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# The membrane potential modulates the ATP-dependent $Ca^{2+}$ pump of cardiac sarcolemma

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**The effect of membrane potential on the activity of the ATP-dependent $Ca^{2+}$ pump of isolated canine ventricular sarcolemmal vesicles was investigated. The membrane potential was controlled by the intravesicular and extravesicular concentration of $K^+$, and the initial rates of $Ca^{2+}$ uptake both in the presence and the absence of valinomycin were determined. The rate of $Ca^{2+}$ uptake was stimulated by a inside-negative potential induced in the presence of valinomycin. The valinomycin-dependent stimulation was enhanced by the addition of $K^+$ channel blocker, tetraethylammonium ion or $Ba^{2+}$. The electrogenicity of cardiac sarcolemmal ATP-dependent $Ca^{2+}$ pump is suggested from the increase of $Ca^{2+}$ uptake by negative potential induced by valinomycin.**

Caroni and Carafoli [1,2] demonstrated the existence of a ATP-driven, calmodulin-activated $Ca^{2+}$ pump in sarcolemmal vesicles prepared from canine ventricles, and other investigators reported on the ATP-dependent $Ca^{2+}$ uptake into highly purified cardiac sarcolemmal vesicles [3–5]. Caroni and Carafoli [6] showed recently that protein kinase-dependent phosphorylation of a sarcolemmal protein stimulates the activity of $Ca^{2+}$ uptake into vesicles. The ATP-driven $Ca^{2+}$ pump in sarcolemma is assumed to regulate the intracellular $Ca^{2+}$ through the stimulation of calmodulin and protein kinase. In cardiac cells, the membrane potential may change the rate of $Ca^{2+}$ transport of the sarcolemmal $Ca^{2+}$ pump if the transport of $Ca^{2+}$ is electrogenic [7]. At present, however, the electrogenicity of the $Ca^{2+}$ pump of the plasma membrane is not established [8–10]. In the present study, the sensitivity of the ATP-dependent $Ca^{2+}$ pump to the membrane potential of sarcolemmal vesicles is examined. We found that an inside-negative potential accelerates the rate of $Ca^{2+}$ uptake, and that $K^+$ channel blockers [11–13], such as tetraethylammonium (TEA) ion or $Ba^{2+}$, could amplify the $K^+$ diffusion-potential dependence of the valinomycin stimulation of $Ca^{2+}$ pump.

Valinomycin was purchased from Sigma Chemical Co., and $^{45}CaCl_2$ was from New England Nuclear. Calmodulin was prepared from rabbit skeletal muscle according to the method of Yazawa et al. [19]. Sarcolemmal vesicles from canine ventricles were isolated by a procedure described previously [5]. The fresh vesicles (SL-4 and SL-5) in Ref. 5) were diluted with 3 vols. of the vesicle-loading solution containing 300 mM (potassium + lithium) chloride and 20 mM imidazole-HCl (pH 7.4). In some cases, 1 mM $BaCl_2$ or 10 mM TEA chloride was added to the vesicle-loading solution.

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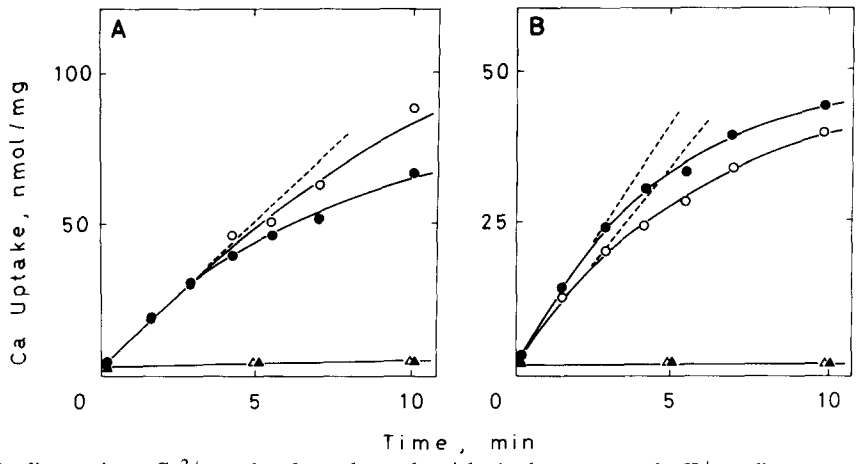


