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Modulation by calcium of the potassium permeability of dog heart sarcolemmal vesicles

(trifluoperazine/quinidine/Na^+/Ca^{2+} exchange)

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ABSTRACT **The movement of K^+ in heart sarcolemmal vesicles has been followed through the opposing movement of the tetraphenylphosphonium ion. Ca^{2+} (5–50 μM) stimulates the efflux of K^+ from K^+-loaded vesicles [$K_m(Ca^{2+}) \approx 10$ μM], and the activation requires that Ca^{2+} be present inside the vesicles together with K^+. The efflux of $^{86}Rb^+$ from K^+-, Rb^+-loaded vesicles is similarly stimulated by 5–50 μM Ca^{2+} [$K_m(Ca^{2+}) \approx 10$ μM]. The Ca^{2+}-induced increase of K^+ permeability does not become spontaneously inactivated. The effects of some inhibitors have been tested under conditions in which Ca^{2+} promotes the entry of K^+ into vesicles. In this system, direct interaction of the drug with the Ca^{2+} and K^+ membrane binding site(s) was therefore studied. Tetraethylammonium ion, which inhibits the potential-dependent K^+ channel, does not interfere with the effect of Ca^{2+} whereas quinidine (IC_{50} = 12 μM) and trifluoperazine (IC_{50} = 8 μM at 50 μg of sarcolemmal protein per ml) inhibit.**

Ca^{2+}-induced changes in the K^+ permeability of the plasma membrane have been described in many cell types (1, 2). These changes are normally attributed to the effect of Ca^{2+} on a K^+ channel that, in some cells, apparently operates in addition to the voltage-sensitive K^+ channel (3, 4). Some nonexcitable cells (e.g., erythrocytes) apparently possess only the Ca^{2+}-dependent channel (5–7). It has been suggested that the voltage-dependent channel is more sensitive to tetraethylammonium ion (8) and to 4-aminopyridine (9) than the Ca^{2+}-dependent channel. The latter, on the other hand, is inhibited by quinine, quinidine (9), high concentrations of chlorpromazine (10), and by trifluoperazine (11). The most detailed studies on the Ca^{2+}-dependent modulation of the K^+ permeability have been carried out in erythrocytes. Interestingly, the Ca^{2+} sensitivity of the K^+-permeation mechanism has been found to vary with the experimental conditions and the physiological state of the cell (12). Calcium-activated outwardly directed (K^+) currents have been described in Purkinje fibers (9, 13–15) and in ventricular muscle fibers (16–18).

Studies on intact cell systems offer advantages but do not permit full control of the experimental conditions at the site of the transport process(es). In addition, they are often complicated by the difficulty of isolating the phenomenon under study from interconnected events. Vesicular preparations of plasma membranes could be more useful, but it is difficult to manipulate membrane potentials in vesicular preparations. As a result, the study of voltage-gated channels, at least in the absence of drugs (e.g., veratridine for the Na^+ channel) that block the gating mechanism in the open position is currently difficult. The Ca^{2+}-dependent K^+ channel has been reported to be voltage insensitive (19) and remains apparently activated for prolonged times (1). Therefore, it is potentially accessible to vesicle studies.

The experiments described in this paper characterized a Ca^{2+}-dependent increase in K^+ permeability in heart sarcolemmal vesicles. The dependence of the activation of K^+ permeability on the Ca^{2+} concentration and the affinity of the transport system for K^+ have been measured. Ca^{2+} and K^+ interact with the channel from the same side of the vesicles. The activating effect of Ca^{2+} is decreased by quinidine and trifluoperazine, whereas Et_4N^+, which inhibits the voltage-dependent K^+ channel, has no effect.

MATERIALS AND METHODS

Chemicals. Phosphatidylcholine and phosphatidic acid were grade 1 from Lipid Products, South Nutfield, England. Quinidine sulfate, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) and valinomycin were from Sigma; Et_4NCl and arsenazo III were from Fluka, Buchs, Switzerland. A23187 was from Eli Lilly. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and trifluoperazine were gifts of P. G. Heytler (Du Pont) and of Smith, Kline & French, respectively. Quinidine, DIDS, and trifluoperazine solutions were prepared freshly and kept protected from light. Dog hearts were obtained from blood donors of the Kantonsspital in Zurich.

