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A PROPOSED MODEL FOR THE Na^+ PUMP

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SUMMARY

A kinetic model of the Na^+ pump is described. Its properties have been investigated by means of extensive calculations using a computer, and it is found that the model reproduces very satisfactorily those properties of the pump which have been investigated experimentally. It is further concluded that it is quite practicable to test even complicated models quantitatively.

INTRODUCTION

There is an enzyme system in cell membranes which transports Na^+ outwards and K^+ inwards. It is known as "($Na^+ + K^+$)-activated ATPase" or simply as the Na^+ pump, and a considerable amount of work has been done on the experimental investigation of its properties[1]. The mechanism of the pump is not known, but several hypothetical mechanisms have been suggested[2–4]. In a previous paper[5] it was pointed out that some of these hypotheses were incompatible with the experimental information, in that they gave incorrect values for the rate reduction ratio (a quantity defined in ref. 5). There are a number of other features of the Na^+ pump which must be reproduced by any proposed mechanism: the stoichiometry, the kinetics of pumping, the characteristics of exchange of internal Na^+ for external Na^+ in zero external K^+, the form of the variation of membrane phosphorylation with varying reagent concentrations, and so on. For some of these criteria, it is quite easy to see whether a given model satisfies them or not—examples are the stoichiometry, and in some models the rate reduction ratio and Na^+–Na^+ exchange. For other criteria, quite detailed calculation may be needed before it is possible to say whether the model fits the observations. The purpose of the present paper is to suggest a simple kinetic scheme for the Na^+ pump which can be shown to reproduce all of the characteristics of the pump which have so far been studied in any detail. I will begin with a description of this scheme and go on to show how it accounts for the various aspects of the pump's behaviour.

THE REACTION SCHEME

It is to be understood that this scheme is offered as a hypothesis. I shall, however, for the sake of clarity and brevity, describe it in more positive terms.

The process is illustrated diagrammatically in Fig. 1. Each pump unit has one carrier which takes successively the three forms X, Y and Z. Each of these forms can take up Na^+ or K^+ with various affinities, but the affinity of Z for Na^+ is assumed to be negligible. X is confined to the inside of the membrane, and has roughly equal affinities for Na^+ and K^+. XNa is converted into a phosphorylated form Y by the enzyme E and ATP. (It seems to be unimportant whether XNa reacts with an $E \cdot ATP$ complex or with a phosphorylated form $E \sim P$.) Y is confined to the outside of the membrane, perhaps to a definite site; it has a higher affinity for K^+ and a lower affinity for Na^+ than X has. It is not clear how the translocation of the carrier is achieved, but it seems evident that the mechanism of the pump must include some step involving translocation and a change in affinity.

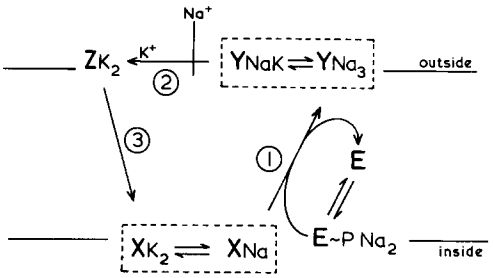

Fig. 1. Outline of the proposed scheme.

Reaction 2 converts Y into Z, which is still phosphorylated. This reaction requires K^+ and is inhibited by Na^+. Finally Z diffuses to the inside of the membrane, where it is hydrolysed to give X and phosphate (Reaction 3). The reverse of this reaction is very slow under normal conditions; Reactions 1 and 2, on the other hand, are quite easily reversible. The rate-limiting step is normally Reaction 1.

