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Chloride and Osmotic Contractures in Skinned Frog Muscle Fibers

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Summary. Single contractures were elicited in segments of skinned frog muscle fibers when the segments were moved from relaxing-loading solutions to various test solutions. The effective test solutions produced an increase in the concentration of chloride ions in the myofilament space, $[\mathrm{Cl}]_{ms}$, and/or presumably caused the sarcoplasmic reticulum to undergo a change in volume. The contractures were quantified in terms of their maximum tension and time-integral. Two outer segments from each fiber underwent a contracture in a control solution (chloride ions were substituted for all of the methanesulfonate ions in the relaxing solution). The mean values of tension and area in the control contractures of each fiber were divided into the corresponding values from a test contracture obtained in the central segment of the same fiber. Test contractures obtained upon increasing $[\mathrm{Cl}]_{ms}$ and increasing the product, $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$, were compared to contractures that were obtained by increasing $[\mathrm{Cl}]_{ms}$ while keeping $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$ constant. The former contractures were greater in magnitude for a given $[\mathrm{Cl}]_{ms}$. Whereas the former solutions may have caused an increase in the volume of the sarcoplasmic reticulum and altered the electrical potential across the membranes of the sarcoplasmic reticulum as well, only a change in potential was presumed to have occurred in the latter solutions. Other types of contractures were investigated to show that both swelling of the sarcoplasmic reticulum and changes in the electrical potential of its membranes can cause release of calcium ions and elicit contractures in skinned fibers.

The processes that immediately precede the release of calcium ions from the sarcoplasmic reticulum during excitation-contraction coupling in skeletal muscle are not known. One possible process is a change in the electrical potential across the membranes of the sarcoplasmic reticulum which leads to or is coincident with the release of calcium ions. Such an occurrence seems to be likely because the events that occur even earlier in the process of excitation-contraction coupling are electrical. The action potential in the transverse tubular system (Costantin, 1970; Bastian & Nakajima, 1974) probably causes movement of electrical charge within the membranes at the junction of the transverse tubules and sarcoplasmic reticulum (Schneider & Chandler, 1973), and

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the movement of charge may allow release of calcium ions directly, or further electrical events may be inititated in the sarcoplasmic reticulum. Therefore, it is important to understand the electrical properties and permeability of the membranes of the sarcoplasmic reticulum.

Ford and Podolsky (1970) found that when skinned muscle fibers whose sarcoplasmic reticulum had been loaded with calcium ions were moved from a solution composed largely of potassium propionate to a solution composed largely of potassium chloride, a transient contracture resulted. Ford and Podolsky suggested that the contracture resulted from a change in the electrical potential across the internal membranes of the fiber, which were assumed to be permeable to chloride ions. The change in potential in turn caused calcium ions to be released from the sarcoplasmic reticulum. Endo and Nakajima (1973) investigated chloride contractures in fibers that were split longitudinally. The split fibers had transverse tubules that were accessible to the bathing solution. Endo and Nakajima proposed that the contractures which they observed upon moving a fiber from a solution of potassium methanesulfonate to a solution composed of potassium chloride were due to a change in potential across the membranes of the sarcoplasmic reticulum and a release of calcium ions. Assuming the interior of the sarcoplasmic reticulum to be analogous to the extracellular space, Endo and Nakajima suggested that the chloride ions caused a depolarization of the sarcoplasmic reticulum.

Meissner and McKinley (1976) investigated the permeability of the membranes of isolated vesicles of sarcoplasmic reticulum. They found that the membranes were almost equally permeable to chloride, potassium, and sodium ions. When the concentrations of chloride and potassium ions in their solutions were varied, Meissner and McKinley observed that the release of calcium ions from vesicles seemed to be independent of whether the change in the electrical potential of the membranes was positive or negative. They proposed that the calcium ions that were released from the vesicles of sarcoplasmic reticulum depended mainly or entirely on the degree of osmotic swelling of the vesicles.

The subject of this paper is two stimuli that appear to be effective in causing release of calcium ions from the sarcoplasmic reticulum in skinned fibers: (i) A change in electrical potential across the membranes such that the interior of the sarcoplasmic reticulum becomes more negative, and (ii) an increase in volume or a swelling of the sarcoplasmic reticulum. If both stimuli occur as suggested, then the results have implications for a model of the sarcoplasmic reticulum as a Donnan equilibrium system. Portions of this work have been described in abstracts (Mobley, 1977; 1978).