Preparation of Dog Heart Sarcolemma. Dog heart sarcolemma was prepared by the method of Jones *et al.* (20) modified as described (21). At the end of the procedure, the vesicles were suspended (5–10 mg of protein/ml) in 160 mM KCl/20 mM Hepes, pH 7.4 ("KCl medium"), 160 mM KOAc/20 mM Hepes, pH 7.4 ("KOAc medium"), or 160 mM NaCl/20 mM Hepes, pH 7.4 ("NaCl medium"). The suspension was immediately frozen in liquid nitrogen and stored at −80°C. Calmodulin-depleted sarcolemmal vesicles were prepared as described (22). Studies of ion fluxes in plasma membrane vesicles would benefit from the availability of populations of uniform polarity. Such populations have been obtained (23, 24) but at the cost of large losses of material. Thus, in this study, no attempt has been made to obtain vesicles of uniform polarity. The experimental conditions chosen, however, have permitted "functional" exclusion of vesicles of unwanted polarity.

Tetraphenylphosphonium ion (Ph_4P^+) Uptake Experiments. Ph_4P^+ uptake was measured with a Ph_4P^+-sensitive electrode (90% response in 0.1 sec) (25) in the presence of 10 μM Ph_4P^+. KCl-loaded vesicles were incubated for 5 min at 30°C in 160 mM KCl/20 mM Hepes/100 μM EGTA/0.5 μM A23187/30 μM Ph_4P^+, pH 7.4 to deplete the vesicles of Ca^{2+}. Ten microliters of these vesicles was added to 1 ml of 160 mM choline chloride

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Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Ph_4P^+, tetraphenylphosphonium ion; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; Cho, choline.

(or 160 mM KCl as indicated)/20 mM Hepes Tris/0.5 μM A23187/10 μM Ph_4P^+ containing a Ca^{2+}/EGTA buffer (100 μM EGTA) calculated to give the free Ca^{2+} desired. However, the latter was estimated with a Ca^{2+}-sensitive electrode (26). The pH was 7.4 and the temperature was 30°C. For experiments in the absence of A23187, the vesicles were incubated for 30 min at 30°C as indicated above but without the ionophore.

$^{86}Rb^+$ Efflux Experiments. Two techniques were used. In the first, the Ca^{2+} concentration in the outside medium was equilibrated with that inside the vesicles by A23187 as in the experiments with Ph_4P^+. In this case, KOAc-loaded vesicles (8 mg of protein/ml) were incubated for 3 hr on ice in the presence of 0.1 mM Tris/EGTA/0.5 μM A23187/1 mM ^{86}RbCl (430 Ci/mol of Rb^+; 1 Ci = 3.7×10^{10} becquerels), pH 7.4. At time zero, the previously incubated ^{86}Rb-loaded vesicles were diluted 1:100 with 250 μl of 160 mM choline chloride/20 mM Hepes Tris/5 mM Et_4N^+/0.5 μM A23187/0.2 μM FCCP, pH 7.4, at 30°C. A Ca^{2+}/EGTA buffer (100 μM EGTA) produced the free Ca^{2+} desired. After 5 and 30 sec, 100-μl portions were removed, filtered through Millipore filters (pore diameter, 0.22 μm), and immediately washed with 2 ml of ice-cold 160 mM choline chloride/20 mM Hepes Tris/10 mM Et_4N^+/5 mM EGTA, pH 7.4. In the second method, Ca^{2+} was equilibrated by passive diffusion across the sarcolemmal membrane. In this case, KOAc-loaded vesicles were incubated with 10 mM Et_4N^+ and various concentrations of Ca^{2+} on ice for 24 hr. ^{86}Rb efflux was measured as described above (no Ca^{2+} ionophore was included in the medium). The results with the two techniques were essentially identical, and Figs. 3 and 4 refer to data obtained with the ionophore technique. The initial $^{86}Rb^+$ content of the vesicles was estimated by diluting the vesicles in the "efflux" medium at 4°C and taking an aliquot after 5 sec. The value corresponded, within experimental error, to the extrapolated "zero value" derived from a first-degree exponential curve. If added, valinomycin was 1 μM. KOAc/$^{86}Rb^+$-loaded liposomes (96% phosphatidylcholine/4% phosphatidic acid) were prepared by a conventional method (27). The loading and efflux media for the liposome experiments were as described above. The concentration of phospholipid in the assay and in the incubation medium was approximately the same for sarcolemma and for liposomes. The $^{86}Rb^+$ content of the liposomes after dilution in the efflux medium was measured by centrifugation through a "dried" (28) Sephadex G-50 column equilibrated with 160 mM choline chloride (ChoCl)/20 mM Hepes Tris pH 7.4.

