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Calcium uptake and release characteristics of the dense tubules of digitonin-permeabilized human platelets

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The kinetics of ATP-driven Ca^{2+} uptake by the dense tubules were studied in digitonin-permeablized human blood platelets. Digitonin at 3 $\mu g/ml$ was shown capable of permeablizing the plasma membrane to lactate dehydrogenase and the cytoplasmic Ca^{2+} indicator Quin2 without increasing the passive permeability of the dense tubular membrane for Ca^{2+}. Experimentation was carried out with platelets treated with 3 $\mu g/ml$ digitonin reisolated and resuspended in detergent-free medium ('digitonin-permeablized' platelets). Active Ca^{2+} accumulation, which occurs over a period of minutes, was monitored by the increase in the fluorescence of chlorotetracycline after the addition of Mg-ATP (37°C). The active uptake is inhibited by 15 μM trifluoperazine. The process is saturable with respect to external $[Ca^{2+}]$, with a K_m of 180 ± 5 nM and a Hill coefficient (n) of 1.40 ± 0.05. Analysis of the maximal uptake in steady state gave similar results ($K_m = 160 \pm 5$ nM, $n = 1.50 \pm 0.05$). The rate of uptake at $[Ca^{2+}] \approx K_m$ is increased when the digitonin-permeablized platelets are preincubated with 100 nM phorbol 12-myristate 13-acetate. Actively accumulated Ca^{2+} is rapidly released (less than 1 min) by addition of D-*myo*-inositol trisphosphate (IP_3). The maximal extent of release is 50%; the EC_{50} for IP_3 is approx. 12 μM. The data are compared with findings for fractionated dense tubular membrane vesicles and for the intact platelet.

Introduction

The human platelet is an extremely accessible example of a Ca^{2+}-regulated excitable cell. It possesses some of the properties of both a muscle and a gland, undergoing a number of internal biochemical processes, a shape change upon stimulation and secreting protein factors and activators which activate other platelets (cf. Refs. 1–4). As with muscle, the concentration of free Ca^{2+} in the cytoplasm ($[Ca^{2+}]_{cyt}$) is the primary determinant of activation. The level of $[Ca^{2+}]_{cyt}$ can be shown to be determined by the balance of multiple Ca^{2+} handling systems in the plasma membrane (Ca^{2+} extrusion system, leakage pathways and receptor-activated channels) and in the dense tubules (Ca^{2+} accumulation and analogous leakage pathways and activated channels.).

There is a strong analogy between the dense tubule and the sarcoplasmic reticulum of skeletal muscle. Adunyah and Dean [5] have studied ATP-driven Ca^{2+} uptake in a vesicular platelet membrane fraction enriched in endoplasmic reticulum markers and devoid of plasma membrane markers. The fraction showed Ca^{2+} uptake kinetics comparable to that of rabbit skeletal sarcoplasmic reticulum. A pump stoichiometry of 2.0 Ca^{2+}/ATP and a saturable $[Ca^{2+}]$ dependence with a K_m of 0.1 μM were determined. They also

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showed that the system has a biphasic dependence on Mg^{2+}-ATP, with K_m values of 9.5 μM and 0.6 mM. Purification and antibody cross-reactivity experiments also suggest that the enzyme is similar to that of rabbit skeletal sarcoplasmic reticulum [6]. The present study will characterize dense tubular Ca^{2+} uptake in digitonin-permeablized platelets.