Materials and Methods

Isolation, Skinning and Loading the Fibers

Single fibers were isolated from semitendinosus muscles of the frog, *Rana pipiens* in one of two ways: (i) Fibers were isolated in Ringer's solution, 113.5 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 5.0 mM imidazole, and 10.0 mM dextrose. (ii) Small bundles of fibers were isolated in Ringer's solution; then a bundle was placed in relaxing solution (Table 1) and single fibers were isolated. The single fibers were moved to or remained in relaxing solution and split longitudinally along their entire length (Endo & Nakajima, 1973). These split or skinned fibers were loaded with calcium ions in one of two ways: (i) Fibers were left in loading solution #1 (relaxing solution, *see* Table 1) for approximately 45 min. (ii) Fibers were placed in loading solution #2 (Table 1) for 1 min and were then returned to relaxing solution for a 10-sec rinse.

Types of Contractures

Following the loading period, single contractures were elicited from each of three adjacent segments of the skinned fibers. The segments were obtained from the central region of each fiber; each segment was 1–2 mm in length. Contractures in the two outer segments served as controls for a single test contracture in the central segment (Fig. 1). The control contractures were chloride contractures where $[Cl]_{ms} = 120$ mM and $[K]_{ms} \times [Cl]_{ms} = 14{,}400$ (*ms* is myofilament space). Test contractures were of five types (Table 1): (i) Chloride contractures: Chloride ions were substituted 1:1 for some or all of the methanesulfonate ions in the relaxing solution. (ii) Chloride contractures when $[K]_{ms} \times [Cl]_{ms} = 300$ (the same magnitude as in the relaxing solution and the loading solutions): Chloride ions were substituted for some or all of the methanesulfonate ions, and choline ions were substituted for some of the potassium ions in the relaxing solution. (iii) Hyposmotic contractures: Some potassium methanesulfonate was removed from the relaxing solution. (IV) Chloride and hyposmotic contractures when $[K]_{ms} \times [Cl]_{ms} = 300$; A combination of types *ii* and *iii* (v) Chloride and hyperosmotic contractures; sucrose was added to test solutions which without sucrose would have produced chloride contractures of type *i*.

Recording and Quantifying the Contractures

The skinned segments were mounted at their slack length (as determined by observation at 12 × magnification) between the arm of a force transducer and a fixed arm. The ends of the segments were fastened to the arms with spring-loaded clamps. The different solutions were contained in different compartments in a plastic chamber; the segments were immersed initially in the relaxing solution. The segments, transducer, and associated apparatus were fixed in position, but the chamber could be lowered and moved with respect to the segments. When the chamber was lowered, the desired compartment was positioned under the segments, and then the chamber was raised. The solutions that bathed the segments could be changed in 1–2 sec.

Table 1. Solutions (mM concentrations)

Relaxing solution and loading solution #1		
K methanesulfonate (KMS)	117.5	
KCl	2.5	$[K]_{ms} \times [Cl]_{ms} = 300$
Imidazole	20.0*	pH = 7.0*
$MgSO_4$	6.0*	*p*Ca = 8.4*
Na_2ATP	4.0*	*T* = 20–24 °C*
Na_2EGTA	0.5*	
Loading solution #2		
Same components and concentrations as in loading solution #1 except:		
Na_2EGTA	1.3	*p*Ca = 7.0
$Na_2CaEGTA$	0.7	
Test solutions		**Contractures**
* All test solutions contain the components and concentrations noted above plus the components listed below		
1. 2.5 − 120 KCl		Chloride contractures
KCl	2.5 − 120.0	
KMS	117.5 − 0.0	
2. 30 − 120 Cl		Chloride contractures when $[K]_{ms} \times [Cl]_{ms} = 300$
KCl	10.0 − 2.5	
Choline Cl (ChCl)	20.0 − 117.5	
Ch methyl sulfate	90.0 − 0.0	
3. −20 −60 KMS		Hyposmotic contractures
KCl	2.5	
KMS	97.5 − 57.5	
4. 60 Cl, −60 KMS; 90 Cl, −30 KMS		Chloride and hyposmotic contractures when $[K]_{ms} \times [Cl]_{ms} = 300$
KCl	5.0, 3.33	
ChCl	55.0, 86.67	
5. 120 KCl, +40 −200 sucrose		Chloride and hyperosmotic contractures
KCl	120.0	
Sucrose	40.0 − 200.0	

The contractures were recorded on a Brush 220 strip chart recorder and on a Nicolet 1074 Instrument Computer. Each contracture was characterized in terms of the maximum tension developed and in terms of the result of integrating the record of tension with respect to time. The maximum tension was measured from the tracing on the recorder, and the integral of each contracture was calculated by the computer. Each parameter of a test contracture in each fiber was divided by the corresponding mean value of the parameters of the two accompanying control contractures in the same fiber.

