distilled water was deionized and redistilled in glass prior to use.

\(^1\)In the present study, *calcium binding* refers to the ability of microsomes to bind calcium in the absence of oxalate; *calcium uptake* or *calcium accumulation* signifies the sequestration of calcium as calcium oxalate from solutions containing oxalate.

\(^2\)Microsome concentrations are expressed as milligrams of microsomal protein.

\(^3\)Tris = tris (hydroxymethyl) amino methane.

**Results**

**EFFECTS OF NaCl AND KCl ON CALCIUM UPTAKE**

The rate of calcium uptake by purified cardiac microsomes was increased by low concentrations of both NaCl and KCl (Fig. 1), whereas higher concentrations of these salts, above approximately 1.0 M, inhibited calcium uptake. Within the lower range of salt concentration, KCl caused greater enhancement of both the initial rate of calcium accumulation and the maximal amount of calcium taken up by the microsomes (Fig. 2). The optimal concentration of KCl was slightly higher than that of NaCl (Fig. 1). At concentrations of microsomes above approximately 0.05 mg/ml, where calcium uptake becomes reduced (11), these effects were reversed and calcium uptake was higher in the presence of Na\(^+\) (Table 1). The latter finding is of doubtful significance, however, because of the impaired calcium uptake.

Comparison of the three major fractions obtained by ultracentrifugation of crude microsomes on density gradients made with

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**FIGURE 1**

Effects of NaCl and KCl on the rate of calcium uptake by purified cardiac microsomes. The amount of calcium taken up after 4 min, approximately half the time required for maximal calcium uptake by 0.028 mg/ml cardiac microsomes, was measured in the presence of varying concentrations of KCl (○) and NaCl (●) in 0.033 mM \(^{45}\text{CaCl}_2\), 2.5 mM Tris oxalate, 5.0 mM MgATP, and 10 mM histidine, pH 7.0 at 25°C. Microsome concentrations are expressed as milligrams of microsomal protein.

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20, 45, and 60% sucrose showed that the top layer, layer I, which corresponds to the purified microsomes (11), had the greatest sensitivity to replacement of K⁺ with Na⁺ (Fig. 3). The middle layer, layer II, showed little or no differential Na⁺-K⁺ sensitivity while the lowest layer, layer III, was without calcium-accumulating ability in either salt.

The possibility that mixtures of NaCl and KCl could potentiate calcium uptake was examined in experiments in which the total concentration of KCl and NaCl was maintained constant, but the proportions of the alkali-metal ions varied. The rates of calcium uptake in the presence of such mixtures of NaCl and KCl failed to show additional effects that could be attributed to the presence of both ions together (Fig. 4).

### EFFECTS OF NaCl AND KCl ON CALCIUM BINDING IN THE PRESENCE OF ATP

At concentrations below approximately 0.12 M, KCl increased the amount of calcium bound to the cardiac microsomes in the presence of ATP (Fig. 5). However, at concentrations above 0.12 M, KCl had no further effect on calcium binding (Fig. 6). These results suggest that the interaction of NaCl and KCl with the cardiac microsomes is complex and may involve both direct and indirect effects on calcium binding. The exact nature of these interactions requires further investigation.
of ATP (Fig. 5). At higher KCl concentrations, however, calcium binding was reduced. These differences, though slight, occurred with a number of microsomal preparations. Comparison of the effects of NaCl and KCl showed no consistent differences in either the increase at lower alkali-metal ion concentrations or the decrease at the higher salt concentrations (Fig. 6). At the level of alkali-metal salt where calcium binding was maximal, no significant effects of replacement of K⁺ by Na⁺ upon either the calcium-binding constant or number of calcium-binding sites were apparent (Fig. 7). Studies of deoxycholate-treated (Table 2) and sonicated (Table 3) microsomes showed no significant differences in the effects of NaCl and KCl on calcium binding.

**FIGURE 4**
Effect of varying mixtures of KCl and NaCl on the rate of calcium uptake of purified cardiac microsomes. In all reactions the total concentration of K⁺ plus Na⁺ was 0.06 M. The calcium uptake of 0.015 mg/ml microsomes was measured in 0.003 mM ⁴⁰CaCl₂, 5.0 mM MgATP, 2.5 mM Tris oxalate and 10 mM histidine, pH 7.0 at 25°C. The vertical lines represent ± 1 standard deviation of the mean of 4 determinations.

**FIGURE 5**
Effects of KCl on calcium binding by cardiac microsomes in the presence of ATP. The binding of 5 μM ⁴⁰CaCl₂ by 0.20 mg/ml purified microsomes was measured at various concentrations of KCl in 5.0 mM MgATP, 2.5 mM Tris oxalate and 10 mM histidine, pH 7.0 at 25°C. The vertical lines represent ± 1 standard deviation of the mean of 4 determinations.

