J. Membrane Biol. 114, 225–230 (1990)

The Journal of
Membrane Biology

Direct Inhibitory Action of EGTA-Ca Complex on Reverse-Mode Na/Ca Exchange in *Myxicola* Giant Axons

R.A. Sjodin, A.A. Mahmoud,* and J.G. Montes
Department of Biophysics, University of Maryland School of Medicine, Baltimore, Maryland 21201

Summary. Giant axons from the marine annelid *Myxicola infundibulum* were internally dialyzed with solutions containing ^{22}Na ions as tracers of Na efflux. In experiments performed in Li-substituted seawater, Na efflux that is dependent on external Ca ion concentration, $[Ca^{2+}]_o$, was measured using dialysis to maintain $[Na^+]_i$ at 100 mM, which enhances the $[Ca^{2+}]_o$-dependent Na efflux component, (i.e., reverse-mode Na/Ca exchange). When dialysis fluid contained EGTA (1 mM) to buffer the internal Ca concentration, $[Ca^{2+}]_i$, to desired levels, Na efflux lost its normal sensitivity to external calcium. The inhibition was not simply due to the Ca-chelating action of EGTA to produce insufficient $[Ca^{2+}]_i$ to activate Na/Ca exchange. The addition of EGTA inhibited Ca_o-dependent Na efflux even when a large enough excess of $[Ca^{2+}]_i$ was present to saturate the EGTA and still produce elevated values of $[Ca^{2+}]_i$. Control experiments showed that these high values of $[Ca^{2+}]_i$ resulted in normal Na/Ca exchange in the absence of EGTA. It is concluded that the presence of EGTA itself interferes with the manifestation of reverse-mode Na/Ca exchange in *Myxicola* giant axons.

Key Words Ca chelators · calcium fluxes · sodium fluxes

Introduction

The efflux of sodium ions in both squid giant axons and in *Myxicola* giant axons is sensitive to the external calcium ion concentration, $[Ca^{2+}]_o$ (Baker et al., 1969; Abercrombie & Sjodin, 1977; Sjodin & Abercrombie, 1978). When the internal Na ion concentration is elevated and the external Na concentration is reduced to nominally zero by replacement with Li ions, the electrochemical gradient for Na ions is reversed from normal, and under these conditions Na efflux has a large component that appears only if Ca ions are present in the external medium. This Ca_o-dependent Na efflux is believed to be a manifestation of the Na/Ca exchange system operating in a direction reversed from normal (Baker et al., 1969).

A frequently applied experimental maneuver is to employ EGTA/Ca-EGTA buffers to stabilize the Ca^{2+} concentration inside axons (Baker & McNaughton, 1976; DiPolo, 1979). A difficulty in the use of EGTA to control the value of $[Ca^{2+}]_i$ in axons has been emphasized by Baker and McNaughton (1976) who point out that EGTA itself inhibits ion flux components. For example, the Ca_o-dependent Na efflux and the influx of Ca ions in squid giant axons are both inhibited by injection of EGTA or Ca-EGTA buffers into the axon (Baker, 1970). Other Ca chelators such as Quin-2, EDTA, and BAPTA have also been shown to selectively inhibit the reversed mode of Na/Ca exchange even when used with sufficient Ca to give a free Ca^{2+} concentration close to the physiological value of 100 nM (Allen & Baker, 1985).

Among various possibilities, two stand out. As the previous studies have not been done at various levels of $[Ca^{2+}]_i$, the Ca chelators may exert an inhibitory action solely by virtue of their Ca-chelating activity. It has been shown that a certain critical level of $[Ca^{2+}]_i$ is required to activate Na/Ca exchange (DiPolo, 1979). The inhibiting chelators may simply reduce $[Ca^{2+}]_i$ to below this critical value. This interpretation has been previously proposed by Allen and Baker (1985). Another possibility is that only the free form of the chelators are inhibitory so that one cannot use them as buffers and they must be saturated with Ca to lose their inhibitory action. The present work addresses these questions. As Na/Ca exchange has been shown to be present in a wide variety of cell types, both in invertebrates and vertebrates, and since Ca chelators are in wide use both as buffers and indicators, it seems important to establish these points and to make any confounding effects of their use as widely known as possible.