Na^+/Ca^{2+} Exchange. Na^+-dependent Ca^{2+} uptake was measured by the method of Reeves and Sutko (29) modified as described (21). If added, valinomycin was 1 μM. Ca^{2+} uptake was monitored at 685–660 nm in an Aminco DW-2 spectrophotometer.

RESULTS AND DISCUSSION

The vesicles used in this study are enriched about 100-fold in sarcolemmal markers as compared with the homogenate, are tightly sealed, and possess electrogenic Na^+/Ca^{2+} exchange (21). The inside-out/right-side-out/leaky vesicle ratio is estimated to be 40:45:15 (21). As will be pointed out below, the results obtained presumably reflect the activity of vesicles of one orientation.

During operation of the Na^+/Ca^{2+} exchanger, an electrical potential, negative inside if internal Na^+ is exchanged for external Ca^{2+}, is generated (25, 21). The initial rate of Na^+-dependent Ca^{2+} uptake shows hyperbolic dependence on the free Ca^{2+} of the medium under conditions of optimal charge compensation [i.e., when the uptake is measured by diluting Na^+-loaded vesicles in a medium containing isoosmotic K^+ and valinomycin (21)]. However, if valinomycin is omitted from the medium, the kinetics becomes biphasic (21). The initial rate of Ca^{2+} uptake in the absence of valinomycin parallels that in its presence at a free medium Ca^{2+} concentration below about 0.5 μM, remains constant at 0.5–5 μM Ca^{2+}, and increases rapidly between 5 and 50 μM Ca^{2+} to a value approaching that observed in the presence of valinomycin. The increment between 5 and 50 μM Ca^{2+} is not observed if the K^+ in the uptake medium is replaced by Cho, suggesting that 5–50 μM Ca^{2+} increases the K^+ permeability of the membrane. The increased K^+ penetration would charge compensate the Na^+/Ca^{2+} exchanger. In experiments of this type, only inside-out vesicles contribute to the increased uptake of K^+, because the K^+ "channel" is activated by intra-, but not by extra-cellular Ca^{2+} (Figs. 1 and 2).