This gives a bald outline of the scheme. However, comparison of the scheme with experiment calls for detailed calculations of the kinetics, which are most conveniently carried out using a computer. For this purpose it is necessary to specify the steps in the process much more precisely. I must emphasize, however, that the very detailed scheme which will be described is set up only for the purpose of obtaining

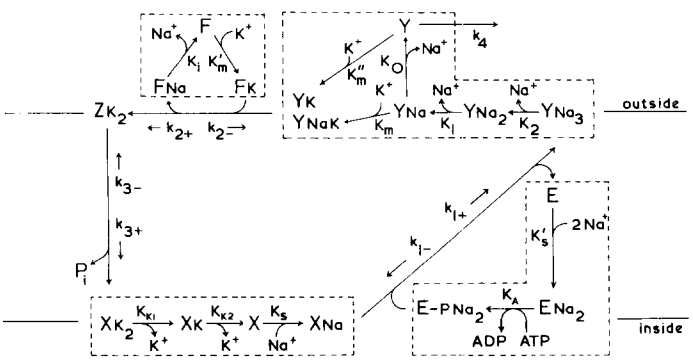

Fig. 2. Detailed scheme used in the calculations. The values of the rate constants and equilibrium constants are listed in Table I.

clear quantitative results for comparison with experiment. One might expect that schemes similar in outline but different in detail would give broadly similar results. Indeed, the mechanism may be different in detail, or perhaps totally different, in different tissues. However it is natural to assume that the mechanism is the same everywhere unless experimental evidence shows this assumption to be untenable.

The detailed scheme is shown in Fig. 2; it involves seven rate constants and twelve equilibrium constants. For the purposes of calculation, it is assumed that reactions involving ions or ATP are fast compared with the major steps of the reaction, so that the groups of reactions enclosed by the dotted lines in Fig. 2 are in equilibrium at all times. It is assumed that the steady-state approximation applies. The effects of Mg^{2+} are not represented explicitly, although it is recognized that Mg^{2+} is essential for the operation of the pump.

The calculation itself is quite straightforward. Consider, for example, the equilibrium involving X. We have $[XNa] = [X]([Na]_i/K_s)$, $[XK] = [X]([K]_i/K_{K_2})$, $[XK_2] = [XK]([K]_i/K_{K1}) = [X]([K]_i^2/K_{K_1}K_{K_2})$, where subscript i denotes internal concentration (and e will denote external concentration). If we write $x = [XNa] + [X] + [XK] + [XK_2]$, we easily obtain (for example)

$$[XK_2] = \left\{\frac{[K]_i^2/K_{K_1}K_{K_2}}{[Na]_i/K_s + 1 + [K]_i/K_{K_2} + [K]_i^2/K_{K_1}K_{K_2}}\right\} x = a_{3-}x, \text{ say.}$$

In other words, a fraction a_{3-} of the concentration x is available to take part in Reaction 3. Since a_{3-} is constant for given reagent concentrations $[Na]_i$ and $[K]_i$, the kinetics are the same as if the whole concentration x was able to react, but with a rate constant reduced from k_{3-} to $a_{3-}k_{3-} = \varkappa_{3-}$, say. Applying this procedure to all the equilibria, one obtains a very simple scheme (Fig. 3) which is easily solved using standard steady-state theory with the condition $x + y + z =$ constant.

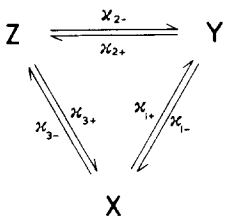

Fig. 3.

RESULTS

A large number of calculations has been done on the scheme described. For this purpose it was necessary to introduce some definite numbers, and after a number of computer experiments the set of values given in Table I was arrived at. The equilibrium constants (the reciprocals of the affinities) are given in mM, except for K_A which is dimensionless, and the rate constants are in arbitrary units. I should emphasize that these values are just an indication of orders of magnitude; one cannot expect exact quantitative agreement with every experiment when the experiments have been done on a variety of tissues, under various experimental conditions and in particular at various temperatures. No attempt has been made to get values re-

presenting a best fit, but the values quoted seem to be generally satisfactory for the red blood cell; for kidney membranes a better fit is obtained if the potassium constants (*i.e.* K_{K1}, K_{K2}, K_m, K_m', and K_m'') are all reduced by a factor of 10.