**FIGURE 6**
Comparison of the effects of KCl and NaCl on the calcium binding by cardiac microsomes in the presence of ATP. The binding of 5 μM ⁴⁰CaCl₂ by 0.20 mg/ml purified cardiac microsomes was measured at various concentrations of KCl (•) and NaCl (○) in 5.0 mM MgATP and 10 mM histidine at pH 7.0.

**FIGURE 7**
Double reciprocal plot of calcium binding by cardiac microsomes versus free calcium concentration in the presence of 0.12 M KCl (○) and 0.12 M NaCl (●). Reactions were carried out in 5.0 mM MgATP and 10 mM histidine at pH 7.0 with 0.1 mg/ml microsomes at 25°C.

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3) microsomes failed to demonstrate a site for Na\(^+\),Ca\(^{2+}\) competition that might have existed on the "inside" of the microsomal vesicle. A double reciprocal plot (11) in which 
\[
\frac{1}{[\text{Ca}^{2+}]_{\text{bound}}} \quad \text{versus} \quad \frac{1}{[\text{Ca}^{2+}]_{\text{free}}}
\]
showed some of the characteristics of competitive binding for a single site in that the differences between the two curves were less at the higher calcium concentrations (Fig. 8). Because the lines failed to intersect where 
\[
\frac{1}{[\text{Ca}^{2+}]_{\text{free}}} = 0
\]
however, the strict criteria for competition at a single binding site were not met.

**TABLE 2**

<table>
<thead>
<tr>
<th>Calcium Binding by Deoxycholate-Treated Microsomes</th>
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<td>Microsomes</td>
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<tr>
<td>Control</td>
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<tr>
<td>Deoxycholate-</td>
</tr>
<tr>
<td>treated</td>
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</table>

All reactions were carried out at a microsome concentration of 0.2 mg/ml in 5 mM 45 CaCl\(_2\), 5 mM MgATP and 10 mM histidine at pH 7.0.

**TABLE 3**

<table>
<thead>
<tr>
<th>Calcium Binding by Sonicated Cardiac Microsomes</th>
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<tbody>
<tr>
<td>Microsomes</td>
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<tr>
<td>Control</td>
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<td></td>
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<tr>
<td>Sonicated 10 sec</td>
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<td>Sonicated 1 min</td>
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All reactions were carried out at a microsome concentration of 0.2 mg/ml in 5 mM 45 CaCl\(_2\), 5 mM MgATP and 10 mM histidine at pH 7.0.

**FIGURE 8**

Double reciprocal plot of calcium binding by cardiac microsomes versus free calcium concentration of 0.12 M KCl (•) and 0.80 M KCl (○). Reactions were carried out in 5.0 mM MgATP and 10 mM histidine at pH 7.0 with 0.1 mg/ml microsomes at 25°C.
FIGURE 9

Effects of ATP on the KCl dependence of calcium binding by cardiac microsomes. The binding of 5 μM ⁴²CaCl₂ by 0.20 mg/ml purified microsomes was measured in 5.0 mM MgATP (•) and 5.0 mM MgCl₂ (○) at various concentrations of KCl in 10 mM histidine at pH 7.0.

TABLE 4

<table>
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<tr>
<th>Alkali metal salt</th>
<th>Calcium binding (μmole/mg microsomal protein)</th>
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<tbody>
<tr>
<td>None</td>
<td>0.0081</td>
</tr>
<tr>
<td>0.2 M KCl</td>
<td>0.0066</td>
</tr>
<tr>
<td>0.2 M NaCl</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

Reactions were carried out with 0.5 mg/ml purified cardiac microsomes, 0.1 mM ⁴²CaCl₂, 5.0 mM MgCl₂ and 10 mM histidine at pH 7.0. Microsomes were collected by ultracentrifugation (see Methods).

Discussion

The effects of Na⁺ and K⁺ on both the binding and uptake of calcium by cardiac microsomes have been examined because, at the present time, it is not certain which of these variables is most closely related to the mechanism which effects relaxation in the intact heart. Although the total calcium uptake, in which calcium is trapped as insoluble calcium oxalate within the microsome vesicles (see ref. 11), greatly exceeds the amount of calcium estimated to be taken up during relaxation, the rate of calcium uptake appears to be too slow to account for relaxation in vivo (11). On the other hand, calcium binding, which is a much more rapid process, is barely sufficient to deplete the contractile proteins of the heart of the calcium believed to be released during excitation (11, 14).

The finding that low concentrations of alkali-metal cations stimulate microsomal calcium uptake (Fig. 1) is in accord with recent data obtained from skeletal muscle microsomes (15). In an earlier study, Fanburg and Gergely noted only inhibition of calcium uptake by NaCl and KCl (16), but these authors examined the effects of a greater addition of salt to a system that already contained significant amounts of Na⁺ and K⁺.

