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Action of Mercurials on the Active and Passive Transport Properties of Sarcoplasmic Reticulum

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Abstract

The effect of Hg^{2+} and CH_3-Hg^+ on the passive and active transport properties of the Ca^{2+}-Mg^{2+}-ATPase-rich fraction of skeletal sarcoplasmic reticulum (SR) is reported. The agents abolish active transport, at 10^{-5} and 10^{-4} *M* concentrations, respectively. Addition of the mercurials was also shown to release actively accumulated Ca^{2+}. The mercurials increase the passive Ca^{2+} and Mg^{2+} permeability in the absence of ATP at the same concentrations at which they inhibit transport. It is proposed that both effects are the result of direct binding of the mercurials to the SH groups of the Ca^{2+}-Mg^{2+}-ATPase pump, altering the conformational equilibria of the pump. The agents were also shown to increase the passive KCl permeability. The SR preparation consists of two vesicle populations with respect to K^+ permeability, one with rapid KCl equilibration faciliated by a monovalent cation channel function and one with slow KCl equilibration. The mercurials increase the rates of KCl equilibration in both fractions, but produce higher rates in the fraction containing the channel function. The results are discussed in terms of pump and channel function and are compared with results for the electrical behavior of the Ca^{2+}-Mg^{2+}-ATPase and other SR proteins in black lipid membranes, as presented by others.

Key Words: Sarcoplasmic reticulum; Ca^{2+} transport; K^+ permeability; mercurials; Ca^{2+}-Mg^{2+}-ATPase; fluorescent probe.

Introduction

The Ca^{2+}-Mg^{2+} activated ATPase of skeletal muscle sarcoplasmic reticulum (SR)[3] is a Ca^{2+} transport enzyme capable of rapid pumping of Ca^{2+} in a reversible and thermodynamically efficient manner (MacLennan and Holland, 1975; Hasselbach, 1978). The SR and the reconstituted enzyme have

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[3]Abbreviations: sarcoplasmic reticulum, SR; 1-anilino-8-naphthalenesulfonate, ANS^-; chlorotetracycline, CTC; ethyleneglycoltetraacetic acid, EGTA.

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been the subject of a large amount of kinetic and biochemical studies aimed at elucidating the pump mechanism (Hasselbach and Makinose, 1963; Weber *et al.*, 1966; Inesi and Scarpa, 1972; MacLennan, 1970; MacLennan and Holland, 1975; Froehlich and Taylor, 1975, 1976; Inesi *et al.*, 1978a, b; Noack *et al.*, 1978). Shamoo and MacLennan (1974, 1975) demonstrated that the Ca^{2+} transport and ATPase functions of the enzyme reconstituted in phospholipid vesicles can be blocked by Hg^{2+} and CH_3-Hg^+. Their study also presented data on the effects of these two agents on Ca^{2+}-dependent conductance of oxidized cholesterol membranes treated with the succinylated enzyme. Inhibition of the Ca^{2+} conductance was observed with Hg^{2+} but not with CH_3-Hg^+. On the basis of this evidence Shamoo and MacLennan (1974, 1975) concluded that Hg^{2+} was capable of inhibiting the ATPase function of the enzyme and blocking the "ionophore" or ion translocator.

