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Possible Mechanisms for the Linkage of Membrane Potentials to Metabolism by Electrogenic Transport Processes with Special Reference to *Ascaris* Muscle

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Introduction

Although electrogenic transport processes in cell membranes are being increasingly invoked to account for certain membrane phenomena (see for example Kernan, 1962; Cross *et al.*, 1965; Kerkut and Thomas, 1965; Moreton, 1969; Brading and Caldwell, 1971; Marmor, 1971) there has been comparatively little discussion of likely mechanisms and of the ways in which these might be linked to metabolism and affect the membrane potential.

A recent example for which electrogenic processes have been postulated is the highly abnormal resting membrane potential of the muscle cells of the pig roundworm *Ascaris lumbricoides* (Brading and Caldwell, 1971). Under normal conditions this potential is remarkably insensitive to changes in the concentrations of extracellular ions (Brading and Caldwell, 1964, 1971; del Castillo *et al.*, 1964) and in many situations the membrane potential, V, is given by the following form of the constant field equation

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i + x}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o + y} \qquad (1)$$

where $x = 290$ and $y = 1300$ if the permeability constant for potassium, $P_K = 1$ and if the ionic concentrations K_o etc. are expressed in mM. The sign of V is that observed on entering the cell. The terms x and y in (1) are large compared with the other terms and if they arise from electrogenic processes it would seem that the *Ascaris* muscle membrane potential is predominantly determined by these processes.

Moreton (1969) and Marmor (1971) have considered the situation in which an electrogenic sodium pump contributes a constant current I to the membrane currents, this current being independent of membrane potential. This leads to the following form of equation (1)

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i + IRT/VF^2}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o + IRT/VF^2} \qquad (2)$$

Although Marmor (1971) has obtained evidence that (2) holds for *Anisodoris* giant neurones, with I tending to remain constant and to be independent of membrane potential, (2) does not appear to hold for *Ascaris* muscle fibres. If (2) held for *Ascaris* muscle fibres, then x and y in (1) should be the same, which they are not, and they should be dependent on V.

In an attempt to account for equation (1) Brading and Caldwell (1971) took into consideration the electrogenic sodium pump mechanism discussed by Cross *et al.* (1965) shown in Fig. 1, and suggested that

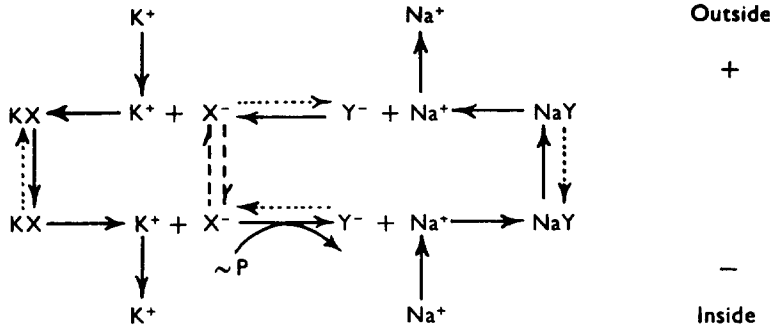

Figure 1. The scheme for an electrogenic sodium pump mechanism proposed by Cross, Keynes and Rybová (1965). Y^- is the sodium carrier, and it is only able to cross the membrane in the form of the complex NaY. X^- is the potassium carrier and it can cross the membrane either on its own or else as the complex KX.

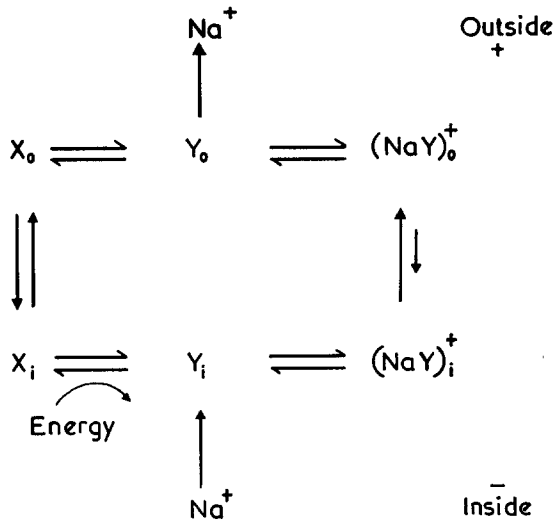

Figure 2. The scheme for the simple electrogenic sodium pump mechanism discussed in the text. Y is the sodium carrier which is uncharged and can only cross the membrane as the complex $(NaY)^+$. X is another form of the carrier which is uncharged, can cross the membrane by itself and does not form a complex with sodium or other ions. The subscripts refer to the amounts of X, Y and $(NaY)^+$ on the inside and outside of the membrane.

