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Topical Review

Electrogenic Properties of the Na : Ca Exchange

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Introduction

The design requirements for an intracellular messenger which can act as an effective switch to change the state of some cellular process from an "off" to an "on" state (or vice versa) are fairly obvious: the messenger must be present in a low concentration when the signal is to be off, so that the addition of a small amount of the messenger to the cytoplasm will cause a large relative change in its concentration; there must be some mechanism for releasing the transmitter within the cell or for admitting it from the exterior; and, finally, the effector molecule sensing the concentration change must do so with a sharp threshold. In many cells intracellular calcium and the signal molecules to which it binds fulfill these three requirements. In those cells in which it has been measured $[Ca]_i$ is of the order of 100 nM at rest, even though the external calcium concentration ($[Ca]_o$) may be 1 or 2 mM and the membrane potential of the order of −70 mV. The distribution of Ca^{2+} ions on either side of the plasma membrane is therefore far from equilibrium, and it is the existence of this strong electrochemical gradient favoring Ca^{2+} entry that allows a change in the Ca^{2+} permeability of the plasma membrane to generate a rapid intracellular Ca^{2+} signal (*see* for example articles in the volume edited by Miller, 1988). In many cells, such as skeletal and cardiac muscle, the calcium influx from the exterior is also greatly augmented by a release within the cell. Finally, the calcium signal can act on a wide range of intracellular calcium sensors, such as the calmodulin family, and these all have in common that two or more calcium ions must bind with some degree of cooperativity in order to cause activation, thereby generating a steep threshold and a low degree of activation in the resting state.

The importance of calcium as an internal messenger controlling many cell functions has encouraged much research into the mechanisms by which intracellular free calcium concentration ($[Ca]_i$) is regulated. The problem can be broken down into three components: the study of the ion channels which admit calcium through the surface membrane, of the mechanisms subserving the storage and release of calcium within the cell, and of the transport mechanisms which eventually must maintain a low intracellular calcium concentration against the steep electrochemical gradient normally existing across the surface membrane. Two mechanisms of Ca^{2+} extrusion have so far been identified. The first is an enzymatic pump which derives the energy for Ca^{2+} extrusion from the coupled hydrolysis of ATP (Schatzmann, 1966, 1986). The second is a carrier which does not depend directly on ATP, but instead couples the efflux of Ca^{2+}, driven against its electrochemical gradient, to the influx of Na^+, flowing down its electrochemical gradient. This Na-dependent mechanism has usually been referred to as the Na : Ca exchange, although it has recently been demonstrated that in vertebrate photoreceptors K^+ is cotransported with Ca^{2+}, so that a better term may be the Na : Ca,K exchange (Cervetto et al., 1989). While it seems possible that an exchange of Na for Ca and K may be a general phenomenon (*see below*), firm evidence is lacking for all but the photoreceptor outer segment, and we shall, for the sake of brevity and familiarity, stick to the usual terminology of "Na : Ca exchange."

Since the Na-dependent transport of Ca^{2+} was first demonstrated in the squid giant axon (Baker et al., 1969; Blaustein & Hodgkin, 1969) and in cardiac muscle (Reuter & Seitz, 1968), the properties and

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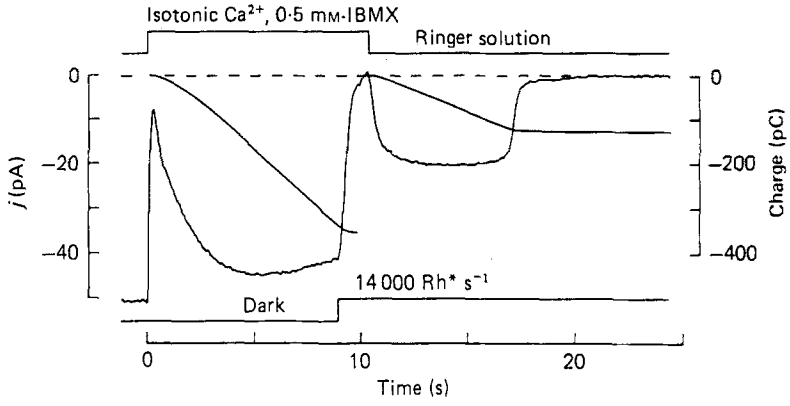

Fig. 1. Na : Ca exchange current recorded in an intact salamander rod after the introduction of a large Ca load into the outer segment. The rod was loaded by exposure to isotonic $CaCl_2$ containing 0.5 mM IBMX, and the exchange current recorded by turning on a bright light 1 sec before returning to Ringer solution. The superposed smooth curves and the right-hand scale show the integrals of the charge transferred during the loading period (353 pC) and during the operation of the exchange in extruding this Ca load (130 pC). (Reproduced from Hodgkin et al. (1987) with permission of the publisher)

role of the exchange have been investigated in a variety of tissues including photoreceptors, smooth muscle and epithelia (for a collection of recent reviews *see* Allen, Noble & Reuter, 1989). Amongst the questions that have attracted the most attention in recent years are (i) the stoichiometry of the exchange, including the related question of its electrogenicity, (ii) the voltage-dependence of the exchange, and (iii) the mechanism of transport. These fundamental properties of the exchange determine its role in the regulation of $[Ca]_i$, both at rest and during electrical activity. The intention of this article is to review recent work that has addressed these issues.

The Na : Ca Exchange is Electrogenic

A major problem in the study of Na-dependent Ca transport has been the separation of ion fluxes or membrane currents through the exchange from those through other membrane carriers or pores. For this reason the idea that the exchange is electrogenic lacked, until recently, any clear experimental evidence to support it (reviewed by Eisner & Lederer, 1985). One obvious approach would be to identify the exchange current using a specific and selective blocker of the carrier mechanism, much as ouabain has been used to identify the current generated by the Na : K pump (Gadsby, Kimura & Noma, 1985). However, although a variety of substances have been shown to inhibit the exchange, their usefulness is limited because they are also active against other membrane conductances (Requena et al., 1985; Lagnado & McNaughton, 1987*c*; Lipp & Pott, 1988*b*). It is only in the last few years that convincing demonstrations of a Na : Ca exchange current have been provided.

Vertebrate Photoreceptors

The first clear evidence for a membrane current associated with the Na-dependent transport of Ca^{2+} was obtained in experiments in which the current through the outer segment of vertebrate rod photoreceptors was recorded (Yau & Nakatani, 1984; Hodgkin, McNaughton & Nunn, 1985, 1987; Hodgkin & Nunn, 1987). This preparation is particularly suited to the study of the Na : Ca exchange because only two mechanisms contribute to current through the outer segment membrane. The first is the light-sensitive conductance, which is gated by cGMP at the internal membrane surface and has an appreciable permeability to Ca^{2+}. These channels can therefore be used to load the outer segment with Ca^{2+}, and are readily closed by shining a bright light onto the outer segment. The current generated by the second electrogenic mechanism, the Na : Ca exchange, is then recorded in isolation. Other sources of current, such as the electrogenic Na : K pump and voltage-dependent channels, are located in the inner segment of the rod and do not need to be blocked because the outer segment impedance is an order of magnitude higher than that of the inner segment, and the current circulating between the inner and outer segments is therefore determined principally by changes in the outer segment conductance (Baylor, Lamb & Yau, 1979; Baylor, Matthews & Nunn, 1984).

Figure 1 shows one of the methods used to introduce a known Ca^{2+} load into the outer segment of a rod and to record the current associated with the extrusion of this Ca load in exchange for external Na. In darkness, the rod is transferred from normal Ringer solution to a solution containing isotonic $CaCl_2$ and the phosphodiesterase inhibitor IBMX. The increase in $[Ca]_o$ causes an initial rapid suppression of the light-sensitive current due to a