* *Present address:* Department of Physiology, Faculty of Medicine, University of Ismailiah, Egypt.

Materials and Methods

Biological Specimens

Giant axons were dissected from specimens of *Myxicola infundibulum* obtained from Marine Research Associates, New Brunswick, Canada. They were kept 5–7°C in aerated artificial seawater reconstituted from Instant Ocean (Aquarium Systems, Mentor, OH).

Internal Dialysis of *Myxicola* Giant Axons

A modified version of the method of Brinley and Mullins (1967) and Forbush (1979) was used. The only significant modification was in limiting the length of the dialysis region to 0.5 cm; this was accomplished by reducing to 0.5 cm the central region of the chamber used for isotope collection, and shortening the porous region of the dialysis capillary to the same extent. This modification was made in order to limit flux measurements to only one internodal segment of the axon. Unlike squid giant axons, *Myxicola* axons possess nodal or constricted regions that are difficult to clear of surface blood vessels during dissection; these regions interrupt the somewhat longer internodal regions, which are relatively easy to clean. Because the nodal regions are much more likely to undergo injury during dissection, a breach in the integrity of the axon membrane is more likely to occur at these regions, thereby producing significant leakages that introduce inaccuracies in the measured fluxes. In preliminary experiments in this laboratory using the earlier, unmodified protocol (i.e., using chambers with longer central regions), nodal regions were observed to coincide often with the region of dialysis. Marginal injuries not leading to collapse of the axons often were manifested as leaky regions during dialysis. This observation led to adoption of a shorter dialysis region within which a very short, undamaged, node-free region of the axon could be centered. Other details of the method and chamber can be found in the aforementioned references. Capillary tubing consisted of a stretch of cellulose acetate tubing (95 i.d. × 190 μm o.d., Fabrius Research, Dedham, MA) that had been made porous along a 0.5-cm stretch by treatment for 2–3 hr in 2N HNO_3 (40°C), and that then had been deacetylated for 14–18 hr in 50 mM NaOH. All experiments were conducted at 13–15°C.

Radioactive Na

^{22}NaCl (New England Nuclear, Boston) was obtained in a carrier-free solution. It was added to dialysis fluid to yield specific activities in the range 2–5 cpm/pmol.

Solutions

External medium was LiSW (lithium-substituted seawater) that was 10 mM KCl, either 0 or 10 mM $CaCl_2$, 410 mM LiCl (if $CaCl_2$ = 10 mM) or 415 mM LiCl (if $CaCl_2$ = 0), 25 mM $MgCl_2$, and 5 mM HEPES, and always contained ouabain (10^{-4} M). Dialysis fluid was always 100 mM Na (aspartate), 1 mM ATP, 3 mM Mg, 200 K (as aspartate) and 50 mM K_2 TES ("standard" dialysis fluid); total Ca concentration, $[Ca]_T$, in experiments using EGTA was almost 0 (standard or control experiment with contaminant Ca only), 1 mM, or 1.5 mM; in some experiments KCN (0.5 mM) and oligomycin (10 μg/ml) were used as metabolic inhibitors. The pH of the dialysis fluid was 7.3. The osmolarity of the dialysis solution was in the range 890–936 mosM. The legends to the figures spell out the particular solutions used according to experiment.

Measurement of Na Efflux

Na fluxes were measured by using a chamber previously described by Brinley and Mullins (1967) in studies of squid giant axons, and adapted by Forbush (1979) to study Na fluxes in *Myxicola* giant axons. After beginning dialysis with fluid containing ^{22}Na, a minimum of 15 min was allowed before making measurements of Na efflux; this was done to allow equilibration of the dialysis fluid with the cellular compartment. An automatic gamma-counter (Searle Model 1185, Searle Analytic, Silver Spring, MD) was used to make counts from samples taken at regular time intervals from the medium surrounding the fibers.

