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KINETIC CHARACTERIZATION OF THE CHLORIDE DEPENDENCE OF SODIUM TRANSPORT IN THE FROG SKIN

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SUMMARY

1. Na^+ transport across the frog skin was studied at different Na^+ concentrations in the presence and absence of Cl^- in the Ringer's solution.

2. The overall kinetic behaviour can be described as the sum of two kinetic components: a saturation component and a linear component.

3. In the absence of Cl^- the linear component is negligible and the K_m of the saturation component increases by a factor of 5.

INTRODUCTION

In a previous paper[1] we showed that Na^+ transport across the isolated frog skin is in some way dependent on the anionic composition of the Ringer's solution bathing both sides of the frog skin preparation. More recently Ferreira *et al.*[2] reported that when Cl^- is replaced SO_4^{2-} in the Ringer's solutions bathing the isolated sheep rumen the net Na^+ flux falls by 30 %, and this suggests that the same effects may be seen in other epithelia. When we reported our results we put forward the suggestion that Na^+ transport across the isolated frog skin preparation might have two components one being anion dependent. Herrera and Curran[3], by studying the effect of Ca^{2+} and antidiuretic hormone on the frog skin, had already separated two parallel pathways for Na^+ transport. Biber and Curran[4], measuring the initial uptake rates of $^{22}Na^+$ across the mucosal barrier of the frog skin, characterized two kinetic components, one obeying saturation kinetics (facilitated diffusion) and the other being a simple diffusional process. Curran *et al.*[5] and Cereijido *et al.*[6] have also produced good evidence in support of the assumption that the rate-limiting step in transepithelial transport of Na^+ across the frog skin is the entrance of this ion across the mucosal barrier. The experimental technique used in the present work is based on this assumption. We studied the kinetics of Na^+ transport across the mucosal barrier in the presence and absence of Cl^-.

METHODS

Frogs of the species *Rana ridibunda* Pallas were used. They were kept in a temperature-controlled room at 6–8 °C, half immersed in running tap water. Frogs

were double pithed and the skin dissected and mounted in Ussing-type chambers. The area of the chambers was 3.14 cm^2, and six pieces of the same skin were used in order to test six different Na^+ concentrations at the same time. Short-circuit currents (s.c.c.) were measured by an automatic device using operational amplifiers and a potentiometric multichannel recorder with calibrated resistances fitted to the input in order to read the current values directly. By using the same Ringer's soluton at both sides of the skin preparation we can use the s.c.c. as a measure of net Na^+ transport. The Ringer's solution used had the following composition (in mM): Na^+, 114; Cl, 121; K^+, 2.4; Ca^{2+}, 2.4. Na^+ was replaced by Mg^{2+} in the Ringer's solution containing a lower Na^+ concentration. Cl^- was replaced by SO_4^{2-} in the Ringer's solution in which the absence of Cl^- was to be tested. Osmolalities were compensated by glucose so that all Ringer's solutions had the same osmolality (220 mosM). The aerated Ringer's solutions were titrated with Tris–HCl buffer to a pH of 8. The composition of the Ringer's solutions were frequently checked. Na^+ and K^+ concentrations were measured with an Eppendorf flame photometer and Cl^- by coulometric titration in an Aminco Cotlove Titrator. pH was checked with a Radiometer 27 pH meter, and osmolalities with an Advanced Osmometer.

We used Mg^{2+} as a substitute for Na^+, because Li^+ is now known to be an inhibitor of Na^+ transport[4,7] and choline and tetraethylammonium have a stimulating effect on s.c.c.[8]. This was confirmed by us in some preliminary experiments.

RESULTS

The effect of different Na^+ concentrations on the s.c.c. was studied in the presence of Cl^- and after replacement of Cl^- by SO_4^{2-} in the Ringer's solution. Six Na^+-concentrations were tested on different pieces of the same frog skin mounted in six different chambers. These experiments were repeated 15 times. The skins were allowed to equilibrate with control Ringer's solution, *i.e.* the solution containing 114 mM Na^+ and 121 mM Cl^-. The s.c.c. under these conditions was used as a control value of 100 %. All other values are given as percentages of these control values. In this way the experimental values from different pieces of the same frog or from different frogs could be compared although the control values for the s.c.c. were not the same. This considerably reduces the scatter of the values. A control period was used before and after the duration of the experiment and a graphic interpolation was made to get a control value during the experimental period. The results of the first group of experiments are summarized in Table I. A direct plot of s.c.c. *versus* Na^+ concentration shows that the curve is not a simple saturation curve. A good fit was obtained by postulating the existence of two kinetic components as described by the equation:

$$\bar{\Phi}\,\% = (\bar{\Phi}\,\%)_{max}\left[\frac{Na_0^+}{Na_0^+ + K_m} - \frac{Na_i^+}{Na_i^+ + k_m}\right] + B(Na_0^+ - Na_i^+)$$

The first part of the equation describes saturation kinetics in steady state with its two parts, influx and outflux, and the second part a diffusion alprocess. $(\Phi\,\%)_{max}$ and K_m have the usual meaning of Michaelis–Menten kinetics, Na_0^+ is the concentration of the Ringer's solution and Na_i^+ is the concentration of the intracellular exchangeable Na^+ pool. This value could be evaluated by considering in agreement with Curran *et al.*[5], a

TABLE I

EFFECT OF DIFFERENT Na^+ CONCENTRATIONS OF THE RINGER'S SOLUTION BATHING BOTH SIDES OF THE FROG SKIN PREPARATION ON THE SHORT-CIRCUIT CURRENT

In this group Ringer's solutions with Cl^- were used. Means and standard deviations are given. Number of experiments is 14.