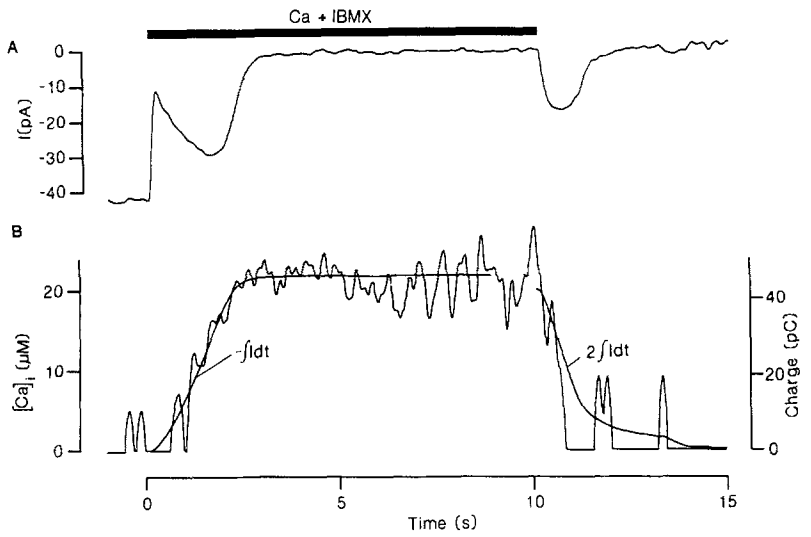

Fig. 2. Measurements of Ca influx, Na:Ca exchange current and intracellular free [Ca] in the outer segment of an intact salamander rod. (*A*) Outer segment membrane current. The period of exposure to isotonic $CaCl_2$ and IBMX is shown by the bar. The Ca^{2+} current flowing during this period was terminated by the rod's response to its own aequorin light emission. The charge carried during the period of Ca influx was 46 pC. On return to Ringer a transient light-insensitive current was activated, carrying a total charge of 20.6 pC. (*B*) Intracellular free [Ca] measured from the aequorin light emission (noisy trace). After the introduction of the Ca load there is no decline in $[Ca]_i$ in the absence of external Na, but on return to Ringer $[Ca]_i$ fell at a rate of 30 μM sec^{-1}. The smooth traces (corresponding to the right-hand ordinate) show the integral of the Ca influx and twice the integral of the Na:Ca exchange current. (Modified from McNaughton et al., 1986)

blocking action of Ca on the light-sensitive channel (Hodgkin et al., 1985), and there is then a slower increase in the inward Ca current because the inhibition of cGMP hydrolysis by IBMX causes the cGMP-gated channels to open. After a delay, a bright light is switched on in order to hold the light-sensitive channels shut and so terminate the influx of Ca. The reasons for these changes in light-sensitive current are in fact unimportant in the present context, and the only piece of information we need to know is the charge carried by the pure Ca^{2+} current, which can be obtained by integration. When the rod is transferred back to a solution containing Na, a transient inward current is activated. Several lines of evidence show that this inward current is due to the electrogenic exchange of external Na^+ for internal Ca.

1. The current is activated by external Na, but not by Li or other monovalent cations (Yau & Nakatani, 1984; Hodgkin et al., 1987). Li is known not to substitute for Na in the activation of Ca efflux in the squid axon and in heart muscle (Blaustein & Hodgkin, 1969; Baker & McNaughton, 1976*a*; Ledvora & Hegyvary, 1983).

2. The duration of the current depends on the size of the preceding Ca load (Yau & Nakatani, 1984; Hodgkin et al., 1987; Lagnado, Cervetto & McNaughton, 1988). With larger Ca loads (as shown in Fig. 1) the current is saturated for a period, and then declines to zero. The time course of this decline is independent of the initial Ca load introduced into the cell.

3. A reduction in $[Na]_o$ reduces the saturating current level and shows its decline (Hodgkin et al., 1987; Lagnado et al., 1988). Similar effects are caused by increasing the external concentration of Ca, Mg or K, all of which are believed to reduce the affinity of the exchange for external Na (Reeves & Sutko, 1983; Hodgkin & Nunn, 1987).

4. The current is inhibited by micromolar concentrations of lanthanum, which is known to be a powerful inhibitor of Na-dependent Ca transport in the squid giant axon (Baker & McNaughton, 1978).

5. The total charge transferred during the operation of the current is directly proportional to the size of the Ca load, which is simply calculated in this sort of experiment by integrating the light-sensitive current flowing during the exposure to isotonic $CaCl_2$. In the example shown in Fig. 1, the charge entering the cell during Ca influx is about 2.7 times that entering during the period in which the exchange current is recorded, an observation consistent with the influx of 0.74 net positive charges in exchange for a single Ca ion (*see below*).

6. The activation of the inward current is correlated with a fall in $[Ca]_i$ (Cervetto, McNaughton &

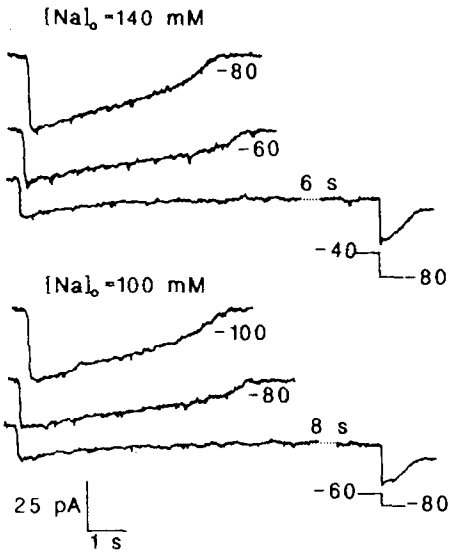

Fig. 3. Effects of a reduction in $[Na]_o$ and membrane depolarization on the transient inward current in an atrial myocyte from the guinea-pig. The upper three traces show inward current recorded with $[Na]_o$ = 140 mM at the membrane potentials indicated. Note that depolarization reduces the maximum amplitude of the inward current and slows its decline. Lower traces recorded with $[Na]_o$ = 100 mM (Na being substituted by Li). The solution in the patch pipette used to make the whole-cell recording was Cs rich. Reprinted by permission from *Nature* Vol. 319, pp. 597–599. Copyright © 1986, Macmillan Magazines Ltd.

Nunn, 1985; McNaughton, Cervetto & Nunn, 1986; Cervetto, Lagnado & McNaughton, 1987). Figure 2 shows the results of an experiment similar to that shown in Fig. 1, except that the outer segment membrane current was recorded whilst simultaneously measuring $[Ca]_i$ using the Ca-sensitive photoprotein aequorin. After the Ca influx was terminated by the closure of the light-sensitive channels $[Ca]_i$ remained at a stable high level whilst the rod remained in isotonic $CaCl_2$. On restoration of external Na the inward exchange current was activated and the free $[Ca]_i$ fell rapidly. The rate of decline of $[Ca]_i$ could be predicted from the integral of the exchange current (Fig. 2*B*). In fact, the exchange current in rods is activated by $[Ca]_i$ in a simple Michaelis fashion with a K_M of 1–2 μM (Cervetto et al., 1987; Lagnado & McNaughton, 1989), which is similar to the activation characteristics of the Na-dependent Ca efflux in squid axons dialyzed with solutions containing ATP (Blaustein, 1977).

The activity of the Na : Ca exchange in rods can be investigated under more physiological conditions by delivering a saturating flash in normal Ringer solution. After shutting the light-sensitive channels a small inward exchange current, decaying with a time constant of about 0.5 sec, is recorded on the plateau of the light response (Yau & Nakatani, 1985; Hodgkin et al., 1985; Cervetto & McNaughton, 1986). The decay of the exchange current is thought to reflect a fall in $[Ca]_i$, caused by the continued extrusion of Ca^{2+} by the exchange after the Ca^{2+} influx through the light-sensitive channels has been terminated (Yau & Nakatani, 1985; McNaughton et al., 1986). The role of the Na : Ca exchange in Ca homeostasis in rods has recently been reviewed by Lagnado and McNaughton (1989).

Heart Muscle

Currents attributable to the countertransport of Na and Ca have been recorded in cardiac muscle using three distinct approaches. Mechmann and Pott (1986), using the whole-cell voltage-clamp technique, have recorded a transient inward current caused by the spontaneous or caffeine-induced release of Ca from the sarcoplasmic reticulum of atrial myocytes. Figure 3 shows that this inward current is reduced in amplitude and has a prolonged time-course when $[Na]_o$ is reduced or when $[Ca]_o$ is increased, suggesting that it is generated predominantly by the extrusion of the released Ca load via the Na : Ca exchange. This conclusion is supported by the lack of a reversal potential for this inward current at membrane potentials up to +75 mV (Lipp & Pott, 1988*a*,*b*). In these studies K^+ channels were blocked using $CsCl_2$, but the inward current is certainly not a pure Na : Ca exchange current. Of the other conductances present in the plasma membrane of heart muscle, the most troublesome in terms of the isolation of the Na : Ca exchange current is a Ca-activated nonspecific cation channel. These channels produce an inward current at hyperpolarized membrane potentials under precisely the same conditions in which the Na : Ca exchange current is activated, that is, when $[Ca]_i$ is raised (Kass et al., 1978; Colquhoun et al., 1981; Ehara, Noma & Ono, 1988).