The data from the ten fibers in Table 2 where $[Cl]_{ms} = 120$ mM served as a control for the procedure of comparing the test contracture in a central segment to the mean values of the control contractures elicited in the outer segments. The expected values of both ratios, tension and area, were 1.00. The ratios observed were 0.96 ± 0.03 SEM and 1.03 ± 0.05 SEM, respectively.

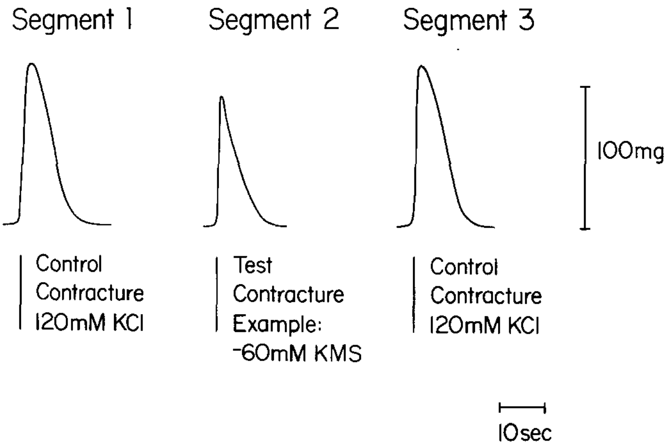

Fig. 1. Three contractures in three segments of a skinned fiber. The first and third contractures were the controls (120 mM KCl). The second contracture was the test (60 mM KMS was removed from the relaxing-loading solution). $T_{\text{test}}/T_{120\text{KCl}}=0.84$; $A_{\text{test}}/A_{120\text{KCl}}=0.58$

Table 2. Chloride contractures

$[\text{Cl}]_{ms}$ (mM)	30	60	90	120
$T_{\text{test}}/T_{120\text{KCl}}$, $A_{\text{test}}/A_{120\text{KCl}}$	0.43, 0.27	0.87, 0.78	0.69, 0.69	1.02, 1.03
	0.48, 0.11	0.68, 0.30	0.94, 0.80	0.93, 0.96
	0.24, 0.12	1.01, 0.85	0.90, 0.77	0.95, 1.10
	0.84, 0.70	0.70, 0.39	1.07, 1.24	0.96, 1.13
	0.30, 0.11	0.86, 0.56	0.89, 0.68	0.85, 0.81
	0.52, 0.19	0.86, 0.65	0.87, 0.51	1.01, 1.18
	0.22, 0.03	0.86, 0.58	0.85, 0.71	1.08, 1.35
	0.69, 0.21	1.17, 0.94	0.81, 0.57	0.86, 0.87
	0.41, 0.26	0.71, 0.48	0.92, 1.01	0.87, 0.83
	0.53, 0.13	0.88, 0.53	0.87, 1.13	1.07, 1.04
Mean	0.47, 0.21	0.86, 0.61	0.88, 0.81	0.96, 1.03
SEM	0.06, 0.06	0.04, 0.06	0.03, 0.08	0.03, 0.05

Results

Chloride Contractures

Table 2 gives the results of a series of chloride contractures in 40 skinned fibers. T represents maximum tension and A represents area (tension × time), the result of integrating the contractures. References to contractures in regard to this and the other tables should be understood to be references to the test contractures; the control contractures were performed in all fibers and they made possible the comparisons of the different types of contractures. The chloride contractures increased in a graded manner with the increase in $[\mathrm{Cl}]_{ms}$, a result obtained earlier by Endo and Nakajima (1973). Table 2 also shows that the mean area of the contractures with respect to $[\mathrm{Cl}]_{ms}$ was never clearly maximal or saturated; while the tension was saturated at $[\mathrm{Cl}]_{ms} = 60$ mM. These results are consistent with the results of Endo and Blinks (1973) which showed that the tension in chloride contractures reached saturation at lower $[\mathrm{Cl}]_{ms}$ than did the elapsed time of the contractures. An additional 10 fibers not shown in Table 2 were tested when $[\mathrm{Cl}]_{ms} = 2.5$ mM, i.e., the loaded segments were simply moved between pools of relaxing solution. No contractures occurred in those fibers.

