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Influence of External Cations on Caffeine-Induced Tension: Calcium Extrusion in Crayfish Muscle

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Summary. The transience of caffeine-induced tensions in crayfish muscle fibers indicates a caffeine-insensitive, Ca-transport system in these fibers. Analysis of the changes in relaxation time of caffeine tensions following ionic substitutions in the crayfish saline leads to the conclusions that this Ca-transport system is located in the sarcolemma and that the extrusion process involves binding of external cations, in particular Na.

It is generally accepted that caffeine exerts its effects on striated muscle of vertebrates (Weber & Herz, 1968; Lüttgau & Oetlicker, 1968) and invertebrates (Chiarandini *et al.*, 1970*a*, *b*) by blocking the absorption of Ca by the sarcoplasmic reticulum (SR). Doses of 2–10 mM caffeine may produce transient tension in muscle fibers of frog (Frank, 1962; Lüttgau & Oetlicker, 1968) and always cause transient tension in crayfish (Chiarandini *et al.*, 1970*a*). If the caffeine tension is caused by Ca released from the SR, the transience of this tension indicates a secondary Ca-transport system in these muscle fibers. In this paper we demonstrate the sensitivity to external ionic conditions of the secondary system in crayfish muscle, and add support to the speculation that the secondary system is located in the plasma membrane (Chiarandini *et al.*, 1970*a*).

Experiments reported below are interpreted in terms of a Ca-extrusion system which is dependent on external cations, primarily Na ions. The major argument for this Na-dependent transport of Ca is based on changes observed in the time course of caffeine tension and in the sensitivity of fibers to caffeine when the cations of the bath are varied. Ca efflux across the plasma membrane has been clearly linked to an opposite movement of Na in cardiac muscle (Reuter & Seitz, 1968) and in squid axon (Blaustein & Hodgkin, 1969).

Since tension appears to be a sensitive function of internal Ca (Ebashi & Endo, 1968), it was used as a monitor for internal Ca changes. The half-time of the caffeine-tension decay, $t_{1/2}$, was assumed to be a measure of the rate of Ca extrusion from the myoplasm by the secondary system. The exact form of the relationship of tension to internal Ca is needed for an exact description of Ca efflux, but not for the exploratory work reported here.

Materials and Methods

All experiments were conducted at room temperature with isolated muscle fibers from crayfish walking legs (*Orconectes* or *Procambarus*). Our methods differ in only a few details from those described by Chiarandini *et al.* (1970*a*). As given in that publication, the control saline, buffered at pH 7.4 with 2 mM Tris, contained 200 mM Na, 5 mM K, and 13.5 mM Ca. In order to minimize problems of drug diffusion, the changes of saline were rapid so that 10 times the chamber volume of 1 ml was flushed through in 3–4 sec.

In some experiments, the impermeant anion propionate (Prop) was substituted for Cl. All cation substitutions were isosmotic, and Na-free saline was made by substitution of Tris for Na, unless otherwise noted. Salines referred to as 0 Ca were maintained Ca-free by addition of 1 mM K_2EGTA.

Caffeine solutions were prepared by dilution of 80 mM caffeine stock solutions with test saline. These caffeine stock solutions were made by dissolution of crystalline caffeine in test saline. Most experiments were carried out with 20 mM caffeine, which produces a maximal caffeine tension (Chiarandini *et al.*, 1970*a*). In a previous series of experiments *(unpublished)* employing many single crayfish fiber preparations, we found that the shape of a maximal caffeine tension ([caffeine] $\geqq$ 10 mM) in control saline is fairly constant. An inflection point occurs at 0.8 of the peak of the caffeine tension and is followed by an exponential decay of tension (Fig. 1). In most cases, we have measured the half-time for relaxation as the time for tension to fall from 0.8 peak to 0.4 peak. The experiments to be described concern the effects of changing the external cations on the $t_{1/2}$ of these tensions. Changes in cation concentration either were simultaneous with application of caffeine or preceded the latter by 30–60 sec. The two procedures yielded nearly identical results. Since the magnitude of $t_{1/2}$ is quite variable from fiber to fiber, changes in $t_{1/2}$ for each fiber were measured relative to the values of $t_{1/2}$ in control saline $(t_{1/2})_c$. These data are presented as the ratio, $\theta = (t_{1/2})_c / t_{1/2}$.