the movements of the negatively charged ion-free carrier X^- would contribute a current to the membrane currents to give the following form of the constant field equation

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i + P_x X_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o + P_x X_o} \tag{3}$$

where P_x is a permeability constant for X^- and X_i and X_o are its concentrations at the inner and outer surfaces of the membrane. If $P_x X_i$ and $P_x X_o$ were larger than the other terms and X_i and X_o were near to electrochemical equilibrium, then the values of x and y in equation (1) could be explained.

The electrogenic sodium pump model shown in Fig. 1 is not the simplest available and attempts to express X_i and X_o in terms of other quantities lead to quite complex expressions, even if considerable simplifications are introduced. The simpler model shown in Fig. 2 is easier to deal with and it will therefore form the basis of the discussion which follows.

Membrane with Potential Determined Solely by a Simple Electrogenic Sodium Pump with a Neutral Carrier

Figure 2 illustrates one of the simplest possible mechanisms for an electrogenic sodium pump. X and Y are neutral, X being able to cross the membrane on its own while Y can only cross in company with sodium. The sodium transport and transfer of charge are accomplished by the outward movement of the sodium:carrier complex $(NaY)^+$ across the membrane. The system can be kept in operation either by displacement of the concentrations of X and Y from equilibrium on the inside of the membrane as illustrated or by displacement on the outside. The displacement from equilibrium is brought about by a suitable coupling with energy yielding metabolic processes such as the splitting of ATP.

It is likely that all but one of the stages shown in Fig. 2 will operate near thermodynamic equilibrium (c.p. Caldwell, 1969), the exception being the movement of the complex $(NaY)^+$ across the membrane. X will move very rapidly compared with $(NaY)^+$ so its internal and external concentrations in the membrane will be almost equal.

Therefore

$$X_o = X_i = \alpha Y_o = \beta Y_i \tag{4}$$

where X_o, X_i, Y_o, Y_i are external and internal concentrations of free X and Y in the membrane, and α and β are constants relating these concentrations. If the amount of $(NaY)^+$ in the membrane is small in relation to the amount of free carrier, i.e., if Y only reacts weakly with Na, then the total carrier ϕ is given approximately by

$$\phi = (X_i + X_o + Y_i + Y_o) \tag{5}$$

Then

$$Y_i = \frac{\alpha\phi}{(2\alpha\beta + \alpha + \beta)} \tag{6}$$

$$Y_o = \frac{\beta\phi}{(2\alpha\beta + \alpha + \beta)} \tag{7}$$

Since only a small proportion of the carrier Y is assumed to be in combination with sodium the concentrations of $(NaY)^+$ at the inner and outer surfaces of the membrane will be given by the equations

$$(NaY^+)_i = \gamma Na_i Y_i \tag{8}$$

$$(NaY)_o^+ = \gamma Na_o Y_o \tag{9}$$

where γ is the product of the partition coefficient of sodium between the aqueous and membrane phases and the association constant for the interaction of the free membrane sodium and Y.

The current carried into the cell by $(NaY)^+$, $I_{(NaY)}$, is given by the following equation (see for example Goldman, 1943; Hodgkin and Katz, 1949) if the sign of the membrane potential V is that observed on entering the cell.

$$I_{(NaY)} = \frac{u_{(NaY)}FV}{a}\left[\frac{(NaY)_o^+ - (NaY)_i^+ e^{FV/RT}}{e^{FV/RT} - 1}\right]$$

$$= P_{(NaY)}\frac{F^2 V}{RT}\left[\frac{Na_o Y_o - Na_i Y_i e^{FV/RT}}{e^{FV/RT} - 1}\right] \tag{10}$$

where $u_{(NaY)}$ is the mobility of $(NaY)^+$ in the membrane, a is the membrane thickness. $P_{(NaY)}$ is a constant which is defined as being equal to $u_{(NaY)}\gamma RT/aF$. It is not strictly comparable with the permeability constants P_K, P_{Na} and P_{Cl} which are introduced later.