Kimura, Noma and Irisawa (1986) used a whole-cell pipette to both voltage clamp and internally perfuse single ventricular cells, in the presence of ouabain to block the Na : K pump, $BaCl_2$ and $CsCl_2$ to block K channels and D600 to bock Ca channels. Under these conditions, an outward current was recorded when Ca was applied to the outside of the cell, provided that Na was present internally but absent externally. This outward current is likely to reflect the reversed mode of the exchange, mediating Ca influx, since it is reduced by a decrease in $[Ca]_o$ or $[Na]_i$ and blocked by La. The activation of the outward current also requires a low concentration of internal Ca, a property of Na-dependent Ca influx observed in the squid axon (Dipolo & Beauge, 1983; Allen & Baker, 1986*b*). Since the exchange process is reversible, one might ex-

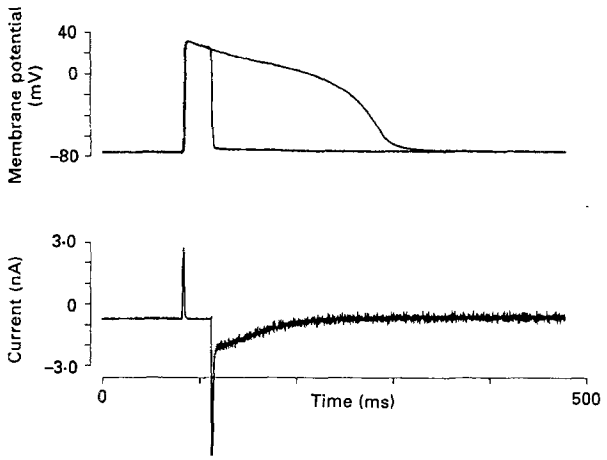

Fig. 4. Slow inward tail current attributable to the Na : Ca exchange recorded in a ventricular myocyte from the guinea-pig. The upper trace shows one normal action potential superimposed on one interrupted by repolarization to the resting potential 20 msec after the upstroke. The lower trace shows the current recorded in association with the interrupted action potential. The inward tail current decays relatively slowly. A single-electrode switch-clamp method was used. (Modified from Egan et al., 1989)

pect that the reversal of the ionic gradients across the membrane will generate an inward current. Using pipette solutions free of Na, an inward current was indeed recorded when superfusing Na with Ca present internally, and, as would be expected of a current generated by Na-dependent Ca efflux, it was reduced by a decrease in $[Na]_o$ (Kimura, Miyamae & Noma, 1987). Although these experiments provide strong evidence for the electrogenicity of the Na : Ca exchange in heart muscle, the conductance of the preparation depends on both $[Na]_i$ and $[Ca]_i$ even when the Na : Ca exchange is inactive, making it difficult to obtain quantitative information.

The Na : Ca exchange current in single ventricular cells has been studied under more physiological conditions by Egan et al. (1989), without the use of pharmacological agents or intracellular perfusion. In these experiments hyperpolarizing voltage-clamp pulses to the resting potential were imposed during the action potential, evoking a slowly declining inward current (Fig. 4). This tail current is believed to reflect Ca efflux through the Na : Ca exchange consequent on the release of Ca from intracellular stores, since it is abolished by buffering intracellular Ca or by the replacement of external Na with Li (*see also* Fedida et al., 1987). The current is also greatly reduced by replacing external Ca with Sr, or by the application of ryanodine, a drug which prevents Ca uptake into the sarcoplasmic reticulum. The inward current appears, therefore, to be activated by an increase in $[Ca]_i$, so its time course can be taken as a measure of the removal of Ca^{2+} from the cytoplasm. Thus, even though part of the current may be passing through Ca^{2+}-activated cation channels, it seems likely that its time course is determined principally by the activity of the Na : Ca exchange in the plasma membrane. The role of the Na : Ca exchange in regulating the contractility of heart muscle has been discussed by Chapman (1989).

Stoichiometry of the Na : Ca Exchange

An important attribute of the Na : Ca exchange is its stoichiometry, that is to say the identity, number and flux direction of the ions transported during the movement of a single Ca^{2+} ion. The stoichiometry of the exchange will to a large extent determine its role in the regulation of $[Ca]_i$ because the exchange is not directly dependent on energy-releasing reactions such as ATP hydrolysis: it is the coupled movement of ions down their electrochemical gradient that provides the energy required to move Ca^{2+} out of the cell against its electrochemical gradient. For instance, the larger the number of Na^+ ions that enter through the exchange, the greater will be the electrochemical gradient against which Ca^{2+} can be translocated.

Blaustein and Hodgkin (1969) noted that in the squid axon the electrochemical gradients for Na^+ and Ca^{2+} are such that a net Ca efflux through the exchange requires the energy equivalent of more than 2 Na^+ ions moving down their electrochemical gradient, and they suggested that 3 Na^+ ions are transported for each Ca^{2+} ion. Mullins (1979) has used similar thermodynamic arguments to suggest that in heart muscle 4 Na^+ ions are transported. The exact number, however, has been the subject of much debate, principally because of the problems involved in the isolation of Na^+ and Ca^{2+} fluxes through the exchange mechanism from those through other membrane pores and carriers. In the squid axon, for instance, flux measurements are consistent with 2–5 Na^+ ions exchanging with each Ca^{2+} ion (Baker et al., 1969; Allen & Baker, 1986*b*).

One Charge Enters the Cell Per Ca^{2+} Extruded

The number of charges translocated per Ca^{2+} extruded can be measured in experiments of the type shown in Figs. 1 and 2, in which a known amount of Ca^{2+} is loaded into a rod outer segment and the charge movement associated with its extrusion is observed. Experiments in intact rods gave values consistent with the net movement of between 0.94

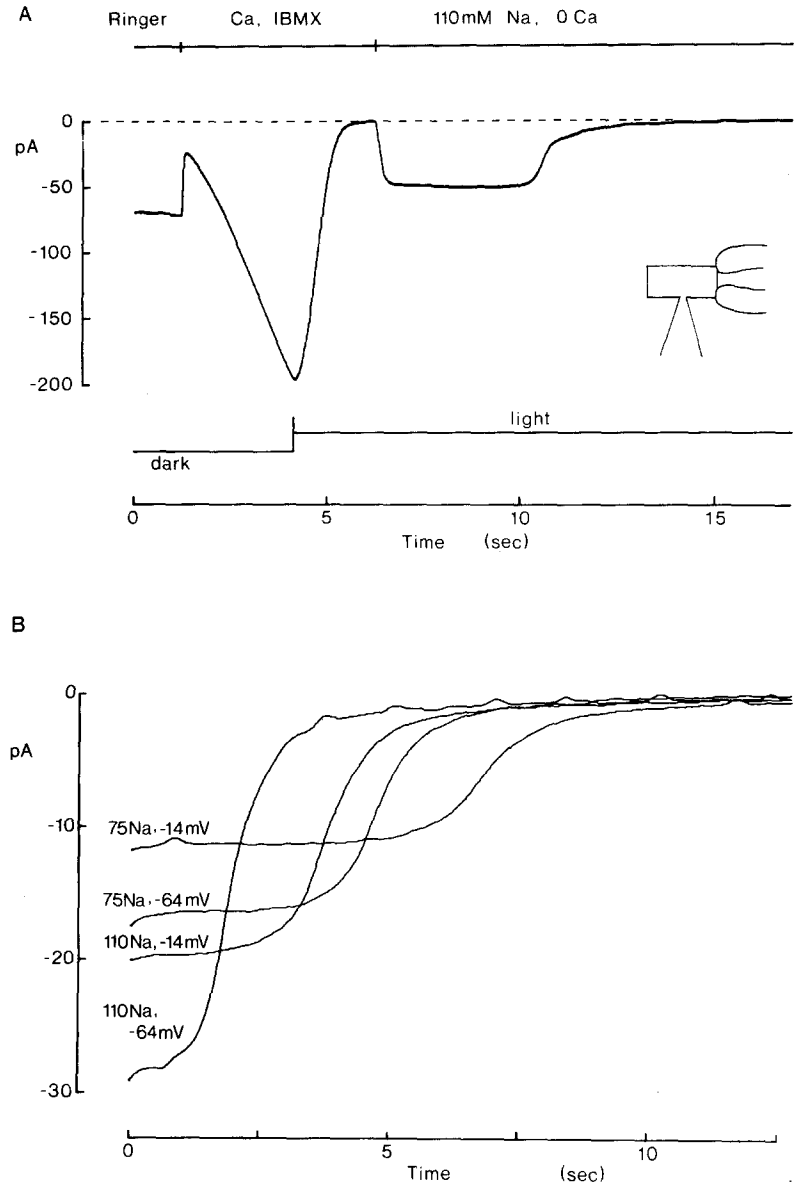

Fig. 5. Measurement of net charge movement by the Na:Ca exchange in isolated salamander rod outer segment under whole-cell voltage clamp. (*A*) The outer segment was loaded with Ca and the exchange current activated using a protocol similar to that shown in Fig. 1. The charge transferred during the loading period was 417 pC, and the charge transferred by the operation of the exchange was 220 pC, so that the ratio of the charge movement during Ca influx and efflux, *r*, was 1.90. Membrane potential clamped to −14 mV throughout. (*B*) The Na:Ca exchange current under various conditions, recorded as in *A* but in a different rod. Note that a reduction in $[Na]_o$ or membrane depolarization reduces the saturated exchange current and slows the removal of a Ca load. Measured values of *r* were (from bottom) 2.02, 2.00, 1.90 and 2.08. The solution in the whole-cell pipette was Na free to prevent Ca influx through the reversed exchange. (Modified from Lagnado et al., 1988)

and 0.74 charges into the cell for every Ca^{2+} ion extruded (Yau & Nakatani, 1984; Hodgkin et al., 1987).