Results

The effects of external cation concentration changes on caffeine tension described in this report were immediate. The effect of Na withdrawal simultaneous with caffeine addition is illustrated in Fig. 1. The first experiments to be described below test some obvious implications of the hypothesis that the decay of a caffeine tension is due to a Na-dependent Ca-extrusion system: the effect of cation changes on $t_{1/2}$, the effect of Na withdrawal on basal tension, and the effect of Na withdrawal on the caffeine dose-response curve.

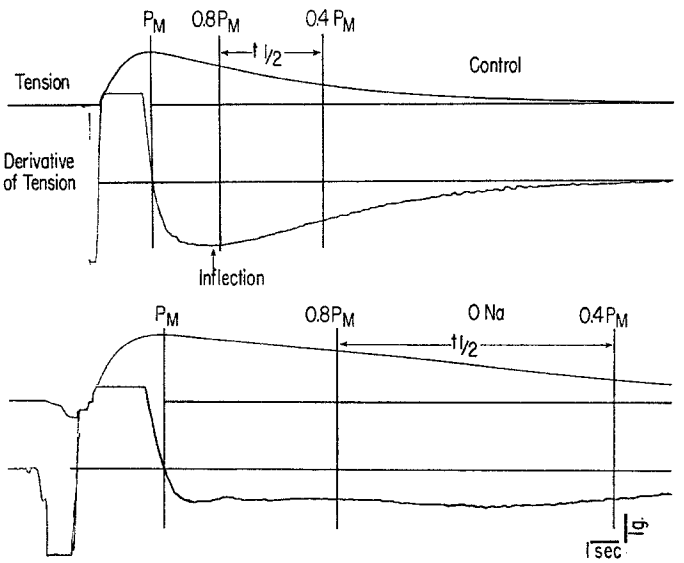

Fig. 1. Time course of 20 mM caffeine-induced tension. Effect of Na-free saline. Upper tracings: control saline. Lower tracings: 0 Na saline

Effect of Cation Changes on $t_{1/2}$

Varying the [K] from 0 to 20 mM in control [Na] produces no change in caffeine tension. Similarly, increasing the [Ca] to 40 mM, or triple the control value, produces only a small prolongation of tension which can be accounted for by the [Na] decrement required to maintain isotonicity. Similar results were reported previously (Chiarandini *et al.*, 1970*a*). Withdrawal of Na, on the other hand, not only prolongs the tension, but also sensitizes the fiber to changes in both [K] and [Ca], as demonstrated in Table 1. The presence or absence of Cl does not affect these conclusions, as similar results were obtained in propionate saline (*see* Fig. 5 and Table 3).

Table 1. *Effect of cation changes on* $t_{1/2}$ *in Cl saline*

$$\theta = \frac{(t_{1/2})_c}{t_{1/2}}.$$

Condition	θ	N	S.E.M.
0 Na	0.40	6	0.05
0 Na + 0 K	0.19	13	0.03
0 Na + 3x Ca	0.22	4	0.02

Effect of Na Withdrawal on Basal Tension

One would anticipate some tension in the absence of caffeine if the Ca efflux rate constant is decreased fivefold by the simultaneous withdrawal of both Na and K, as indicated in Table 1. This implication was tested. Recorder sensitivity was increased by a factor of 10, and the basal tension in 0 K solutions of various [Na] was examined. Oscillatory tensions were observed when [Na] was reduced to between 20 and 100 mM in different fibers. In the experiment of Fig. 2, oscillations appeared when the [Na] was reduced to 20 mM, and were suppressed when the [Na] was returned to 200 mM.