If a membrane contained the electrogenic mechanism shown in Fig. 2 but had no passive permeability to ions, then charge would only be carried by $(NaY)^+$. In the absence of an applied potential the membrane would reach a stable potential when $I_{(NaY)} = 0$. When $I_{(NaY)} = 0$, $Na_o Y_o - Na_i Y_i e^{FV/RT} = 0$, and, from equations (6) and (7), the stable potential, V, is given by

$$V = \frac{RT}{F}\ln\frac{Na_o Y_o}{Na_i Y_i} = \frac{RT}{F}\ln\frac{\beta Na_o}{\alpha Na_i} \tag{11}$$

Equation (11) shows the way in which the membrane potential would be determined if the simple type of electrogenic mechanism shown in Fig. 2 was the only factor involved.

The potential is determined by the concentrations of sodium ion on the two sides of the membrane and by the values of α and β. If no energy is supplied to the mechanism then $\alpha = \beta$ and (11) reduces to the simple

Nernst equation for a membrane permeable to sodium. In these circumstances the electrogenic mechanism becomes equivalent to a channel permeable to sodium. If on the other hand energy is fed into the mechanism so that either α is changed relative to β or β relative to α, then (11) shows that the membrane potential depends on β/α as well as on Na_o/Na_i. In principle any value of the membrane potential can be obtained providing a suitable coupling of metabolism to the mechanism can produce the appropriate value of β/α.

Membrane with Potential Determined by a Simple Electrogenic Sodium Pump with Neutral Carriers and by the Passive Movement of Potassium, Sodium and Chloride

The mechanism to be considered is the same as that in Fig. 2 with the addition of passive movements of potassium, sodium and chloride. The inward currents carried by potassium, sodium and chloride, if the sign of V is that observed on entering the cell, are given by the following equations (see for example Hodgkin and Katz, 1949)

$$I_K = P_K \frac{F^2 V}{RT}\left[\frac{K_o - K_i e^{FV/RT}}{e^{FV/RT} - 1}\right] \tag{12}$$

$$I_{Na} = P_{Na} \frac{F^2 V}{RT}\left[\frac{Na_o - Na_i e^{FV/RT}}{e^{FV/RT} - 1}\right] \tag{13}$$

$$I_{Cl} = P_{Cl} \frac{F^2 V}{RT}\left[\frac{Cl_i - Cl_o e^{FV/RT}}{e^{FV/RT} - 1}\right] \tag{14}$$

A stable potential is reached when the total current across the membrane, $I_{NaY} + I_K + I_{Na} + I_{Cl}$, is zero so that, from 10, 12, 13 and 14

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i + P_{NaY} Na_o Y_o}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o + P_{NaY} Na_i Y_i} \tag{15}$$

From 6, 7 and 15

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i + P_{NaY} Na_o \beta\phi/(2\alpha\beta + \alpha + \beta)}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o + P_{NaY} Na_i \alpha\phi/(2\alpha\beta + \alpha + \beta)} \tag{16}$$

The effect of the electrogenic component in equation (16) depends on the size of the terms $P_{NaY}\beta\phi/(2\alpha\beta + \alpha + \beta)$ and $P_{NaY}\alpha\phi/(2\alpha\beta + \alpha + \beta)$ in relation to P_K, P_{Na} and P_{Cl}. If these terms are a good deal larger than P_K, P_{Na} and P_{Cl} then (16) reduces to (11). If they are a good deal smaller then (16) reduces to the conventional constant field equation.

The term $P_{NaY} Na_i \alpha\phi/(2\alpha\beta + \alpha + \beta)$ will tend to remain fairly constant and, if $P_{NaY}\alpha\phi/(2\alpha\beta + \alpha + \beta)$ is large, it could contribute the large constant term y needed in equation (1) to account for the resting

membrane potential of *Ascaris* muscle. The term $P_{NaY}Na_o\beta\phi/(2\alpha\beta+\alpha+\beta)$ will vary with Na_o, although it can be a good deal smaller than $P_{NaY}Na_i\alpha\phi/(2\alpha\beta+\alpha+\beta)$ if $\beta \ll \alpha$. If β is very small then $P_{NaY}Na_o\beta\phi/(2\alpha\beta+\alpha+\beta)$ will be much smaller than $P_{Na}Na_o$ and can be neglected. If it is larger then the effective permeability constant for external sodium ions will be increased to $(P_{Na}+P_{NaY}\beta\phi/(2\alpha\beta+\alpha+\beta))$. It seems possible that in *Ascaris* muscle $P_{NaY}\beta\phi/(2\alpha\beta+\alpha+\beta) \approx 10P_{Na}$ since treatment with γ amino-butyric acid, which seems to inactivate the electrogenic mechanisms, drastically reduces the values of x and y needed in equation (1) and simultaneously reduces the value needed for P_{Na} from 1 to 0·1.