Fig. 1. The effect of valinomycin on $Ca^{2+}$ uptake of sarcolemmal vesicles in the presence of a $K^+$ gradient across the membrane. The reaction was started by the addition of 8 $\mu$l of vesicles into 592 $\mu$l of the reaction mixture and the $Ca^{2+}$ uptake was measured by the filter-assay method. (A) Vesicles loaded with 4 mM KCl, 296 mM LiCl and 20 mM imidazole-HCl (pH 6.8) were assayed under the following conditions: 50.9 $\mu$g/ml protein, 298 mM KCl, 4 mM LiCl, 50 $\mu$M $^{45}CaCl_2$, 3 mM $MgCl_2$, 1 $\mu$g/ml calmodulin and 20 mM imidazole-HCl (pH 6.8). (B) Vesicles loaded with 300 mM KCl and 20 mM imidazole-HCl (pH 6.8) were assayed under the following conditions: 46.5 $\mu$g/ml protein, 4 mM KCl, and 296 mM LiCl. Other conditions were the same as in (A). ●, ▲, 0.3 $\mu$M valinomycin; ○, △, without valinomycin; ●, ○, 2 mM ATP; ▲, △, without ATP.

The vesicles were collected by centrifugation at 69400 × $g$ for 25 min and the resultant precipitate was washed twice with the vesicle-loading solution. The washed precipitate was suspended in the vesicle-loading solution at a protein concentration of 2–4 mg/ml and used immediately. The protein was determined by the method of Bensadoun and Weinstein [20] with bovine serum albumin as the standard. $Ca^{2+}$ uptake reaction was carried out at 37°C in a medium containing 30–60 $\mu$g/ml vesicles, 300 mM (potassium + lithium) chloride, 3 mM $MgCl_2$, 2 mM Tris-ATP, 50 $\mu$M $^{45}CaCl_2$, 1 $\mu$g/ml calmodulin and 29 mM imidazole-HCl (pH 6.8) in the presence or absence of 0.3 $\mu$M

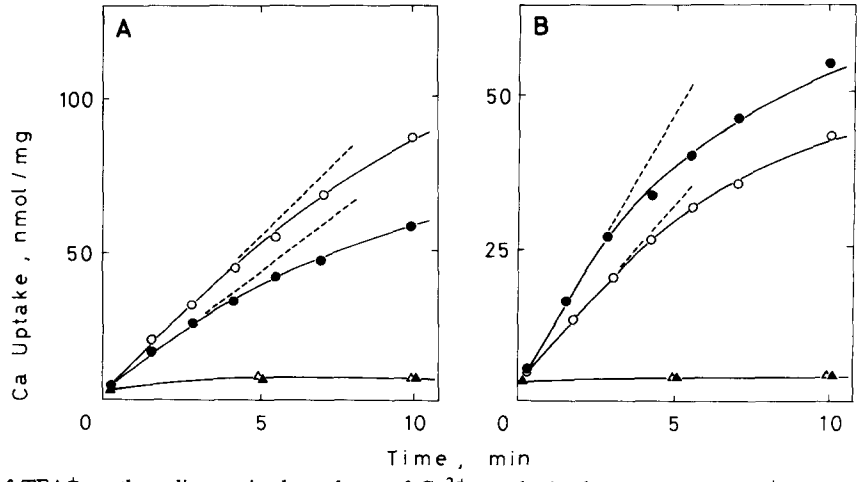


Fig. 2. The effect of $TEA^+$ on the valinomycin dependence of $Ca^{2+}$ uptake in the presence of a $K^+$ gradient. The experimental conditions were the same as described for Fig. 1 except that 10 mM TEA-Cl was added to the vesicle-loading solution and to the reaction mixture. (A) 61.3 $\mu$g/ml protein, 4 mM $[K^+]_i$, 296 mM $[K^+]_o$. (B) 59.1 $\mu$g/ml protein, 300 mM $[K^+]_i$, 4 mM $[K^+]_o$.

valinomycin. In some cases, 1 mM $BaCl_2$ or 10 mM TEA chloride was added to the medium. The reaction was started by the addition of 8 $\mu$l of vesicle suspension. At different times, 100 $\mu$l of the sample was pipetted on to a type HA 0.45-$\mu$m Millipore filter, and immediately washed 5 ml of chilled solution containing 300 mM KCl and 20 mM Imidazole-HCl (pH 7.4 at 4° C). The radioactivity retained on the filter was measured as described previously [5].