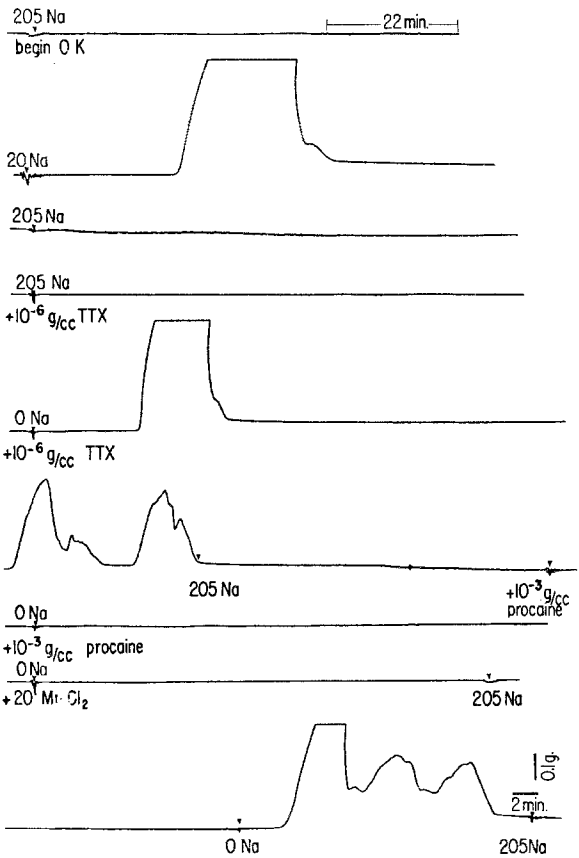

Fig. 2. Spontaneous tension of crayfish fiber in low-Na salines. Continuous recording of tension at high gain. The first three tracings demonstrate the spontaneous tension that occurred in low-Na saline. The remaining tracings show the affect of various drugs on 0 Na-induced tensions. Exposure of the fiber to 205 mM Na + drug is only shown for TTX (tracing 4). The ineffectiveness of TTX on the 0 Na tension is shown in tracings 5 and 6. The reversible block of these tensions by procaine and Mn is demonstrated in the remaining tracings. Time scale is 5 mm/min unless noted

The effects of various drugs were also determined, as shown in Fig. 2. The blockader of Na conductance, tetrodotoxin (TTX), had no effect on the tensions, while Mn reversibly blocked the tensions. Mn has been demonstrated to block Ca influx in crustacean muscle fibers (Hagiwara & Nakajima, 1966; Takeda, 1967), and to have no effect on caffeine-induced tensions except to prolong the refractory period (Chiarandini *et al.*, 1970*a*, *b*). Procaine, which reversibly blocks both K- and caffeine-induced tensions (Chiarandini *et al.*, 1970*a*), reversibly blocked the Na-withdrawal tensions.

Effect of Na Withdrawal on the Caffeine Dose-Response Curve

A shift of the dose-response curve toward the left (lower caffeine concentration) is clearly expected if Ca, released from the SR by a given dose of caffeine, is impeded in its extrusion from the fiber. Such is the case when Na is withdrawn from the bathing saline. This dramatic alteration in caffeine sensitivity of the muscle tension is illustrated by the inset to Fig. 3. Here, the threshold dose for this fiber in control saline elicited greater than 90% maximum tension in 0 Na. Dose-response data for two fibers are plotted in Fig. 3. The 0 Na curve has shifted to the left of the control curve.

Concomitant with the effects on caffeine tension, variations in [Ca] and [K] may induce large changes in membrane potential, since 0 Ca in the

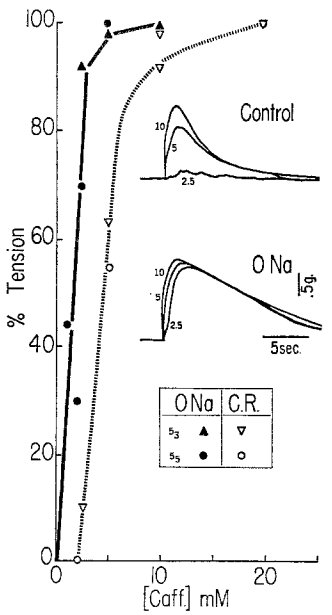

Fig. 3. Dose-response curve for caffeine-induced tension. Effect of Na-free saline

presence of Na depolarizes the fiber (Reuben, Brandt, Girardier & Grundfest, 1967*a*), as does high [K]. To avoid the complications caused by KCl redistribution that accompanies membrane potential changes in Cl saline, these depolarizing conditions were examined in fibers bathed in propionate saline.

Effect of 0 Ca on Caffeine Tension

We have confirmed the findings of Chiarandini *et al.* (1970*a*) on crayfish and of Caputo (1966) on frog fibers that reduction of external [Ca] has little or no effect on caffeine tension. In a series of experiments, submaximal tensions (2.5 mM caffeine) were elicited in a 0 Ca, KProp saline. After several fibers produced caffeine tensions unaffected by short exposures (10 min) to 0 Ca, two fibers were exposed for 30 min to a Ca-free saline prior to caffeine addition. As demonstrated in Fig. 4, there was no apparent effect on the caffeine tension even though the 0 Ca medium was able to block recovery after a caffeine tension, as previously reported (Chiarandini *et al.*, 1970*b*). The small tensions produced on return to 13.5 mM Ca from 0 Ca are similar to Ca tensions reported by Reuben, Brandt, Garcia and Grundfest (1967*b*).

