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Calcium efflux and cycling across the synaptosomal plasma membrane

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Ca^{2+} efflux from intact synaptosomes is investigated. Net efflux can be induced by returning synaptosomes from media with elevated Ca^{2+} or high pH to a normal medium. Net Ca^{2+} efflux is accelerated when the Na^+ electrochemical potential gradient is collapsed by veratridine plus ouabain. Under steady-state conditions at 30°C, Ca^{2+} cycles across the plasma membrane at 0.38 nmol·min^{-1}·mg^{-1} of protein. Exchange is increased by 145% by veratridine plus ouabain, both influx and efflux being increased. Increased influx is probably due to activation of voltage-dependent Ca^{2+} channels, since it is abolished by verapamil. The results indicate that, at least under conditions of low Na^+ electrochemical gradient, some pathway other than a Na^+/Ca^{2+} exchange must operate in the plasma membrane to expel Ca^{2+}.

In recent years it has become apparent that the plasma membranes of excitable cells possess two pathways, a Ca^{2+}-translocating ATPase and a Na^+/Ca^{2+} exchanger, capable in theory of extruding Ca^{2+} from the cell against the considerable Ca^{2+} electrochemical potential gradients ($\Delta\mu_{Ca^{2+}}$) that exist across the membranes under physiological conditions (reviewed in Carafoli, 1982).

The isolated nerve terminal (synaptosome) is one system in which both transport pathways have been investigated. Early studies with intact synaptosomes emphasized a role for Na^+/Ca^{2+} exchange in extruding Ca^{2+} from the cytosol (Swanson *et al.*, 1974; Blaustein & Oborn, 1975; Blaustein & Ector, 1976). However a Na^+-dependency for Ca^{2+}-efflux could only be demonstrated in the unphysiological conditions of external Ca^{2+}-depletion (Blaustein & Ector, 1976). At the same time it was found that uncharacterized inverted synaptosomal membrane preparations possessed ($Ca^{2+}+Mg^{2+}$)-stimulated ATPase activity and accumulated Ca^{2+} in an ATP-dependent manner (Duncan, 1976; Ichida *et al.*, 1976; Blaustein *et al.*, 1978*a*,*b*; Rahamimoff & Abramovitz, 1978; Sobue *et al.*, 1979). The enzyme was initially assumed to be localized on internal membranes (Blaustein *et al.*, 1978*a*,*b*; Rahamimoff & Abramovitz, 1978) but has recently been shown to be primarily on the plasma membrane (Michaelis *et al.*, 1983) where it coexists with the Na^+/Ca^{2+} exchange activity (Gill *et al.*, 1981).

There is considerable uncertainty as to the role of the two Ca^{2+}-translocating pathways, since synaptosomal plasma membrane vesicles, while advantageous for establishing the presence of transport mechanisms, can give little information on ion fluxes under more physiological conditions.

We have previously shown that depletion of ATP in depolarized synaptosomes increases the net accumulation of Ca^{2+}, consistent with the inhibition of an ATP-dependent Ca^{2+}-efflux pathway such as the Ca^{2+}-ATPase (Åkerman & Nicholls, 1981*b*). In the present paper we assess the ability of intact synaptosomes to exchange and extrude the cation when collapse of the Na^+ electrochemical potential gradient ($\Delta\mu_{Na^+}$) by veratridine plus ouabain would prevent a Na^+/Ca^{2+} antiport from transporting Ca^{2+} out of the synaptosome.

Experimental

Synaptosomes

Synaptosomes were obtained from the forebrains of Dunkin–Hartley strain guinea-pigs of either sex by Ficoll density gradient centrifugation as described previously (Nicholls, 1978; Scott & Nicholls, 1980). They were stored as pellets in 0.25 M-sucrose/5 mM-Tes (sodium salt), pH 7.4, at 0°C for not more than 4 h before use. Protein was

Abbreviations used: $\Delta\mu_{Ca^{2+}}$, $\Delta\mu_{Na^+}$, the electrochemical potential gradients across the synaptosomal plasma membrane of Ca^{2+} and Na^+ respectively; $\Delta\psi_m$, mitochondrial membrane potential; $\Delta\psi_p$, plasma membrane potential.

quantified by the biuret method (Gornall *et al.*, 1949).