The levels of $[Ca^{2+}]_{cyt}$ in the resting and activated state have been studied in the activated state using the fluorescence indicator, Quin2 [7–9]. Changes in the free ('ionized') Ca^{2+} level in the dense tubular lumen can be conveniently monitored in the intact platelet by the fluorescent probe chlorotetracycline (CTC; Refs. 2,10–12). The latter reference details the coupled reactions which give CTC fluorescence a linear dependence on the free $[Ca^{2+}]$ in the dense tubular lumen. The demonstration of coupling is based on studies in isolated sarcoplasmic reticulum [13,14] and quantitative comparisons of CTC fluorescence and $^{45}Ca^{2+}$ retention in cardiac sarcolemmal vesicles [15]. Previous studies have shown that the Ca^{2+} in the ADP storage granules (dense granules) does not make an appreciable contribution to the CTC signal [12] and that contributions associated with mitochondrial Ca^{2+} uptake are not important for cytoplasmic Ca^{2+} concentrations below 1 μM [22].

The present study applies the CTC technique to monitor Ca^{2+} uptake and release in digition-permeablized platelets. The latter technique has been applied to a number of cell types, including platelets. Low and controlled concentrations of digitonin have been used to selectively permeabilize the plasma membranes of a number of cell types, allowing the investigator to manipulate the cytoplasm and study intracellular processes. In platelets the method has been applied to defining the resting $[Ca^{2+}]_{cyt}$ [16], differentiation of cytoplasmic and noncytoplasmic ATP [17] and in the study of secretion processes [18,19].

Materials and Methods

Phorbol 12-myristate 13-acetate and D-*myo*-inositol trisphosphate (IP_3) were purchased from Sigma Chemical Co., St. Louis MO. The latter (No. I-4009) is a mixture, containing approx. 75–80% 1,4,5-IP_3 and approx. 20–25% 2,4,5-IP_3.

The extracellular Ca^{2+} concentration was controlled by a Ca/EGTA buffer ($[EGTA]_T = 2.5$ mM). The reported free Ca^{2+} concentrations for the study pH 7.0, 37°C) were based on an apparent association constant of $3.77 \cdot 10^6$ M^{-1} calculated with the help of a computer program using published EGTA complexation and association constants [20], corrected for temperature using the enthalpy values given.

Blood was drawn from normal donors into anticoagulant citrate dextrose and washed platelets were prepared as described previously [21]. To chemically permeablize the platelets, one part of concentrated platelet suspension ($4 \cdot 10^8$ platelets/ml in Tyrode's buffer) was added to 9 parts of 'skinning solution' consisting of 3 μg/ml digitonin, 140 mM NaCl, 3 mM KCl, 2 mM ATP, 1 mM KH_2PO_4, 2 mM EGTA, 10 mM Hepes, 10 mM Tris (pH 7.0) and 0.1% bovine serum albumin. (For the experiment of Fig. 1 the digitonin concentration was varied.) After 10 min incubation, the treated platelets were centrifuged at 600 $\times g$ for 7 min. The pellet was resuspended in 5 parts 'resuspension solution' consisting of 20 mM NaCl, 80 mM KCl, 1 mM KH_2PO_4, 2 mM EGTA, 20 mM Hepes, 40 mM Tris (pH 7.0) and 0.1% bovine serum albumin. Lactate dehydrogenase activity was measured using Sigma Diagnostic Kit No. 228-00, which is based on the NAD/NADH lactate/pyruvate couple. The reaction was measured at 340 nm.

The fluorescence instrumentation, the use of CTC and the description of its mechanism of response have been described earlier [21]. All experimentation was done at 37°C with 600 rpm stirring. All reported results are based on at least four observations in four separate preparations. Presented traces are the data from a particular experiment; data plotted as points are the average of at least four experiments.

Results

Experiments were carried out to identify a digitonin concentration which will permeablize the plasma membrane without causing the release of accumulated dense tubular Ca^{2+}. Fig. 1 shows an experiment which establishes the digitonin threshold for the latter process. Intact platelets