Quantitative Comparison of Na Effluxes in Reverse Na/Ca Exchange

So that statistical comparisons of reverse Na/Ca exchange could be made between the experiments in which the dialysis fluid was varied with respect to its concentration of Ca and EGTA, the following procedure was implemented: (*i*) for $[Ca^{2+}]_o = 0$, a calculated average Na efflux was obtained by averaging the measured, individual flux values of all points for the entire duration, except for the first 6 min, that the axon was maintained in the same concentration of external Ca; (*ii*) the same was done starting at 6 min from the time the medium surrounding the axon was changed to $[Ca^{2+}]_o$ = 10 mM, (*iii*) a ratio of the two average effluxes, i.e., the average Na efflux in $[Ca^{2+}]_o$ = 10 mM divided by the average Na efflux in $[Ca^{2+}]_o = 0$, was computed for each experiment, and when experiments were repeated, a standard error of the mean (SEM) of the ratio was calculated for each type of experiment. Only first-change data were used (i.e., going from 0 Ca to 10 mM Ca, but not back to 0 Ca again); second-change data (*not shown*) gave similar, but less pronounced, results. This approach gave consistent, satisfactory numbers from which to draw conclusions.

Results

It was previously shown that Na efflux in *Myxicola* giant axons microinjected with additional Na to increase $[Na^+]_i$ is sensitive to the external calcium ion concentration, $[Ca^{2+}]_o$ (Sjodin & Abercrombie, 1978). The magnitude of the Ca_o-dependent Na efflux under these conditions is comparable to that observed in squid giant axons (Baker et al., 1969). The present study grew out of an investigation of Na efflux in internally dialyzed *Myxicola* giant axons. It seemed important in establishing the adequacy of the dialysis method for *Myxicola* giant axons to show that dialyzed axons have the same Na efflux components as are observed in microinjected axons. The results of a typical experiment are illustrated in Fig. 1, which is taken from a number of

Table Stimulation by external Ca of reverse Na/Ca exchange in *Myxicola* axons dialyzed with different concentrations of EGTA and Ca[a]

Concentration in dialysis fluid		Estimated $[Ca^{2+}]$ in axoplasm	Na efflux (pmol/cm² sec)		Ratio of fluxes (SEM) 10 Ca/0 Ca	Number of axons
Total EGTA	Total Ca		0 Ca	10 Ca		
0	contaminant	1–10 μM[b]	24.3	37.9	1.8 (0.07)	5
0	10 μM	10–20 μM[c]	14.6	23.0	1.9 (0.33)	3
0	150 μM	~150 μM	12.5	23.1	1.9	1
0	0.5 mM	~0.5 mM	24.7	36.7	1.5 (0.01)	2
0	1 mM	~1 mM	30.3	45.0	1.5 (0.07)	2
1 mM	contaminant	<0.002 μM[d]	21.2	29.1	1.4	1
1 mM	1 mM	12–18 μM[e]	50.3	59.8	1.3 (0.09)	4
1 mM	1.5 mM	~0.5 mM	41.6	43.5	1.1 (0.12)	2

[a] Average Na efflux (*see* Materials and Methods for details) is shown for $[Ca^{2+}]_o$ = 0 *vs.* 10 mM in experiments in which axons were first immersed in 0 Ca (20–40 min) and then in 10 mM Ca for a comparable period. The ratio of fluxes refers to the average Na efflux in 10 mM Ca divided by the average Na efflux in 0 Ca. (*See* figure legends or Materials and Methods for additional characterization of external media and dialysis solutions; SEM = standard error of the mean.)

[b] Empirical measurements by spectrophotometric methods of dialysis solutions yielded contaminating levels of Ca in excess of 1 μM but less than or about 10 μM.

[c] The upper limit of 20 μM arises from the sum of the intentional 10 μM Ca in the dialysis solution and the nominally maximum contaminating level of Ca = 10 μM.