Membrane potentials

$\Delta\psi_p$ and $\Delta\psi_m$ were measured simultaneously by incubating the synaptosomes in the presence of 10 μM-$^{86}Rb^+$ (0.3 μCi/ml), 1 mM-[^{14}C]sucrose (0.4 μCi/ml), 1 μM-[^{3}H]triphenylmethylphosphonium bromide (0.4 μCi/ml) and 5 mM-tetraphenylboron as described previously (Scott & Nicholls, 1980), with the modification that the synaptosomes were separated by oil centrifugation as described below (Åkerman & Nicholls, 1981*a*).

Ca^{2+} transport

For total Ca^{2+}, synaptosomes were resuspended at 1.5 mg of protein/ml in a medium at pH 7.4 and 30°C containing 122 mM-NaCl, 3.1 mM-KCl, 1.2 mM-$MgSO_4$, 0.4 mM-KH_2PO_4, 5 mM-$NaHCO_3$, 20 mM-Tes (sodium salt), 10 mM-D-glucose and 50 μM-[^{3}H]sucrose (1.5 μCi/ml). After 15 min preincubation, 1.3 mM-$^{45}Ca^{2+}$ (0.5 μCi/ml of incubation) was added. At defined times 150 μl aliquots were taken and mixed in an Eppendorf tube with 150 μl of Ca^{2+}-free incubation medium containing additionally 4 mM-EGTA and 5 μM-Ruthenium Red and overlaying 250 μl of a 1:1 (v/v) mixture of Dow–Corning 550 silicone oil and dinonylphthalate. After 5 s to remove superficially bound Ca^{2+} (Scott *et al.*, 1980), the tubes were centrifuged for 60 s in an Eppendorf model 5412 microcentrifuge. Pellets were extracted in 12% (w/v) $HClO_4$ and transferred to scintillation vials, mixed with 5 ml of Scintran cocktail T scintillation fluid (BDH) and counted for ^{3}H and $^{45}Ca^{2+}$ on a LKB 1216 Rackbeta 11 liquid scintillation counter. On preparation, synaptosomes contain less than 0.6 nmol of Ca^{2+}/mg of protein (Scott *et al.*, 1980). There is thus little error in equating isotopic and total Ca^{2+} contents.

Synaptosomal Ca^{2+} content was increased either by incubation at pH 8.0 (Fig. 2), or by incubation at 7.5 mg of protein/ml for 30 min with 6.5 mM-$^{45}Ca^{2+}$ (2.5 μCi/ml) after which they were diluted with 4 vol. of Ca^{2+}-free incubation medium to give 1.5 mg of protein/ml of incubation and to lower the external Ca^{2+} to its normal value of 1.3 mM (Fig. 3).

For inward exchange of $^{45}Ca^{2+}$, synaptosomes were incubated as above, but with 1.3 mM-Ca^{2+} for 30 min to achieve near steady-state conditions. $^{45}Ca^{2+}$ (1 μM; 0.5 μCi/ml) was then added to initiate exchange, and samples were taken and analysed for ^{3}H and Ca^{2+} as above.

For outward exchange, synaptosomes were incubated in the presence of 1.3 mM-$^{45}Ca^{2+}$ (2 μCi/ml) at a concentration of 6 mg of protein/ml of incubation. After 30 min in the presence of the isotope, the incubation was diluted with 3 vol. of incubation medium containing 1.3 mM-Ca^{2+} to decrease the specific radioactivity of the external Ca^{2+} pool. Samples were taken and analysed as above.

Materials

[^{3}H]Triphenylmethylphosphonium was obtained from New England Nuclear, Dreiech, Germany. All other radioisotopes were from The Radiochemical Centre, Amersham, Bucks., U.K. Ficoll was obtained from Pharmacia (Uppsala, Sweden) and was exhaustively dialysed against water before use. Silicone fluid (Dow–Corning 550) and dinonylphthalate were obtained from Hopkin and Williams (Chadwell Heath, Essex, U.K.). All other reagents were obtained from Sigma (London) Chemical Co. (Poole, Dorset, U.K.) or BDH (Poole, Dorset, U.K.).