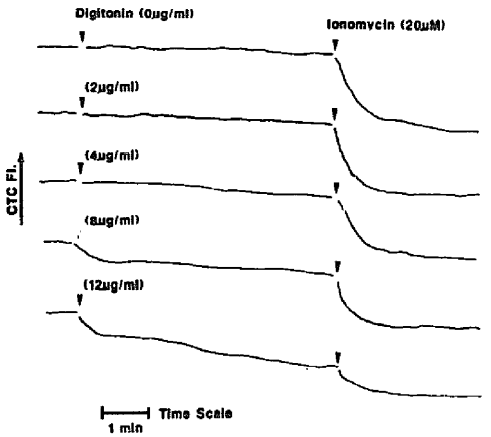

Fig. 1. Effect of variable digitonin concentrations on kinetics of Ca^{2+} efflux from the dense tubules of intact platelets. The behavior of digitonin addition on the rate of decline of CTC fluorescence is presented. Other experimental details are described in the text.

were first equilibrated with 1 mM external Ca^{2+} and 20 μM CTC to for 30 min to allow for establishment of about 110 nM $[Ca^{2+}]_{cyt}$ and maximal dense tubular uptake for this condition (cf. Refs. 12, 21, 22). Stable CTC fluorescence levels were established and then 2.5 mM EGTA was added to remove the extracellular Ca^{2+}. Fig. 1 shows the behavior of the CTC fluorescence after completion of the rapid phase of fluorescence decline associated with the removal of the Ca-CTC complex from the outer surface of the plasma membrane. The very slow decline of CTC fluorescence is due to slow leakage of Ca^{2+} from the dense tubules to the cytoplasm (and its subsequent extrusion from the cell by active transport processes). This is comparable to the behavior observed previously (Fig. 2B, Ref. 12). Fig. 1 shows that addition of digitonin at 4 μg/ml or lesser concentration does not increase the rate of decline of fluorescence, whereas challenge with 20 μM ionomycin gives a rapid decline. Thus, 7 min exposure to a maximum of 4 μg/ml digitonin does not change the basal Ca^{2+} permeability of the dense tubules. The figures shows that 8 μg/ml or higher digitonin causes Ca^{2+} leakage and that subsequent challenge with ionomycin causes correspondingly less release. The dose–response behavior of the digitonin-induced dense tubular release is also plotted in Fig. 2.

The release of lactate dehydrogenase and the cytoplasmic indicator Quin2 was studied as a measure of the degree of plasma membrane permea-

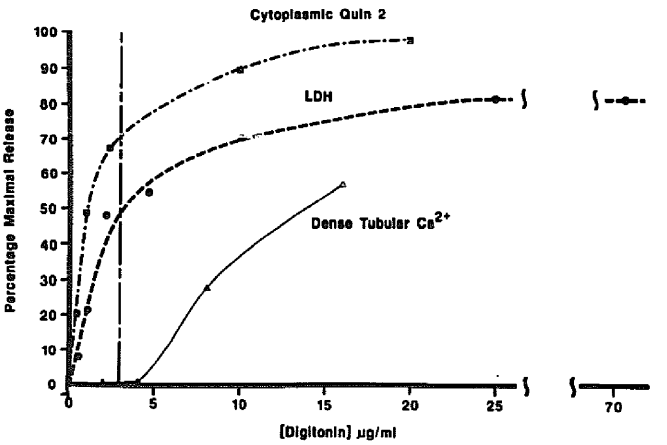

Fig. 2. Digitonin concentration dependence of release of LDH from the cytoplasm and release of Ca^{2+} from the dense tubules. The LDH release data were obtained by performing the permeabilization procedure (Materials and Methods) at variable digitonin concentrations (37°C incubation), isolating the supernatant, adding 0.1% Triton X-100 and assaying for total activity. Residual activity in the pellet was determined by resuspending the pellet, adding 0.1% Triton X-100 and assaying. The sum of the released and residual activities was found to be invariant and was taken as 100%.

bilization as described under Materials and Methods. Fig. 2 shows the effect of digitonin concentration in the 'permeablizing medium' on percentage release of lactate dehydrogenase to the supernate before isolation of the permeablized platelets. Half-maximal release of the 140 kDa enzyme is observed at 3.5 μg/ml. The figure shows that release of the smaller cytoplasmic indicator Quin2 is half-maximal at 0.8 μg/ml digitonin.