In the next series of experiments, the influence of 0 Ca on relaxation from a maximal tension was investigated. Caffeine tensions were recorded in both NaProp and KProp saline with either 13.5 mM Ca or 0 Ca. Control caffeine tensions for each experiment were elicited in 0 Ca, NaProp. The

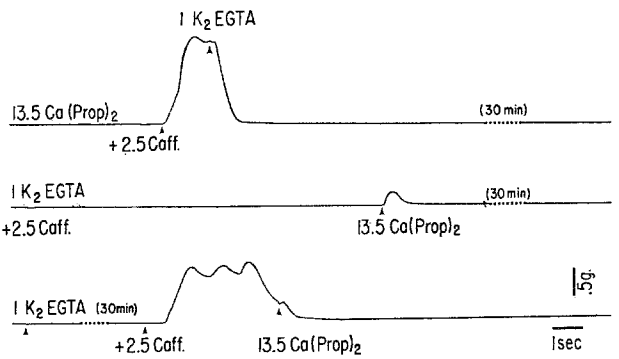

Fig. 4. The effect of Ca-free saline on caffeine tension in KProp and the recovery of response. Successive exposures of a fiber to caffeine are shown by succeeding traces. The initial caffeine tension was terminated with a Ca-free saline containing 1 mM K_2EGTA. After a second exposure to caffeine, which produced no tension, the fiber was returned to 13.5 mM Ca saline for 30 min prior to an equal time in Ca-free saline. A final exposure to caffeine produced the tension shown on the bottom tracing. Time scale is 1 cm/sec except where noted

Table 2. *The absence of significant change in $t_{1/2}$ in Ca-free salines: Control tension in Na Prop + 1 mM K_2EGTA*

Condition	θ(13.5 mM Ca)	θ(0 Ca)	*t*-test	
			t, d.f.	$t_{0,95}$
KProp (*Proc.*)	0.62	0.55	0.92, 2	4.3
KProp (*Orc.*)	0.30	0.33	0.33, 5	2.6
NaProp (*Orc.*)	0.90	1	0.25, 3	3.2

$t_{1/2}$ of these tensions was $(t_{1/2})_c$. No significant effect of the Ca-free condition on θ was found, as indicated in Table 2. Since the fiber is at its normal resting potential in NaProp and is depolarized in 0 Ca, NaProp (Reuben *et al.*, 1967*a*), row 3 of Table 2 also demonstrates that depolarization has little effect on the Ca-efflux system. This is consistent with the results of Chiarandini *et al.* (1970*a*) on the absence of an effect of maintained K depolarization on caffeine tension.

Summary of Cation Effects in Propionate Saline

Since tensions in 0 Ca vs. 13.5 mM Ca showed no significant difference, data from the two conditions were pooled to give the values of θ shown in Table 3. The data are from experiments on two *Procambarus* fibers and four *Orconectes* fibers in propionate salines. Although *Procambarus* fibers were less sensitive to cation changes than *Orconectes* fibers, qualitative results are the same for both; relaxation takes about twice as long in KProp as in NaProp, and twice as long again in TrisProp as in KProp.

The phenomena in propionate saline described above are illustrated by the record in Fig. 5. Comparison of the first two tracings shows the insensitivity of the caffeine tension to Ca withdrawal. Tension elicited in NaProp is smaller and much briefer than in KProp, whereas the final tension in TrisProp is more prolonged than that in KProp.

Table 3. *Effect of cation on $t_{1/2}$: Orconectes vs. Procambarus in propionate*

Species	θ(NaProp)	θ(KProp)	θ(TrisProp)
Procambarus	1	0.59	0.31
Orconectes	1	0.31	0.15