Results

Our initial experiments to characterize the significance of the Ca^{2+}-ATPase and Na^+/Ca^{2+} pathways in the extrusion of Ca^{2+} from synaptosomes centred on attempts to induce a specific inhibition of one pathway by using agents reported to be effective in other systems. Doxorubicin (adriamycin E) has been reported to be a specific inhibitor of Na^+/Ca^{2+} exchange at the sarcolemmal membrane, 50% inhibition requiring about 10 μM (Caroni *et al.*, 1981). When preincubated at 20 μM with polarized synaptosomes, we found no effect of doxorubicin on the net transport of $^{45}Ca^{2+}$ across the plasma membrane (results not shown). This however cannot be used as evidence against the role of Na^+/Ca^{2+} exchange in the absence of firm information on the tissue specificity of the inhibitor.

Vanadate is a potent inhibitor of brain Ca^{2+}-stimulated ATPase activity (Robinson, 1981; Michaelis *et al.*, 1983). However, even at 30 μM, we found that vanadate was without effect on synaptosomal Ca^{2+} transport or plasma membrane potential (results not shown). Since vanadate inhibits the $(Na^+ + K^+)$-dependent ATPase, the absence of a ouabain-like depolarization of the plasma membrane indicates that vanadate does not permeate across the plasma membrane to reach the inhibitory sites of the ATPases.

In the absence of suitable inhibitors, the alternative approach of collapsing $\Delta\mu_{Na^+}$ was tried. The sodium electrochemical potential gradient across the synaptosomal plasma membrane is given by:

$$\Delta\mu_{Na^+} = \Delta\psi_p + 60\log_{10}([Na^+]_{out}/[Na^+]_{in}) \quad (1)$$

Under the present conditions, $\Delta\mu_{Na^+}$ has a value of

102mV, of which 62mV is due to $\Delta\psi_p$ and 40mV to the Na^+ concentration gradient (Åkerman & Nicholls, 1981*a*). The combination of ouabain, to inhibit the Na^+ pump, and veratridine, to activate the voltage-dependent Na^+ channel, is an effective means of collapsing both the membrane potential and concentrative terms of $\Delta\mu_{Na^+}$ (Åkerman & Nicholls, 1981*a*).

Fig. 1 shows the rate and extent of plasma membrane depolarization, and the absence of an effect of $\Delta\psi_m$ caused by veratridine plus ouabain under the conditions employed in the subsequent experiments. The partial antagonism of veratridine action by the Ca^{2+} channel inhibitor verapamil (Galper & Catterall, 1979) is also shown.

Net efflux

It should be possible to induce a net efflux of Ca^{2+} by exposing synaptosomes to conditions which cause them to take up excess Ca^{2+} and then restoring the normal milieu. Fig. 2(*a*) shows that synaptosomes incubated at pH8.0 double their Ca^{2+} content relative to controls at pH7.4. Addition of acid to restore the normal pH produces a net efflux of Ca^{2+}, the content slowly approaching that of the control. Synaptosomes pretreated with ouabain plus veratridine show enhanced Ca^{2+} uptake at pH7.4 (Blaustein, 1975; Åkerman & Nicholls, 1981*c*) and this is further increased by 100% at pH8.0 (Fig. 2*b*). On restoring the pH to 7.4, there is a rapid Ca^{2+} efflux.

Control experiments (results not shown) confirm that the intrasynaptosomal mitochondria remain polarized in both experiments. Brain mitochondria possess a Ca^{2+} uniport whose activity increases sharply with Ca^{2+} concentration (Nicholls, 1978; Nicholls & Scott, 1980). As a result of the Ca^{2+} cycle across the mitochondrial inner membrane between the uniporter and the Na^+-dependent

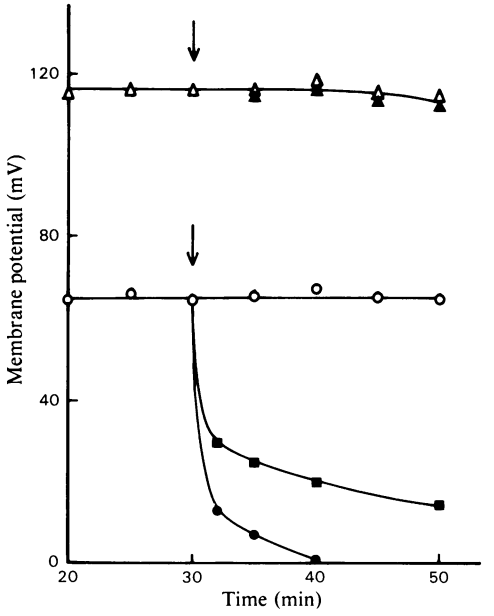

Fig. 1. *Effect of veratridine plus ouabain on $\Delta\psi_m$ and $\Delta\psi_p$; influence of verapamil*

