J. Physiol. (1971), **218**, *pp.* 599–608
With 5 text-figures
Printed in Great Britain

THE INDEPENDENCE OF ELECTROGENIC SODIUM TRANSPORT AND MEMBRANE POTENTIAL IN A MOLLUSCAN NEURONE

By MICHAEL F. MARMOR*

From the Laboratory of Neuropharmacology, Division of Special Mental Health Research, National Institute of Mental Health at Saint Elizabeths Hospital, Washington, D.C. 20032, U.S.A.

(*Received 22 March 1971*)

SUMMARY

1. The current–voltage relations of the *Anisodoris* giant neurone (G cell) were studied in the presence and absence of Na pump activity.

2. Inhibition of the electrogenic Na pump with ouabain had no effect on either the presence at warm temperatures (10–15° C), or absence at cold temperatures (0–5° C), of inward-going rectification.

3. Abolition of inward-going rectification in the warm, by replacement of external K with Rb, did not affect the electrogenic Na pump.

4. The current generated by the electrogenic pump was essentially constant between the membrane potentials of -30 and -100 mV.

5. The potential produced by the electrogenic pump can be predicted by a modification of the constant field equation.

6. It is estimated that the energy required to extrude Na was between 3160 and 3700 cal/g-atom, and that uncoupled Na efflux during pump activity was typically between 0·2 and 4·0 p-mole/cm^2.sec.

INTRODUCTION

Several authors have proposed that Na pump activity in muscle fibres varies with electrochemical gradient for Na (Horowicz & Gerber, 1965; Mullins & Awad, 1965; Fozzard & Kipnis, 1967; Rapaport, 1970). However, studies on the squid axon (Hodgkin & Keynes, 1955; Brinley & Mullins, 1970) indicate that Na transport is largely independent of membrane potential. When Na-K exchange is not one for one, the pump is electrogenic and changes in pump activity can affect the membrane potential. If electrogenic Na transport diminished progressively during

* Present address: Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, Mass. 02114.

hyperpolarization, the current–voltage (I–V) relation would be non-linear and would appear to show 'inward-going' rectification (see Noble, 1965).

The I–V relation of the *Anisodoris* giant neurone (G cell) shows inward-going rectification only at temperatures greater than 5° C in the presence of external potassium (Marmor, 1971). These two conditions are also necessary for electrogenic sodium transport in the G cell (Gorman & Marmor, 1970*a*). However, the present experiments indicate that inward-going rectification is independent of electrogenic sodium transport, and that the sodium pump in the G cell functions essentially as a constant current source over a broad range of physiologic membrane potentials. Preliminary reports of this material have been presented elsewhere (Marmor, 1970; Marmor & Salmoiraghi, 1970).

METHODS

The techniques used, and the notation for ions, were identical to those described elsewhere (Marmor, 1971). Ouabain (Mann Research Labs., N.Y.) and acetylcholine (Calbiochem, Los Angeles) were dissolved directly in the artificial sea water (ASW). K-free sea waters, 0 K ASW and 10 Rb (0 K) ASW, were prepared by replacing all K with 10 mM of Na or Rb respectively. All I–V relations illustrated were generated by biphasic current ramps that were initially depolarizing, and lasted 100–350 sec. In the records shown, no attempt was made to fully reproduce action potentials.

RESULTS

The effects of ouabain

The Na pump in the G cell is inhibited by cold temperatures, the removal of external K, or the addition of ouabain to the bathing medium (Gorman & Marmor, 1970*a*). The first two conditions eliminate inward-going rectification (Marmor, 1971), but are not specific for the Na pump since many aspects of membrane behaviour may be affected by temperature and changes in the ionic medium. In contrast, ouabain is quite specific as an inhibitor of the Na-K transport mechanism (Glynn, 1964).

Fig. 1 demonstrates that inhibition of the Na pump with 5×10^{-4} M ouabain did not affect inward-going rectification. At 15° C the addition of ouabain to the bath caused a prompt depolarization, consistent with inhibition of an electrogenic process, but did not alter the inward-going rectification which was present during hyperpolarization (Fig. 1*A*). The oscilloscope records in Fig. 1*B* confirm these conclusions, and show for comparison the elimination of inward-going rectification by cooling. It should be emphasized that rectification in the G cell is related to the *absolute* membrane potential, rather than the resting potential (see Marmor, 1971). Ouabain does not alter this relationship, but may change the pattern of

rectification near *resting* potential by depolarizing the membrane from a region where inward-going rectification is present to one where it is absent (this is evident in Fig. 1 and shown graphically in Fig. 5).