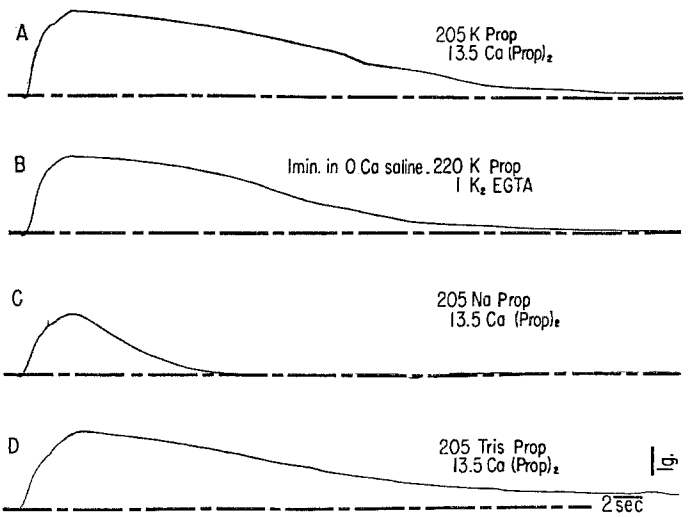

Fig. 5. The effect of cations on 20 mM caffeine tension in propionate saline

Quantitative Relation between $t_{1/2}$ and [Na]

The preceding data indicates that Ca is effluxed from the muscle fiber by a process that is: (1) primarily dependent on external Na, but can function at a reduced rate when Na is withdrawn (Tables 1 & 3); and (2) inhibited by elevated external [Ca] when Na is withdrawn (Table 1).

Efflux sites which take up internal Ca only when some number, n, of monovalent cations from the saline are bound would behave in this manner if they were selective for Na and had a slight affinity for Ca.

A simple model based on these concepts is developed in Appendix I. Three states are posited for an efflux site; empty and Ca-bound states are inactive for net Ca efflux, but the third state in which n monovalent cations are bound is active. The fraction of sites in the third state, θ_3, is assumed proportional to the Ca-efflux rate. This model is schematically represented in Fig. I-1. The equations deduced in the appendix can be used to describe the experimental data if one assumes that $t_{1/2}$ is proportional to the time constant for Ca efflux, and that $(t_{1/2})_c$ can be used to estimate the minimum value for $t_{1/2}$. Then θ_3 of the appendix can be equated to θ, and Eqs. (I-3a, b) relating S to θ_3 can be rewritten as Eqs. (1) and (2) below, where S is Na concentration.

$$\theta = \frac{\theta_0 + \alpha S^n}{1 + \alpha S^n}, \tag{1}$$

$$\frac{\theta - \theta_0}{1 - \theta} = \alpha S^n. \tag{2}$$

As shown in Appendix I, the contribution of secondary, monovalent cations can be represented by a single term, A, which is one of three parameters in addition to n that define the system. The other two parameters are the binding constant for Ca, K_c, and the binding constant for n Na ions, K_s. In the following discussion, all concentrations will be given in relative terms, i.e., $S=[\mathrm{Na}]/[\mathrm{Na}]_c$, $C=[\mathrm{Ca}]/[\mathrm{Ca}]_c$. Thus the binding constants are also dimensionless, and will be denoted with primes: $K_s'=K_s[\mathrm{Na}]_c^n$, $K_c'=K_c[\mathrm{Ca}]_c$. These parameters are simply related to the empirical parameters, α and θ.

The two parameters n and K_s' can be determined from the response of the fiber to variations in [Na]. The parameter K_c' can similarly be found from the variation of θ_0 with changes in [Ca], while A is computed from θ_0. The relationships used for these calculations are derived in Appendix II, and rewritten in terms of θ below.

$$\frac{(1+K_c'C_1)}{(1+K_c'C_2)}=\left(\frac{1-\theta_0}{\theta_0}\right)_1\left(\frac{\theta_0}{1-\theta_0}\right)_2, \tag{3a}$$

$$A=(1+K_c'C)\left(\frac{\theta_0}{1-\theta_0}\right), \tag{3b}$$

$$K_s'=\frac{\alpha(1+K_c'C)}{(1-\theta_0)}. \tag{3c}$$

In order to increase the range of S, a series of experiments was conducted in hyperosmotic ($2\times$) salines as well as normal ($1\times$) salines. We examined the effects of a threefold increase in Ca ($C=1$ vs. $C=3$) in normal and $2\times$ salines. As in the earlier experiments, $CaCl_2$ was isosmotically substituted for Tris-Cl in the $2\times$ saline. However, in the normal salines, ionic strength as well as osmotic pressure was kept constant by using sucrose to maintain isosmoticity while balancing ionic strength with changes in Tris-Cl. The effects observed in the normal ($1\times$) saline were essentially the same as those observed in the earlier experiments, where there was a small change in ionic strength (about 5%). The average θ_0 (four fibers) decreased from 0.44 to 0.29 in normal saline (compare to Table 1), but only from 0.39 to 0.34 in $2\times$ salines. The latter change, although in the expected direction, is not statistically significant ($t=0.96$, d.f. $=3$, $p<0.4$). A crude estimate of K_c' was made by substitution of these values, 0.39 and 0.34, into Eq. (3a), $K_c'=0.2$. If θ_0 at $C=1$ is used with this value of K_c' in Eq. (3b), one calculates $A=1.8$.