Synaptosomes were incubated as described in the Experimental section. △, ▲, $\Delta\psi_m$; ○, ●, ■, $\Delta\psi_p$. At the time indicated by the arrow the following additions were made: ▲, ●, 100μM-veratridine plus 0.2mM-ouabain; ■, 0.1mM-veratridine, 0.2mM-ouabain and 0.2mM-verapamil; ○, control. A representative experiment is shown.

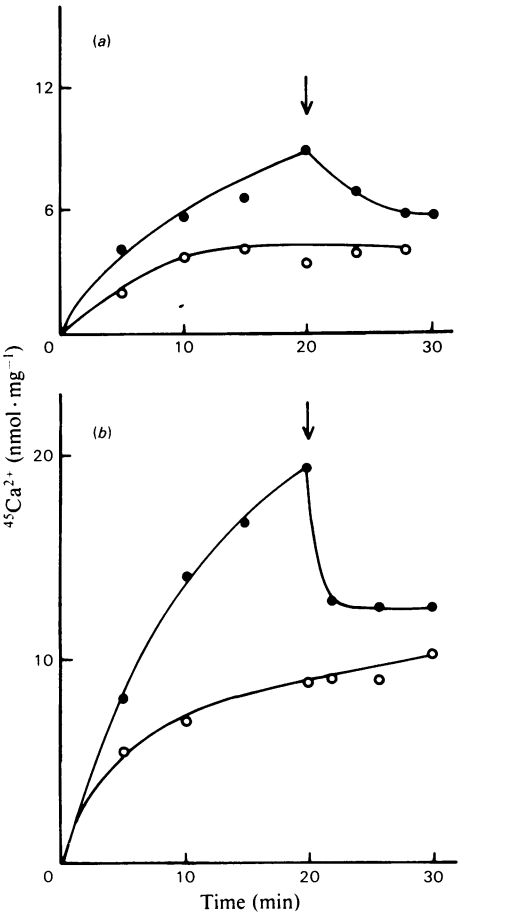

Fig. 2. *Effect of a pH jump on the net Ca^{2+} transport across the synaptosomal plasma membrane in the presence and absence of $\Delta\mu_{Na^+}$*

Synaptosomes were incubated as described in the Experimental section at either pH7.4 (○) or pH8.0 (●). (*a*), Control; (*b*), 100μM-veratridine and 0.2mM-ouabain initially present for both traces. Where indicated by the arrow, sufficient HCl was added to restore the pH to 7.4. A representative experiment is shown.

mitochondrial efflux pathway, they rapidly accumulate Ca^{2+} until the free concentration in their environment is no more than micromolar (Nicholls & Scott, 1980). This setting of an upper limit to the cytosolic free Ca^{2+} only fails if the mitochondria become damaged and depolarize (Nicholls & Scott, 1980). The maintenance of a high $\Delta\psi_m$ is thus an indirect indication that the cytosolic free Ca^{2+} is low, and by extension that the extrusion of Ca^{2+} into a medium containing millimolar Ca^{2+} is an uphill process.

Fig. 3 shows that incubation of synaptosomes in a concentrated suspension in the presence of a 5-fold elevated Ca^{2+} leads to an increased uptake. Dilution with 4 vol. of Ca^{2+}-free medium lowers the external Ca^{2+} to the normal value of 1.3mM. Synaptosomes with polarized plasma membranes show a slow net efflux of Ca^{2+} on dilution (Fig. 3*a*); however those loaded in the presence of veratridine and ouabain extrude Ca^{2+} at twice the control rate (Fig. 3*b*). The mitochondrial membrane potential remained high in both these experiments (results not shown).