Previous experiments (Gorman & Marmor, 1970*b*) have suggested that ouabain does not affect membrane potential or permeability, except through inhibition of the Na pump. Similarly, the persistence of inward-going rectification at warm temperatures in the presence of ouabain cannot be

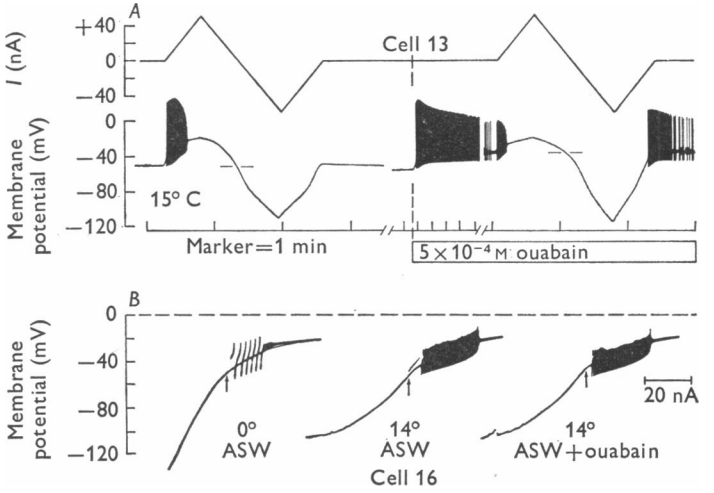

Fig. 1. Effects of Na pump inhibition on inward-going rectification. The electrogenic pump was blocked with 5×10^{-4} M ouabain where indicated. *A*, penwriter record with current (*I*) indicated above membrane potential. Note the changes in paper speed. *B*, oscilloscope records of continuous *I–V* relations at two temperatures, with and without ouabain. Membrane potential is on the vertical axis; applied current on the horizontal axis; resting potential is indicated by an arrow.

attributed to any direct effect of ouabain itself: At 0° C, or at warmer temperatures in the absence of external K, the *I–V* relation did not show inward-going rectification and was not significantly altered by ouabain (Fig. 2).

The effects of Rb

The preceding section demonstrated that inhibition of the Na pump did not block inward-going rectification. Conversely, Fig. 3 shows that inward-going rectification may be blocked (by replacement of external K with Rb) without inhibition of the Na pump. First, 10 Rb (0 K) ASW did not block the normal hyperpolarization in response to warming the cell, as did 0 K ASW (Fig. 3*A*). And in contrast to the prompt depolarizations produced by 0 K ASW or ouabain at 15° C, 10 Rb (0 K) ASW had only minor

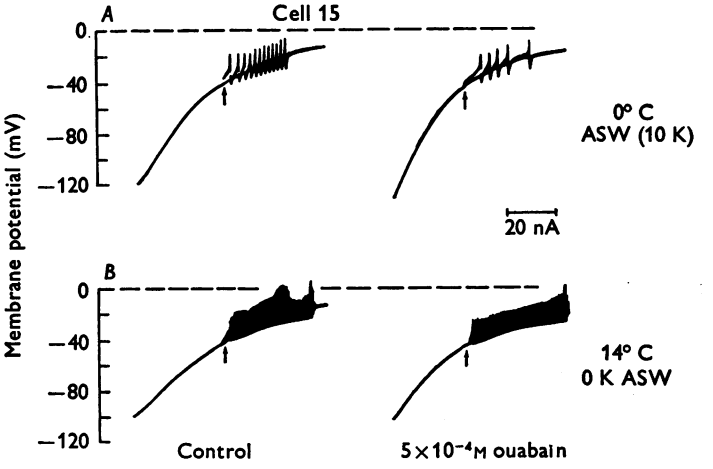

Fig. 2. Effects of 5×10^{-4} M ouabain on the $I-V$ relation in the cold, and in the absence of external K. Oscilloscope records as in Fig. 1*B*. *A*, 0° C in normal artificial sea water (10 mM-K). *B*, 14° C in K-free sea water.