TABLE I

NUMERICAL VALUES USED IN THE CALCULATIONS

For explanation refer to text and Fig. 2.

Rate constants (arbitrary units)		*Equilibrium constants (mM)*			
		Interior		*Exterior*	
k_{1+}	2	K_{K1}	90	K_m	1
k_{1-}	200	K_{K2}	10	K_m'	2
k_{2+}	40	K_s	0.5	K_m''	0.4
k_{2-}	20	K_s'	5	K_0	0.5
k_{3+}	20	K_A	2	K_1	60
k_{3-}	0.05			K_2	200
k_t	4			K_t	150

The following properties of the pump have been investigated.

1. Stoichiometry and electrogenicity

According to the scheme presented here, the pump transports three Na^+ out of the cell and two K^+ into it for every molecule of ATP split. This stoichiometry is in agreement with the rather uncertain experimental evidence[6]. It is evident that the model transports positive charge to the outside of the cell, and that it is therefore capable of setting up a potential difference across the membrane. Since one or both of ZK_2 and YNa_3 must be charged, a change in potential would also affect the kinetics: for example, if YNa_3 is positively charged and ZK_2 neutral, then an increased potential will reduce k_{1+} and perhaps increase k_{1-}, with effects which are in principle observable. If YNa_3 were neutral and ZK_2 negatively charged, the effects of varying the potential would be different.

It is possible that the stoichiometry is variable, though there is no clear evidence for this. The model could easily be modified to allow for variable stoichiometry; for example by supposing that YNa_2 can also cross the membrane, with rate constant k_{1+}', but that $k_{1+}' \ll k_{1+}$ under normal conditions. If now the membrane potential is increased, k_{1+} will be reduced but k_{1+}' will not (assuming YNa_3 carries one positive charge) and a higher proportion of pump cycles will extrude two Na^+ instead of three. This sort of mechanism would allow active transport to continue in the presence of a high potential.

2. Rate reduction ratio ρ

This quantity was defined in ref. 5, and gives an indication of the nature of the interaction of two reagents in a many-step cyclic reaction. In this case the significant reagents are internal Na^+ and external K^+, and the experimental evidence indicates that ρ is less, but not much less, than 1. It was pointed out in ref. 5 that the models of SHAW[3] and OPIT AND CHARNOCK[4] gave values of 4/3 and are therefore inadequate in their simplest forms, though they might work with modifications. For the present model, a simple analysis is not possible, and the value of ρ

must be calculated numerically. It turns out to depend somewhat on the reagent concentrations, and to be sensitive to the value of k_{1-}. Satisfactory values can be obtained by adjustment of k_{1-}.

3. Exchange of internal Na^+ for external Na^+

It is observed[7,8] that in the absence of external K^+, internal Na^+ can be exchanged for external Na^+. The process requires, but does not consume, ATP, and there is no net loss of Na^+ from the cell. The striking feature of this exchange is that it occurs when the internal Na^+ concentration is low, but not when it is high. No previously proposed model accounts for this observation, but the present one does. The exchange occurs through Reaction 1, and is prevented if Reaction 1 is inhibited. This inhibition may be caused by high external $[K^+]$ (which moves the Y equilibrium away from YNa_3) or by high $[Na^+]_i$ or high $[ATP]/[ADP]$, both of which tend to move the E equilibrium away from E.