Chloride Contractures when $[K]_{ms} \times [Cl]_{ms} = 300$

Table 3 shows the results of chloride contractures elicited when $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms} = 300$. Significant contractures were observed when $[\mathrm{Cl}]_{ms} = 90$ mM; while Table 2 showed that chloride contractures occurred when $[\mathrm{Cl}]_{ms}$ was as low as 30 mM. Both the tension and the area of the contractures in Table 3 were smaller for a given $[\mathrm{Cl}]_{ms}$ than the contractures in Table 2. The contractures in Table 3 were quantitatively equivalent when $[\mathrm{Cl}]_{ms} = 90$ and 120 mM. Figure 2 shows the results from one of the fibers in Table 3 when $[\mathrm{Cl}]_{ms} = 120$ mM. Seven additional fibers not shown in Table 3 were tested at $[\mathrm{Cl}]_{ms} = 30$ mM; no contractures were elicited in that solution. Figure 3 summarizes the data in Tables 2 and 3.

Table 3. Chloride contractures when $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms} = 300$

$[\mathrm{Cl}]_{ms}$ (mM)	60	90	120	
$T_{\mathrm{test}}/T_{120\mathrm{KCl}}$, $A_{\mathrm{test}}/A_{120\mathrm{KCl}}$	0.10, 0.11	0.62, 0.28	0.63, 0.28	0.90, 0.73
	0.25, 0.25	0.86, 0.68	0.62, 0.16	0.38, 0.08
	0.08, 0.01	0.75, 0.30	0.40, 0.40	0.64, 0.25
	0.04, 0.04	0.62, 0.17	1.09, 0.38	0.88, 0.40
	0.00, 0.00	0.49, 0.15	1.00, 0.49	0.67, 0.29
	0.18, 0.09	0.64, 0.25	0.90, 0.55	0.32, 0.15
	0.00, 0.00	0.61, 0.19	0.49, 0.15	0.66, 0.41
	0.00, 0.00	0.56, 0.17	0.84, 0.29	0.64, 0.16
	0.01, 0.00	0.48, 0.22	0.53, 0.09	0.67, 0.34
	0.00, 0.00	0.88, 0.46	0.74, 0.26	0.93, 0.44
Mean	0.07, 0.05	0.65, 0.29	0.70, 0.31	
SEM	0.03, 0.03	0.04, 0.05	0.05, 0.04	

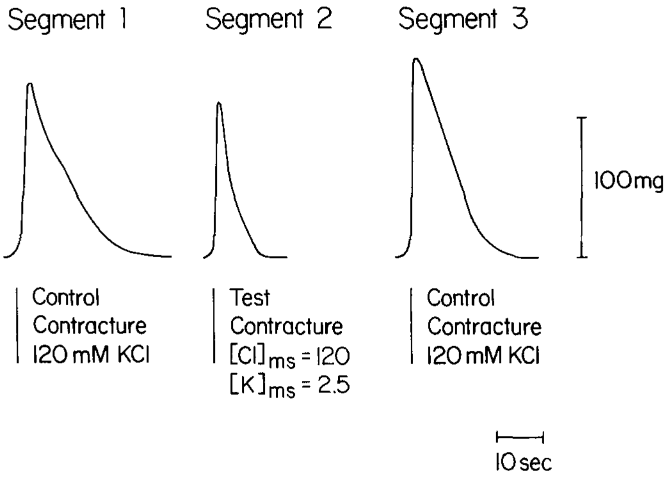

Fig. 2. Three contractures in three segments of a skinned fiber. The first and third contractures were the controls (120 mM KCl). The second contracture was the test ($[\mathrm{Cl}]_{ms} = 120$ mM; $[\mathrm{K}]_{ms} = 2.5$). The central segment exhibited a smaller contracture in terms of tension and area than the controls. This was characteristic of the contractures at constant $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$. $T_{\mathrm{test}}/T_{120\mathrm{KCl}} = 0.84$; $A_{\mathrm{test}}/A_{120\mathrm{KCl}} = 0.29$