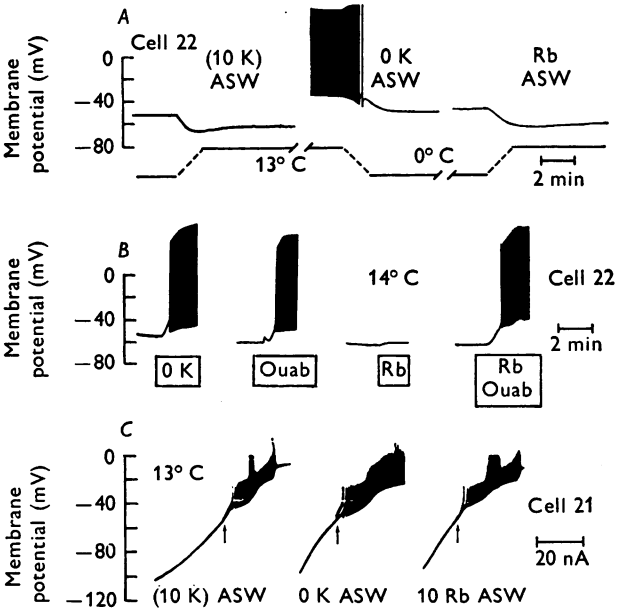

Fig. 3. Effects of Rb on electrogenic Na transport and inward-going rectification. *A*, penwriter record showing the temperature sensitivity of the membrane potential in ASW, 0 K ASW, and 10 Rb (0 K) ASW. *B*, penwriter record at 14° C showing the relative depolarization (i.e. pump inhibition) produced by 0 K ASW, ASW with 5×10^{-4} M ouabain (ouab), 10 Rb (0 K) ASW, and 10 Rb (0 K) ASW with 5×10^{-4} M ouabain. *C*, oscilloscope records (as in Fig. 1*B*) showing that inward-going rectification at 13° C in ASW is eliminated by both 0 K ASW and 10 Rb (0 K) ASW.

effects upon the membrane potential (Fig. 3*B*). Secondly, 10 Rb (0 K) ASW eliminated inward-going rectification in the warm as effectively as 0 K ASW (Fig. 3*C* and Marmor, 1971). These findings are in agreement with data from muscle fibres (Adrian, 1964; Adrian & Slayman, 1966), and suggest that the mechanism by which the removal of external K blocks inward-going rectification is not related to inhibition of the electrogenic Na pump.

The Na pump as a constant current generator

To determine whether the current generated by the Na pump was truly independent of membrane potential, the effects of ouabain were compared with those of acetylcholine (ACh) which does not inhibit the Na pump (Gorman & Marmor, 1970*b*). ACh depolarizes the G cell by increasing the relative Na permeability of the membrane (A. L. F. Gorman and M. F. Marmor, unpublished observations). Since ionic currents are voltage dependent, according to the constant field hypothesis (Hodgkin & Katz, 1949), the effects of ACh should not be identical to a constant current source. In fact, if the only effect of ACh is to change the Na/K permeability ratio, the following proportionality can be derived from the constant field equations:

$$(I_{\text{control}} - I_{\text{ACh}}) \propto V/(1 - e^{VF/RT}) \qquad (1)$$

where I is applied current, V is membrane potential, and R, T and F have their usual meanings.

I–V relations were determined at 15° C in the presence and absence of ouabain (Fig. 4*A*) and in the presence and absence of sufficient ACh to produce the same depolarization (Fig. 4*B*). The distance between the curves with and without drugs represents the current needed (ΔI), at any given potential, to balance the effect of the drug. In the case of ouabain, ΔI is equivalent to the electrogenic pump current and was essentially independent of potential (Fig. 4*C*). In the case of ACh, ΔI represents the additional Na leakage allowed by the drug, and was observed to vary continuously with the membrane potential in accordance with proportionality 1 (Fig. 4*D*).

Data from three other cells are shown in Fig. 5. The plots on the right-hand side show that the magnitude of the electrogenic pump current (ΔI) differed from cell to cell, but was quite constant within each cell between the membrane potentials of -30 and -100 mV. Beyond this range there was considerably variability, and no conclusions can be drawn because the measurement of electrogenic activity was inaccurate when the membrane conductance was large (during delayed or inward-going rectification). The plots on the left-hand side of Fig. 5 show the slope conductance ($\Delta I/\Delta V$) in the presence and absence of ouabain, with the resting potential indi-

cated by an arrow. Despite the large depolarization caused by ouabain in all of these cells, the slope conductance and the pattern of rectification remained essentially unchanged at any given absolute membrane potential.