Ca^{2+}-Depleted vesicles loaded with KCl and Ph_4P^+ were diluted in a ChoCl/Ph_4P^+-containing medium. Since the sarcolemmal membrane is much less permeable to Cho^+ than to K^+ (21), an electrical potential (negative inside) develops if K^+ flows out of the vesicles and will be monitored by uptake of the lipophilic cation Ph_4P^+ (Fig. 1). To eliminate membrane potential-independent partition of Ph_4P^+ in the sarcolemmal membranes, the vesicles were first incubated with exactly the amount of Ph_4P^+ that produced no further "uptake" when KCl-loaded vesicles were diluted in KCl medium (i.e., in the absence of any diffusion potential) (Fig. 1). The free Ca^{2+} concentration of the outside medium was then varied, and A23187 (28) was added to rapidly equilibrate Ca^{2+} across the sarcolemmal membrane. Figs. 1 and 2 show that Ca^{2+} increased the rate of Ph_4P^+ uptake into the vesicles in the same concentration range at which the increase in K^+ permeability was observed in the Na^+/Ca^{2+} exchange experiments (21). Half-maximal activation was observed at a free external Ca^{2+} concentration of about 10 μM. This value is probably an underestimation, because the method used does not permit reliable quantitative estimation of uptake rates at Ca^{2+} concentrations in excess of about 50 μM. The possibility of an electrogenic K^+/Ca^{2+} exchange was ruled out, because KCl-loaded vesicles did not accumulate Ca^{2+} when diluted with a Ca^{2+}-containing ChoCl medium. In principle, alkalinization of the intravesicular space by the $Ca^{2+}/2\ H^+$ exchange catalyzed by A23187 could also have promoted the uptake of Ph_4P^+. This was ruled out, however, because increasing the buffering power inside and outside the vesicles by increasing the Hepes concentration from 20 to

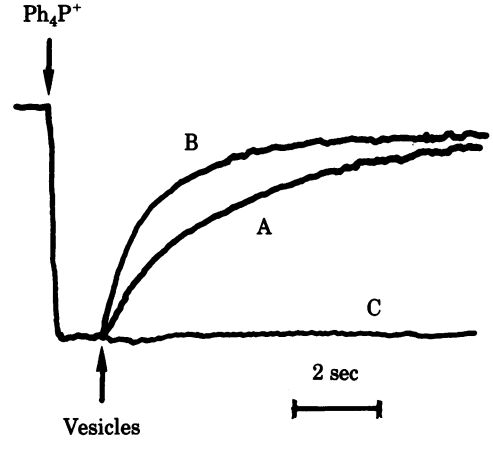

FIG. 1. Time course of Ph_4P^+ uptake. K^+-loaded vesicles were incubated with medium containing 0.2 nmol of Ph_4P^+ for 5 min at 30°C and then added to various isoosmotic solutions containing 10 μM Ph_4P^+. Curves: A, 160 mM ChoCl/20 mM Hepes Tris/0.5 μM A23187/100 μM EGTA; B, as for curve A, but 100 μM Ca^{2+} instead of 100 μM EGTA; C, as for curve A, but 160 mM KCl instead of 160 mM ChoCl.

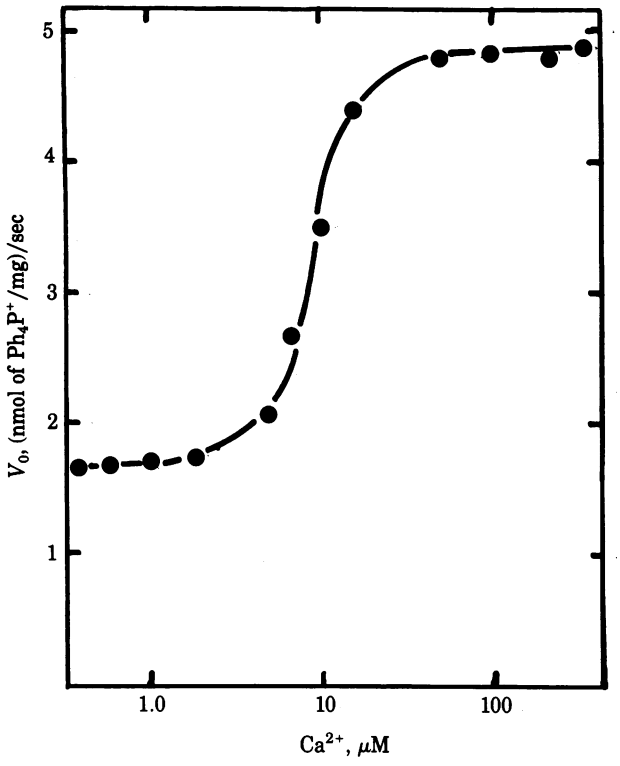

FIG. 2. Ca^{2+} dependence of Ph_4P^+ uptake (V_0) promoted by efflux of K^+.