[d] Obtained by application of the stability constant for EGTA/Ca, $K_D = 1.4 \times 10^{-4}$ mM, to the maximum level of contaminating Ca in the dialysis fluid, 10 μM.

[e] Calculated from the K_D, using range of contaminating Ca in dialysis solution, 1–10 μM.

experiments summarized in the Table. The experiment begins in a Ca-free, Li-substituted seawater and shows that addition of external Ca at the usual concentration of 10 mM brings about a prompt and marked increase in the Na efflux. Removal of external Ca at a subsequent time results in a decline in Na efflux to its initial value. The magnitude of the Ca_o-dependent Na efflux in this particular experiment was about 20 pmol/cm² sec. In many such experiments the magnitude of Ca_o-dependent Na efflux in dialyzed axons is of this order, and a readily detectable Ca_o-dependent Na efflux is always seen under these conditions.

In experiments performed to determine the influence of $[Ca^{2+}]_i$ on the Ca_o-dependent Na efflux, EGTA/Ca-EGTA buffer mixtures were used to obtain various values of $[Ca^{2+}]$ in the dialysis solution with which the value of $[Ca^{2+}]_i$ would eventually equilibrate. It was soon noticed that whenever EGTA was present in the dialysis solution, the magnitude of the Ca_o-dependent Na efflux appeared to be reduced or absent. A typical experiment in which EGTA was present in dialysis fluid at a total concentration of 1 mM is illustrated in Fig. 2 (*see also* the Table). The total Ca concentration was also 1 mM and the calculated value of $[Ca^{2+}]_i$ from the dissociation constant of Ca-EGTA in *Myxicola* axoplasm (Abercrombie et al., 1981) was 12–18 μM. In this particular experiment, the initial response to $[Ca^{2+}]_o$ = 10 mM was prevented or significantly reduced (Ca_o-dependent Na efflux = about 5 pmol/cm² sec) when compared to what was seen with similar $[Ca^{2+}]_i$ in the absence of EGTA (*cf. also*

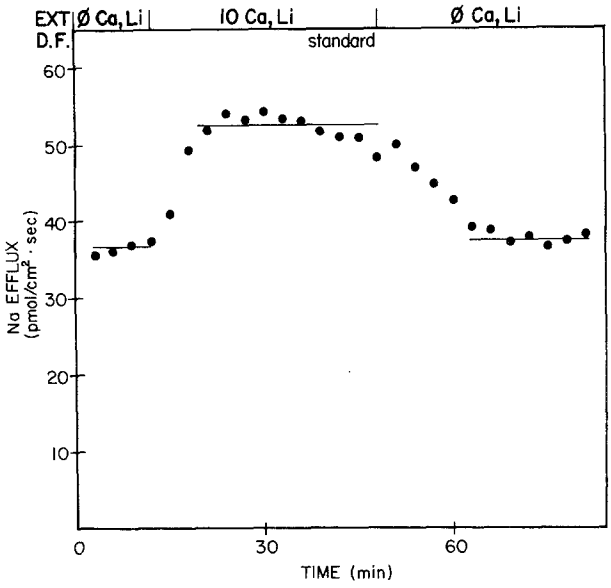

Fig. 1. Na/Ca exchange in dialyzed fibers is readily detectable. Na efflux increases significantly in the presence of 10 mM Ca. This is the same result as obtained in injected fibers. D.F. = dialysis fluid; EXT = external medium. $[Na^+]_{D.F.}$ = 100 mM; $[ATP]_{D.F.}$ = 1 mM; dialysis fluid was nominally Ca-free. External medium was Li-substituted seawater, with or without calcium, and contained ouabain (10^{-4} M)