When using intact rods it is not possible to be sure that all of the Ca load remains in the outer segment and that none leaks into the inner segment. A further drawback is the lack of control over the membrane potential. A simpler preparation which has recently been developed is the rod outer segment isolated from the inner segment and the rest of the cell (Lagnado & McNaughton, 1987*a*,*b*). The outer segment membrane current is recorded using a whole-cell pipette under conditions of voltage clamp, and the ionic environment on either side of the membrane is under the control of the experimenter (Lagnado et al., 1988; Cervetto et al., 1989; Lagnado & McNaughton, 1989). Figure 5 shows the use of this preparation to measure the charge movement per exchange cycle under several conditions. The experimental protocol is similar to that shown in Fig. 1, and gives a more accurate value of 1.005 ± 0.01 charges (mean ± SEM, 84 measurements; L. Lagnado & P.A. McNaughton, *in preparation*) entering the rod outer segment per Ca^{2+} ion extruded.

These results clearly demonstrate that the carrier transports one net positive charge into the cell in exchange for each Ca^{2+} ion. It has also been shown that the charge transferred is independent of $[Na]_o$ (220–35 mM), of membrane potential (−64 to +16 mV), and of the presence or absence of external K^+ (2.5 mM) or Mg^{2+} (1.6 mM) (Lagnado et al., 1988; Lagnado & McNaughton, 1988). It appears,

therefore, that the movement of ions through the exchange mechanism is tightly coupled according to a stoichiometry which is fixed over a wide range of conditions.

An investigation of the net charge movement per exchange cycle is obviously more difficult to carry out in other tissues. In heart muscle, for instance, the experiment is complicated by the difficulties in isolating the Ca^{2+} current and Na : Ca exchange current, and by the uncertainty as to whether all of the Ca^{2+} that enters through the plasma membrane is removed by the exchange. Bridge and Spitzer (1989), studying isolated ventricular myocytes, have used caffeine to prevent Ca^{2+} uptake and release from intracellular stores, and the Ca^{2+} channel blocker nifedipine to isolate the Ca^{2+} current. Preliminary experiments indicate that the charge entering the cell through the Ca^{2+} channel is about twice that moved by the exchange in expelling the Ca^{2+} load, as was found in photoreceptors.

K and Ca are Cotransported in Photoreceptors

The observation that one net positive charge enters the rod in exchange for a single Ca^{2+} ion was initially interpreted as showing that the exchange stoichiometry was $3Na^+ : 1Ca^{2+}$ (Yau & Nakatani, 1984; Hodgkin et al., 1987; Lagnado et al., 1988). The only other possibility consistent with experiments of this type would be a mechanism such as $4Na^+ : 1Ca^{2+},1K^+$, in which Ca^{2+} leaves the cell in association with K^+, since all ions other than Na^+ can be removed from the external solution without inhibiting the exchange, and since K^+ was the only small ion in the solution used for internal perfusion in the experiments of Lagnado et al. (1988). Recent evidence has, however, overturned the assumption that Na^+ is the only ion coupled to the flux of Ca^{2+}. The reversed mode of the exchange—in which Ca^{2+} enters the cell in exchange for internal Na^+—has been found to depend on the presence of external K^+, apparently because K^+ must be cotransported with Ca^{2+}.

This surprising observation was made when we attempted to characterize the reversed exchange using isolated outer segments internally perfused with patch solutions containing a high [Na] (Cervetto et al., 1989). Figure 6*A* shows that reducing $[Ca]_o$ to 10 μM activates a transient inward current similar to that observed during the extrusion of a Ca load (*see* Figs. 1, 2 and 5). Restoring $[Ca]_o$ to 1 mM activates a transient outward current due to the operation of reversed exchange in re-establishing the previous equilibrium internal [Ca]. The integrals of the inward and outward currents are equal, showing that the charge moved by the exchange per Ca^{2+} ion transported is equal in both the forward and reversed modes of operation. Figure 6*B* shows that the outward exchange current, reflecting Ca influx, can also be activated by a reduction in $[Na]_o$, but only if K^+ is present in the external solution along with Ca^{2+}. But does K^+ merely act as a catalyst, perhaps assisting Ca^{2+} to bind, or is it actually transported by the exchange? If K is co-transported with Ca then a change in the K gradient will perturb the equilibrium level of $[Ca^{2+}]_i$ reached by the exchange, while if the ion is simply acting as a catalyst a change in the K gradient will inhibit or promote the forward and reversed modes of the exchange equally, leaving the equilibrium unaffected. The crucial evidence against the latter idea is provided by the observation that changes in $[K]_o$ activate transient exchange currents, and therefore perturb the equilibrium value of $[Ca]_i$, showing that K must contribute energy to the exchange process by being transported across the membrane. The trace labeled *Na = 110* in Fig. 7*A* shows that a reduction in $[K]_o$, with no change in $[Ca]_o$ or $[Na]_o$, causes a charge influx, corresponding to a movement of Ca from the cell, followed by an efflux of charge as $[K]_o$ is increased again and the previous equilibrium level of $[Ca]_i$ is restored. The change in $[K^+]_o$ causes no change in membrane potential, of course, because the cell is voltage clamped.

The remaining traces in Fig. 7*A* show the effects of simultaneous reductions in $[Na]_o$ and $[K]_o$. If n Na^+ ions exchange for one K ion, then the exchange will remain at equilibrium if the ratio $[Na]^n/[K]$ is maintained constant. For a tenfold change in $[K^+]_o$ we therefore expect equilibrium to be maintained at $[Na^+]_o$ = 62 mM if n = 4, and at $[Na^+]_o$ = 51 mM if n = 5. Figure 7*A* shows that the equilibrium is maintained at $[Na]_o$ = 62 mM, while when $[Na]_o$ = 51 mM there is a net charge efflux, with a corresponding influx on return to $[Na]_o$ = 110 mM. The charge movements on return to $[Na]_o$ = 110 mM are plotted as a function of $[Na]_o$ in Fig. 7*B*. The intersection with the horizontal axis occurs near the value of 61.9 mM expected if 4 Na^+ ions exchange for one K^+ ion. Similar results were obtained in experiments where (i) a change in $[Ca^{2+}]_o$ was balanced by the change in $[Na^+]_o$ expected for the exchange of 4 Na^+ and 1 Ca^{2+}; (ii) a change in $[K^+]_o$ was balanced by the change in membrane potential expected for an exchange of 1 K^+ for one net positive change; and (iii) a change in $[Ca^{2+}]_o$ was balanced by the change in $[K^+]_o$ expected for a cotransport of one K^+ and one Ca^{2+} (Cervetto et al., 1989). The only exchange stoichiometry consistent with all these results, together with the known countertransport of one positive charge for every