In discussing this aspect of the pump's behaviour it is necessary to define one's terms rather carefully. In most conditions, there is a ouabain-sensitive efflux of Na^+ from the cell *via* Reaction 1_1, and also a ouabain-sensitive influx *via* Reaction 1_-. The net pumping rate is the difference of these:

$$\langle rate \rangle = \langle efflux \rangle - \langle influx \rangle$$

Now it is possible to measure all of these experimentally: $\langle rate \rangle$ is proportional to the rate of ouabain-sensitive ATP breakdown, and $\langle efflux \rangle$ and $\langle influx \rangle$ can be measured directly by tracer techniques. Under normal conditions, with $\langle rate \rangle > 0$, $\langle influx \rangle$ is the rate at which internal Na^+ is replaced by external Na^+, and is therefore the natural theoretical measure of Na^+ exchange. The experiments[7,8] gave values of $\langle efflux \rangle$ in K^+-free media, but under these conditions $\langle rate \rangle = 0$, so $\langle efflux \rangle = \langle influx \rangle$ and the theoretical measure coincides with the experimental quantity. In some experiments, $\langle influx \rangle$ was measured directly.

Fig. 4 shows the calculated dependence of $\langle influx \rangle$ on $[Na^+]_i$, for various values of $[K^+]_e$. It can be seen that in zero K^+, the exchange has a maximum near $[Na^+]_i = 10$ mM, and that exchange is cut off quite sharply by increasing either $[Na^+]_i$ or $[K^+]_e$. The K_m for inhibition of exchange by external K^+ is about the same as the K_m for activation of pumping. All these results are in gratifying agreement with experiment[7]. The variation of $\langle influx \rangle$ with $[Na^+]_e$ has also been studied using the model (see Fig. 4), and there is satisfactory agreement with experiment here too.

The effects of varying [ATP], [ADP] and $[P_i]$ have also been studied. The variation of the ratio [ATP]/[ADP] has much the same effect as a variation of $[Na^+]_i$, and the model seems to be in agreement with experiment here. The effects of P_i, however, do not all seem to be reproduced. It is observed[7] that in a cell with low $[Na^+]_i$ and high $[P_i]$, the Na^+ efflux into zero K^+ is slightly higher than that into 10 mM K^+. The model gives the same result. For cells with high $[Na^+]_i$, however, there seems to be disagreement. Experimentally, it is observed that addition of P_i to cells containing much Na^+ and moderate amounts of ATP increases the efflux into zero K^+. The model shows no such effect. However, it is possible that the large amount of P_i interferes with the action of ATP in some way, thus allowing exchange to occur more freely. Further investigation of this point would be very useful, especially as it is the only case where the model has been found to disagree with experiment.

4. Exchange of internal K^+ for external K^+

Little experimental work has been done on this phenomenon as yet. However, the present model would predict its occurrence. The rate of exchange would be expected to be low, since Reaction 3_- is slow, but it would be increased by large amounts of P_i.

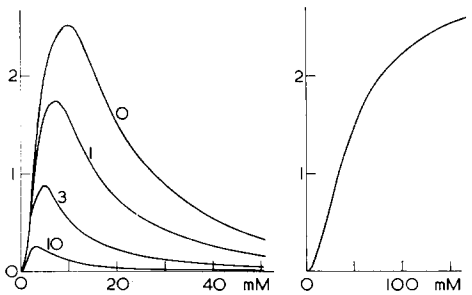

Fig. 4. *Left.* Calculated dependence of Na^+–Na^+ exchange on $[Na^+]_i$, for the values of $[K^+]_e$ shown. $[K^+]_i$ = 120 mM, $[Na^+]_e$ = 140 mM, [ATP]/[ADP] = 17, $[P_i]$ = 0.1 mM. *Right.* Calculated dependence of Na^+–Na^+ exchange on $[Na^+]_e$, $[K^+]_i$ = 120 mM, $[K^+]_e$ = 0, $[Na^+]_i$ = 10 mM, [ATP]/[ADP] = 17, $[P_i]$ = 0.1 mM.