Exchange

Fig. 4 and Table 1 report the inward exchange of $^{45}Ca^{2+}$ into synaptosomes preincubated for 30min to achieve near steady-state conditions prior to addition of the isotope. Since the total Ca^{2+} content does not change significantly after 30min incubation in the presence or absence of veratridine plus ouabain (Fig. 3), the influx of $^{45}Ca^{2+}$ implies an equal and opposite efflux of Ca^{2+}. Exchange is considerably enhanced in the presence of ouabain plus veratridine, and this increase is sensitive to the Ca^{2+} channel inhibitor verapamil. Graphical analysis of each exchange indicates two kinetic pools. A rapidly exchanging pool with a rate constant of less than 1min was somewhat variable in replicate experiments. It is likely that it represents residual superficial Ca^{2+} which is not removed by 5s exposure to EGTA prior to centrifugation (see the Experimental section), since omission of this treatment increased the size of the fast pool from a mean of 1.7nmol·mg^{-1} of protein to 14nmol·mg^{-1} of protein (results not shown). After correcting for this pool, the control (Table 1) $^{45}Ca^{2+}$ exchanges at 0.38nmol·min^{-1}·mg^{-1} of protein. Collapse of $\Delta\mu_{Na^+}$ increases this to 0.93nmol·min^{-1}·mg^{-1} of protein (Table 1).

The exchange of Ca^{2+} in the presence of the inhibitors indicates that both influx and efflux are increased. The increased influx could be due to the Na^+/Ca^{2+} exchanger operating in the direction of Ca^{2+} influx, or to operation of the voltage-dependent Ca^{2+} channel. The increased efflux could be due to an activation of the Ca^{2+}-ATPase either by the raised cytosolic Ca^{2+} (Åkerman &

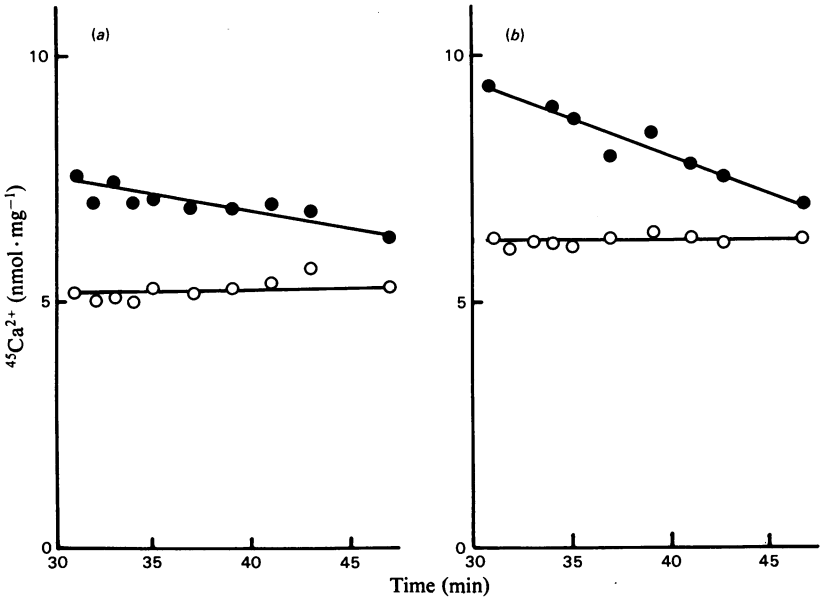

Fig. 3. *Net Ca^{2+} efflux from synaptosomes in the presence and absence of $\Delta\mu_{Na^+}$*
Synaptosomes were Ca^{2+}-loaded by preincubation for 30min at 7.5mg of protein/ml in the presence of 6.5mM-$^{45}Ca^{2+}$. At $t = 30$min they were diluted into Ca^{2+}-free medium to lower the Ca^{2+} to 1.3mM (●). Controls (○) were preincubated throughout in 1.3mM-$^{45}Ca^{2+}$. (*a*), No further additions; (*b*), 0.1mM-veratridine plus 0.2mM-ouabain added at $t = 0$. A representative experiment is shown.