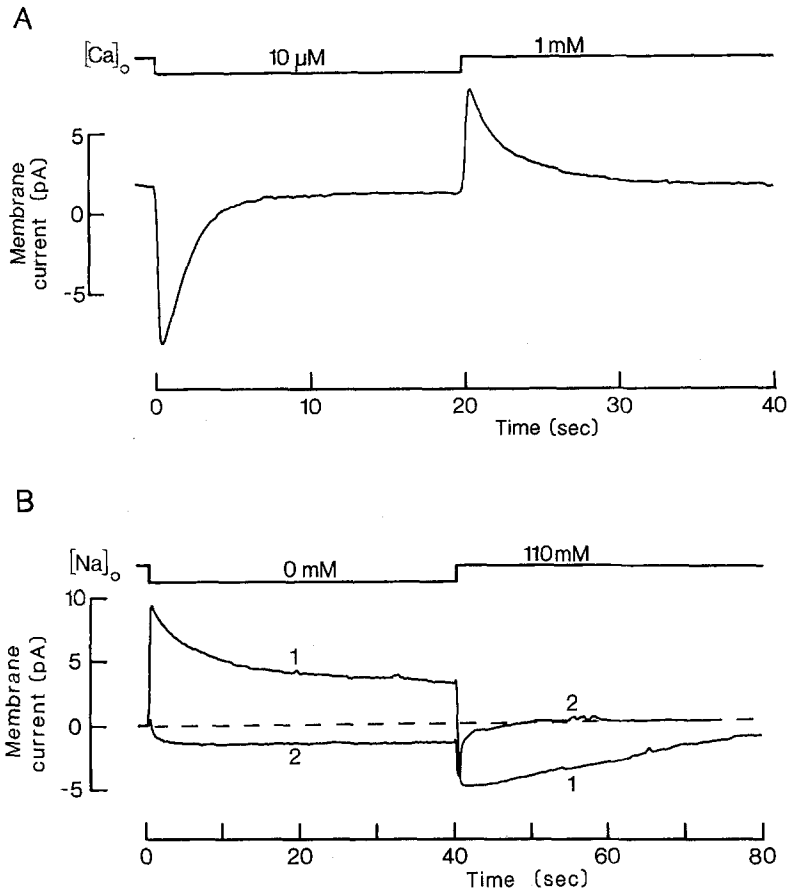

Fig. 6. Exchange currents associated with the forward and reversed modes of the Na : Ca exchange in an isolated rod outer segment. (*A*) The outer segment was transferred from Ringer containing 1 mM [Ca] to a solution containing 10 μM [Ca], activating an inward exchange current due to Ca efflux. An outward exchange current associated with Ca influx is recorded on returning to 1 mM $[Ca]_o$. Charge entering the outer segment during Ca efflux was 21.8 pC, and the charge leaving during Ca influx was 21 pC. (*B*) The dependence of the reversed Na : Ca exchange current on external K. Trace *1* shows the outward current activated by replacing external Na with Li in a solution containing 5 mM K and 1 mM Ca. The withdrawal of external Na causes a Ca influx through the exchange, so that on restoration of external Na an inward exchange current is recorded whilst the previous equilibrium level of $[Ca]_i$ is established. Trace *2* shows the absence of any exchange currents when Na is withdrawn in the absence of external K. The small inward current is believed to be generated by a shift in the junction potential between the patch electrode and the bath electrode. The difference between traces *1* and *2* gives the net exchange current. The net K-sensitive charge efflux was 215 pC, and the charge influx on restoration of external Na was 190 pC. The membrane potential was clamped to −14 mV throughout, and the solution in the whole-cell pipette included 100 mM Na and 28.5 mM K. (Modified from Cervetto et al., 1989, and reprinted by permission from *Nature* Vol. 337, pp. 740–743. Copyright © 1989, Macmillan Magazines Ltd.)

Ca^{2+} ion, is $4Na^+ : 1Ca^{2+},1K^+$, where the colon indicates countertransport and the comma cotransport (Cervetto et al., 1989). The idea of Ca,K cotransport in photoreceptors is also supported by the results of flux measurements in bovine rod outer segments, where it has been found that external K stimulates Ca uptake via the reversed exchange, and internal K stimulates the component of Ca efflux which is dependent on external Na (Schnetkamp, 1986; Schnetkamp, Szerescei & Basu, 1988).

Evidence for a Cotransport of Ca and K in Nerve and in Heart Muscle

In the squid axon, the forward mode of the exchange is inhibited by 70% on removal of internal K (Dipolo & Rojas, 1984), implying an interaction between K and the exchange, although the possibility remains open that K is not transported. The reversed mode of the exchange, mediating Ca influx, is activated by alkali metal cations in the external solution, with K being considerably more effective

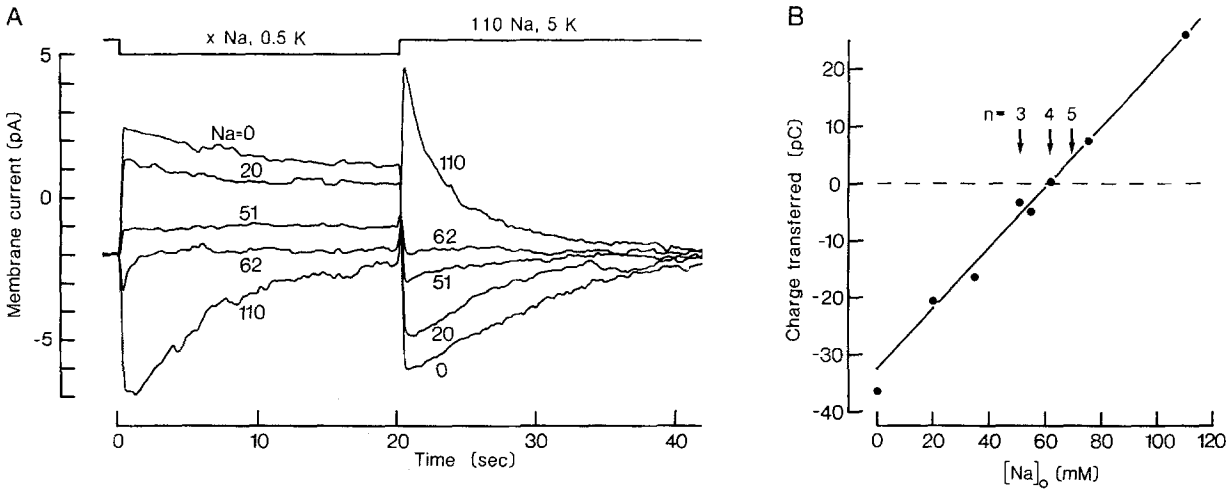

Fig. 7. Transient Na:Ca exchange currents caused by changes in $[K]_o$ and $[Na]_o$. (*A*) Currents observed in an isolated rod outer segment during a 20-sec exposure to a solution containing 0.5 mM K, 1 mM Ca and the Na concentration shown. The rod outer segment was otherwise maintained in 110 mM Na, 5 mM K and 1 mM Ca. Note that when $[K]_o$ is reduced and $[Na]_o$ held constant (the trace labeled 110 mM Na) an inward exchange current is recorded, reflecting Ca efflux through the Na:Ca exchange. (*B*) Relation between $[Na]_o$ during the exposures shown in *A* and the charge flow on return to 110 mM $[Na]_o$. The straight line, drawn by eye, crosses the axis at $[Na]_o$ = 62 mM. The arrows show the value of $[Na]_o$ which would balance the effect of the tenfold reduction in $[K]_o$ for exchange stoichiometries of nNa$^+$: 1K$^+$, where n = 3, 4 or 5. (Modified from Cervetto et al., 1989, with permission of *Nature* Vol. 337, pp. 740–743. Copyright © 1989, Macmillan Magazines Ltd.)

than Li, Na or Rb (Baker et al., 1969; Allen & Baker, 1986*a*). Allen and Baker (1986*a*) observed these effects under conditions of voltage clamp, showing that they are not due to an indirect effect of K on the membrane potential. An activation of the reversed exchange by external K has also been noted in brain synaptosomes (Coutinho, Carvalho & Carvalho, 1983). Barzilai and Rahamimoff (1987*a*) measured the bidirectional fluxes of Na^+ and Ca^{2+} in a similar preparation and found that between four and five Na^+ ions exchange for every Ca^+. These results suggest that the cotransport of K and Ca through the Na:Ca exchange may be a general phenomenon in nerve, although the observation that Li can substitute at least partially for K, while in the rod outer segment Li is inactive, implies that the K-binding site has a lower selectivity than the corresponding site in the rod outer segment exchange.

At present it is not clear whether a 4Na$^+$: 1Ca^{2+},1K$^+$ exchange is also operating in other cell types. In an elegant series of experiments using cardiac sarcolemmal vesicles, Reeves and Hale (1984) found a stoichiometry of 3 Na^+ ions cotransported with one positive charge, a result which is not consistent with the findings in the rod outer segment. The approach used in their experiments was to vary the Na^+ gradient and membrane potential across the vesicles until the equilibrium condition, with no net Ca^{2+} flux in either direction, was attained. Either the exchange in these cardiac sarcolemmal vesicles is different from that in rods, perhaps because the properties of the exchange mechanism were altered during the isolation procedure, or in their experiments the carrier was not truly at equilibrium, possibly because of Ca fluxes through other pathways.