The data of Fig. 2 show that 3 μg/ml digitonin causes release of 50% of the lactate dehydrogenase 70% of the trapped Quin2 and 0% of the dense tubular Ca^{2+}. This concentration was chosen as the standard for preparation of digitonin-permeablized platelets for further studies. Additional experiments showed that the plasma membrane remained permeabilized after the final centrifugation and resuspension in detergent-free medium. The above experimentation provides the basis for control the concentration of Ca^{2+} to which the dense tubular pump is exposed *.

Fig. 3 shows that permeablized platelets are capable of ATP supported Ca^{2+} uptake measurable by CTC fluorescence. Permeablized platelets were introduced into a cuvette containing the high K^+/low Na^+'resuspension solution', with 2.5 mM EGTA and added Ca^{2+} to buffer the free $[Ca^{2+}]$ at $4 \cdot 10^{-7}$ M, 10 μg/ml antimycin A and 10 μg/ml oligomycin. The latter two agents were added to ensure antimycin A and 10 μg/ml oligomycin. The latter two agents were added to ensure that the mitochondria could make no contribution to active Ca^{2+} uptake. The experiment was conducted according to protocols worked out for isolated sarcoplasmic reticulum or other Ca^{2+}-accumulating vesicles [13–15]. The above-cited publications show that the slow increase observed upon initiation of transport is a linear measure of the free internal $[Ca^{2+}]$ in the vesicle or organelle lumen.

Fig. 3 shows that platelet addition produces a small light-scattering artifact. Addition of 10 μM

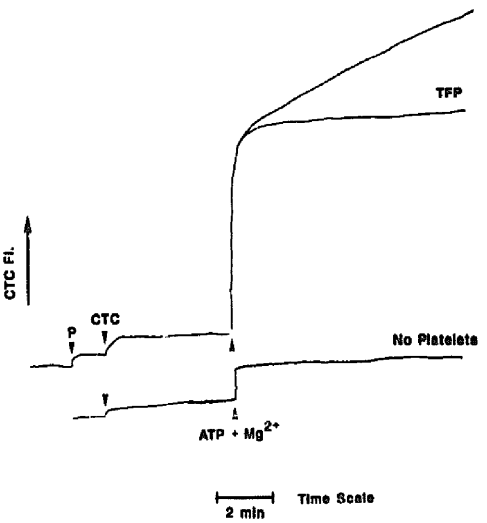

Fig. 3. Increase in CTC fluorescence associated with ATP-driven Ca^{2+} uptake in permeablized platelets. Mitochondrial inhibitors and other experimental details are given in the text. For the curve labeled trifluoperazine (TFP), the digitonin-permeablized platelets were preincubated with 15 μM trifluoperazine.

CTC gives a small increase due to fluorescence contributions from aqueous CTC and a small amount of membrane-bound species. After addition of 1.0 mM Mg-ATP a large biphasic increase is seen. The initial (instantaneous) phase arises from the formation of Mg-ATP and Mg^{2+} complexes of CTC in the medium and on the exposed membrane surfaces. The control trace (no platelets) show a fast phase due to aqueous Mg-ATP and CTC complexes. In the experimental trace with permeablized platelets, a slow time-resolved increase of considerable amplitude is observed. Addition of 0.5 μM ionomycin or omission of Mg-ATP (not shown) abolishes this phase, indicating that it is due to ATP-driven Ca^{2+} accumulation. The time-course and maximal fluorescence increase of the slow phase are similar to those found for dense tubular uptake in the intact platelet [12,21]. The figure also shows that preincubation of the permeablized platelets with 15 μM trifluoperazine abolishes the dense tubular uptake reaction. This concentration blocks dense tubular uptake in intact platelets [12].