Equation (16) can therefore account for the large constant y in equation (1) and the variable value of P_{Na}. It does not account however for the smaller constant x. For this the independent electrogenic transport of an anion, possibly chloride, must be postulated.

Membrane with Potential Determined by Simple Electrogenic Sodium and Chloride Pumps with Neutral Carriers and Passive Diffusion of Potassium, Sodium and Chloride

Suppose that chloride is transported as well as sodium but by an independent electrogenic mechanism, analogous to that in Fig. 2, with chloride replacing sodium. If carriers X^1 and Y^1 are involved, if the constant total amount of carrier is θ and if $X^1_o = X^1_i = AY^1_o = BY^1_i$, then

$$Y^1_o = B\theta/(2AB+A+B) \tag{17}$$

$$Y^1_i = A\theta/(2AB+A+B) \tag{18}$$

The inward current $I_{(ClY^1)}$ carried by the mechanism will be given by

$$I_{(ClY^1)} = P_{(ClY^1)}\frac{F^2 V}{RT}\frac{Cl_i Y^1_i - Cl_o Y^1_o e^{FV/RT}}{e^{FV/RT}-1} \tag{19}$$

where $P_{(ClY^1)}$ is a constant, the definition of which is similar to that for $P_{(NaY)}$. If the current given by (19) is added to the currents given by (10), (12), (13) and (14) the following expression is obtained

$$V = \frac{RT}{F}\ln\frac{P_K K_o + P_{Na}Na_o + P_{Cl}Cl_i + P_{NaY}Na_o\beta\phi/(2\alpha\beta+\alpha+\beta) + P_{ClY^1}Cl_i A\theta/(2AB+A+B)}{P_K K_i + P_{Na}Na_i + P_{Cl}Cl_o + P_{NaY}Na_i\alpha\phi/(2\alpha\beta+\alpha+\beta) + P_{ClY^1}Cl_o B\theta/(2AB+A+B)} \tag{20}$$

If $P_{ClY^1}B\theta/(2AB+A+B)$ is small compared with P_{Cl} so that $P_{ClY^1}Cl_oB\theta/(2AB+A+B)$ can be neglected, (20) can be used to explain many of the features of the normal resting membrane potential of *Ascaris* muscle fibres.

$P_{\mathrm{Na}Y}\mathrm{Na_i}\,\alpha\phi/(2\alpha\beta+\alpha+\beta)$ and $P_{\mathrm{Cl}Y'}\mathrm{Cl_i}\,\mathrm{A}\theta/(2\mathrm{AB}+\mathrm{A}+\mathrm{B})$ will tend to remain fairly constant and since they can have different values they can be made equivalent to y and x in equation (1). Further, the term $P_{\mathrm{Na}Y}\mathrm{Na_o}\beta\phi/(2\alpha\beta+\alpha+\beta)$ can contribute to the effects of external sodium and its reduction, due to inactivation of the electrogenic mechanisms, can be postulated to explain the reduction in the effective value of P_{Na} observed in the presence of γ amino-butyric acid.

Membrane with an Electrogenic Sodium Pump with Negatively Charged Carriers and Passive Diffusion of Potassium, Sodium and Chloride

The electrogenic transport models which have been discussed so far can account quite well for the behaviour of the membrane potential of *Ascaris* muscle fibres under various conditions. They do not however lead to a condition under which the current contributed by the electrogenic sodium pump is constant and independent of membrane potential. As was mentioned earlier this situation has been discussed in connection with *Helix* neurones by Moreton (1969) and experimental evidence for it has been obtained for *Anisodoris* neurones by Marmor (1971).