Electrophysiological evidence for a 3Na$^+$: Ca^{2+} exchange in heart muscle has been obtained by Ehara, Matsuoka and Noma (1989). These workers used isolated ventricular myocytes to measure the reversal potential (V_{rev}) of the Na:Ca exchange current under fixed external and internal ionic conditions. Most other membrane conductances were blocked and the exchange current was identified by the subtraction of the *I-V* relations obtained in the presence and absence of 2 mM Ni^{2+}, which is known to inhibit the exchange. Figure 8*A* shows such *I-V* relations, obtained with $[Na]_o$ between 30 and 140 mM, and the arrows indicate the reversal potential of the Ni^{2+}-sensitive current. It can be seen that V_{rev} is shifted to more positive potentials as $[Na]_o$ is increased. It is difficult to assess the contribution of the nonspecific cation conductance to the estimate of V_{rev} since Ni^{2+} is certainly not a specific blocker for the Na:Ca exchange. Nonetheless, the dependence of V_{rev} on $[Na]_o$ can be very well accounted for by an exchange stoichiometry of 3Na$^+$: 1Ca^{2+} (Fig. 8*B*). Similar experiments in which V_{rev} was measured as a function of $[Ca]_o$ also agreed with this stoichiometry.

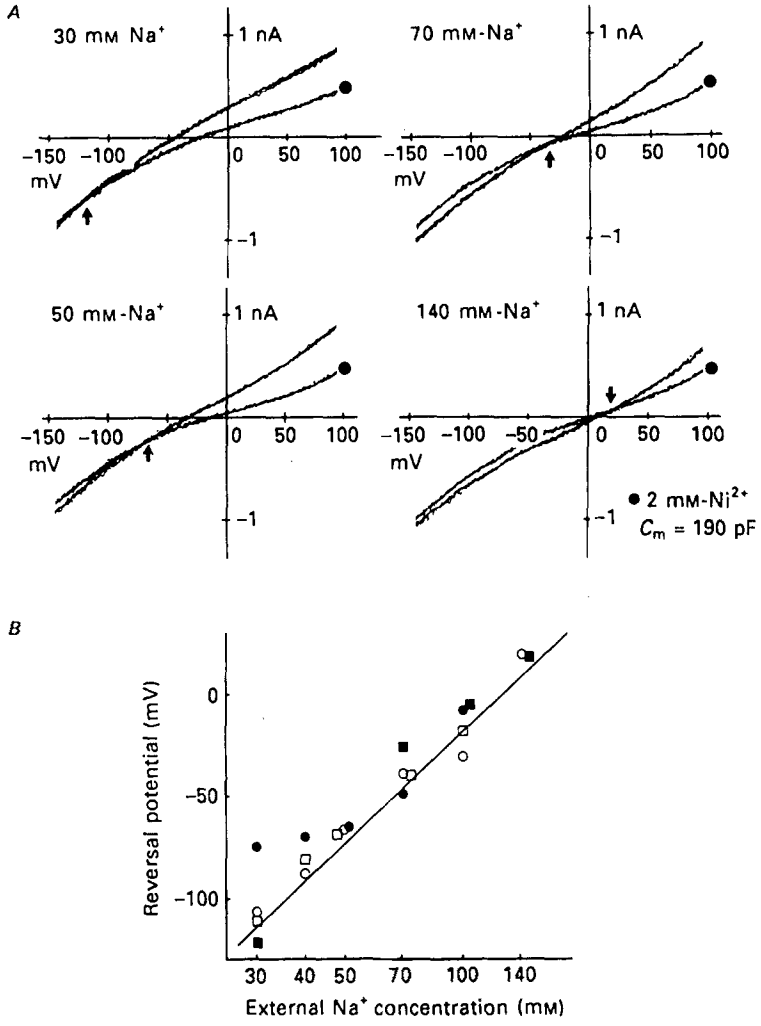

Fig. 8. (*A*) *I-V* relations of the current in isolated cardiac myocytes induced by various $[Na]_o$ before and during (marked by the circle) application of 2 mM Ni^{2+}. $[Na]_i$ = 10 mM, $[Ca]_i$ = 253 nM and $[Ca]_o$ = 0.5 mM. Intersection of the two *I-V* relations is shown by the arrows. (*B*) Reversal potential of the Ni^{2+}-sensitive current plotted against $[Na]_o$ on a semilogarithmic scale. Straight line shows the expected reversal potential for a $3Na^+ : 1Ca^{2+}$ exchange. (Reproduced from Ehara et al. (1989) with permission of the publisher)

It should also be pointed out that Ehara et al. (1989) were able to record outward exchange currents in the absence of external K^+, showing that the reversed exchange in heart muscle is not absolutely dependent on this ion. The outward exchange current may have been activated by Cs^+, which was present externally, and it will be interesting to see if Cs^+ can replace K^+ in the photoreceptor exchange (preliminary experiments by R.J. Perry and P.A. McNaughton indicate that Cs is indeed a weak activator of the K-binding site in photoreceptors). Measurements of the stoichiometry of the exchange in photoreceptors and heart muscle are, however, clearly at variance and, at least at the moment, there seems to be no obvious way to reconcile the two sets of results. This may be simply because the carriers in the two tissues are different. Before accepting such a conclusion, however, further investigation of the stoichiometry of the exchange, with particular reference to the possible involvement of K^+, are clearly desirable.

Cotransport of Ca and K will Increase the Power of the Exchange

A stoichiometry of $4Na^+ : 1Ca^{2+},1K^+$ would allow the exchange to maintain a Ca^{2+} efflux against a much steeper Ca gradient than a $3Na^+ : 1Ca^{2+}$ exchange. Increased energy for Ca^{2+} efflux will be provided both by the influx of an extra Na^+ ion and by the efflux of a K^+ ion down its electrochemical gradient. The equilibrium level of $[Ca]_i$ for a $4Na^+ : 1Ca^{2+},1K^+$ exchange is given by (Hodgkin et al., 1987; Cervetto et al., 1989)

$$[Ca^{2+}]_i = [Ca^{2+}]_o \frac{[Na^+]_i^4}{[Na^+]_o^4} \frac{[K^+]_o}{[K^+]_i} \exp(V_m F/RT). \quad (1)$$

Taking as an example the rod outer segment in bright light, V_m is about −55 mV (Baylor et al., 1984), and taking $[Na^+]_i$ = 10 mM (Torre, 1982) and $[K^+]_i$ = 110 mM, we obtain an equilibrium level of

$[Ca^{2+}]_i = 1.8 \times 10^{-10}$ M in Ringer containing 110 mM $[Na]_o$, 2.5 mM $[K]_o$ and 1 mM $[Ca]_o$. This level of $[Ca]_i$ is nearly 500 times lower than the value of 8.5×10^{-8} M which would be attained at equilibrium by a $3Na^+ : 1Ca^{2+}$ exchange. While it seems unlikely that this level will be reached in practice, the cotransport of Ca^{2+} and K^+ should enable the exchange to maintain a Ca efflux down to much lower levels of $[Ca]_i$ than was previously supposed. In photoreceptors this may be of particular importance because the fall in $[Ca]_i$ in response to light is thought to mediate light adaptation by relieving the inhibition of a guanylate cyclase which is not fully active until $[Ca]_i$ has fallen to about 2×10^{-8} M (Koch & Stryer, 1988).

The stoichiometry of the exchange also has important implications for its role in the regulation of $[Ca]_i$ during electrical activity. It has been suggested, for instance, that depolarization during the cardiac action potential may cause Ca influx through the reversed exchange (Mullins, 1979; Campbell et al., 1988). The reversal potential of a $4Na^+ : 1Ca^{2+},1K^+$ exchange is given by

$$E_{Na:Ca} = \frac{RT}{F} \ln \frac{[Ca]_i[Na]_o^4[K]_i}{[Ca]_o[Na]_i^4[K]_o} \qquad (2)$$
$$= 4E_{Na} - E_K - 2E_{Ca}.$$

Taking the ionic concentrations listed above, and assuming that $[Ca]_i$ reaches 1 μM (Cannell et al., 1987), the calculated value of $E_{Na:Ca}$ is +104 mV, which is far above the peak depolarization attained during an action potential. Such an exchange will therefore produce a net Ca^{2+} efflux under all physiologically relevant situations. In contrast, $E_{Na:Ca}$ for a $3Na^+ : 1Ca^{2+}$ exchange would be about +7 mV. The plateau of the ventricular action potential is positive to this value, implying that an exchange with this stoichiometry could carry Ca^{2+} into the cell during excitation.

The involvement of K^+ in the regulation of $[Ca]_i$ may be important in several situations. One example is heart muscle, where the positive ionotropic effects of cardiac glycosides have been attributed to the rise in $[Na]_i$ caused by the inhibition of the Na : K pump. This rise in $[Na]_i$ is believed to cause a rise in $[Ca]_i$ by reducing the driving force for Ca efflux through the Na : Ca exchange (Glynn, 1969; Baker et al., 1969). If a $4Na^+ : 1Ca^{2+},1K^+$ exchange is also operating in the heart then not only will the effects of the reduction in the Na gradient be more powerful than previously supposed, but the increase in $[K]_o$ adjacent to the membrane, also caused by the inhibition of the Na : K pump, will in addition reduce the K gradient and cause a further rise in $[Ca]_i$, an effect that could be as significant as that caused by the increase in $[Na]_i$. A rise in $[K]_o$ is also known to be involved in mediating the Ca-dependent death of neurones in cerebral ischemia, hypoglycemia and epilepsy (Walz & Herz, 1983). Choi (1988) has noted that the reversed Na : Ca exchange may be an important route of Ca^{2+} influx under such conditions, due to the rise in $[Na]_i$ and membrane depolarization that is the caused by the release of excitatory amino acids. If K^+ is cotransported with Ca^{2+}, the rise in $[K]_o$ will further contribute to the rise in $[Ca]_i$ that leads to autolysis.