* In the unlikely case that a fraction of the platelets have resealed, that fraction would not make a contribution to our observations, since none of the added constituents would then be available to the dense tubules. With intact platelets for $[Ca^{2+}]_0 \leq 10^{-6}$ M minimal dense tubular uptake is observed [12].

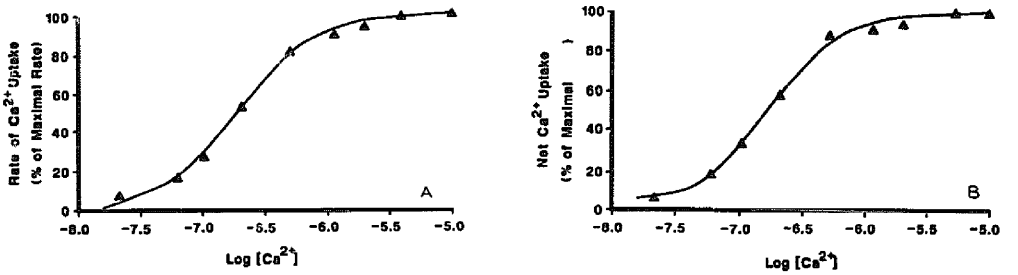

Fig. 4. Effect of $[Ca^{2+}]$ on the rate and extent of ATP-driven Ca^{2+} uptake. The ATP-driven Ca^{2+} uptake experiments were performed as in Fig. 3, with variable $[Ca^{2+}]_0$, controlled by the Ca/EGTA buffer. The data are the average of four experiments on four preparations. The standard deviations of the mean were about 0.05 of the measured value. Panel A, initial rate data; panel B, maximal extent of uptake in steady-state, determined at $t = 20$ min.

The experiment of Fig. 3 was repeated in four preparations at variable free Ca^{2+} concentrations set by the Ca/EGTA buffer. Fig. 4 presents the dependence of the initial rate and maximal extent of the Ca^{2+}-transport-associated fluorescence increase as a function of $-\log [Ca^{2+}]$. The continuous curves are the result of a computer fit to the equation:

$$\text{rate}/\text{rate}_{max} = \frac{[Ca^{2+}]_o^n}{[Ca^{2+}]_o^n + K_m^n} \quad (1)$$

where K_m is the $[Ca^{2+}]_0$ for half-maximal rate (or extent) of uptake and where n is the power of the Ca^{2+} dependence. The fitted parameters are presented in Table I. That data show and optimal fit with $K_m = 180$ nM, $n = 1.4$ for the rate data and $K_m = 160$ nM, $n = 1.5$ for the maximal uptake data. Agreement between the two measures of uptake has been reported for isolated skeletal sarcoplasmic reticulum [23].

It has been proposed [24,25] that phorbol ester (phorbol 12-myristate 13-acetate) activates platelets by increasing their sensitivity to cytoplasmic Ca^{2+}. Fig. 5 shows the effect of phorbol ester on ATP-supported uptake in the permeablized platelet at 200 nM external Ca^{2+}. The agent was added to the platelets prior to initiation of the reaction by ATP. The figure shows that the initial rates in the phorbol ester-treated and control preparations are identical but that after 1 min the two traces diverge, with the phorbol-ester-treated preparation sustaining higher rates than the control. Since $[Ca^{2+}]_0$ was near the K_m, the effect encompasses both K_m and V_m effects. This observation was also

TABLE I

KINETIC PARAMETERS OF DENSE TUBULAR PUMP AND MAXIMAL UPTAKE

The fits were performed on the data of Fig. 4. The curve-fitting to Eqn. 1 was done using ASYSTANT@ (Macmillan Software Company). The uncertainties of the fitted constants were determined as the degree of change necessary to obtain significant degradation of the R^2 parameter.