The present report compares the activities of these two agents on three permeability properties of an ATPase-rich fraction of SR (Meissner, 1975): (a) passive permeability to Ca^{2+} and Mg^{2+}, (b) passive permeability to K^+ (Cl^-), and (c) active transport. The SR membrane has an appreciable permeability to Ca^{2+} and Mg^{2+}, with half-times for isotope or net flux in the 100–200 sec range (Jilka *et al.*, 1975; Inesi, 1979; Chiu and Haynes, 1980a, b). We have reported kinetic studies which suggest that the slow passive transport is mediated by the Ca^{2+}-Mg^{2+}-ATPase using reaction steps not involving the high-energy phosphoenzyme form of the membrane (Chiu and Haynes, 1980a). The inhibitory effect of the mercurial compounds on the active transport reaction would be expected to alter the passive permeability to Ca^{2+} and Mg^{2+} if the latter property is indeed Ca^{2+}-ATPase mediated. The present report also shows that the mercurials increase the passive permeability of SR to K^+ and Cl^-. The work of McKinley and Meissner (1977, 1978) has shown that a fraction of the vesicles (Type I) is highly K^+-permeable, by virtue of a monovalent cation channel. The remainder of the vesicles (Type II) lack this channel and are not K^+-permeable. SR vesicles were also shown to have an electrically active anion permeability (e.g., Cl^-, phosphate) (Kometani and Kasai, 1978). We have used the response of 1-anilino-8-naphthal enesulfonate (ANS^-) to confirm these findings and to show that Type II vesicles are devoid of electrically active permeability to Cl^- and P_i (Chiu and Haynes, 1980a; Haynes, 1982). Both Type I and Type II vesicles have an electrically-active H^+ permeability (Meissner and Young, 1980; Haynes, 1982). The present study will show that the KCl permeability of both types of vesicle is increased by the mercurials.

Methods and Experimental Procedures

The low-density fraction (Meissner, 1975) of rabbit skeletal SR was prepared as described previously (Chiu *et al.*, 1980). The use of 1-anilino-

8-naphthalenesulfonate (ANS^-) to measure Ca^{2+} transport and passive equilibration of monovalent and divalent cations has been described previously (Chiu and Haynes, 1980a, b). The comparison of the ANS^- technique and the chlorotetracycline (CTC) technique has been detailed (Millman *et al.*, 1980; Haynes, 1982). Upon initiation of Ca^{2+} transport, chlorotetracycline accumulates in the SR interior as a Ca^{2+}-CTC complex which binds to the inner surface of the membrane with a marked increase in fluorescence (Caswell and Hutchison, 1971; Caswell, 1972). CTC experiments are carried out using a Perkin-Elmer spectrofluorometer. In these experiments, the SR is preincubated with the CTC in a Ca^{2+}-EGTA buffer and other cofactors, and the reactions were initiated by adding ATP. The fluorescence increases, reaches a plateau, and remains there until the ATP is exhausted and Ca^{2+} is slowly released. At low vesicle concentrations the ATP-dependent amplitude in the plateau phase is proportional to the free Ca^{2+} concentration in the SR lumen. However, the time course of the fluorescence increase is slower than the time course of the actual uptake due to the necessity of CTC permeation, which has a $t_{1/2}$ of approximately 30 sec (Millman *et al.*, 1980). The method has much better time resolution for the Ca^{2+} release process which does not require the transport of CTC. The CTC method is favored for studies of the effects of serial addition of reagents on the steady-state levels of internal Ca^{2+}.

For the study of active uptake kinetics, the 1-anilino-8-naphthalenesulfonate (ANS^-) technique is the method of choice. The binding of ANS^- to the inner surface increases the internal Ca^{2+} concentration and the $[K^+]_o/[K^+]_i$ ratio. In the presence of catalytic concentration of valinomycin the response is rapid. The kinetics of Ca^{2+} uptake are faithfully resolved in stopped-flow experiments in which SR is rapidly mixed with ATP. The use of valinomycin as a diagnostic for electrically active cation and anion permeabilities has also been described (Kometani and Kasai, 1978; Chiu and Haynes, 1980a; Haynes, 1982). The use of 10 mM histidine buffer suppresses changes in internal pH resulting from transient movement of H^+, following a KCl jump (Haynes, 1982).

Rapid mixing experiments with ANS^- were performed with an Aminco-Morrow stopped-flow apparatus (Catalog No. 4-8409). The apparatus mixes equal volumes of two solutions. The excitation monochromator was set at 368 nm, and a Schott GG420 cutoff filter was placed in front of the photomultiplier. Kinetic and mechanistic criteria described on previous studies (Chiu and Haynes, 1980a, b) were applied to the experiments monitoring ANS^- fluorescence.