Table 1. *Inward exchange of $^{45}Ca^{2+}$ into synaptosomes under steady-state conditions: influence of veratridine plus ouabain and verapamil*

Synaptosomes were incubated as described in the Experimental section. Data for a variable, rapidly exchanging pool, believed to represent residual superficial Ca^{2+} (see the text), is not shown. Results are given as the mean ± S.D. for three to six experiments.

Experimental conditions	Pool size (nmol of Ca^{2+}·mg^{-1})	Time constant (τ) (min)	Exchange flux (nmol·min^{-1}·mg^{-1})
Control ($n = 6$)	4.2 ± 0.6	11.3 ± 1.5	0.38 ± 0.09
200 μM-Ouabain + 100 μM-veratridine ($n = 5$)	7.3 ± 1.8	8.0 ± 1.1	0.93 ± 0.25
200 μM-Ouabain + 100 μM-veratridine + 200 μM-verapamil ($n = 3$)	4.7 ± 0.6	10.8 ± 1.4	0.45 ± 0.12

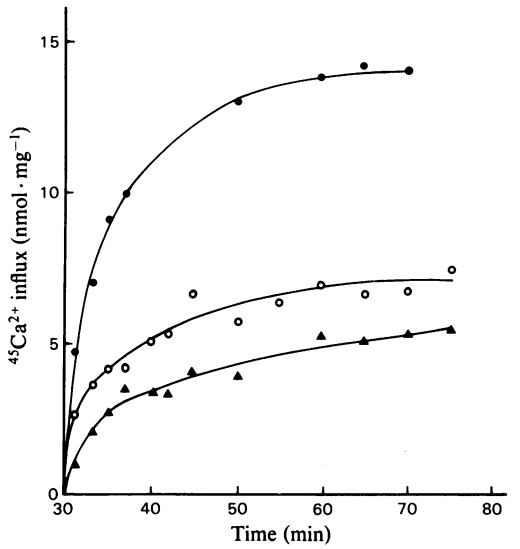

Fig. 4. *Inward exchange of $^{45}Ca^{2+}$ into synaptosomes in the presence and absence of $\Delta\mu_{Na^+}$*

Synaptosomes were preincubated for 30 min in the presence of 1.3 mM-Ca^{2+} to approach steady-state conditions. At $t = 30$ 1 μM-$^{45}Ca^{2+}$ (0.5 μCi/ml) was added and exchange was followed for a further 45 min. ▲, No further additions; ●, 100 μM-veratridine and 200 μM-ouabain added at $t = 0$; ○, 100 μM-veratridine, 200 μM-ouabain and 200 μM-verapamil added at $t = 0$.

Heinonen, 1983; Ashley *et al.*, 1984) or to a decreased thermodynamic back-pressure for Ca^{2+} efflux due to the collapse of $\Delta\psi_p$. To distinguish between these possibilities, verapamil was added in addition to ouabain and veratridine (Fig. 4). The exchangeable pool is not significantly different from the control and the exchange flux is reduced to control values, even though the plasma membrane is still largely depolarized (Fig. 1). This suggests that increased influx is due to entry through the voltage-dependent Ca^{2+} channel and that elevated cytosolic Ca^{2+}, rather than decreased $\Delta\psi_p$, is primarily responsible for the increased efflux.

In Fig. 5, the exchange of $^{45}Ca^{2+}$ out of the synaptosome is followed, to confirm the data from the inward flux of isotope. The same features are apparent. First there is an extremely rapidly exchanging pool which is enhanced by omitting the EGTA pretreatment of the synaptosomes prior to centrifugation, and second the outward $^{45}Ca^{2+}$ exchange is increased in the presence of ouabain and veratridine.

Discussion

Ca^{2+} extrusion in the absence of $\Delta\mu_{Na^+}$

Synaptosomes do not require a $\Delta\mu_{Na^+}$ in order to maintain a large Ca^{2+} gradient across their plasma membrane for the duration of most experiments. Thus measurements with arsenazo III (Åkerman & Heinonen, 1983) or quin-2 (Ashley *et al.*, 1984) show that while veratridine increases cytosolic free Ca^{2+}, the increase does not continue indefinitely but stabilizes at a sub-micromolar value (Ashley *et al.*, 1984). Furthermore the increase in total Ca^{2+} (Åkerman & Nicholls, 1981*a*) and cytosolic free Ca^{2+} (Ashley *et al.*, 1984) induced by veratridine appears to be due to operation of the voltage-dependent Ca^{2+} channel, rather than to failure or reversal of Ca^{2+} efflux mechanism, since the increases in both are blocked by verapamil.