Process	K_m (nM)	Hill coefficient	R^2
Initial rate	180 ± 5 nM	1.40 ± 0.05	0.9989
Maximum steady-state uptake	160 ± 5 nM	1.50 ± 0.05	0.9990

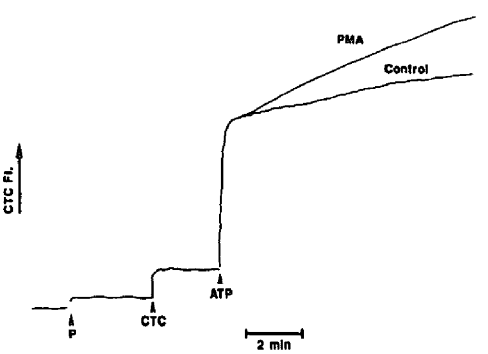

Fig. 5. Effect of phorbol ester on uptake kinetics in permeablized platelets. The phorbol ester concentration was 100 nM and was added before uptake was initiated. The Ca^{2+} concentration in this experiment was 200 nM.

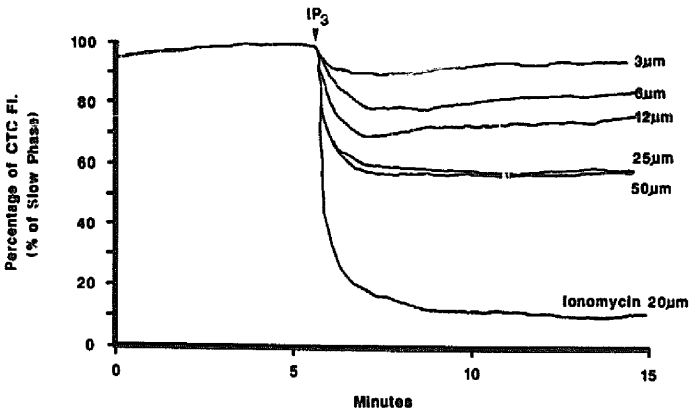

Fig. 6. IP_3-triggered release of actively accumulated Ca^{2+} in permeablized platelets. Active uptake was initiated as in Fig. 3. The rapid phase of increase after Mg-ATP addition is not shown. The $[Ca^{2+}]_0$ was buffered at 150 nM. Addition of 50 μM IP_3 after ionomycin gave no change (data not shown).

repeated four times with four separate preparations.

The permeablized platelet system was tested to determine whether inositol trisphosphate (IP_3) can cause Ca^{2+} release. Fig. 6 shows an initial plateau fluorescence due to ATP-driven uptake at 150 nM external Ca^{2+}. At this point variable concentrations of IP_3 were added. The figure shows that addition of IP_3 releases actively-accumulated Ca^{2+} in less than 1 min. The extent of release was dependent on the concentration of IP_3 added, with half-maximal release at approx. 12 μM. The figure shows that the maximal extent of the release of IP_3-triggered release seen at high concentrations represents 50% of the actively accumulated Ca^{2+} as judged by the fluorescence amplitude of the accumulation phase and its complete reversal by 20 μM ionomycin. The figure shows that the release is followed by reaccumulation at the lower IP_3 concentrations. This behavior is reminiscent of the dense tubular response to variable concentrations of thrombin in intact platelets (panel D, Fig. 2 of Ref. 21).

Discussion

Our study has shown that the plasma membrane can be permeablized with 3 μg/ml digitonin without affecting the Ca^{2+} permeability of the dense tubular membrane. Active, ATP-supported Ca^{2+} uptake into the dense tubules was studied in this digitonin-permeablized preparation. The results are in good agreement with both in situ studies of dense tubular function in intact platelets and with in vitro studies of platelet membrane vesicles of dense tubular origin.