It has been shown elsewhere (Marmor, 1971) that the constant field equation for ionic current (Hodgkin & Katz, 1949) can be applied to the G cell. According to the present data, the electrogenic Na pump may be

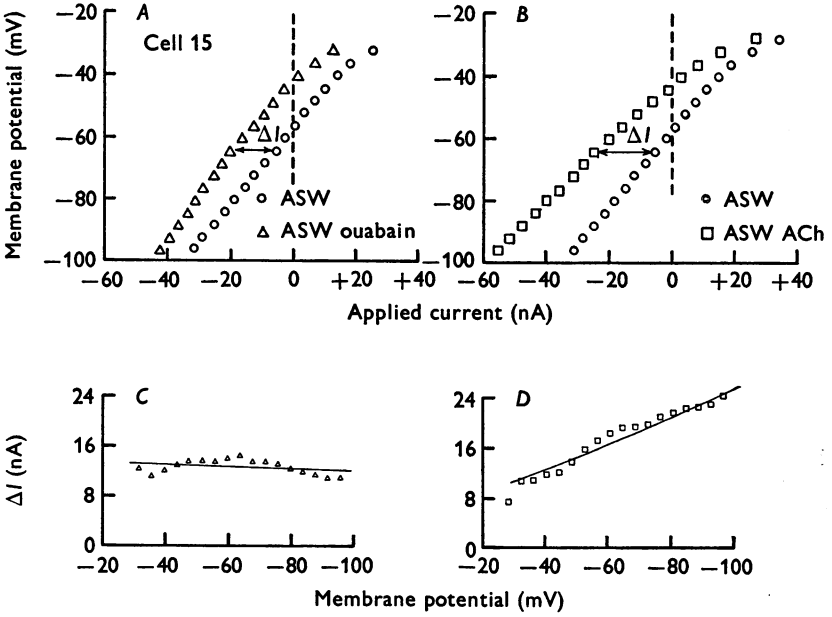

Fig. 4. Membrane current generated by the electrogenic Na pump and by acetylcholine. Data from one cell at 14° C, showing the effects of comparable depolarization with 5×10^{-4} M ouabain (*A* and *C*) and $5{\cdot}5 \times 10^{-5}$ M acetylcholine (*B* and *D*). *A* and *B*, *I–V* relations, digitized at 4 mV intervals (see Marmor, 1971); the dashed line at zero current indicates the resting potential. ΔI represents the difference in applied current, at each membrane potential, between the *I–V* relations with and without drugs. *C* and *D*, plots of ΔI *vs.* membrane potential, derived from *A* and *B* respectively. The straight line in *C* is a least-squares fit. The curved line in *D* was drawn from proportionality 1 in the text.

represented (over a broad range of potentials) by a term of fixed value added onto the passive ionic current density. Thus

$$I = \frac{VF^2P_K[K_o + (P_{Na}/P_K)Na_o - K_i e^{VF/RT}]}{RT(1 - e^{VF/RT})} + I_p, \qquad (2)$$

where I is the over-all ionic current density, I_p is the current density generated by the pump, P_K and P_{Na} are the membrane permeabilities to K and Na, and subscripts i and o indicate internal and external ionic con-

centrations. This equation is of limited practical use in predicting the I–V relations of the G cell, because inward-going rectification is present under most conditions where the Na pump is active (Marmor, 1971). However, the contribution of the electrogenic Na pump to the resting potential may be calculated in terms of the pump current, I_p, by setting $I = 0$ in eqn. (2) and rearranging terms. Thus

$$V = \frac{RT}{F} \ln \left\{ \frac{K_o + (P_{Na}/P_K)Na_o + I_p RT/VF^2 P_K}{K_i + I_p RT/VF^2 P_K} \right\}. \quad (3)$$

This same expression may also be derived in terms of ionic fluxes (Moreton, 1969).