Dose-response curves for Na, θ vs. S, were run on four fibers in $2\times$ salines. Values of $S=0$, 0.5, 1, and 2 were used since our primary interest was in the value of θ at $S=1$ for comparison with fibers in normal saline.

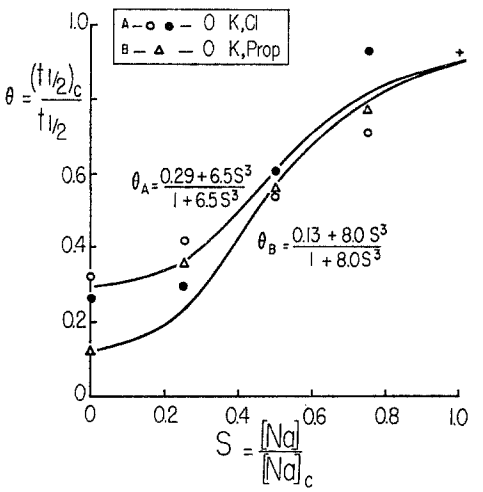

Fig. 6. The relation between half-time for relaxation of caffeine tension, $t_{1/2}$, and [Na]. Solid lines are calculated relations. + represents average data at $S=1$ of experiments in hyperosmotic ($2x$) salines

Eq. (2) was put into a linear form for least squares analysis, Eq. (4). From Eq. (3c) it can be seen that, $b=\ln\left(\frac{K_s'}{1+K_c'C}\right)$.

$$\ln\left[\frac{\theta-\theta_0}{(1-\theta_0)(1-\theta)}\right]=b+n\ln(S). \tag{4}$$

A reasonable correlation was found ($r=0.8$) with $n=2.89$ and $b=2.92$. If $K_c'=0.2$, this value of b implies $K_s'=1.2\exp(2.92)=22$. There was very little change in θ between $S=1$ and $S=2$; at $S=1$, $\theta=0.93$. Since θ at $S=1$ is nearly one, $(t_{1/2})_c$ can be used to estimate the saturation value of $t_{1/2}$.

Dose-response curves for Na in normal saline were also run. In this condition, values of $S=0$, 0.25, 0.5, 0.75, and 1 were used. Experiments were run on two fibers in Cl saline with θ_0 values of 0.25 and 0.33, and one fiber in Prop saline with $\theta_0=0.13$. These relatively low values of θ_0 were necessary for examination of the effects produced by these relatively small Na changes. Eq. (1) was successful in correlating the data from these fibers ($r=0.9$) with $n=2.99$ and $b=2.22$.

We now repeat the calculations of parameters for the normal saline data that were made for 2× saline. If we take the average values in normal saline, $\theta_0=0.44$ at $C=1$ and $\theta_0=0.29$ at $C=3$, Eq. (3a) gives $K_c'=0.87$. Substitution of the values for θ_0 at $C=1$ and $C=3$ into Eq. (3b) gives $A=1.47$. Similarly, Eq. (3c) gives $K_s'=1.87\exp(2.22)=17$.

Fig. 6 presents the data for these three fibers in the form θ vs. S as well as the average value of θ at $S=1$ from the hyperosmotic saline data. The

curves were calculated from Eq. (1) with a value of $n=3$. The curve marked θ_A was calculated based on a θ_0 of 0.29 and $\alpha=(1-\theta_0)\exp(2.22)=6.53$. This curve closely fits the data from the two fibers in Cl saline. The θ_B curve was calculated with the θ_0 of the third fiber, 0.13, and $\alpha=(1-\theta_0)\exp(2.22)=8.03$.

The quantitative agreement is sufficiently strong to justify an estimate of the fractions of Ca-transport sites in the various ionic states in control saline. From the above calculations, one can say that $K_c'<1$, $A\simeq 1$, $K_s'\simeq 20$. From this it follows that about 90% of the sites are Na bound, while 3 to 4% of the sites are in each of the other states.