The ANS^- method can be used to monitor passive Ca^{2+} transport across the membrane by Ca^{2+} jump experiments. The transport of KCl can also be measured in KCl jump experiments. The ANS^- concentration in the SR lumen and the degree of ANS^- binding to the inner surface are responsive to

the $[K^+]_o/[K^+]_i$ ratio. After a KCl jump, the ANS^- fluorescence increases with a $t_{1/2}$ of approximately 10 msec in a process representing establishment of a new binding equilibrium set by the initially high $[K^+]_o/[K^+]_i$ ratio. This is followed by a slower phase of fluorescence decrease as ANS^- binding decreases in response to the lowered $[K^+]_o/[K^+]_i$ ratio as KCl moves across the membrane and comes to equilibrium. We have used this technique to differentiate Type I and Type II SR (Chiu and Haynes, 1980a). All experimentation was at 23°C, with SR protein concentrations of 0.08 or 0.1 mg/ml.

Results

Effects of Mercurials on Active Uptake

Both Hg^{2+} and CH_3-Hg^+ were found to attenuate active uptake in the ATPase-rich fraction of SR. This was demonstrated in experiments using both the ANS^- and CTC techniques. Figure 1 shows that preincubation of the SR with Hg^{2+} concentrations as low as 1×10^{-6} M (1.25×10^{-5} mole/g SR) greatly attenuates the Ca^{2+} uptake reaction as measured by the increase in CTC fluorescence. Complete abolition of active uptake was observed with 1×10^{-5} M Hg^{2+} (1.25×10^{-4} mole/g SR). Figure 2 shows that the cation is also effective at bringing about the rapid release of Ca^{2+} already accumulated.

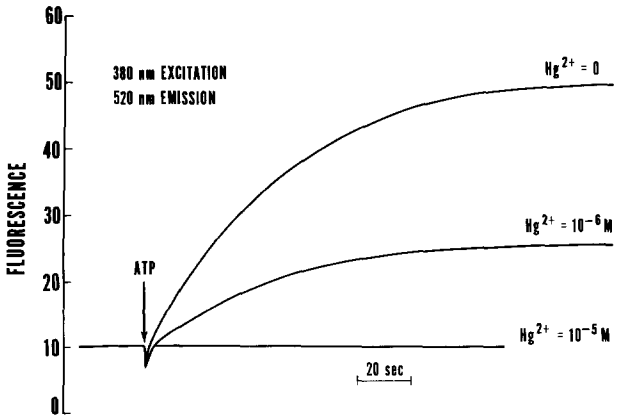

Fig. 1. Hg^{2+} inhibits the active transport of Ca^{2+} by the SR as measured by chlorotetracycline fluorescence. SR (0.08 mg/ml) was preincubated with Hg^{2+} for 5 min at the indicated concentration in a medium consisting of 250 mM sucrose, 50 mM KCl, 250 μM Ca-EGTA, 10 mM histidine buffer at pH 7.0, and 10 μM chlorotetracycline. Active uptake was initiated by addition of Mg-ATP to a final concentration of 250 μM.

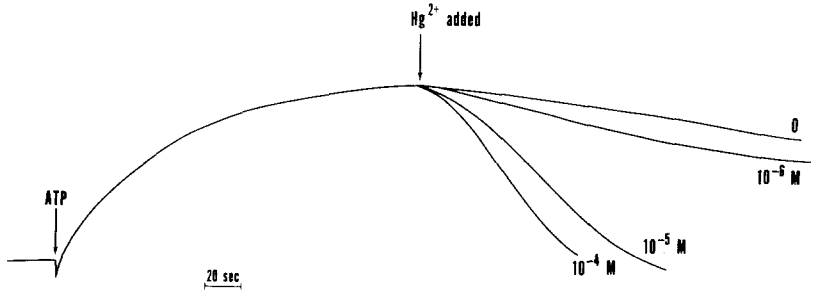

Fig. 2. Ca^{2+} uptake and Hg^{2+}-induced release measured by chlorotetracycline fluorescence. Incubation medium consisted of 250 mM sucrose, 50 mM KCl, 250 μM $MgCl_2$, 50 μM $CaCl_2$, 250 μM Ca-EGTA, 10 μM chlorotetracycline, and SR (0.08 mg protein/ml). Mg-ATP (250 μM) and $HgCl_2$ were added where indicated.