The gradient of $K^+$ across the vesicular membrane was produced by dilution of the vesicle suspension. The ATP-dependent $Ca^{2+}$ uptake of the vesicle preparation was measured in the presence and the absence of valinomycin. The ionic strength was maintained constant with a compensating amount of $Li^+$, which does not drive the $Na^+/Ca^{2+}$ exchange system [14]. Under this experimental conditions the measurements of $Ca^{2+}$ uptake into the vesicles give the activity of the ATP-driven $Ca^{2+}$ pump. We assumed that the membrane potential is dominantly determined by the diffusion potential of $K^+$ in the presence of valinomycin, because the preliminary measurements indicated the much greater permeability of $K^+$ compared to that of $Li^+$ or $Cl^-$ (data not shown). Figs. 1, 2 and 3 show the time course of ATP-dependent $Ca^{2+}$ uptake. When the intravesicular $K^+$ concentration ($[K^+]_i$) was 4 mM and the extravesicular $K^+$ concentration ($[K^+]_o$) was 296 mM, valinomycin did not affect the initial rate of $Ca^{2+}$ uptake in the absence of $TEA^+$ and $Ba^{2+}$ (Fig. 1), whereas the inhibition by valinomycin became clear in the presence of 10 mM $TEA^+$ or 1 mM $Ba^{2+}$ (Figs. 2A and 3A). On the other hand, when $[K^+]_i$ was 300 mM and $[K^+]_o$ was 4 mM, valinomycin activated the initial rate of $Ca^{2+}$ uptake. The activating effect of valinomycin increased in the presence of 10 mM $TEA^+$ or 1 mM $Ba^{2+}$ (Figs. 2B and 3B).

Two possible explanations for the effect of $TEA^+$ and $Ba^{2+}$ were considered. First, $TEA^+$ or $Ba^{2+}$ could change the membrane potential of vesicles in the absence of valinomycin. The membrane potential was dominated by the opening of intrinsic $K^+$ channels under $Ca^{2+}$ uptake conditions even without valinomycin; the existence of $Ca^{2+}$-dependent $K^+$ channels has been reported in sarcolemmal vesicles [15–18]. When $K^+$ channels of the sarcolemmal vesicles are blocked by $TEA^+$ or $Ba^{2+}$, the $K^+$ channel-dependent membrane potential should be diminished. Therefore, the effect of valinomycin to $K^+$ diffusion potential in the presence of $TEA^+$ or $Ba^{2+}$ will be larger than that in the absence of $TEA^+$ or $Ba^{2+}$.

It is also possible to assume that the modification of the activity of the $Ca^{2+}$ pumps by $TEA^+$ or $Ba^{2+}$ might depend on the membrane potential. $TEA^+$ and $Ba^{2+}$ inhibited, respectively weakly and strongly, the $Ca^{2+}$ uptake. The inhibitory effects of these reagents increased with increasing concentration, but the activation by valinomycin did