Discussion

The data we have presented seem to indicate the existence of a Ca-efflux system in the crayfish muscle fiber. It may be helpful to summarize the argument.

1. Transience of caffeine tension implies the existence of a caffeine-insensitive, Ca-uptake system (Chiarandini *et al.*, 1970*a*).

2. Immediate prolongation of tension in response to Na withdrawal (Fig. 1) implies that the Ca-uptake system is in the sarcolemma.

3. Repriming of the SR after a caffeine tension is reversibly blocked by Ca-free saline (Fig. 4, also Chiarandini *et al.*, 1970*b*), which implies that the Ca taken up by the sarcolemma during relaxation is lost to the cell.

That efflux of Ca involves surface sites with bound monovalent cations, in particular Na, is indicated by the following:

1. The relaxation is slowed by lowering the [Na] (Table 1, Fig. 6), and slows much more when Tris is substituted for Na than when K is substituted for Na (Table 3, Fig. 5).

2. In Na-free salines, but not at $[Na]_c$, high [Ca] prolongs caffeine tension (Table 1). This is indicative of a competition between Na, Ca, and other cations (K, H) for efflux sites.

The simple model of Appendix I, based on the concept of Ca efflux through sites to which several cations can bind, gives an adequate description of these cation effects on caffeine tension. Although we have clearly demonstrated the existence of a Na-dependent, Ca-efflux system in the crayfish muscle, little can be said about the mechanism of transport from the physiological data. Tracer and chemical studies are required in order to determine the mechanism of this process.

A similar Ca-efflux system may exist in frog muscle, since caffeine tension in frog may also be transient (Lüttgau & Oetliker, 1968) and is accompanied by increased Ca efflux (Bianchi, 1961). Also, recovery of responsiveness after a caffeine tension is blocked by a Ca-free medium in frog muscle (Frank, 1962; Caputo, 1966). Although we lack comparative data describing such a system in skeletal muscle, there are other superficial Ca-extrusion systems reported in the literature. A Na vs. Ca exchange has been clearly demonstrated both in cardiac muscle, where a dependence of flux on $[Ca]/[Na]^2$ was found (Reuter & Seitz, 1968; Niedergerke, 1963), and in squid axon, where a [Na] dependence with a power of two or three was suggested (Blaustein & Hodgkin, 1969). On the other hand, a Ca-extrusion system in the red blood cell, independent of alkali cation concentration, has been reported (Schatzmann, 1966; Lee & Shin, 1969; Schatzmann & Vicenzi, 1969).

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Appendix I

Kinetics of Ca Efflux

A simple model for Ca efflux was constructed in order to guide interpretation of the effects of external cations on tension decay. We hypothesized a number, L, of Ca-efflux sites distributed in the surface membrane. Each site exists in one of three states. In two of these, the site is not available for Ca-efflux, i.e., the empty site and the Ca-bound site. The third state, however, a site containing n adsorbed monovalent cations, is available for Ca efflux. For simplicity, we assumed that a site can only bind n identical cations. We can represent this model diagramatically as shown in Fig. I-1.

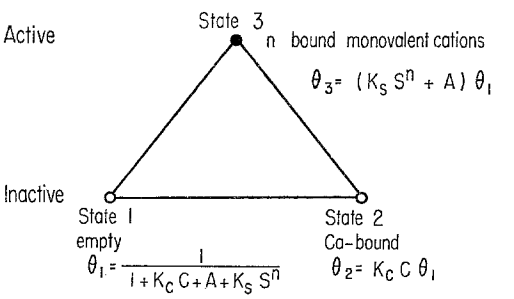

Fig. I-1. A diagramatic representation of the states in which Ca-efflux sites may exist

Table I-1. *Definitions*

Ionic species	Dominant ion	j^{th} secondary ion	Ca
Binding constant	K_s	K_j	K_c
Concentration	S	$[N_j]$	C
		$A=\Sigma K_j[N_j]^n$	

Four assumptions are proposed:

1. Ca efflux is proportional to $[Ca]_{in}$;

$$J_C=\frac{v[Ca]_{in}}{\tau} \tag{I-1}$$

where τ = the time constant for Ca efflux, and v = the cell volume.

2. Ca efflux is proportional to the number of efflux sites with n, bound, monovalent cations;

$$\tau^{-1}=kL\theta_3 \tag{I-2}$$

where L = total number of efflux sites, and θ_3 = fraction of sites with n, bound, monovalent cations.