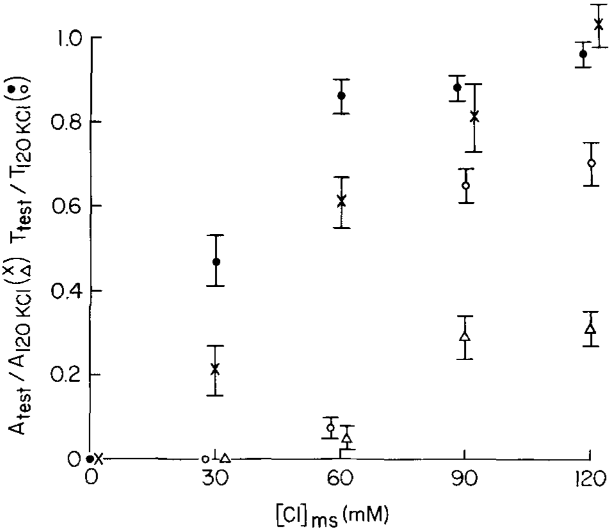

Fig. 3. This figure summarizes the data in Table 2 on chloride contractures (tension ratios, ●; area ratios, ×) and the data in Table 3 on chloride contractures when $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms} = 300$ (tension ratios, ○; area ratios, △)

Hyposmotic Contractures

Endo and Thorens (1975) showed that contractures were elicited when fibers loaded with calcium ions were placed in solutions that were hyposmotic with respect to the relaxing solution. They noted that the

Table 4. Hyposmotic contractures

$-[\mathrm{KMS}]_{ms}$ (mM)	30	40	60
T_{test}/T_{120KCl}, A_{test}/A_{120KCl}	0.13, 0.04	0.61, 0.25	0.83, 0.39
	0.27, 0.21	0.49, 0.38	0.72, 0.43
	0.52, 0.29	0.97, 0.76	0.72, 0.37
	0.48, 0.20	0.50, 0.25	0.65, 0.44
	0.15, 0.04	1.07, 0.65	0.80, 0.47
	0.14, 0.03	0.71, 0.66	1.09, 1.02
	0.01, 0.00	0.43, 0.34	1.04, 1.00
	0.42, 0.17	0.60, 0.27	0.74, 1.10
	0.71, 0.56	0.94, 0.56	1.03, 0.77
	0.45, 0.16	0.52, 0.23	0.84, 0.58
Mean	0.33, 0.17	0.68, 0.44	0.85, 0.69
SEM	0.07, 0.05	0.07, 0.06	0.05, 0.09

magnitudes of the contractures were graded and increased with the decline in concentration of $[\mathrm{KMS}]_{ms}$. The results in Table 4 are similar to the results of Endo and Thorens. Four additional fibers not shown in Table 4 were tested in −20 mM $[\mathrm{KMS}]_{ms}$; no contractures occurred in those fibers.

In another set of experiments the segments were loaded as usual and moved to a test solution of choline methyl sulfate; i.e., $[\mathrm{K}]_{ms}$ was reduced to 0 mM. No contractures occurred in the seven fibers tested.

Chloride and Hyposmotic Contractures when $[K]_{ms} \times [Cl]_{ms} = 300$

Table 5 shows the results of contractures when $[\mathrm{Cl}]_{ms}$ was raised at constant $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$ while simultaneously $[\mathrm{KMS}]_{ms}$ was lowered. These contractures were larger than the contractures produced either by raising $[\mathrm{Cl}]_{ms}$ at constant $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$ (Table 3) or by lowering $[\mathrm{KMS}]_{ms}$ (Table 4).

Chloride and Hyperosmotic Contractures

Experiments were performed when sucrose was added to test solutions that without sucrose would have produced contractures like the ones in Table 2. The results are presented in Table 6. They show that these contractures were smaller than the contractures in Table 2 for the same

Table 5. Chloride and hyposmotic contractures when $[K]_{ms} \times [Cl]_{ms} = 300$

$[Cl]_{ms}$, $-[KMS]_{ms}$ (mM)	60−60	90−30
T_{test}/T_{120KCl}, A_{test}/A_{120KCl}	1.22, 1.47	1.11, 1.29
	1.06, 1.07	1.18, 1.19
	1.04, 1.17	1.06, 1.09
	0.75, 0.72	1.08, 1.06
	1.31, 1.29	1.02, 0.98
	1.04, 1.17	1.04, 0.78
	1.05, 0.98	1.07, 0.89
	1.29, 1.57	1.22, 0.78
	1.31, 1.31	0.70, 0.64
	0.89, 0.89	0.92, 0.39
Mean	1.10, 1.16	1.04, 0.91
SEM	0.06, 0.08	0.05, 0.09