Basic pump kinetics

The present results show that the rate of uptake (measured as the rate of increase in free Ca^{2+} in the dense tubular lumen) has saturation kinetics with respect of external Ca^{2+}. They supersede our previous estimates of these parameters made using an ionophore permeabilization stategy [12]. The present rate data are fit well by Eqn. 1 with $K_m = 180 \pm 5$ nM and $n = 1.40 \pm 0.05$. Our results also show that the maximal dense tubular uptake achieved in the steady state has a similar dependence, with $K_m = 160$ nM and $n = 1.5$. Agreement between initial rate and maximal steady-state measurements has been previously shown in studies of isolated skeletal sarcoplasmic reticulum [23].

The measured K_m values are in good agreement with those determined in platelet membrane vesicles of dense tubular origin by Adunyah and Dean (Ref. 5). They showed saturation kinetics with a K_m of 0.1 μM (30°C). Fig. 5B of their paper shows that the rate increases from 10% of 90% of maximal over an approx. 1.5 unit change in pCa^{2+}, suggesting an n value of approx. 1.3.

Our measured K_m and n values are also in good agreement with assessments of these parameters made in intact platelets. We have made parallel measurements of the resting $[Ca^{2+}]_{cyt}$ and free dense tubular Ca^{2+} (CTC measurements) in a large number of washed platelet samples from normal volunteers and from patients with arterial thrombosis [22]. The patients had elevated values of both parameters, a condition which was correctable by ex vivo treatment with verapamil and attributable to opened Ca^{2+} channels in the plasma membrane [22]. The paired $[Ca^{2+}]_{cyt}$ and CTC data of 21 normals and 28 patients defined a continuum which could be fit by the equation:

$$R = R_{max} \frac{[Ca^{2+}]^2_{cyt}}{K_m^2 + [Ca^{2+}]^2_{cyt}} \quad (2)$$

where R is a measure of the resting dense tubular Ca^{2+} level (ratio of slow to fast phase of CTC response after addition of 2 mM external Ca^{2+}), where R_{max} is its maximal value (1.5) and where the remaining terms have the same meaning as used here. A K_m value of 180 nM was obtained from the fit. This is very close to the correlate value of 160 nM obtained from Fig. 4B. These dense tubular values can be compared with our estimate of the analagous constants for the plasmalemmal Ca^{2+} extrusion pump (K_m approx. 81 nM; $n = 1.7$) from in situ measurements of extrusion pump activity in platelets overloaded with Quin2 [37]. In the Quin2-overloaded condition the indicator becomes a true and linear measure of the numbers of Ca^{2+} extruded and its is possible to determine pump velocity vs. $[Ca^{2+}]_{cyt}$ characteristics in situ [37].

The data of the present communication show that the combination of the digitonin permeablization and CTC fluorescence techniques is a valid and convenient method for characterizing the dense tubular Ca^{2+} transport system. However, one idiosyncrasy of the CTC technique is that the fluorescence response is a linear measure of the concentration of free Ca^{2+} in the vesicle lumen [14,15]. The (electron-) dense tubules probably contain low-affinity Ca^{2+} binding proteins analogous to the calsequestrin of sarcoplasmic reticulum (cf. Refs. 26, 27) and transported Ca^{2+} which is bound to these proteins will not be reported by CTC. Our quantitative comparisons of decreases in CTC fluorescence and increases in Quin2 fluorescence during thrombin activation [21] suggest a method for quantitating total dense tubular Ca^{2+} and assessing this factor.

Modifiers of pump kinetics

Trifluoperazine has been shown to affect a number of platelet functions [28]. In a previous study in intact platelets [12] we showed that 15 trifluoperazine is the optimal concentration for inhibition of dense tubular Ca^{2+} uptake. Nonspecific effects on passive Ca^{2+} permeability of the plasma membrane were seen in the 30–50 μM range. Brass [29] showed that 20 μM trifluoperazine decrease the size of the slowly exchangeable $^{45}Ca^{2+}$ pool associated with the dense tubules. The present finding that 15 μM trifluoperazine abolishes dense tubular Ca^{2+} uptake in the chemically permeabilized platelet is in agreement with the above. Our studies do not show whether this is due to direct inhibition of the pump or inhibition of calmodulin stimulation. Relevant to this question are the studies of Dean and Sullivan [30] on a platelet membrane fraction of probable dense tubular origin. For their EGTA-washed membranes, 0.5 μM calmodulin increased the Ca^{2+}-ATPase activity 150% and 100 μM chlorpromazine decreased the activity 37%.