The different stimulatory effects of NaCl and KCl upon calcium uptake, and particularly the greater rate and extent of calcium uptake in the presence of K⁺ than of Na⁺ (Fig. 2), could be of significance in the control of contractility in the intact myocardium. It is well-established that exchange of extracellular Na⁺ for intracellular K⁺ occurs during the enhancement of myocardial...
contractility brought about by a variety of procedures (9, 10). The present findings indicate that this ionic shift could directly impair calcium uptake by the sarcoplasmic reticulum. The resulting increase in intracellular concentration of free Ca\(^{2+}\) would act to increase the activity of the cardiac actomyosin (2, 3, 16). Such an impairment of calcium uptake by the sarcoplasmic reticulum during relaxation would also be expected to prolong relaxation during positive inotropic procedures, such as in the positive rate staircase. However, because relaxation, as well as contraction, is accelerated under these conditions (17), a direct causal link between Na\(^+\) and K\(^+\) exchange observed in vivo and the effects seen in the purified cardiac microsomes is not obvious. Thus, the present findings may not be relevant to the events occurring in the intact heart.

The relationship between the ATP-dependent microsomal calcium uptake and the (Na\(^+\) + K\(^+\))-activated ATPase enzyme system isolated from the heart under somewhat different conditions (18-23) remains obscure. Although microsomal calcium uptake was stimulated by either Na\(^+\) and K\(^+\), the effects when both alkali-metal ions are present are those expected solely on the basis of the individual effects of each ion (Fig. 4).

Both NaCl and KCl have dual actions on the calcium binding by cardiac microsomes. Low concentrations of either salt enhance, while higher concentrations depress, the binding of calcium by microsomes (Figs. 5 and 6). In accord with earlier studies of skeletal muscle microsomes (24), we have found that ATP increases the quantity of calcium bound to the cardiac microsomes (Fig. 9). Because these microsomes contain nonradioactive calcium at the time of purification, the possibility that ATP and salts facilitate exchange of this calcium with \(^{45}\)Ca cannot be excluded. The present studies also demonstrate that the alkali-metal ion concentration at which calcium binding reached its maximum is higher in the presence of ATP (Fig. 9). Inhibition of skeletal muscle microsomal calcium binding by high concentrations of KCl and NaCl in the absence of ATP has been demonstrated previously (25), and Carvalho (12) has presented evidence that Na\(^+\), K\(^+\) and Ca\(^{2+}\) compete for a single binding site on these microsomes. Our findings with cardiac microsomes indicate that the reduction of calcium binding by high concentrations of KCl in the presence of ATP may also reflect competition for a single binding site. However, a double reciprocal plot of the dependence of calcium binding upon free calcium concentration failed to demonstrate classical competitive binding (Fig. 8). The latter finding may reflect an additional nonspecific effect of the altered ionic strength on the conformation of the microsomal lipoproteins, or on the Debye radius of the charged groups on the microsomes. In general, therefore, although the microsomal membranes have certain characteristics of an ion exchanger (26), these interactions are more complex in that, at low ionic strengths, binding of Ca\(^{2+}\), particularly in the presence of ATP, is facilitated by addition of alkali-metal salts.

The present findings, which fail to demonstrate differences between the effects of Na\(^+\) and K\(^+\) on calcium binding by microsomes (Figs. 6 and 7, Tables 2-4), do not support the view that intracellular exchanges of these alkali-metal ions directly affect the ability of the sarcoplasmic reticulum to bind calcium. Previous studies of the intact myocardium have suggested the existence of a site at which Na\(^+\) competes specifically for Ca\(^{2+}\) (27-29). Higher concentrations of the alkali-metal ions do reduce calcium binding (Figs. 5, 6, and 9) by a mechanism which appears to be, in part at least, competitive (Fig. 8). However, the failure of the purified cardiac microsomes to distinguish between Na\(^+\) and K\(^+\) in vitro makes it unlikely that these membranes, which presumably are exposed to a high concentration of intracellular K\(^+\) in vivo, represent the site of this physiologically important Na\(^+\)–Ca\(^{2+}\) competition.

Addendum

In a recent paper (J. Gen. Physiol. 50: 1327, 1967), Carvalho and Leo confirmed their
previous finding (12) that calcium binding by skeletal microsomes in the absence of ATP is inhibited by high concentrations of other cations. Additional data in their paper show that K\(^+\) inhibits calcium binding in the presence of ATP as well. Carvalho and Leo also demonstrate that the presence of cations such as K\(^+\), approximately 1000 times as much Ca\(^{2+}\) is needed to achieve an extent of calcium binding in the presence of 1.0 mM ATP. Our experiments were carried out at relatively high Ca\(^{2+}\) concentrations. Under these conditions, less difference between calcium binding in the presence and absence of ATP would be expected.

References


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