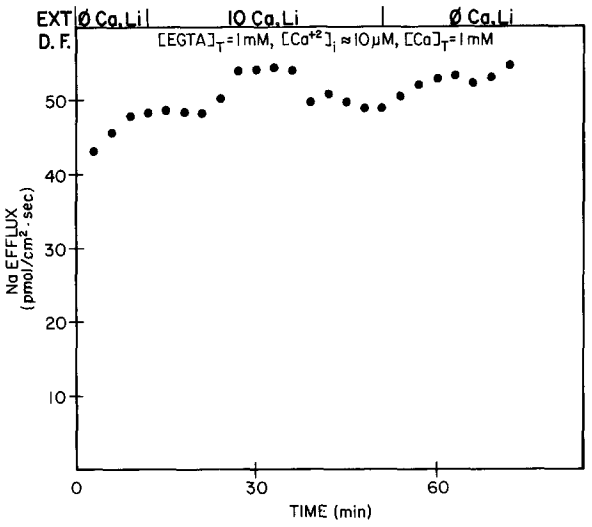

Fig. 2. Na/Ca exchange is significantly affected by internal EGTA. When EGTA is present inside the axon, there is a null or much lower response of Na efflux to external Ca^{2+}. D.F. = dialysis fluid; EXT = external medium. $[Na]_{D.F.}$ = 100 mM; $[ATP]_{D.F.}$ = 1 mM; dialysis fluid contained EGTA (1 mM); $[Ca^{2+}]_i \approx 10$ μM; and $[Ca]_T$ = 1 mM. External medium was Li-substituted seawater, with or without calcium, and contained ouabain (10^{-4} M)

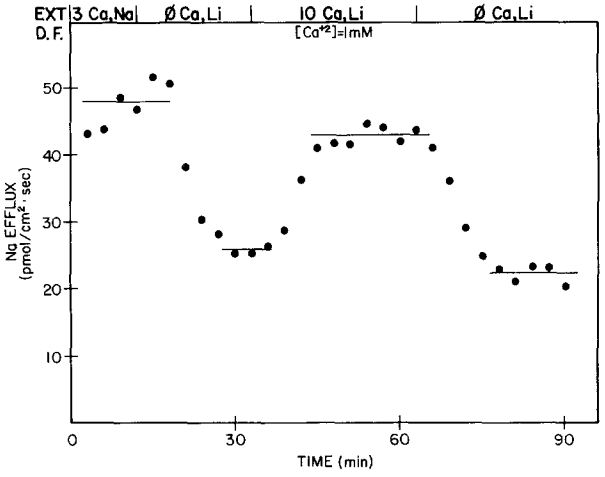

Fig. 3. The presence of high $[Ca^{2+}]$ in the dialysis fluid did not alter the manifestation of Na/Ca exchange in the absence of EGTA. Experimental conditions were as in Fig. 1, except that dialysis fluid contained $[Ca^{2+}]$ = 1 mM

rows 2 and 7 in the Table), and Na efflux did not decline when Ca was again removed from the external medium. Figure 2 can be compared with Figs. 1 and 3 where EGTA was absent and normal responses of Na efflux to external Ca were observed. Figure 2 illustrates the difficulty in accurately determining any Ca_o-dependent Na efflux in the presence of EGTA in this preparation. Nevertheless, the procedure described in Materials and Methods was consistently applied to get the results presented in the Table.