5. Membrane phosphorylation

POST, SEN AND ROSENTHAL[9] have carried out a number of experiments on a phosphorylated intermediate in kidney membranes. They believe this intermediate to be part of the Na^+ pump. It has been suggested[10] that the evidence for this belief is not very strong, but it seems likely to be true and I have assumed that it is. POST, SEN AND ROSENTHAL[9] used a broken-cell preparation, so that the concentrations "inside" and "outside" the membrane are the same. The results of particular interest are those summarized in Figs. 5, 6 and 7 of their paper and reproduced in Figs. 5, 6 and 7 of this paper together with calculated curves. Each figure is a plot of the amount of phosphorylated intermediate against the turnover rate, showing the way that these

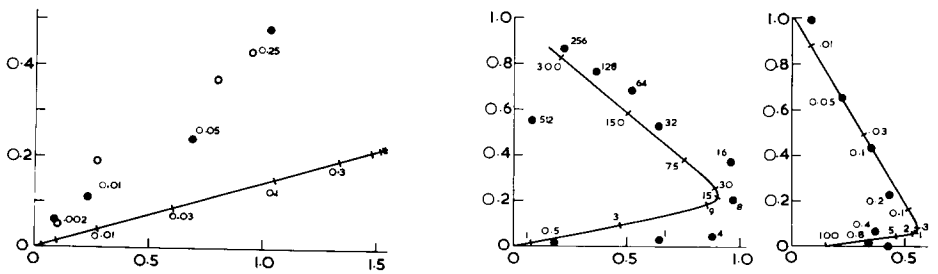

Fig. 5. *Left.* Relation between phosphorylation (ordinate) and turnover rate (abscissa) with varying [ATP] in broken cells. ●, ○, experimental (for details see ref. 9); —+—, calculated. $[Na^+]$ = 150 mM, $[K^+]$ = 0.6 mM, [ATP] as indicated.

Fig. 6. *Centre.* Relation between phosphorylation and turnover rate with varying $[Na^+]$ in broken cells. ●, experimental (ref. 9); —+—, calculated. $[K^+]$ = 0.1 mM, [ATP] = 0.1 mM, $[Na^+]$ as indicated.

Fig. 7. *Right.* Relation between phosphorylation and turnover rate with varying $[K^+]$ in broken cells. ●, experimental (ref. 9); —+—, calculated. $[Na^+]$ = 15 mM, [ATP] = 0.03 mM, $[K^+]$ as indicated.

vary with the concentration of each of the three reagents ATP, Na^+ and K^+. In the calculations, the concentration of phosphorylated intermediate is taken as $(y + z)$.

It can be seen that the general trend of the experimental results is very satisfactorily reproduced, except for some differences in scale which are probably attributable to the different conditions in the three sets of experiments. The calculation was done with the same conditions throughout, and although the units of turnover rate are arbitrary, they too are the same throughout.

An interesting point arises in connection with Fig. 5. If it is supposed that E takes up Na^+ after phosphorylation instead of before, it is found that Fig. 5 cannot be reproduced. The relation between phosphorylation and rate is still linear, but the affinity for ATP is much too high, and cannot be reduced without spoiling the agreement with experiment of other properties of the pump. This is an example of the way in which a particular property of the pump can give information about a particular detail of the mechanism, once the general scheme has been postulated, and shows how it is not usually necessary to adjust the entire scheme in order to fit a given experimental result. It also illustrates the importance of quantitative calculations; this point would not have been apparent if qualitative arguments alone had been used.

6. Available free energy and Na^+ efflux

CALDWELL AND SCHIRMER[11] have studied the dependence of the ratio $r =$ (Na^+ efflux in zero K^+)/(Na^+ efflux in 10 mM K^+) on the free energy ΔG available to the pump from ATP hydrolysis, and have found that r decreases (roughly linearly) as ΔG increases. The present calculations agree with this, provided that ΔG is varied by changing [ATP]/[ADP]. If [P_i] is varied, r is unaffected except at very high [P_i], when it starts to decrease. This is not in conflict with CALDWELL AND SCHIRMER's[11] results, partly because their points show a fairly large scatter, and partly because they did not vary [P_i] directly, but it suggests that the relation between r and ΔG is very indirect.