Table 6. Chloride and hyperosmotic contractures

$[Cl]_{ms}$, $+[\text{sucrose}]_{ms}$ (mM)	120+40	120+80	120+120	120+160	120+200
T_{test}/T_{120KCl}, A_{test}/A_{120KCl}	1.02, 0.76	1.09, 0.73	0.69, 0.40	0.52, 0.14	0.19, 0.03
	1.09, 0.84	1.03, 0.91	0.72, 0.31	0.30, 0.06	0.34, 0.08
	0.92, 0.83	1.32, 1.24	0.51, 0.21	0.77, 0.46	0.91, 0.46
	0.94, 0.90	0.91, 0.93	0.72, 0.49	0.13, 0.06	0.00, 0.00
	0.96, 0.96	0.83, 0.73	0.15, 0.03	0.37, 0.09	0.16, 0.04
	0.88, 0.78	0.84, 0.69	0.66, 0.33	0.74, 0.62	0.63, 0.31
	1.13, 0.91	0.72, 0.55	0.79, 0.59	0.28, 0.07	0.45, 0.16
	1.04, 0.96	0.90, 0.77	0.40, 0.09	0.55, 0.21	0.00, 0.00
	0.88, 0.95	0.84, 0.77	0.02, 0.01	0.45, 0.14	0.06, 0.01
	0.98, 1.03	0.94, 0.77	0.35, 0.10	0.82, 0.42	0.46, 0.13
Mean	0.98, 0.89	0.94, 0.81	0.50, 0.26	0.49, 0.23	0.32, 0.12
SEM	0.03, 0.03	0.05, 0.06	0.08, 0.06	0.07, 0.06	0.09, 0.05

$[Cl]_{ms}$; however, contractures were completely eliminated in only a few fibers as a result of the added sucrose. These results are different from those of Thorens and Endo (1975) which showed that 40 mM or higher concentrations of sucrose completely inhibited chloride contractures.

Effect of Sodium Ions

Table 7 shows the effect of raising $[Cl]_{ms}$ to 120 mM and substituting sodium ions for potassium ions in a test solution. Both the tension and the area of these test contractures were slightly larger than the

Table 7. Chloride contractures in 120 NaCl

$[NaCl]_{ms}$ (mM)	120
T_{test}/T_{120KCl}, A_{test}/A_{120KCl}	1.36, 1.78
	1.24, 1.89
	0.85, 0.70
	1.05, 0.98
	1.24, 1.53
	1.09, 1.06
	1.19, 1.09
	0.93, 0.94
	1.12, 1.50
	0.96, 1.24
Mean	1.10, 1.27
SEM	0.05, 0.12

Table 8. Chloride contractures when $[Na+K]_{ms} \times [Cl]_{ms} = 324$

$[Na]_{ms}$, $[K]_{ms}$, $[Cl]_{ms}$ (mM)	1.7, 1.0, 120
T_{test}/T_{120KCl}, A_{test}/A_{120KCl}	0.73, 0.39
	0.88, 0.60
	0.45, 0.32
	0.47, 0.22
	1.03, 0.89
	0.86, 0.59
	0.71, 0.16
	0.62, 0.18
	0.82, 0.34
	0.57, 0.08
Mean	0.71, 0.38
SEM	0.06, 0.08

corresponding parameters of the contractures in Table 2 when $[KMS]_{ms}$ was 120 mM. The contractures summarized in Table 8 were elicited in test solutions where $[Cl]_{ms} = 120$ mM and $[Na+K]_{ms} \times [Cl]_{ms} = 324$ (approximately the same magnitude as in relaxing solution). There was no apparent difference between these contractures and the contractures in Table 3 when $[Cl]_{ms} = 120$ mM and $[K]_{ms} \times [Cl]_{ms} = 300$.

Discussion

The purpose of these experiments was to distinguish between the release of calcium ions from the sarcoplasmic reticulum in skinned fibers by a change in electrical potential and by a change in volume of the sarcoplasmic reticulum. The former is conceivably related to a physiological event; the latter is not. The small sizes of the components of the sarcoplasmic reticulum in muscle fibers would seem to make it impossible to obtain direct measurements of the membrane potential and volume of the sarcoplasmic reticulum. Therefore predictions were made and tested regarding the relative magnitudes of contractures that were to be produced in various test solutions. Implicit in the predictions were the assumptions that the test solutions would cause the sarcoplasmic reticulum to undergo changes in membrane potential and/or changes in volume to different degrees, and that the changes would in turn lead to different degrees of calcium release and contracture. The maximum tension and the time-integral of the contractures were used as semi-quantitative indicators of the calcium ions that produced the contractures.