It was clear from our results that EGTA was, for some reason, inhibitory to Ca_o-dependent Na efflux. It was not clear, however, whether the combined or uncombined form of the chelator was responsible for the inhibition. For this reason, experiments were performed in which a large excess of internal Ca was present to essentially combine all of the EGTA with Ca ions to see if inhibition still persisted. To achieve this condition, dialysis fluid contained 1 mM EGTA and 1.5 mM total Ca, which results in essentially complete combination of EGTA with Ca and a residual $[Ca^{2+}]$ of about 0.5 mM. The experimental result (summarized in the Table for two experiments) was that the inhibition of Ca_o-dependent Na efflux persisted. As the value of $[Ca^{2+}]_i$ was elevated in experiments of this type, it had to be established that high $[Ca^{2+}]_i$ *per se* is not inhibitory to Ca_o-dependent Na efflux. To address this point, experiments were performed in which the value of $[Ca^{2+}]_i$ was elevated in the absence of EGTA (*see* Fig. 3). The internal calcium ion concentration was raised by making the value of $[Ca^{2+}]$ in the dialysis solution 1 mM in the absence of any EGTA. The value of $[Ca^{2+}]_i$ in axoplasm will not, of course, immediately come to be 1 mM, because of the operation of endogenous, intrafibrillar Ca buffers. Some optical measurements, using the indicator dyes arsenazo III and antipyrilazo III, were made of the value of $[Ca^{2+}]_i$ reached in the axoplasm after beginning dialysis with a solution of $[Ca^{2+}]$ = 1 mM. The optical experiments affirmed that equilibration of axoplasmic $[Ca^{2+}]$ with dialysis fluid had occurred before external solution changes were made to measure Ca_o-dependent Na efflux. The results in Fig. 3 show that Na/Ca exchange could still be readily discerned, even though the calcium level in the dialysis fluid, $[Ca^{2+}]_{D.F.}$, was highly elevated. In some experiments (*data not shown*), KCN and oligomycin were introduced to inhibit mitochondrial Ca buffering. Identical results were obtained indicating that endogenous intracellular Ca buffers are not a problem at such high Ca loads.

In view of these results, one can be certain that it is the EGTA that interferes with the manifestation of Ca_o-dependent Na efflux, and not the elevated values of $[Ca^{2+}]_i$. It is also evident that the completely combined form of EGTA is inhibitory. It would be difficult to show that the completely uncombined form of EGTA is inhibitory due to the difficulty in achieving this condition without lowering the value of $[Ca^{2+}]_i$ below that required for activation of Na/Ca exchange.

The following conclusions can be drawn from the data:

1. The percentage change in flux due to "reverse" Na/Ca exchange as deduced from the ratio of fluxes column in the Table indicates an inhibitory effect of EGTA or Ca-EGTA (statistically significant at $P < 0.001$).

2. The inhibitory effect of the complexed form of EGTA may be higher than that for EGTA with zero or contaminant Ca in dialysis fluid as deduced from ratio tests, though not enough experiments were done to make this conclusion firm.

3. The inhibition of Ca_o-dependent Na efflux by the complexed form of EGTA occurs at an elevated value of total ouabain-insensitive Na efflux so that the baseline flux against which ratio comparisons are made is higher. The system behaves as though the Ca_o-stimulated Na efflux has already largely occurred in the absence of external Ca at high internal Ca-EGTA levels which results in less additional stimulation from added Ca_o.

Discussion

The results show that internal EGTA in some way interferes with the effect of Ca_o on Na efflux in dialyzed *Myxicola* giant axons, confirming in another preparation what has been previously observed in squid giant axons (Baker & McNaughton, 1976). On the other hand, the inhibitory action of EGTA on Ca_o-activated Na efflux persisted even when enough Ca was added to the dialysis fluid to complex essentially all of the EGTA (*see* the Table). It seems clear that caution must be exercised in the interpretation of results obtained in investigations of Na/Ca exchange in which the EGTA/Ca-EGTA buffer system has been used to control the internal calcium ion concentration.

The results also bear on another point. Though internally introduced EGTA apparently inhibits Ca_o-dependent Na efflux in giant axons, Na_o-dependent Ca efflux is apparently normal in the presence of internal EGTA (Baker & McNaughton, 1976). The Ca_o-dependent Na efflux is usually believed to be due to the Na/Ca exchange mechanism operating in the reverse of its normal direction, which is a Na-driven Ca extrusion. If this is so, as seems likely, the results with EGTA would suggest that the forward mode of Na/Ca exchange is unaffected by EGTA, whereas the same process operating in reverse is strongly affected by EGTA. If only one transport protein assembly is responsible for Na/Ca exchange, then blocking transport for one direction of operation should also block the reverse direction according to any simple model for the transport. One possibility is that the observed Ca_o-dependent Na efflux does not represent a simple reversal of Na/Ca exchange. This point should be looked at more critically in excitable cells in view of the facts discussed. For example, an apparent Na/Mg exchange has been reported in human red cells (Lüdi & Schatzmann, 1987) and this process cannot be reversed. Alternative models have been proposed by these authors to account for irreversibility of Na/Mg exchange and these models may be helpful in explaining the apparently contradictory results encountered with Ca chelators in giant axons. The Ca chelators may be effective in blocking the access of Na to a site on the inside of the membrane but not the access of Ca ions to Ca sites. Another fact suggests that Ca_o-dependent Na efflux in giant axons may not represent a simple reversal of Na/Ca exchange. The voltage dependence of so-called "reverse" Na/Ca exchange in squid giant axons is considerably less than the voltage dependence of "forward" Na/Ca exchange (Allen & Baker, 1986). These authors consider the possibility that the Ca_o-dependent Na efflux may consist of a reversed Na/Ca exchange with normal voltage sensitivity plus a voltage-insensitive component due to a different process.