These experiments do not however prove that the plasma membrane can extrude Ca^{2+} in the absence of a $\Delta\mu_{Na^+}$, since if there was only a slow inward leakage of Ca^{2+} across the plasma membrane the low cytosolic Ca^{2+} could be maintained by uptake into the internal mitochondria (Scott *et al.*, 1980; Åkerman & Nicholls, 1981*c*). In order to

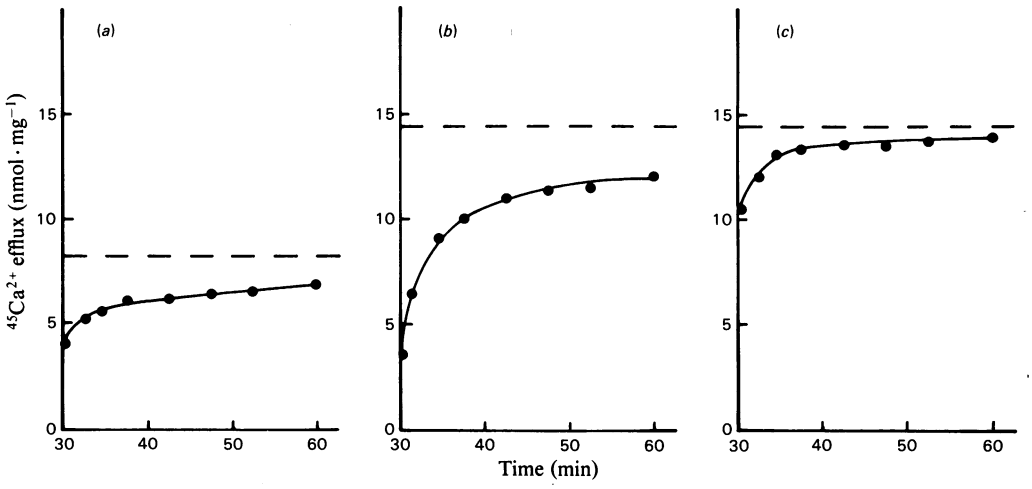

Fig. 5. *Outward exchange of $^{45}Ca^{2+}$ from synaptosomes in the presence and absence of $\Delta\mu_{Na^+}$*
Synaptosomes were preincubated for 30 min at 6 mg of protein/ml of incubation in a medium containing 1.3 mM-$^{45}Ca^{2+}$ (2 μCi/ml). The incubations were then diluted with 4 vol. of medium containing 1.3 mM-Ca^{2+} to decrease the specific radioactivity of the external pool. The broken line represents the theoretical efflux of $^{45}Ca^{2+}$ after complete equilibration. (*a*) Control; (*b*), 100 μM-veratridine and 200 μM-ouabain added at $t = 0$; (*c*), EGTA and Ruthenium Red omitted from sample processing.

prove that a separate Ca^{2+} efflux pathway is functioning it is necessary to show that synaptosomes can catalyse either net efflux or exchange of Ca^{2+} across the plasma membrane against the $\Delta\mu_{Ca^{2+}}$ when no energy is available from $\Delta\mu_{Na^+}$. The present paper provides such proof, and, since the only other efflux mechanism which has been described is the Ca^{2+}-translocating ATPase, is consistent with a predominant role for this latter mechanism.

Does the Na^+/Ca^{2+} exchanger function under near physiological conditions?

The existence of a Na^+/Ca^{2+} antiport activity in synaptosomal plasma membrane vesicles is well established (Gill *et al.*, 1981; Schellenberg & Swanson, 1982). However demonstration of a comparable activity in intact synaptosomes appears more elusive. The earliest reports showed not Ca^{2+} efflux, but an enhanced Ca^{2+} uptake into synaptosomes suspended into Na^+-free medium containing millimolar Ca^{2+} (Swanson *et al.*, 1974; Blaustein & Oborn, 1975). Blaustein & Ector (1976) only showed a Na^+-dependency for Ca^{2+} efflux from synaptosomes when external Ca^{2+} was chelated by EGTA. A further limitation with such Na^+-replacement experiments is that any effect on the voltage dependent Ca^{2+} channel due to Na^+ removal (through inhibition of the Na^+ pump and hence collapse of $\Delta\psi_p$) is generally ignored. Neither of these conditions therefore approach the physiological, where Ca^{2+} must be pumped from a cytosol at 0.1–1 μM to an external phase at 1 mM. The low affinity of the antiporter also poses a problem, since Gill *et al.* (1981) have estimated the K_m for Ca^{2+} of the antiport to be 40 μM.