3. There is an equilibrium distribution of cations between efflux sites and the external medium. The binding constant for Ca is K_c. The monovalent cation which is most strongly adsorbed has a binding constant K_s. Other monovalent cations, N_j, have binding constants K_j.

4. Mixed sites, e.g., different monovalent cations on the same site, have a negligible effect on Ca efflux.

The sites were divided into four fractions (*refer* to Fig. I-1): empty sites θ_1, Ca-bound sites θ_2, Na-bound sites θ_s, and other cation-bound sites θ_A. Only those sites with n, bound, monovalent cations contribute to θ_3, the fraction of sites that are active (State 3 of Fig. I-1). Since equilibrium exists between bound ions and those in solution, the following relations between the θ_i hold (*refer* to Table I-1 for definitions):

$$\theta_2=K_c C\theta_1$$
$$\theta_A=(\Sigma K_i[N_i]^n)\theta_1=A\theta_1$$
$$\theta_s=K_s S^n\theta_1$$
$$\theta_3=\theta_s+\theta_A.$$

After the sum of the fractions was equated to one, the resulting equation was solved for θ_1, and θ_3 was calculated from the preceding equations.

$$\theta_1=(1+K_c C+A+K_s S^n)^{-1}$$
$$\theta_3=\frac{K_s S^n+A}{1+K_c C+A+K_s S^n}. \tag{I-3}$$

If the dominant cation is removed from the medium, θ_3 is decreased to $\theta_{3,0}$.

$$\theta_{3,0}=\frac{A}{1+K_c C+A}. \tag{I-4}$$

Eq. (I-3) adopts more useful forms if numerator and denominator are divided by $1+K_c C+A$.

Define

$$\alpha=\frac{K_s}{1+K_c C+A}. \tag{I-5}$$

Two variations of Eq. (I-3) utilizing $\theta_{3,0}$ and α convenient for data reduction are Eqs. (I-3a) and (I-3b).

$$\theta_3=\frac{\theta_{3,0}+\alpha S^n}{1+\alpha S^n}, \tag{I-3a}$$

$$\alpha S^n=\frac{\theta_3-\theta_{3,0}}{1-\theta_3}. \tag{I-3b}$$

Application of these equations requires a relation between θ_3 and τ. Since θ_3 is a fraction, its upper bound is one. If we name the smallest possible time constant τ_∞, we see from Eq. (I-2) that $\tau_\infty^{-1}=kL$. Therefore, we can rewrite Eq. (I-2) as,

$$\theta_3=\frac{\tau_\infty}{\tau}. \tag{I-2a}$$

Appendix II

Application of the Kinetic Model to Data Reduction

Relation Between Parameters, α and $\theta_{3,0}$ and Binding Constants. Eq. (I-4) can be solved for A.

$$\begin{aligned} A&=\frac{\theta_{3,0}}{1-\theta_{3,0}}(1+K_c C)\\ 1+A+K_c C&=\frac{1+K_c C}{1-\theta_{3,0}}, \end{aligned} \tag{II-1}$$

and Eq. (I-5) becomes

$$\alpha=\frac{K_s(1-\theta_{3,0})}{1+K_c C}. \tag{II-2}$$

Computation of K_c and A. The quantity $\theta_{3,0}$ is both sensitive to the variation of C and readily measurable; therefore, it is an appropriate source for the estimate of K_c. If $\theta_{3,0}$ is measured at two levels of C, i.e., C_1 and $C=mC_1$, then from Eq. (II-1):

$$\frac{1+m K_c C_1}{1+K_c C_1}=\left(\frac{1-\theta_{3,0}}{\theta_{3,0}}\right)_m\left(\frac{\theta_{3,0}}{1-\theta_{3,0}}\right). \tag{II-3}$$

Defining the right-hand side by M, then

$$K_c C_1=\frac{M-1}{m-M}. \tag{II-4}$$

The quantity A can now be computed from Eq. (II-1).

Computation of K_s. The variation of θ_3 with S is required to determine K_s. If Eq. (II-2) is substituted into Eq. (I-3b), an appropriate equation for computation of K_s is obtained.

$$\frac{K_s S^n}{1+K_c C} = \frac{\theta_3 - \theta_{3,0}}{(1-\theta_3)(1-\theta_{3,0})}. \tag{II-5}$$

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