100 mM had no effect. Figs. 1 and 2 (as well as experiments described below) thus indicate a specific effect of Ca^{2+} on a mechanism that translocates K^+. Cho was not accepted by the K^+-translocating system but the problem of whether the system discriminates between Na^+ and K^+ was not considered, because the Na^+/Ca^{2+} exchanger of the vesicles would prevent a direct test of possible Na^+ movement through the translocation system.

When A23187 was omitted from the medium, the Ca^{2+} dependence of Ph_4P^+ uptake was lost. This shows that Ca^{2+} activates from the side of the membrane at which K^+ becomes engaged in the translocating pathway. Accordingly, no A23187 was required for the Na^+/Ca^{2+} exchange experiment reported in ref. 21 because, in this case, Ca^{2+} was transported in the same direction as K^+ (i.e., from outside to inside). Finally, to eliminate the possibility that Ca^{2+} might increase the rate of Ph_4P^+ uptake by decreasing the contribution of Cl^- to the charge-compensating process, experiments with DIDS, which inhibits Cl^- permeability (30), were performed. The amount and velocity of Ph_4P^+ uptake in the presence of 0.8 μM DIDS were indeed higher, supporting earlier indications (21) for a role of Cl^- in charge-compensating electrogenic transport processes in sarcolemma, but the Ca^{2+} sensitivity of the process remained the same.

The Ca^{2+} dependence of the K^+ permeability was demonstrated directly by studying the effect of Ca^{2+} on the efflux of Rb^+ from ^{86}Rb-loaded vesicles. Rb^+ can substitute for K^+ in the Na^+/Ca^{2+} exchange experiments as well as in the Ph_4P^+ uptake experiments (data not shown). Vesicles first loaded with KOAc and then equilibrated with $^{86}RbCl$ were diluted in isoosmotic ChoCl. Low concentrations of the protonophore FCCP were included in the release medium. Because undissociated acetic acid generally permeates biological membranes, FCCP was included to permit return of the protons transported outward by acetic acid into the vesicles, thus leaving a net negative charge outside. This would permit the flow of KOAc out of the vesicles without the generation of a membrane potential (31). Cl^- was inadequate for this experiment because the Cl^- permeability of the sarcolemmal membrane is relatively low (21). As mentioned for the Na^+/Ca^{2+} exchange experiment, only vesicles of one polarity were monitored, in this case, those with a right-side-out orientation.

The $^{86}Rb^+$ efflux data were interpreted by assuming first-order kinetics to permit extraction of the rate constants from the equation $K^+(t) = K^+(t = o)e^{-kt}$, where $K^+(t)$ is the amount of K^+ inside the vesicles at a given time [t (in sec)] and k is the rate constant of K^+ ($^{86}Rb^+$) efflux in sec^{-1}. The curve fits the data reasonably well during the first part of the efflux (Fig. 3). The Ca^{2+} dependence of the rate of $^{86}Rb^+$ efflux at 30°C is shown in Fig. 3. Again, Ca^{2+} stimulates the rate at 2–50 μM, and half-maximal activation occurs at about 10 μM ($k = 9.2 \times 10^{-2}$ sec^{-1}). For comparison, when the efflux is measured at 4°C in the absence of Ca^{2+}, $k = 5.5 \times 10^{-4}$ sec^{-1} and, when valinomycin is included in the efflux medium (at 30°C), $k = 7.8 \times 10^{-1}$ sec^{-1}. Interestingly, as long as the Ca^{2+} concentration was kept constant, no spontaneous inactivation of the efflux mechanism was observed; i.e., the Ca^{2+} dependence of $k(t)$ was essentially independent of t. Thus, this K^+ efflux mechanism is turned on and off exlusively by changes in the Ca^{2+} concentration. The rate of $^{86}Rb^+$ efflux is somewhat less than that expected from conductance measurements of Ca^{2+}-activated K^+ channels *in vivo*. Under our experimental conditions, however, quantitative estimates of expected rates are difficult because no information is available as to the number of functional systems present per vesicle. In addition, the properties of the K^+ permeability system may have been altered by the isolation procedure. Control experiments have been carried out by using