The rate of the fluorescence increase in the CTC experiment is limited by the half-time of permeation of the probe (30 sec; cf. Millman *et al.*, 1980); thus Fig. 1 does not resolve the kinetics of the active uptake reaction. The experiment was repeated using the ANS^- method as a rapid means of fluorometric readout. Table 1 shows that active uptake of Ca^{2+} into the ATPase-rich SR under conditions of low KCl concentration occurs with two processes, a rapid one ($t_{1/2} \approx 50$ msec) of small amplitude and a slow one ($t_{1/2} \approx 7.5$ sec). We have analyzed the phenomena (Chiu and Haynes, 1980b) and have shown that the rapid phase corresponds to the first half of a pump

Table I. Concentration Dependence of Inhibition of Active Uptake by Hg^{2+} and CH_3-Hg^{+a}

		Rapid phase		Slow phase	
Inhibitor	Concentration	$t_{1/2,1}$ (msec)	A_1	$t_{1/2,2}$ (sec)	A_2
—	—	45	0.2	7.5	1.25
Hg^{2+}	1×10^{-8} M	50	0.2	7.5	1.25
Hg^{2+}	1×10^{-7} M	50	0.2	7.5	1.25
Hg^{2+}	1×10^{-6} M	50	0.2	12.0	0.84
Hg^{2+}	1×10^{-5} M	—	0.0	—	0.0
Hg^{2+}	1×10^{-4} M	—	0.0	—	0.0
CH_3-Hg^+	1×10^{-8} M	50	0.2	7.5	1.25
CH_3-Hg^+	1×10^{-7} M	50	0.2	7.5	1.25
CH_3-Hg^+	1×10^{-6} M	50	0.2	7.5	1.25
CH_3-Hg^+	1×10^{-5} M	50	0.2	14.0	0.75
CH_3-Hg^+	1×10^{-4} M	—	0.0	—	0.0

[a] Active uptake was monitored by the increase in ANS^- fluorescence observed after rapid mixing of SR with ATP to final concentrations of 0.1 mg/ml and 1×10^{-4}, respectively. The basic medium contained 10^{-4} M Mg^{2+}, 5 mM KCl, 1×10^{-4} M Ca^{2+}, 1×10^{-4} M EGTA, 3×10^{-5} M ANS^-, 0.25 M sucrose, and 10 mM histidine, pH 7.2. The SR reservoir also contained 6 μM valinomycin. Two phases of fluorescence increase was observed (Chiu and Haynes, 1980b). When the experiments were repeated without ATP, no fluorescence response was observed in the time region 0–20 sec. The inhibitors were preincubated with the SR for 5 min and were also present in the ATP reservoir at the indicated concentration.

cycle, at the end of which the Ca^{2+} is released to the interior, reaching a nearly inhibitory concentration. A slow second phase results from slow turnover in the subsequent cycles due to inhibition by internal Ca^{2+}. Table I shows that 1.0×10^{-6} M Hg^{2+} (1.0×10^{-5} mole/g SR) and 1.0×10^{-5} M CH_3-Hg^+ (1.0×10^{-4} mole/g SR) reduced by 30–50 percent both the amplitude of the slow transport process and the rate of its attainment. The rate and extent of the rapid reaction are not affected at these concentrations of mercurials. When the concentrations of mercurials are increased to 10^{-5} (1.0×10^{-4} mole/g) and 10^{-4} M (1.0×10^{-3} mole/g) respectively, both phases of the active transport reaction are abolished. This is the direct effect of the mercurials on the Ca^{2+} transport function of the SR. Control experiments showed that the valinomycin-mediated ANS^- permeability and the ability of the probe to respond to changes in internal Ca^{2+} concentration were not impaired by the mercurials. The half-time and the amplitude of the initial fluorescence increase (valinomycin-assisted ANS^- permeation) are not appreciably affected by preincubation with 1.0×10^{-4} M Hg^{2+} or CH_3-Hg^+. Table II shows that ANS^- fluorescence responds to a Ca^{2+} jump in the presence of 1.0×10^{-4} M Hg^{2+} or CH_3-Hg^+.