It is of interest to compare the present results with those of several other laboratories. It appears that no authors report any significant inhibition or other problem due to EGTA or Ca-EGTA when the normal or forward direction of Na/Ca exchange is studied by measuring either Na influx, Ca efflux, or both (Blaustein & Russell, 1975; Baker & McNaughton, 1976). As this component of Na/Ca exchange was not measured in the present work, there is no conflict with these previous results. With regard to the reverse mode of Na/Ca exchange (Ca influx, Na efflux, or both), some comparisons can be made. An important relevant fact is the finding that micromolar amounts of Ca_i are required for operation of "reverse" Na/Ca exchange in squid giant axons (DiPolo, 1979; DiPolo & Beaugé, 1986, 1987). Thus, a possible interpretation of an inhibitory effect of EGTA is that it complexes Ca to reduce $[Ca^{2+}]_i$ to below levels required for activation of "reverse" Na/Ca exchange. This possible explanation has already been discussed (Allen & Baker, 1985). As our results are in general agreement with their findings and those of Baker and McNaughton (1976), a similar explanation might suffice at first consideration to account for our results. The key question is, does EGTA or Ca-EGTA inhibit in a manner that cannot be accounted for by simply a reduction in the value of $[Ca^{2+}]_i$? The data in the Table indicate that a reduction in $[Ca^{2+}]_i$ cannot be the sole explanation for the inhibition observed.

The data obtained in the presence of 1 mM EGTA with $[Ca^{2+}]_i$ about 0.5 mM (500 μM) is in the range well above the saturation level of $[Ca^{2+}]_i$ which for squid begins at $[Ca^{2+}]_i$ equal to about 100 μM (DiPolo & Beaugé, 1987). Thus, if activation of Na/Ca exchange in *Myxicola* axons follows kinetics close to those holding for squid axons, we would expect no inhibition due to the presence of EGTA if Ca chelation is the only factor involved, which is contrary to the large degree of inhibition observed (last entry in the Table). On the other hand, if EGTA has a real inhibitory effect in squid axons, as it seems to have in *Myxicola* axons, then the activation curves presented in Fig. 11 in the paper of DiPolo and Beaugé (1987) cannot reflect the true kinetics of activation by $[Ca^{2+}]_i$, as the results would then be a composite of activation by Ca_i and some inhibition by EGTA and Ca-EGTA. In the absence of EGTA, for example, more activation by $[Ca^{2+}]_i$ = 1 mM might occur than that cited by these authors and the rate of "reverse" exchange may be significant at values of $[Ca^{2+}]_i$ that are less than those supposed. Clearly, some experiments with and without EGTA at similar values of $[Ca^{2+}]_i$ are required to settle this point in squid axons.