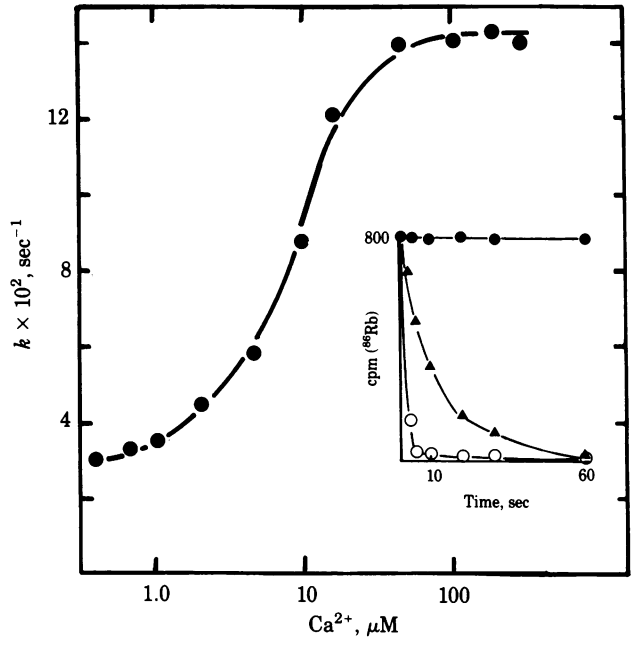

FIG. 3. Ca^{2+} dependence of the rate of ^{86}Rb efflux from KOAc/^{86}Rb-loaded vesicles. k values were derived from three experiments (SEM ≈ 5%) and calculated according to an assumed first-order exponential decay. Aliquots were removed 5 sec after dilution of the vesicles in the efflux medium. The free Ca^{2+} concentration of the efflux medium was adjusted with a Ca^{2+}/EGTA (100 μM EGTA) buffer and A23187 was present during the incubation of the vesicles and in the efflux medium to permit rapid equilibration of Ca^{2+} across membrane. (*Inset*) Typical time courses of K^+ ($^{86}Rb^+$) efflux from which k values were calculated. Efflux was measured at 4°C (●) or at 30°C in the presence of 10 μM (▲) or 1 μM (○) valinomycin. Values on the abscissa are corrected for binding.

liposomes equilibrated with Ca^{2+} outside with the Ca^{2+} ionophore technique. At the concentrations and phospholipid ratio used in the experiments described above, no Ca^{2+}-dependent $^{86}Rb^+$ leak from ^{86}Rb-loaded liposomes was observed. In liposomes, the $^{86}Rb^+(K^+)$ permeability was the limiting factor since the addition of valinomycin induced a 200-fold increase in k at 30°C.

The Ca^{2+}-sensitive portion of the $K^+(^{86}Rb^+)$ efflux, represented by the difference between the efflux rate at 50 μM Ca^{2+} and that at 0.5 μM Ca^{2+}, was insensitive to 0.1–10 mM Et_4N^+, was only slightly sensitive to 0.1–1 mM quinidine, and was inhibited about 10% by 40 μM trifluoperazine at a vesicle concentration of 50 μg of protein per ml. In these experiments, the drugs, which are only poorly permeant across membranes, were added to the side of the membrane that opposes the K^+ and Ca^{2+} binding side.

The dependence of the Ca^{2+}-sensitive K^+ ($^{86}Rb^+$) efflux on the intravesicular K^+ concentration inside the vesicles is shown in Fig. 4. The vesicles were equilibrated with various mixtures of K^+ ($^{86}Rb^+$) and Cho and the efflux rate was measured in ChoCl. The K^+ ($^{86}Rb^+$) efflux [loss of K^+ ($^{86}Rb^+$) during the first 2 sec] is a saturable function of the K^+ concentration, indicating the involvement of a carrier (or channel). Assuming a selectivity ratio of 1 for K^+ and Rb^+, the K_m (K^+) of the system is *ca.* 60 mM.