An alternative approach is to use the model in Fig. 2 but to assume that X and Y carry single negative charges as they do in Fig. 1. If the movement of negatively charged X is assumed to be very rapid so that it can carry a current equivalent to the net outward movement of sodium but remain at approximately equilibrium concentrations so that $X_\mathrm{i}=X_\mathrm{o}e^{FV/RT}$ then equation (4) becomes

$$X_\mathrm{i}=X_\mathrm{o}e^{FV/RT}=\beta Y_\mathrm{i}=\alpha Y_\mathrm{o}e^{FV/RT} \tag{21}$$

From (5) and (21) it can be shown that

$$Y_\mathrm{i}=\frac{\alpha\phi\,e^{FV/RT}}{(\beta+\alpha\beta+\alpha\beta\,e^{FV/RT}+\alpha\,e^{FV/RT})} \tag{22}$$

$$Y_\mathrm{o}=\frac{\beta\phi}{(\beta+\alpha\beta+\alpha\beta\,e^{FV/RT}+\alpha\,e^{FV/RT})} \tag{23}$$

Since the current, I_x, carried by X is equivalent to the net movement of sodium

$$I_x=\mathrm{KNa_i}\,Y_\mathrm{i}-\mathrm{KNa_o}\,Y_\mathrm{o} \tag{24}$$

where K is a constant and Y is far from saturation.

I_x as defined by (24) is normally an outward current. If the value of I_x given by (24) is subtracted from the potassium, sodium and chloride currents given by (12), (13) and (14), then

$$V=\frac{RT}{F}\ln\frac{P_\mathrm{K}\mathrm{K_o}+P_\mathrm{Na}\mathrm{Na_o}+P_\mathrm{Cl}\mathrm{Cl_i}+\mathrm{K}(\mathrm{Na_i}\,Y_\mathrm{i}-\mathrm{Na_o}\,Y_\mathrm{o})\,RT/VF^2}{P_\mathrm{K}\mathrm{K_i}+P_\mathrm{Na}\mathrm{Na_i}+P_\mathrm{Cl}\mathrm{Cl_o}+\mathrm{K}(\mathrm{Na_i}\,Y_\mathrm{i}-\mathrm{Na_o}\,Y_\mathrm{o})\,RT/VF^2} \tag{25}$$

The values of Y_i and Y_o given by equations (22) and (23) can be substituted in (25) to give a rather complex expression for V.

A simplification can be introduced if both $e^{FV/RT}/\beta$ and α are very large and β is small so that $Y_i \approx \phi$.

(25) then becomes

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i + KNa_i \phi RT/VF^2}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o + KNa_i \phi RT/VF^2} \quad (26)$$

Since ϕ is constant, $KNa_i\phi$ will tend to remain constant and equal to the roughly constant outward current, independent of membrane potential, which the electrogenic mechanism will now generate. (26) is in fact equivalent to (2), which is Moreton's equation, and implies the condition of a constant outward current independent of membrane potential found by Marmor (1971) in *Anisodoris* neurones.

Conclusion

The foregoing discussion shows that certain of the features of membrane systems in which electrogenic processes may operate can be described by quite simple models if a few reasonable assumptions are made. Since the model based on neutral carriers seems to explain the behaviour of *Ascaris* muscle fibre membranes, whereas the model based on negative carriers may be more appropriate for *Helix* and *Anisodoris* neurones, it seems that more than one type of electrogenic transport mechanism may exist. The need to postulate an electrogenic chloride pump in the case of *Ascaris* muscle also raises the possibility that ions other than sodium may be subject to electrogenic transport.

From the point of view of bioenergetics, one of the most interesting points which has been raised is the possibility that the membrane potential could be linked directly and rapidly to the free energy made available from metabolism through an electrogenic sodium pump. Equation (11) shows that if the membrane potential is generated almost exclusively by the type of mechanism shown in Fig. 2, then it is in principle possible to obtain a membrane potential of any value. This could be accomplished by a suitable gearing of the electrogenic pump mechanism to free energy yielded by metabolism to produce the required value of β/α. A case of particular interest would arise if $Na_o/Na_i \approx 10$ and $\beta/\alpha \approx 0\ 01$. Equation (11) shows that under these circumstances the resting membrane potential would be about −58 mV even though it arose from a mechanism based on the movements of sodium rather than potassium. If the supply of free energy to the mechanism was momentarily cut off, the value of β/α would change to 1·0 and the membrane potential would change to that given by the Nernst equation for sodium ions, namely about +58 mV. The electrogenic sodium pump mechanism shown in Fig. 2 could therefore give rise to membrane potentials

corresponding to normal resting and action potentials without any contribution by other ions to the membrane currents.

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