The effects of cAMP and cAMP-dependent phosphorylation were not investigated in the present study. Kaeser-Glanzmann et al. [31] reported that cAMP-dependent protein kinase increases the rate of phosphate-supported active Ca^{2+} uptake in platelet membrane vesicles.

Phorbol ester activates platelets [32] and increases the sensitivity of the aggregation reaction to cytoplasmic Ca^{2+} [24,25]. Phorbol esters and diacylglycerols activate protein kinase C, resulting in protein phosphorylation which has been linked to serotonin release [32]. The effects of phorbol ester can be pro-aggregatory or anti-aggregatory, depending on the agonist and conditions [33]. Our test on dense tubular Ca^{2+} uptake was done was 100 mM phorbol ester, a concentration which gives nearly maximal serotonin release and phosphorylation of a 40 kDa protein (cf. Fig. 5, Ref. 32). We have found that 100 nM phorbol ester increases the rate of dense tubular uptake at 200

nM Ca^{2+}. As noted earlier, at this low $[Ca^{2+}]_{cyt}$ the observation could be either the result of a lowered K_m, lowered Hill coefficient or raised V_m. Our results also show that the maximal Ca^{2+} accumulation at steady-state is increased by phorbol ester. Since this would tend to increase the rate of clearance of Ca^{2+} from the cytoplasm, the effect would be anti-aggregatory. Thus it seems that increased dense tubular Ca^{2+} uptake is an important negative feedback effect of phorbol ester. Our results suggest a mechanism for the observation [34] that addition of phorbol ester after stimulation with low-dose thrombin at the instant when peak $[Ca^{2+}]_{cyt}$ is achieved causes an increase in the rate that $[Ca^{2+}]_{cyt}$ returns to baseline (cf. Fig. 5 vs. Ref. 34).

The present results do not determine whether or not the phorbol ester stimulation of dense tubular Ca^{2+} uptake represents a direct effect on the Ca^{2+}-ATPase. Studies of Adunyah and Dean [35] in a platelet membrane vesicle preparation devoid of protein kinase C activity have shown that the diacylglycerol increases the rate of the Ca^{2+}-ATPase reaction, but also increases the passive permeability and decreases the Ca^{2+} uptake.

IP_3-triggered release

Our experiments show that IP_3 can release about 50% of the actively accumulated Ca^{2+} from permeablized platelets. The EC_{50} based on the external medium is approx. 12 μM. These results can be compared with those reported by Adunyah and Dean [36] for membrane vesicles of dense tubular origin. They found that IP_3 can elicit a maximal release of 12% of the actively accumulated Ca^{2+}, with an EC_{50} of maximally 1 μM (cf. Fig. 1, Ref. 36). Our EC_{50} may be higher than the intrinsic or intracytoplasmic value, since the exogenous IP_3 must run the gauntlet of hydrolyzing enzymes before arriving at the dense tubules. It is possible that a pulsatile increase is most efficient in bringing about dense tubular release. However, the large (50%) extent of the release which we observed is probably reflective of the in vivo situation. A high dose of thrombin will release 35% of the accumulated dense tubular Ca^{2+} in intact platelets as measured by CTC (panel D, Fig. 2 Ref. 21). With both agents, the release is countered by reuptake. Whether each dense tubule contains IP_3-gated channels, the degree of platelet heterogeneity, whether Ca^{2+}-triggered Ca^{2+} release plays a role and whether the density of channels is sufficient to overwhelm the Ca^{2+}-ATPase pumps are interesting questions which remain to be answered.

Acknowledgements

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