The data here show that there is a good correlation between the effect of Ca^{2+} on the sarcolemmal K^+ permeability and the stimulatory effect of Ca^{2+} on the initial rate of the Na^+/Ca^{2+} exchange. The two procedures used (Ph_4P^+ uptake and $^{86}Rb^+$ efflux) have the disadvantage that the presumed Ca^{2+} and K^+ binding sites face the inside space of the vesicles and are therefore not readily accessible. It can be assumed that only right-side-out vesicles contribute to the effects of Ca^{2+} on Ph_4P^+ uptake and $^{86}Rb^+$ efflux. By contrast, only inside-out vesicles would participate in the effect of external Ca^{2+} on the rate of the Na^+/Ca^{2+} exchanger in the presence of K^+ because, in this case, both Ca^{2+} and K^+ interact from outside. That Ca^{2+} influences the K^+ permeability *in vivo* by interacting only with the inner side of the sarcolemmal membrane is a permissible assumption, because the inner side is exposed to changes in the concentration of free Ca^{2+} of 0.1–10 μM whereas the interstitial Ca^{2+} is constant at the millimolar level.

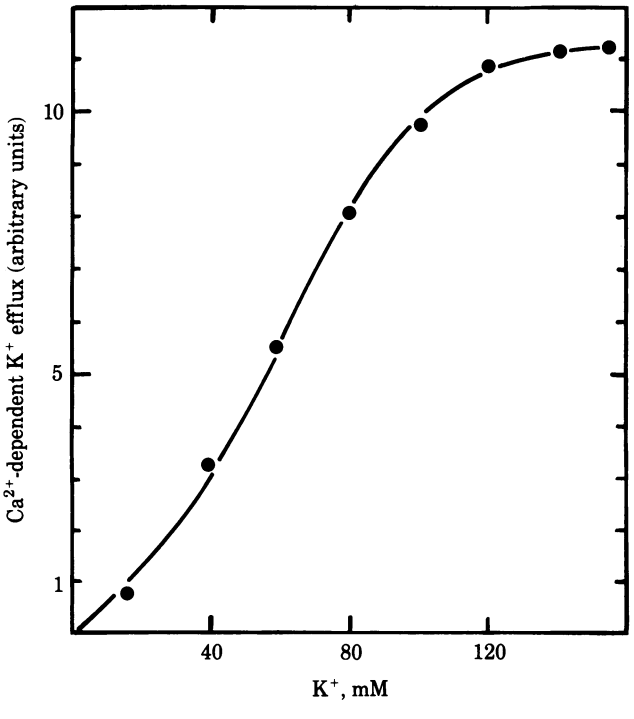

FIG. 4. Dependence of the Ca^{2+}-induced K^+ release on the intravesicular K^+ concentration. Values represent the difference between the 2-sec efflux values in the presence (100 μM Ca^{2+}) and in the absence (100 μM EGTA) of Ca^{2+}. The vesicles were loaded with different ratios of KOAc/^{86}Rb to ChoCl. Total osmolarity was kept at 320 mOsm. The experiment is based on the assumption of a K^+/Rb^+ selectivity ratio of 1 for the Ca^{2+}-dependent K^+ permeability mechanism.