Effects of Mercurials on Passive Ca^{2+} and Mg^{2+} Permeability

Abolition of the active transport reaction could be explained by the induction of a passive leak to Ca^{2+} (and other ions) or by interference with the ATPase function of the pump as suggested by Shamoo and MacLennan (1974, 1975). Both mechanisms could result in an alteration of the passive Ca^{2+} and Mg^{2+} permeability of the ATPase-rich SR. The data of Table II show that this is the case. The control experiments (Nos. 1, 2 and 7, 8) repeat our observation that the passive influx of Ca^{2+} and Mg^{2+} occurs with two phases. A rapid phase with a $t_{1/2}$ of ca. 50 msec and a small amplitude is followed by a slow phase ($t_{1/2}$ = 100–150 sec). The latter phase accounts for the major portion of the Ca^{2+} moved. CH_3-Hg^+ has only a small effect on the rapid phase but produces a large increase in the rate of the slow phase, decreasing its $t_{1/2}$ value to the range of 2.5 to 15 sec (experiments Nos. 5, 6, 11, 12). The relationship between the amplitudes of the two phases was not affected. Treatment with Hg^{2+} resulted in a different pattern. A large acceleration of the bulk of the influx was observed, with measured half-times in the range of 400 to 600 msec (experiments Nos. 3, 4, 9, 10). However, at low KCl concentrations, the amplitude remaining in the slow phase appeared with a half-time which was 2.0–3.5 times longer than in the control experiments (experiments Nos. 3, 4). At high K^+ concentrations, only a moderate increase in the rate of the slow phase over the control value was observed (experiments Nos. 7, 8, 9, 10).

Neither of the agents was found to affect the Mg^{2+} permeability

Table II. Effect of Hg^{2+} and $(CH_3\text{-}Hg)^+$ on the Rates of Passive Ca^{2+} and Mg^{2+} Permeation

Experiment number	Divalent cation jumped	Agent	KCl (mM)	A_1	$t_{1/2,1}$ (msec)	A_2	$t_{1/2,2}$ (msec)	A_3	$t_{1/2,3}$ (sec)	Total fluorescence
1	Ca^{2+}	—	10	0.15	40	—	—	1.6	100	11.3
2	Mg^{2+}	—	10	0.15	40	—	—	1.8	150	10.2
3	Ca^{2+}	Hg^{2+}	10	—	—	1.8	400	0.5	350	11.3
4	Mg^{2+}	Hg^{2+}	10	—	—	1.4	600	0.5	300	9.5
5	Ca^{2+}	$(CH_3\text{-}Hg)^+$	10	0.2	20	—	—	1.7	6.5	8.8
6	Mg^{2+}	$(CH_3\text{-}Hg)^+$	10	0.2	20	—	—	2.0	13	8.2
7	Ca^{2+}	—	100	0.15	30	—	—	2.0	100	8.5
8	Mg^{2+}	—	100	0.15	30	—	—	2.0	150	8.5
9	Ca^{2+}	Hg^{2+}	100	1.6	20	—	—	0.5	70	8.8
10	Mg^{2+}	Hg^{2+}	100	1.1	20	—	—	0.35	100	8.5
11	Ca^{2+}	$(CH_3\text{-}Hg)^+$	100	0.5	10	0.8	400	0.3	15	8.4
12	Mg^{2+}	$(CH_3\text{-}Hg)^+$	100	0.3	10	0.7	600	0.4	2.5	8.4
13	—	—	10	0.0	—	0.0	0.0	0.0	0.0	7.0
14	—	—	100	0.0	—	0.0	0.0	0.0	0.0	5.5