The experiments in the present paper do not prove that Na^+/Ca^{2+} exchange does not occur across the polarized synaptosomal plasma membrane, since it is possible to argue that the increase in cytosolic Ca^{2+} caused by veratridine (Ashley *et al.*, 1984) activates the Ca^{2+}-ATPase to an extent which more than compensates for a reversal of Ca^{2+} flux through the Na^+/Ca^{2+} exchanger. However, taken together with an earlier demonstration that the increased Ca^{2+} influx following potassium or veratridine-induced depolarization can be completely blocked by the Ca^{2+} channel inhibitor verapamil (Åkerman & Nicholls, 1981*a*) it does mean that there is still no direct evidence that the exchanger operates in the direction of Ca^{2+} efflux in the normally polarized synaptosome.

The significance of Na^+/Ca^{2+} exchange is also being reassessed in the squid axon, which provides an easier system for the study of ion fluxes than the synaptosome, although being less accessible to protein chemistry. DiPolo & Beaugé (1980) and Baker & Singh (1981) both provide evidence that the Na^+-independent Ca^{2+} efflux pathway, probably identifiable as a Ca^{2+}-translocating ATPase (DiPolo & Beaugé, 1979), is the more important

pathway at low, physiologically relevant, internal free Ca^{2+} concentrations.

Steady-state cycling of Ca^{2+} across the plasma membrane

Under steady-state conditions, Ca^{2+} exchanges across the plasma membrane of polarized synaptosomes at $0.38\,nmol \cdot min^{-1} \cdot mg^{-1}$ of protein (Table 1). The enhanced exchange when $\Delta\mu_{Na^+}$ is collapsed indicates enhancement of both the efflux and influx of Ca^{2+}. Since the increased influx is verapamil-sensitive, it is most likely due to a stable activation of the voltage-dependent Ca^{2+} channel. The increased efflux could be due either to the raised cytosolic Ca^{2+} (Åkerman & Heinonen, 1983; Ashley *et al.*, 1984) or to the decreased $\Delta\psi_p$ (Fig. 1), since both lower the electrochemical gradient against which the Ca^{2+} must be pumped. The ability of verapamil to lower the exchange rate to control values indicates that it is most likely the cytosolic free Ca^{2+} which activates the Ca^{2+}-translocating ATPase in the veratridine-depolarized condition.

The present results are thus consistent with a continuous, steady-state cycling of Ca^{2+} across the synaptosomal plasma membrane. The regulatory advantages of such a system compared with an equilibrium distribution through a single pathway have already been discussed in relation to mitochondrial Ca^{2+} transport, where a uniporter whose activity is highly dependent on Ca^{2+} concentration and an independent efflux pathway together provide a sensitive means of regulating the upper limit of the cytosolic free Ca^{2+} (reviewed in Nicholls & Åkerman, 1982). Thus at the plasma membrane the cycle would seek to lower the cytosolic free Ca^{2+} to the level at which uptake and efflux were equal and opposite. Any increase in cytosolic Ca^{2+} would be countered by an activation of the Ca^{2+}-translocating ATPase which would eventually restore the resting level. The simultaneous presence of the mitochondrial Ca^{2+}-cycle, with its relatively high 'set-point' at which uptake and efflux balance, but its very high capacity and activity (reviewed in Nicholls & Åkerman, 1982) would provide a means of rapidly lowering the cytosolic Ca^{2+} to a level insufficient for exocytosis (Åkerman & Nicholls, 1981*d*) following a burst of neuronal activity, allowing the plasma membrane cycle to restore a resting free Ca^{2+} in the region of $0.1\,\mu M$ (Ashley *et al.*, 1984).

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