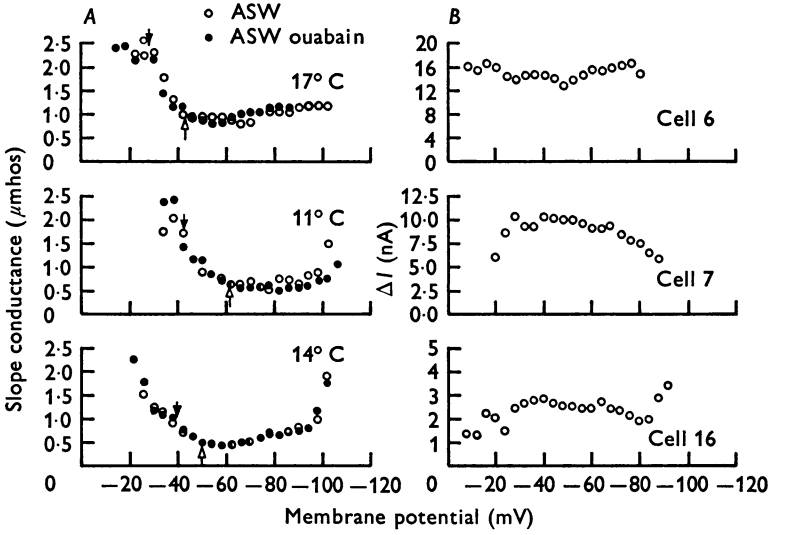

Fig. 5. Rectification and the electrogenic Na pump current as a function of membrane potential. Data from three cells. *A*, plots of slope conductance ($\Delta I/\Delta V$, calculated at 4 mV intervals) *vs.* membrane potential, before (○) and after (●) inhibition of the Na pump with 5×10^{-4} M ouabain. Resting potential is indicated by an arrow with open or filled head to correspond with the data points. *B*, plots of the electrogenic pump current (ΔI, determined as in Fig. 5) *vs.* membrane potential, from the same data as the plots in *A*.

DISCUSSION

Fom the results in this paper, it is concluded that electrogenic Na transport in the G cell functions as a constant current source, at least between −30 and −100 mV, and thus does not affect inward-going rectification. However, as mentioned above, rectification is a function of the absolute membrane potential so that the behaviour of the membrane at *resting* potential may indeed be altered by electrogenic pump activity. Similarly,

measurements of electrogenic activity at different membrane potentials must take the presence or absence of rectification into account or else the pump current will appear incorrectly to vary with potential. These data agree with evidence from the squid axon that Na efflux is largely independent of potential (Hodgkin & Keynes, 1955; Brinley & Mullins, 1970), but differ with reports on muscle fibres which suggest that Na efflux depends upon the electrochemical gradient for Na (Horowicz & Gerber, 1965; Mullins & Awad, 1965; Fozzard & Kipnis, 1967).

The total electrogenic current generated by the Na pump in the G cell varied with temperature and the state of Na loading. However, typical values would be in the range of 10–20 nA (see Fig. 5). The effective area of the G cell ranges from about 0·05–0·5 cm^2 (Marmor, 1971) and using these figures the uncoupled Na efflux would be 0·2–4·0 p-mole/cm^2.sec. These values are considerably lower than those (10–25 p-mole/cm^2.sec, depending upon loading) for uncoupled Na efflux from the squid axon (Hodgkin & Keynes, 1955; Sjodin & Beaugé, 1968), but cause a much greater contribution to potential in the G cell because of the higher membrane resistance (see Hodgkin & Keynes, 1955; Carpenter, 1970; Marmor, 1971).

The Na pump in the G cell can maintain its steady electrogenic current up to a membrane potential of at least -100 mV. Since the Na equilibrium potential is approximately $+60$ mV, judging from the spike overshoot (A. L. F. Gorman and M. F. Marmor, unpublished observations), the over-all energy barrier for Na against which the pump can work without diminishing its current is greater than 160 mV, which is equivalent to 3700 cal/g-atom. However, the pump probably requires less energy than this because Na movement is usually coupled, in part, to K movement (see Caldwell, 1969). At membrane potentials greater than the K equilibrium potential (which is near -77 mV for the G cell (Gorman & Marmor, 1970*a*)), energy expended for coupled Na efflux will be partially matched by the downhill entry of K. Thus, the energy barrier for the Na pump is probably closer to 137 mV or 3160 cal/g-atom than 3700 cal/g-atom. Both of these values are larger than the 'critical energy barrier' (near 2000 cal/g-atom) proposed by Conway, Kernan & Zadunaisky (1961) for Na-loaded muscle fibres, but are close to the energy requirement (3440 cal/g-atom) calculated by Caldwell (1969) for active Na efflux in the squid axon. Furthermore, even the larger figure for the G cell is well below the 4630 cal/g-atom available from hydrolysis of ATP, on the assumption that three Na ions are transported per ATP split (see Caldwell, 1969).

The Na pump obviously cannot surmount an infinite potential difference so that some definable energy barrier must exist. However, the present data emphasize that when the potential difference against which the pump

must work is less than the 'critical' level, factors *other* than potential may control the rate of pump activity. For example, the rate-limiting steps in the transport process may be intermediate ones which depend upon temperature, ATP or internal Na, but do not 'recognize' the membrane potential. From a functional standpoint, the G cell maintains a steady efflux of Na which can respond to Na loading and the metabolic condition of the cell, but which is not affected by every fluctuation in potential during cellular activity.

I am grateful to Drs A. L. F. Gorman and M. Mirolli for encouragement and counsel. I also thank Drs F. Bloom, D. Carpenter and F. Weight for comments on earlier drafts of this paper; Mrs M. Marti-Volkoff for technical assistance; and R. Coates and H. Poole for help with the illustrations.

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