[a]SR vesicles were rapidly mixed with $CaCl_2$ to final concentrations of 0.1 mg/ml and 30 mM, respectively, at 23°C, and the progess curve was analyzed. The basic medium contained 0.25 M sucrose, 10 mM Hepes buffer, pH 7.1, 3×10^{-5} M ANS, 1×10^{-4} M $HgCl_2$ or $(CH_3\text{-}Hg)$-Cl where indicated, and KCl at the indicated concentration. The SR was preincubated with 6 μM valinomycin.

differently than the Ca^{2+} permeability. The rates of the major phase of Mg^{2+} equilibration were always slower than the corresponding rates for Ca^{2+} equilibration.

Effect of Mercurials on Passive KCl Permeability

We have shown previously how the kinetics of KCl equilibration across the SR membrane can be monitored by ANS^- fluorescence (Chiu and Haynes, 1980a). A KCl jump results in a rapid rise in ANS^- fluorescence corresponding to an increase in the internal ANS^- concentation due to the high $[K^+]_o/[K^+]_i$ ratio, followed by two phases of fluorescence decrease with half-times of ca. 2 and 85 sec, corresponding to KCl equilibration (lowering the $[K^+]_o/[K^+]_i$ ratio across the membranes of the Type I and Type II vesicles, respectively). Table III shows that Hg^{2+} and CH_3-Hg^+ increase the rate of KCl permeation, measured by the $t_{1/2}$ values of these two phases. Preincubation with 1×10^{-6} M Hg^{2+} brings about a measurable decrease in the $t_{1/2}$ of the rapid phase. Elevation of the Hg^{2+} concentration to 1×10^{-5} M reduces the $t_{1/2}$ to 45 msec, its minimal value. The slow phase is affected similarly by these Hg^{2+} concentrations. Its $t_{1/2}$ decreases from 80 to 4 sec. The relationship between the amplitudes of the two phases is not altered by preincubation with Hg^{2+}, indicating that the identity of the phases (Type I and Type II vesicles) is not changed. Also, the increase in the rates of the two processes occurs in continuous or graded manner with increasing mercurial concentration, further supporting this assumption. Preincubation with CH_3-Hg^+ brought about a less dramatic increase in the rate of the fast phase but had a larger effect on the rate of the slow phase than did Hg^{2+}. At high Hg^{2+} and CH_3-Hg^+ concentrations, a fourth process was observed with a positive amplitude and $t_{1/2}$ values of 300 and 150 sec, respectively. The direction of this process could be reversed if the mercurials were omitted from the K^+ reservoir. Also, a similar response with a $t_{1/2}$ in this time range could be observed when the mercurial concentrations were jumped from 0 to 1×10^{-5} or 10^{-4} M (data not shown). This suggests that the fourth process results from imbalance of the free concentrations of the mercurials in the K^+ and SR reservoirs, due to mercurial binding to the latter.

Discussion

The present study shows that Hg^{2+} and CH_3-Hg^+ inhibit active Ca^{2+} transport in the SR and increase the passive Ca^{2+}, Mg^{2+}, and K^+ permeability. The inhibition of active transport is in agreement with the finding of Shamoo and MacLennan (1975) that these agents inhibit the ATP hydrolysis function of the enzyme. However, we find Ca^{2+} transport to be more sensitive

Table III. Effect of Hg^{2+} and $(CH_3\text{-}HG)^+$ on Passive Permeability[a]