The problem of the sidedness of K^+ and Ca^{2+} interaction can be extended to the action of inhibitory drugs such as quinidine, Et_4N^+, and trifluoperazine. The results of testing these drugs by using the Na^+/Ca^{2+} exchange as a tool (i.e., under conditions in which they could react from outside, at the same membrane side at which Ca^{2+} and K^+ react) are summarized in Table 1. To show that the drugs acted on the Ca^{2+}-dependent K^+ permeation and not on other parameters influencing the rate of Na^+/Ca^{2+} exchange, two controls were carried out. They were based on the following reasoning: (*i*) that the drug that proved effective in a valinomycin-free KCl medium at 50 μM free Ca^{2+} had to be ineffective in the presence of valinomycin, because optimal charge compensation in this case was already provided, and (*ii*) that the same drug had to be ineffective when the initial rate of Na^+-supported Ca^{2+} uptake in a valinomycin-free KCl medium was assayed at 0.5 μM free Ca^{2+}, a concentration at which the K^+ permeability is not activated (Figs. 2 and 3). As shown in Table 1, Et_4N^+ had no effect on the Ca^{2+}-dependent K^+ permeability, whereas quinidine was strongly inhibitory (IC_{50}, 12 μM at 50 μg of sarcolemmal protein per ml). It can thus be concluded that quinidine inhibits the Ca^{2+}-dependent K^+ permeation and acts at the side of the membrane at which K^+ and Ca^{2+} interact. Table 1 also shows that the calmodulin antagonist trifluoperazine inhibited the Ca^{2+}-dependent K^+ channel. Conclusions on the role of calmodulin based on the effect of trifluoperazine must necessarily be taken as tentative. A direct test (i.e., by calmodulin depletion and readdition) was prevented by the fact that the calmodulin-depletion procedure rendered the vesicles leaky (22) and, thus, only weakly sensitive to valinomycin in the Na^+/Ca^{2+} exchange experiment. However, the fact that trifluoperazine inhibited the process at low concentrations and at a high vesicle/trifluoperazine ratio (IC_{50} = 8 μM at 50 μg of sarcolemmal protein per ml) suggests calmodulin involvement.

Table 1. Ca^{2+}-Dependent compensation by K^+ of the charge separation generated by the Na^+/Ca^{2+} exchanger: Inhibition by quinidine and trifluoperazine

	Initial rate of Na^+-dependent Ca^{2+} uptake		
	50 μM Ca^{2+}		0.5 μM Ca^{2+}
Addition	With valinomycin	Without valinomycin	(without valinomycin)
None	10.1	12.1	2.5
ChoCl (160 mM)	4.2	4.2	2.4
Quinidine (20 μM)	5.2	11.9	2.4
Trifluoperazine (10 μM)	6.8	11.7	2.3
Et_4N^+ (5 mM)	10.4	12.0	2.5

Na^+-dependent Ca^{2+} uptake into sarcolemmal vesicles in the presence of various additions was measured by the arsenazo III method. The basic composition of the extravesicular medium was 160 mM KCl/20 mM Hepes/50 μM arsenazo III, pH 7.4. Where indicated, 160 mM ChoCl was used in place of 160 mM KCl. When added, valinomycin was 1 μM. Free Ca^{2+} concentrations refer to concentrations in the outside medium. Results are expressed as (nmol of Ca^{2+}/mg of protein)/sec and represent the mean of at least three experiments; SEM ≈ 5%.

In conclusion, a Ca^{2+}-dependent K^+ permeability mechanism probably involving a channel has been demonstrated in heart sarcolemmal vesicles. Its properties recall those of Ca^{2+}-activated K^+ channels and the Ca^{2+}-activated Me^+ channel recently demonstrated in heart (32). Indeed, one parameter that has not been defined here is the Na^+/K^+ selectivity of the system. In the preparation used in this study, Na^+ would have moved across the membrane in the presence of Ca^{2+} on the Na^+/Ca^{2+} exchanger, thus obscuring possible Na^+ movements through the system. The possibility that the system described here does not discriminate between Na^+ and K^+ is thus still open.

Considering only the movements of K^+ described above, the concentration range at which Ca^{2+} activation occurs is physiologically relevant [Ca^{2+} activities of up to 10 μM have been recorded during heart contraction (33)] and is compatible with the development of an outwardly directed K^+ flux on entry of Ca^{2+} into the heart cell. The demonstration and characterization of a Ca^{2+}-dependent K^+ permeability system (channel) in a vesicular preparation of heart plasma membrane makes its properties amenable to direct experimentation and is a step toward its functional reconstitution.

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