In barnacle muscle cells, on the other hand (Rasgado-Flores & Blaustein, 1987), and in mammalian heart cells (Kimura et al., 1987), use of internal EGTA to buffer cell calcium apparently leads to no special problems when "reverse" Na/Ca exchange rates are measured. A possibility exists, therefore, that the inhibitory effects of EGTA presently observed are peculiar to the preparation. The fact that EGTA somehow can inhibit "reverse" Na/Ca exchange without influencing the "forward" mode in squid giant axons (Baker & McNaughton, 1976; Allen & Baker, 1985) suggests, however, that some caution be exercised when using EGTA to buffer Ca_i in nerve cells. Whatever the mechanism of the inhibition by Ca chelators of Ca_o-dependent Na efflux, the present work clearly shows that the action cannot be due solely to a low value of $[Ca^{2+}]_i$ produced by chelation.

This work was supported by grants from the National Institutes of Health (RO1-NS07626) and from the National Science Foundation (PCM-8022820) to R.A.S.

References

Abercrombie, R.F., Masukawa, L.M., Sjodin, R.A., Livengood, D. 1981. Uptake and release of ^{45}Ca by *Myxicola* axoplasm. *J. Gen. Physiol.* **78:**413–429

Abercrombie, R.F., Sjodin, R.A. 1977. Sodium efflux in *Myxicola* giant axons. *J. Gen. Physiol.* **69:**765–778

Allen, T.J.A., Baker, P.F. 1985. Intracellular Ca indicator Quin-2 inhibits Ca^{2+} inflow via Na_i/Ca_o exchange in squid axon. *Nature (London)* **315:**755–756

Allen, T.J.A., Baker, P.F. 1986. Comparison of the effects of potassium and membrane potential on the calcium-dependent sodium efflux in squid axons. *J. Physiol. (London)* **378:**53–76

Baker, P.F. 1970. Sodium-calcium exchange across the nerve cell membrane. *In:* Calcium and Cellular Function. A.W. Cuthbert, editor. pp. 96–107. Macmillan, London

Baker, P.F., Blaustein, M.P., Hodgkin, A.L., Steinhardt, R.A. 1969. The influence of calcium on sodium efflux in squid axons. *J. Physiol. (London)* **200:**431–458

Baker, P.F., McNaughton, P.A. 1976. Kinetics and energetics of calcium efflux from intact squid giant axons. *J. Physiol. (London)* **259:**103–144

Blaustein, M.P., Russell, J.M. 1975. Sodium-calcium exchange and calcium-calcium exchange in internally dialyzed squid giant axons. *J. Membrane Biol.* **22:**285–312

Brinley, F.J.,Jr., Mullins, L.J. 1967. Sodium extrusion by internally dialyzed squid axons. *J. Gen. Physiol.* **50:**2303–2331

DiPolo, R.J. 1979. Calcium influx in internally dialyzed squid giant axons. *J. Gen. Physiol.* **73:**91–113

DiPolo, R., Beaugé, L. 1986. Reverse Na-Ca exchange requires internal Ca and/or ATP in squid axons. *Biochim. Biophys. Acta* **854:**298–306

DiPolo, R., Beaugé, L. 1987. Characterization of the reverse Na/Ca exchange in squid axons and its modulation by Ca_i and ATP. *J. Gen. Physiol.* **90:**505–525

Forbush, B., III. 1979. Sodium and potassium fluxes across the dialyzed giant axon of *Myxicola*. *J. Membrane Biol.* **46:**185–212

Kimura, J., Miyamae, S., Mowa, A. 1987. Identification of sodium-calcium exchange current in single ventricular cells of guinea-pig. *J. Physiol. (London)* **384:**199–222

Lüdi, H., Schatzmann, H.J. 1987. Some properties of a system for sodium-dependent outward movement of magnesium from metabolizing human red blood cells. *J. Physiol. (London)* **390:**367–382

Rasgado-Flores, H., Blaustein, M.P. 1987. Na/Ca exchange in barnacle muscle cells has a stoichiometry of 3 Na^+/1 Ca^{2+}. *Am. J. Physiol.* **252:**C499–C504

Sjodin, R.A., Abercrombie, R.F. 1978. The influence of external cations and membrane potential on Ca-activated Na efflux in *Myxicola* giant axons. *J. Gen. Physiol.* **71:**453–466

Received 10 July 1989; revised 31 October 1989