Chloride Contractures

If the membranes of sarcoplasmic reticulum were permeable to chloride ions alone, then the release of calcium ions in chloride contractures could be ascribed entirely to a change in the electrical potential across the membranes of the sarcoplasmic reticulum. However, it is known that the membranes of sarcoplasmic reticulum are permeable to chloride and potassium ions (Meissner & McKinley, 1976); therefore it is possible that the sarcoplasmic reticulum can be described as a double Donnan equilibrium system that is analogous to the muscle fiber and sarcolemma (Boyle & Conway, 1941; Macknight & Leaf, 1977). In that case, when it was exposed to the solutions in Table 2, the sarcoplasmic reticulum would swell or change its shape to accommodate the entry of water, potassium, and chloride ions. Therefore the release of calcium ions that produced the contractures in Table 2 could have been the result of a change in the electrical potential across the membranes of the sarcoplasmic reticulum, an increase in volume of the sarcoplasmic reticulum, or both. The experiments in this paper were performed to determine the stimuli for the release of calcium ions in chloride contractures and thereby to discern the properties of the sarcoplasmic reticulum and its membranes.

Chloride Contractures when $[K]_{ms} \times [Cl]_{ms} = 300$

If the chloride contractures in Table 2 were produced by a change in electrical potential, then chloride contractures produced when $[Cl]_{ms}$ was raised and $[K]_{ms} \times [Cl]_{ms} = 300$ should be equivalent to or larger than the chloride contractures produced when $[K]_{ms} \times [Cl]_{ms}$ was increased. If the chloride contractures were produced by a swelling of the sarcoplasmic reticulum, there would be no contractures produced at constant $[K]_{ms} \times [Cl]_{ms}$. While if chloride contractures were the result of both a swelling and a change in potential in the sarcoplasmic reticulum, then the chloride contractures produced at constant $[K]_{ms} \times [Cl]_{ms}$ would be smaller than the chloride contractures produced at increased $[K]_{ms} \times [Cl]_{ms}$. A comparison of Tables 2 and 3 showed that the latter expectation was the result observed. It seems that more calcium was released from the sarcoplasmic reticulum when the product, $[K]_{ms} \times [Cl]_{ms}$, was raised and when swelling probably occurred in addition to a change in potential than when the product was held constant as $[Cl]_{ms}$ was increased and only a change in electrical potential was expected.

The maximum changes in electrical potential that could have occurred across the membranes of the sarcoplasmic reticulum when each test solution was introduced are as follows: 30 mM $[Cl]_{ms}$, −60 mV; 60 mM $[Cl]_{ms}$, −80 mV; 90 mM $[Cl]_{ms}$, −90 mV; 120 mM $[Cl]_{ms}$, −98 mV where the potential inside the sarcoplasmic reticulum is determined with respect to the myofilament space.

Hyposmotic Contractures

The results in Table 4 confirm previous observations (Endo & Thorens, 1975) that solutions that cause the sarcoplasmic reticulum to increase in volume also cause the sarcoplasmic reticulum to release calcium ions. The experiments in which the test solution contained choline methyl sulfate showed that a reduction in $[K]_{ms}$ and the change in potential that accompanied it did not cause the release of calcium ions; therefore only a minor, if any, part of the contractures in Table 4 could have been caused by a change in potential.

It should be noted that the magnitude of the contractures in Table 4 were probably a function of the ionic strength of the test solutions as well as a function of the osmolarity. Perhaps as much as 10% of the total tension was due to the lowered ionic strength (Gordon et al., 1973).

Chloride and Hyposmotic Contractures when $[K]_{ms} \times [Cl]_{ms} = 300$

If larger contractures can be produced by a combination of swelling and a change in potential than can be produced by either stimulus alone, as Tables 2, 3, and 4 would indicate, then a test solution that would raise $[Cl]_{ms}$ while keeping $[K]_{ms} \times [Cl]_{ms}$ constant and in addition was hyposmotic due to the removal of some remaining KMS would be expected to produce contractures that were similar to those in Table 2 for a given $[Cl]_{ms}$. The results in Table 5 confirmed this expectation. Interpolating and averaging the results of tension and area showed that a test solution of 60 Cl, −38 KMS and 90 Cl, −22 KMS would have given contractures that would have closely approximated the chloride contractures when $[\mathrm{Cl}]_{ms} = 60$ and 90 mM, respectively.