The Na : Ca Exchange Rate is Sensitive to Membrane Potential

The electrical activity of nerve and muscle cells controls such Ca-dependent processes as secretion and contraction, partly by regulating Ca influx through ion channels, but also by altering Ca efflux through the Na : Ca exchange (Allen & Baker, 1986*a*,*b*; Pott, 1986; Egan et al., 1989). In Fig. 5, for instance, it can be seen that depolarization reduces the inward exchange current generated during Ca efflux and so slows the removal of a Ca load. Quantitative information on the interaction between membrane potential and the exchange is therefore essential to an understanding of the control of $[Ca]_i$ in excitable cells.

In squid axons, the component of ^{45}Ca efflux that is dependent on external Na^+ has been found to have a roughly exponential dependence on membrane potential, usually with an *e*-fold change in about 50 mV (Mullins & Brinley, 1975; Baker & McNaughton, 1976*b*; Allen & Baker, 1986*b*). Kimura et al. (1987) have used isolated heart muscle cells in which most membrane conductances have been blocked, and have measured the *I-V* relation of the preparation in the presence of internal Ca^{2+} before and after the replacement of external Li^+ by Na^+. The *I-V* relation of the exchange current activated by 140 mM Na^+ was then obtained by subtraction, and usually showed an *e*-fold reduction for a depolarization of about 35 mV, confirming that Ca^{2+} efflux through the Na : Ca exchange is inhibited by depolarization. In this study, more complete data was obtained for the outward exchange current, which was increased *e*-fold by a 71 mV depolarization (Kimura et al., 1987).

The voltage sensitivity of Ca efflux through the Na : Ca exchange in isolated rod outer segments has been measured by recording the inward exchange current (Lagnado & McNaughton, 1987*b*; Lagnado et al., 1988). The validity of this approach depends on the demonstration that there is an invariable exchange of a single positive charge for a single Ca^{2+}

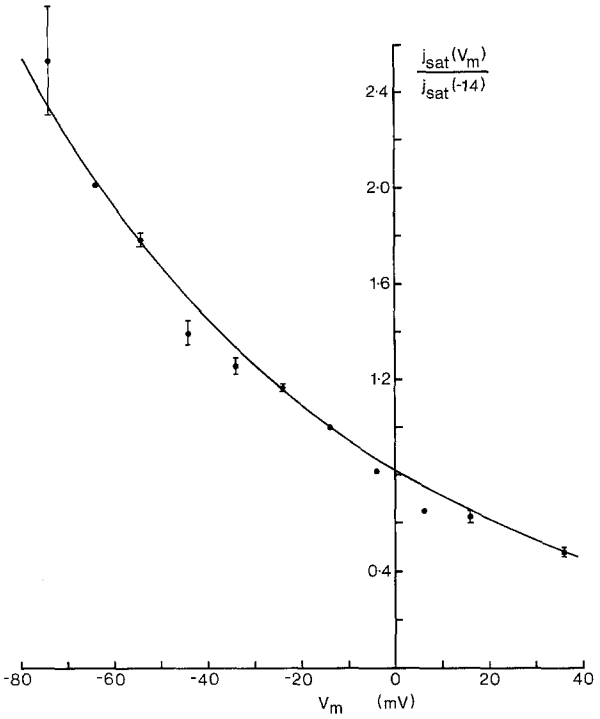

Fig. 9. Voltage dependence of the inward Na : Ca exchange current in isolated rod outer segments. The maximum exchange current, j_{sat}, recorded after the introduction of a Ca load sufficient to saturate the internal Ca^{2+}-binding site, is expressed relative to the value observed at $V_m = -14$ mV. All measurements were made in normal Ringer solution. The continuous line is $j_{sat}(V_m)/j_{sat}(-14 \text{ mV}) = \exp[(-V_m + 14)/70]$. (Reproduced from Lagnado et al. (1988) with permission of the publisher)

ion (Lagnado & McNaughton, 1988; Lagnado et al., 1988; *see above*), implying that the effects of changes in membrane potential on the exchange current are not due to changes in the stoichiometry of the exchange, but to a direct effect on one or more partial reactions of the exchanger. The magnitude of the inward exchange current is therefore a direct index of the rate of Ca^{2+} extrusion by the exchange.

Collected results from a large number of experiments are shown in Fig. 9. The voltage-dependence of the exchange current when the exchange is extruding Ca^{2+} in normal amphibian Ringer solution is well fitted by the relation

$$j(V_2) = j(V_1) \exp\{(V_1 - V_2)/70\}. \quad (3)$$

This relation is valid for all $[Ca]_i$, since the binding of internal Ca^{2+} has been found to be independent of membrane potential (Lagnado et al., 1988; Lagnado & McNaughton, 1989). In photoreceptors, therefore, this relation provides a useful definition of the voltage sensitivity of the exchange over the normal physiological range of membrane potentials and ionic conditions.

It is worth noting that the voltage dependence of the inward exchange current (i.e., when the exchange is extruding Ca^{2+}) measured in rods is less steep than that measured in heart muscle cells by Kimura et al. (1987). The cause of the difference is not clear at present, although it may be related to the observation, described below, that the voltage dependence of the exchange depends on the ionic conditions in which it is measured.

Why is the Na : Ca Exchange Voltage Dependent?

It may seem intuitively obvious that Ca efflux through the Na : Ca exchange should be inhibited by depolarization, since this mode of the exchange transports charge into the cell. The exponential relation between the exchange current and membrane potential suggests a simple model in which the voltage sensitivity of the exchange results from the movement of a charged species, perhaps the exchange molecule loaded with ions, across a rate-limiting energy barrier located part way across the membrane electric field (Baker & McNaughton, 1976*b*; DiFrancesco & Noble, 1985; Kimura et al., 1987). On this basis, the voltage sensitivity observed in photoreceptors, an *e*-fold change per 70 mV, could be explained by a single charge crossing a fraction $\gamma = 0.37$ of the membrane field to reach the top of the energy barrier (Lagnado & McNaughton, 1987*b*). This model, although attractive, has been tested by Lagnado et al. (1988) and found to fail in several important respects.

The model accounts for the voltage-dependence of transport simply through an effect of the membrane field on the rate of ion translocation, whilst the binding of ions at the external and internal membrane surfaces is assumed to be unaffected by membrane potential. However, although the binding of Ca^{2+} ions at the internal membrane surface was found to be independent of membrane potential, the affinity of the exchange for external Na^+ was found to be increased when the rod was hyperpolarized. The activation of the inward exchange current by external Na^+ was first characterized in the absence of all other external cations (curve 1 in Fig. 10) and found to be well fitted by a Hill equation of form

$$\frac{j}{j_{max}} = \frac{[Na]_o^h}{\{[Na]_o + K_{Na}\}^h} \quad (3)$$

with a Hill coefficient, h, of 2.26. At the same membrane potential of -14 mV, the addition of Mg^{2+} and K^+ externally was found to cause a shift in the