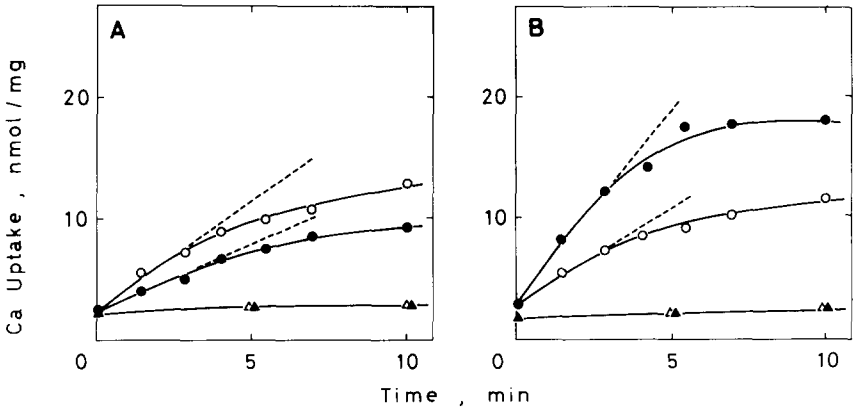


Fig. 3. The effect of $Ba^{2+}$ on the valinomycin dependence of $Ca^{2+}$ uptake in the presence of a $K^+$ gradient. The experimental conditions were the same as described for Fig. 1 except that 1 mM $BaCl_2$ was added to the vesicle-loading solution and to the reaction mixture. (A) 25.7 $\mu$g/ml protein, 4 mM $[K^+]_i$, 296 mM $[K^+]_o$. (B) 38.4 $\mu$g/ml protein, 300 mM $[K^+]_i$, 4 mM $[K^+]_o$.

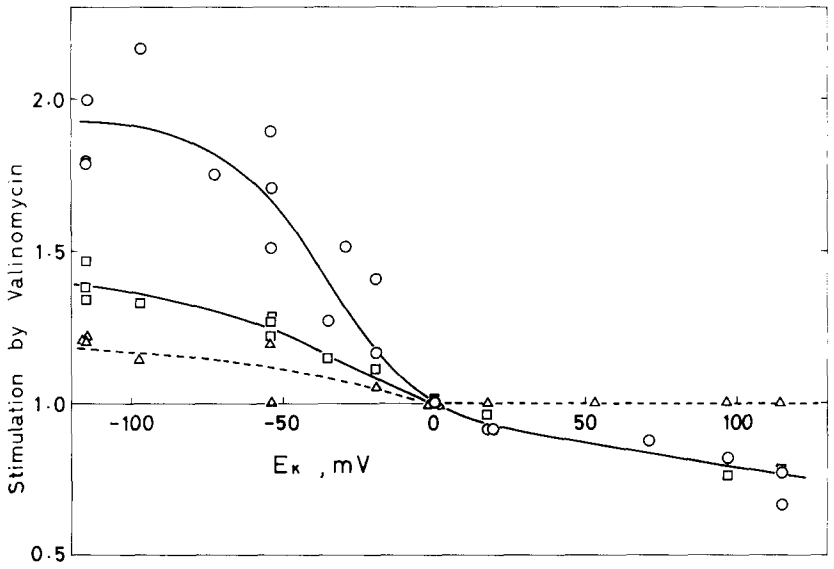


Fig. 4. The effect of the membrane potential on the stimulation of the $Ca^{2+}$ pump in the presence or absence of a $K^+$-channel blocker. Stimulation was expressed by the ratio of the initial rate of $Ca^{2+}$ uptake in the presence of valinomycin to the initial rate of $Ca^{2+}$ uptake in the absence of valinomycin. The membrane potential ($E_K$) was calculated from the $K^+$ gradient, using Nernst's equation; $E_K = 61.5 \log ([K^+]_o/[K^+]_i)$. The $K^+$ carried over to the assay with the vesicles and the dilution of the reaction mixture were taken into account for the calculation of $[K^+]_o$. Values of $[K^+]_i$ were 300 mM, 150 mM, 20 mM and 4 mM, respectively, for $E_K$ values from −115 mM to 0 mV, from −54 to 18 mV, 72 mV, and from 97 to 115 mV, respectively. △, without $K^+$-channel blocker; □, 10 mM TEA-Cl; ○, 1 mM $BaCl_2$.

not change between 5 and 50 mM $TEA^+$ or between 0.1 and 2 mM $Ba^{2+}$ when measured at 300 mM $[K^+]_i$ and 4 mM $[K^+]_o$ (data not shown). As shown in Fig. 4, the increase of the uptake rate induced by a negative potential and the decrease of the uptake rate induced by a positive potential were almost in balance in the presence of $TEA^+$. On the other hand, the effect on the uptake rate of a negative potential was clearly larger than that of a positive potential in the presence of $Ba^{2+}$. The effects of $TEA^+$ and $Ba^+$ showed some difference. The $Ca^{2+}$ pump was activated by a negative potential change by valinomycin even in the absence of $TEA^+$ of $Ba^{2+}$ (Fig. 1B). It is not probable that valinomycin modulates only the inhibition of $Ca^{2+}$ uptake by $TEA^+$ or $Ba^{2+}$.