Chloride and Hyperosmotic Contractures

The sucrose added to the test solutions in Table 6 was expected to prevent or moderate the swelling of the sarcoplasmic reticulum but was not expected to significantly affect the change in potential across the membranes of the sarcoplasmic reticulum or the calcium released by the change in potential. The results in Table 6 confirmed this prediction. The solutions with 40 and 80 mM sucrose had only a minor effect in reducing the contractures. The contractures produced when sucrose was 120 and 160 mM may have been due entirely to changes in potential. The greater shrinkage produced by 200 mM sucrose may have affected the release of calcium ions that was due to the change in potential, but it did not eliminate the release of calcium except in two fibers.

Effects of Sodium Ions

The experiments performed when the concentrations of sodium and chloride ions were increased by replacing potassium and methanesulfonate ions (Table 7) confirm the results of Meissner and McKinley (1976) that the sarcoplasmic reticulum is permeable to sodium ions; they indicate that the sodium ions can enter the sarcoplasmic reticulum just as the potassium ions did in the experiments in Table 2. There is no indication from these experiments what the electrical effect of the sodium ions may have been.

Because the membranes of the sarcoplasmic reticulum were permeable to sodium and chloride ions, there was a possibility that swelling could have occurred in the experiments in Table 3 where $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$ was 300, because the product, $[\mathrm{Na}+\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$, was increased in those test solutions. The experiments in Table 8 tested whether the results of experiments at constant $[\mathrm{K}]_{ms} \times [\mathrm{Cl}]_{ms}$ may have been affected by swelling. The results in Table 8 closely approximate the results in Table 3 when $[\mathrm{Cl}]_{ms}$ was 120 mM, and they indicate that swelling of the sarcoplasmic reticulum was not a significant factor in the contractures in Table 3.

Problems and Alternative Interpretations

Endo and Blinks (1973) showed that the photoluminescent protein, aequorin, was not completely suitable as a quantitative indicator of calcium ions in skinned fibers. Therefore the simpler though also inadequate method of measuring the parameters of the contractures was employed for use as an indicator of the relative amount of calcium ions released.

Some uncertainty persists as to the state of the transverse tubules in the type of skinned fibers used in these experiments and as to the effect of the test solutions on the junction of the transverse tubules and sarcoplasmic reticulum. Some evidence and arguments that the transverse tubules are open in these fibers and that the site of action of the solutions is not at the *T-SR* junction are presented by Endo and Nakajima (1973). Transverse tubular membranes are much more permeable to potassium than to chloride ions (Eisenberg & Gage, 1969). A similar relationship of permeabilities was not apparent in the active membranes in these experiments.

As noted in Table 1, the concentrations of $MgSO_4$, Na_2ATP, imidazole and Na_2EGTA were effectively the same in all experiments. Therefore, while some of these components might have affected the sarcoplasmic reticulum or myofibrils in a peculiar and specific manner, the results of these experiments could not be explained on the basis of those effects. The concentrations of chloride ions and the membrane potential of the sarcoplasmic reticulum were not constant throughout the experiments. Endo and Nakajima (1973) showed that chloride ions inhibit only slightly the uptake of calcium by the sarcoplasmic reticulum. Stephenson (1978) suggested that depolarization of the sarcoplasmic reticulum inhibited the uptake of calcium ions. Neither of the hypotheses, inhibition of calcium uptake by chloride ions or by depolarization, could explain the results of Tables 2 and 3.

The concentration of $MgSO_4$ (6 mM) was always higher than the concentration of Na_2ATP (4 mM) in these experiments. This difference was maintained to reduce or eliminate the regenerative release of calcium by calcium (Endo, Tanaka & Ogawa, 1970; Ford & Podolsky, 1970). Although the possibility of regenerative release could not be ruled out, neither could the data be explained on that basis.

Somlyo, Shuman and Somlyo (1977) found that the chloride and potassium contents in the terminal cisternae of the sarcoplasmic reticulum were similar to and slightly higher than the respective contents of the sarcoplasm in frog skeletal muscle. The results of Somlyo, Shuman and

Somlyo must at present be taken as evidence against either the skinned fiber as a physiological model of the sarcoplasm and sarcoplasmic reticulum or the interpretation of the experiments in this paper.

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