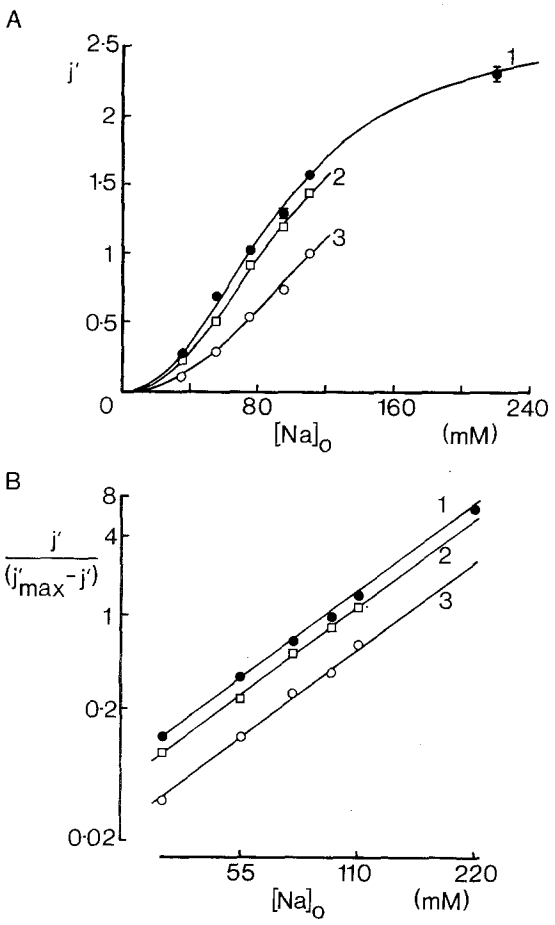

Fig. 10. Activation of the inward Na : Ca exchange current by $[Na]_o$ and the effects of hyperpolarization. (*A*) The parameter j' is the saturated exchange current expressed relative to its value in 110 mM Na, 1.6 mM Mg, 2.5 mM K, 0 Ca at −14 mV. Curve *1* was obtained with V_m = −14 mV in the absence of all external cations except Na and Li (which substituted for Na). Curves *2* and *3* were obtained at V_m = −64 and −14 mV, respectively, in the presence of 1.6 mM Mg and 2.5 mM K. All data could be fitted by a Hill equation (*see* Eq. (7)) with h = 2.26 and j'_{max} = 2.66. Values of $K_{1/2}$ were 93 mM (curve *1*), 103 mM (curve *2*) and 139 mM (curve *3*). (*B*) Hill plot of the data shown in *A*. Straight line fits all drawn using j'_{max} = 2.66. (Reproduced from Lagnado et al. (1988) with permission of the publisher)

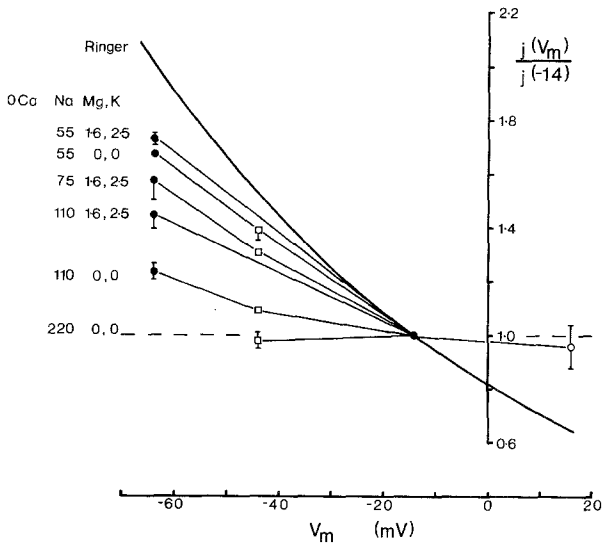

Fig. 11. Voltage dependence of the inward Na : Ca exchange current under various external ionic conditions. The exchange current at a membrane potential V_m is expressed relative to its magnitude at V_m = −14 mV. Note that the steepness of the curve depends on the degree of saturation of the external Na^+-binding site. (Reproduced from Lagnado et al. (1988) with permission of the publisher)

activation curve to the right (curve 3). A good straight-line fit to a Hill plot was obtained assuming that j_{max} was unchanged, which is consistent with simple competitive inhibition of Na^+ binding by Mg^{2+} and K^+. A 50-mV hyperpolarization caused a shift in the activation curve to the left (curve 2), and this effect could be accounted for by an increase in the affinity for external Na^+ without any effect on the maximum rate of exchange or on the Hill coefficient of activation.

Two surprising predictions can be made from these results, both of which run counter to the single energy barrier model. The first is that the voltage sensitivity of the exchange should depend on the degree to which the external Na^+-binding site is saturated. The second prediction is that the rate of Ca^{2+} efflux through the exchange will become voltage independent when the external Na^+-binding site is fully saturated. Both predictions are borne out by the results shown in Fig. 11, where it can be seen that the voltage sensitivity of the exchange is not a constant property, being reduced by an increase in $[Na]_o$ or by the removal of competing cations (Ca^{2+}, Mg^{2+} or K^+). When the Na^+-binding site is close to saturation, no voltage sensitivity is observed, showing that the rate of ion translocation becomes independent of the membrane electric field. The simplest interpretation of these results is that the voltage sensitivity of Ca^{2+} efflux through the Na : Ca exchange is due to the voltage-dependent binding of external Na^+ ions. Hyperpolarization therefore increases the apparent affinity of the Na^+-binding site by increasing the effective concentration of Na^+ ions at the site, and so, at external Na^+ concentrations below saturation, increases the degree to which the exchange is activated (Läuger, 1987). Such a voltage-dependent binding of Na^+ ions would be expected if the external Na^+-binding site were located within the membrane sensing a fraction of the transmembrane potential (Lagnado et al., 1988). The demonstration that the rate of ion translocation by the Na : Ca exchange is independent of voltage when the Na^+ binding site is fully

occupied may seem counterintuitive, but it simply indicates that the rate-limiting step does not involve the movement of charge across the membrane electric field. It seems likely that the exchange cycle involves a complex sequence of events and there is no reason to assume that a step involving ion translocation is rate limiting.

The Na:Ca Exchange Molecule

This review has focused mainly on the way in which changes in ion gradients and in the membrane potential determine the role of the Na:Ca exchange in the regulation of intracellular calcium. Although flux studies and electrophysiological techniques have contributed much to our understanding of these properties of the exchange, the process is still treated very much as a 'black box' since little is known about its molecular mechanism. For instance, does the transport of ions occur simultaneously or are different species transported in a particular sequence? How do the ions cross the membrane? Do they pass through an aqueous channel formed by the protein or are they attached to a mobile part of the molecule? What are the mechanisms that make the exchange selective for certain ions? How is the activity of the exchange modulated biochemically? Of particular interest is recent evidence to suggest that the affinity of the Na:Ca exchange for internal Ca^{2+} is increased by an ATP-dependent phosphorylation requiring Ca^{2+} ions (DiPolo & Beaugé, 1987; Caroni & Carafoli, 1983).

The investigation of these questions would be aided greatly if the exchange molecule could be isolated, a goal towards which several groups are working, even though the task is made difficult by the lack of any specific high-affinity ligands. Early estimates of the molecular weight of the exchange molecule varied widely (de la Pena, Hale & Reeves, 1985; Longoni & Carafoli, 1987; Barzilai & Rahamimoff, 1987*b*), although a degree of consensus has been achieved recently with reports that the Na:Ca exchange is a single protein of about 220 kDa in both cardiac sarcolemmal vesicles (Hale et al., 1988) and bovine rod outer segments (Cook & Kaupp, 1988). The Ca^{2+} fluxes mediated by the molecule isolated from rods have properties similar to those expected from electrophysiological studies, being activated by external Na^+ in a cooperative manner with a Hill coefficient of between 2 and 3. Attempts to clone the gene coding for the cardiac Na:Ca exchange are also under way (Sigel et al., 1988), and so we may not be far from obtaining the amino acid sequence of the carrier.

It would be particularly interesting to compare the structure of the Na:Ca exchange with other transport systems energized by both Na and K gradients. For instance, glutamate is transported into the basolateral membrane of the kidney proximal tubule (Burckhardt et al., 1980; Sacktor et al., 1981) and into retinal glial cells (Barbour, Brew & Attwell, 1988) by a process so similar to the Na:Ca exchange that it seems probable that both exchange systems are descended from a common progenitor. In both carriers the normal forward mode (uptake of glutamate or efflux of calcium) is activated by external sodium and by internal potassium. In both the exchange is electrogenic, with charge being transported into the cell during forward operation. The exchange stoichiometry of the glutamate uptake must therefore be at least $3Na^+$,$1glut^-$: $1K^+$ (Murer et al., 1980; Barbour et al., 1988), although the possibility that 4 Na^+ ions may be involved has not been ruled out. The glutamate uptake carrier seems to be able to operate in a K-independent mode at a lower rate (Burckhardt et al., 1980), resembling in this respect the Na:Ca exchange in squid axon (Blaustein, 1977; Dipolo & Beauge, 1983). Not all Na-linked exchange systems depend on K, though; the Na-dependent glucose uptake mechanism, for instance, seems to be K independent (Burckhardt et al., 1980). K dependence may offer a "power boost" when a substance has to be transported against a particularly unfavorable concentration gradient, and may not be needed in all circumstances.

After the discovery of the Na:Ca exchange in the late 1960's a series of papers, mainly using tracer flux methods, characterized many of the properties of this important exchange mechanism. Recently there has been a resurgence of interest in the Na:Ca exchange with the demonstration that its activity can be recorded directly from the membrane current produced by its operation. The whole-cell clamp has proved invaluable for studying ionic channels, and is now in turn being used to investigate the transport mechanisms that maintain ion gradients.

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