7. Variation of external K^+ and Na^+

Various workers (for references see ref. 12) have experimented on the effects of varying the external Na^+ and K^+ concentrations. The competition between K^+,

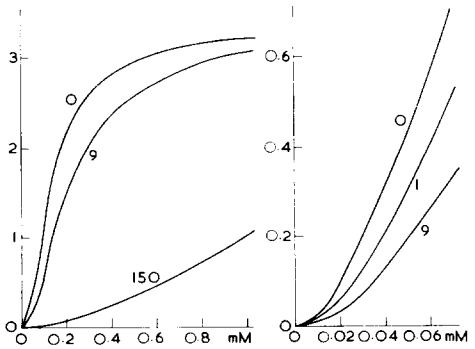

Fig. 8. Variation of pumping rate (ordinate) with $[K^+]_e$ (abscissa) at the various external Na^+ concentrations indicated. The scales are expanded in the right-hand diagram to show the behaviour at the foot of the curve. $[K^+]_i$ = 120 mM, $[Na^+]_i$ = 10 mM, [ATP]/[ADP] = 17, [P_i] = 0.1 mM.

which activates the pump, and Na^+, which inhibits it, is satisfactorily reproduced by the model in an approximately quantitative way (see Fig. 8).

8. Effect of ouabain

The effect of a large dose of ouabain is to stop the pump altogether—indeed the pump is defined experimentally as that phenomenon which is stopped by ouabain. Its mode of action is uncertain, and probably complicated. Concentrations of ouabain which are insufficient to stop the pump completely seem to reduce the affinity for external K^+, and to reduce the maximum pumping rate[13,14]. It has been suggested that the latter effect is a consequence of slower translocation across the membrane[9], in which case it should be possible to observe a change in the rate reduction ratio[5]. On the other hand, recent experiments have shown[15] that in a poisoned cell the action of ouabain is partly reversible, which indicates that ouabain reacts with the carrier at the outside of the membrane. In a poisoned cell, with no ATP available, all the carriers would be at the inside. But it seems pointless at present to try to account in detail for the effects of ouabain, and no attempt has been made to do so.

9. Reversal of the pump

GARRAHAN AND GLYNN[16] have shown that the pump in red cells is driven backwards if there is much P_i and K^+ and little Na^+ inside the cell, and much Na^+ and no K^+ outside. The [ATP]/[ADP] ratio in their experiments was about 3. They found that the backward rate under these conditions was about 2 % of the forward rate under physiological conditions, and that with the same concentrations of ATP, ADP and P_i but a normal distribution of cations the pump went forward at about 4 % of the normal rate. These results are reproduced by the model, which gives the correct directions of operation and relative rates correct to within a factor of about 2.

10. Loss of Na^+ accompanied by anions

BAKER[17] has shown that at very low external K^+ and Na^+ concentrations, Na^+ accompanied by anions is lost from crab nerve cells. The mechanism of this process is likely to be complicated, but one may suppose that Y decomposes spontaneously if it has no cations attached. This supposition has been incorporated into the scheme (see Fig. 2) and accounts satisfactorily for the observations. Presumably Y is regenerated by further reactions, but no attempt has been made to include these.

CONCLUSION

The above list shows that the proposed model accounts in a qualitative, and to a large extent quantitative, way for many of the properties of the Na^+ pump. Whether or not it can account for them all remains to be seen; it is certainly unlikely to survive without at least some modifications of detail. One might speculate on the nature of the carriers X, Y and Z, but it seems profitless to do so at this stage.

One conclusion of the present work which is independent of the success of the particular model discussed is that it is practicable to investigate the characteristic of a model quantitatively, even though the details of the model may be quite complicated. At the same time, there is no doubt that such quantitative analysis is necessary, in that a scheme may have quantitative inconsistencies which cannot be

revealed by any purely qualitative analysis. In my experience, it is quite easy to invent a superficially plausible scheme, but very much more difficult to find one which stands up to the sort of detailed analysis which is described in this paper.

ACKNOWLEDGEMENT

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