Perturbant	ANS equilibration		KCl movement, Type I		KCl movement, Type II		Mercurial dependent process	
	A_1	$t_{1/2,1}$ (msec)	A_2	$t_{1/2,2}$	A_3	$t_{1/2,3}$ (sec)	A_4	$t_{1/2,4}$ (sec)
—	+1.6	10	−0.4	1.0 sec	−0.2	80	—	—
1×10^{-6} M Hg^{2+}	+1.6	10	−0.3	200 msec	−0.2	20	—	—
1×10^{-5} M Hg^{2+}	+1.6	10	−0.3	45 msec	−0.2	4	+0.2	300
1×10^{-4} M Hg^{2+}	+1.6	10	−0.3	45 msec	−0.2	4	+0.5	300
1×10^{-6} M $(CH_3\text{-}Hg)^+$	+1.6	10	−0.3	1.0 sec	−0.2	80	—	—
1×10^{-5} M $(CH_3\text{-}Hg)^+$	+1.6	10	−0.3	0.7 sec	−0.2	10	+0.2	150
1×10^{-4} M $(CH_3\text{-}Hg)^+$	+1.6	10	−0.3	0.3 sec	−0.2	2	+0.5	150

[a]SR vesicles were preincubated in standard medium with 2 μM valinomycin and Hg^{2+} or $CH_3\text{-}Hg^+$ where indicated (perturbant). The experiment was initiated by jumping KCl to a final concentration of 100 mM. The time course of the ANS^- fluorescence was monitored. At the concentration of valinomycin used, the K^+ permeability of Type I vesicles is about twice its intrinsic value, and the Cl^- permeability is still limiting to the overall rate of KCl equilibration (Chiu and Haynes, 1980a). The SR concentration is 0.1 mg/ml.

to Hg^{2+} than to CH_3-Hg^+, whereas Shamoo and MacLennan (1975) reported the opposite. The difference may be the result of any one of a number of differences in experimental protocol. Their preincubations were done at pH 8, 37°C, in absence of added Ca^{2+}. Ours were done at pH 7, 23°C, at a Ca^{2+} concentration well above the K_m of the pump and the K_d of the Ca^{2+} binding site. Their assays were based on oxalate loading while ours were based on the ability of the pump to produce and maintain internal free Ca^{2+} concentrations in the millimolar range. We have shown that 10^{-4} Hg^{2+} can bring about the release of actively accumulated Ca^{2+} with a half-time of ca. 40 sec (Fig. 2). This can be compared with a half-time of several minutes for the reversal of transport by ADP addition (Dixon *et al.*, 1982). It would thus appear that the Hg^{2+}-induced increase in passive Ca^{2+} permeability contributes substantially to the block in transport.

Our finding that the passive Ca^{2+} and Mg^{2+} permeability is increased by the mercurials can be readily explained by their interactions with the SH groups of the Ca^{2+}-Mg^{2+}-ATPase. The enzyme has a number of SH groups whose reactivity is affected by nucleotides and Ca^{2+} (Murphy, 1978; Yamada and Ikemoto, 1978), and electron spin resonance probes attached to SH groups have been shown to respond to Ca^{2+} and nucleotide binding and to Ca^{2+} translocation (Coan and Inesi, 1977; Inesi *et al.*, 1978a,b; Coan *et al.*, 1979). It is probable that changes in SH reactivity and environment also involve changes in conformational energy and that Hg^{2+} or CH_3-Hg^+ binding alters this and results in both the decreased ATPase activity and the increased passive permeability of the pump. The effects on active transport occur at Hg^{2+} and CH_3-Hg^+ to SR ratios of 1.25×10^{-5} and 1.25×10^{-4} moles/g, respectively. Sodium dodecylsulfate gels of our ATPase-rich SR fraction show that 95% of the protein is Ca^{2+}-Mg^{2+}-ATPase. The molecular weight is 105,000 daltons (MacLennan, 1970). Thus we can calculate that our effects are seen at mercurial/ATPase stoichiometries of 1.32 and 13.2, respectively. Full inhibition of active transport was observed at 10-fold higher stoichiometries. For the case of Hg^{2+}, it appears that active transport can be abolished by binding on just a few SH groups. This is in agreement with the observation that blockage of 2–4 SH groups with *N*-ethylmaleimide can give complete inhibition of the ATPase and Ca^{2+}-uptake functions of the enzyme (Hasselbach and Seraydarian, 1966; Yoshida and Tonomura, 1976).