Fig. 4 shows the effect of a $K^+$ diffusion potential on the stimulation of $Ca^{2+}$ uptake in the presence of valinomycin. The diffusion potential was calculated according to Nernst's equation. The voltage dependence of the stimulation was clearly enhanced by $TEA^+$ or $Ba^{2+}$. The increase of the valinomycin-stimulated $Ca^{2+}$ uptake observed at a negative potential suggests that the sarcolemmal $Ca^{2+}$ pump is electrogenic. If the $Ca^{2+}$ pump were electrogenic and no charged ion except $Ca^{2+}$ moved, then $Ca^{2+}$ uptake should induce an inside-positive membrane potential, and this membrane potential should inhibit the $Ca^{2+}$ uptake. In Fig. 4, the rate of $Ca^{2+}$ uptake was not activated when the voltage was clamped at 0 mV by $K^+$-valinomycin. This result shows that a positive potential was not induced by ATP-dependent $Ca^{2+}$ uptake under the experimental conditions. However, it does not necessarily show electroneutrality of $Ca^{2+}$ transport process by the $Ca^{2+}$ pump. The rapid passage of $K^+$ and $Cl^-$ across the membrane of vesicles could short-circuit any potential originating from the $Ca^{2+}$ pump [16,18].

An alternative explanation for Fig. 4 is also possible. The inside negative potential may change the conformation of $Ca^{2+}$ pump which causes activation of $Ca^{2+}$ uptake.

## References

1 Caroni, P. and Carafoli, E. (1980) Nature 283, 765–767.
2 Caroni, P. and Carafoli, E. (1981) J. Biol. Chem. 256, 3263–3270.

3 Trumble, W.R., Sutko, J.L. and Reeves, J.P. (1980) Life Sci. 27, 207–214.
4 Trumble, W.R., Sutko, J.L. and Reeves, J.P. (1982) J. Biol. Chem. 256, 7101–7104.
5 Kuwayama, H. and Kanazawa, T. (1982) J. Biochem. 91, 1419–1426.
6 Caroni, P. and Carafoli, E. (1981) J. Biol. Chem. 256, 9371–9273.
7 Zimniak, P. and Racker, E. (1978) J. Biol. Chem. 253, 4631–4637.
8 Niggli, V., Sigel, E. and Calafoli, E. (1982) J. Biol. Chem 257, 1–15.
9 Rossi, J.P.F.C. and Schatzman, H.J. (1982) J. Physiol. 327, 1–15.
10 Gimble, J.M., Waisman, D.M., Gustin, M., Goodman, D.B.P. and Rasmussen, H. (1982) J. Biol. Chem. 257, 10781–10788.
11 Hagiwara, S. and Saito, N. (1959) J. Physiol. 148, 161–179.
12 Sperelakis, N. and Lehmkuhl, D. (1966) J. Gen. Physiol. 49, 867–895.
13 Hermann, A. and Gorman, A.L.F. (1981) J. Gen. Physiol. 78, 87–110.
14 Reeves, J.P. and Sutko, J.L. (1979) Proc. Natl. Acad. Sci. USA 76, 590–594.
15 Bartschat, D.K., Cyr, D.L. and Lindenmayer, G.E. (1980) J. Biol. Chem. 255, 10044–10047.
16 Caroni, P., Reinlib, L. and Carafoli, E. (1980) Proc. Natl. Acad. Sci. USA 77, 6354–6358.
17 Caroni, P. and Carafoli, E. (1982) Proc. Natl. Acad. Sci. USA 79, 5763–5767.
18 Coronado, R. and Latorre, R. (1982) Nature 298, 849–852.
19 Yazawa, M., Kuwayama, H. and Yagi, K. (1978) J. Biochem. 84, 1253–1258.
20 Bensadoun, A. and Weinstein, D. (1976) Anal. Biochem. 70, 241–250.