Our finding that Hg^{2+} and CH_3-Hg^+ increase the passive Ca^{2+} permeability in SR would appear to run contrary to the findings of Shamoo and MacLennan (1975) in black lipid membranes treated with succinylated Ca^{2+}-ATPase. They found that Hg^{2+} inhibited the ATPase-induced Ca^{2+} conductance while CH_3-Hg^+ was without effect. We found that both agents increase the passive permeability for Ca^{2+}. The bilayer and the native SR systems differ in a number of important features, any one of which may be

sufficient to explain the differing results. First, the lipid bilayer results measure the electrically-active Ca^{2+} permeability, while we measure the net flux of Ca^{2+} which we believe to occur by electrically silent $Ca^{2+}/2K^+$ exchange. Second, the lipid bilayer conductivity experiments involved detergent extraction and succinylation of the enzyme and depended upon "correct" insertion of the Ca^{2+}-ATPase into the bilayer, whereas our measurements were performed *in situ*. Third, the inhibition of the conductivity required higher Hg^{2+} concentrations (1×10^{-4} M) and was observed at lower protein concentrations (5×10^{-4} mg/ml) than studied here. The effect of Hg^{2+} on the bilayer conductance induced by the solubilized enzyme was attributed to Ca^{2+} occupation of the ionophore (translocator) site (Shamoo and MacLennan, 1975).

The present study shows that the mercurials increase the KCl permeability of the SR. As described in the Introduction, our preparations contain two populations of vesicles; two-thirds are Type I containing K^+ and Cl^- channels and one-third are Type II lacking these channels. The experiments reported here suggest that the mercurials can increase the KCl permeability via their effects on the channel and on the ATPase, with the effects on the channel making the largest contribution in Type I vesicles. The data presented in Table III show that 1×10^{-6} M Hg^{2+} and 1×10^{-5} M CH_3-Hg^+ increase the rates of KCl permeation for both types of vesicle. Maximal rates are obtained with 1×10^{-5} and $\geq 1 \times 10^{-4}$ M Hg^{2+} and CH_3-Hg^+, respectively. The relative sizes of the two populations are not changed by the treatment with the mercurials, and the rate of KCl equilibration increases with increasing mercurial concentration in a graded manner. The rate of KCl permeation is increased to the greatest extent in the Type I vesicles, with half-times reduced to 45 and 300 msec for Hg^{2+} and CH_3-Hg^+, respectively. The effects can be attributed to direct effects on the macromolecules responsible for the cation and anion channel function. The half-time observed for Hg^{2+} treatment is substantially lower than the half-time observed in control experiments, even when the K^+ permeability is maximized by valinomycin and the Cl^- permeability is rate-limiting to the overall process (cf. Kometani and Kasai, 1978, and Chiu and Haynes, 1980a, Table 8). Therefore, Hg^{2+} may have increased the Cl^- permeability as well. The effect of CH_3-Hg^+ addition is explicable in terms of an increase in K^+ permeability alone. A possible mechanism for the increase could be reaction with the SH groups to increase the channel diameter.

Our results on the effects of the mercurials on the K^+ channel function are at variance with the results and conclusions of Miller and Rosenberg (1979) concerning monovalent channel behavior. They treated black lipid membranes with SR and discovered a voltage-dependent monovalent conductance which was *inhibited* by mercurials. The lack of correspondence between

our results and theirs suggests that the conductance studied by Miller and Rosenberg (1979) may not be identical to the permeability function which equilibrates KCl across two-thirds of the SR vesicles. Alternatively, the mercurials perturb the channels differently in the black lipid membrane than in the native SR membrane.

The mercurials also increase the KCl permeability of Type II vesicles, but to values ($1/t_{1/2}$) an order of magnitude less than for Type I vesicles. Type II vesicles do not contain a K^+ channel function, and we have suggested that the low K^+ permeability of this fraction may be due to the passive properties of the Ca^{2+}-Mg^{2+}-ATPase. It is plausible that the mercurials increase the passive K^+ permeability by changing the conformational energetics of the pump in the same manner as proposed for the effect of Ca^{2+} permeability.

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