$[Na^+]$ (mM)	$I/[Na^+] \times 10^2$	s.c.c. (%)	$I/s.c.c.$ (%) $\times 10^2$
6.8	14.7	28.2 ± 13.5	3.55
16.0	6.3	41.1 ± 14.8	2.43
28.5	3.5	49.2 ± 8.2	2.03
45.6	2.2	62.7 ± 7.7	1.59
79.8	1.25	80.7 ± 8.1	1.24
114.0	0.88	98.0 ± 3.2	1.02

linear relationship between this parameter and the s.c.c. in the range of our concentrations. Therefore, $Na_i^+ = \Phi\%/A$ and can be substituted in the above equation. P is the slope of the linear component ($\Phi\%_{max}$, K_m, B and A were determined by the least mean square method using an H.P. 2115A computer. In Fig. 1 we have plotted the mean

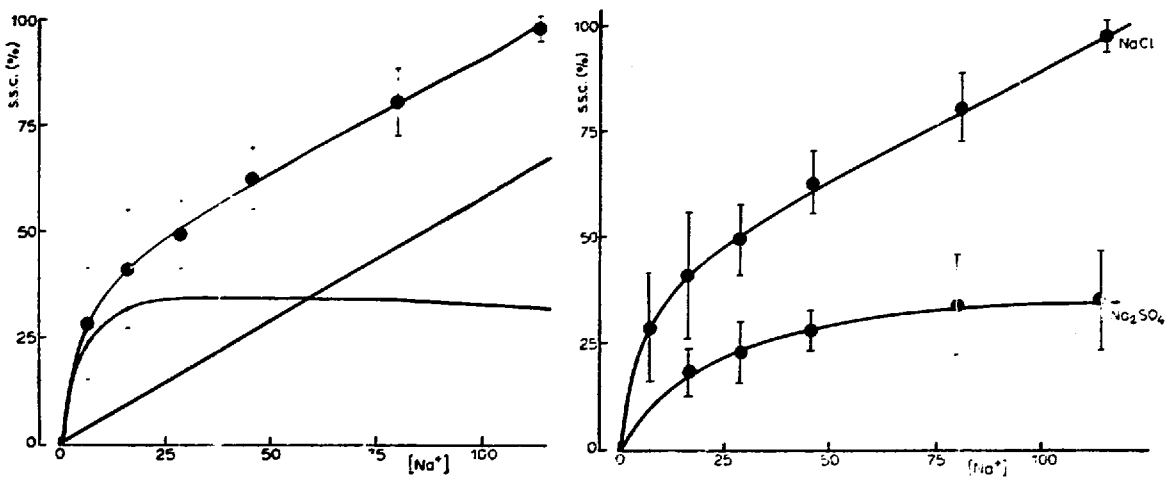

Fig. 1. Direct plot of % of s.c.c. against Na^+ concentration of the Ringer's solution. The points are mean values of the experimental values. The best fits were obtained using the least square method and testing the following equation:

$$\Phi\% = \Phi\%_{max}\left[\frac{Na_0^+}{Na_0^+ + K_m} - \frac{Na_i^+}{Na_i^+ + K_m}\right] + B(Na_0^+ - Na_i^+)$$

The separate kinetics were also computed.

Fig. 2. Direct plot of % of s.c.c. against Na^+ concentration of the Ringer's solution. The upper plot is the result with Ringer's solution containing chloride. The lower plot shows the results in the absence of Cl^-.

values of the experimental results with their standard deviations and the three computed curves, the mixed kinetics, the saturation and the linear component.

The results of the second group of experiments are summarized in Table II and Fig. 2. Here the effect of Na^+ concentration on the s.c.c. in the absence of Cl^- was studied. During the experimental period the Ringer's solution contained SO_4^{2-} and

TABLE II

EFFECT OF DIFFERENT Na^+ CONCENTRATION OF THE RINGER'S SOLUTION BATHING BOTH SIDES OF THE FROG SKIN PREPARATION ON THE SHORT-CIRCUIT CURRENT IN THE ABSENCE OF Cl^-

Means and standard deviations are given. Number of experiments in parentheses.

$[Na^+]$ (*mM*)	$1/[Na^+] \times 10^2$	*s.c.c.* (%)	$1/s.c.c.$ (%) $\times 10^2$
16	6.3	17.7 ± 5.6 (13)	5.6
28.5	3.5	23.0 ± 7.5 (14)	4.3
45.6	2.2	27.5 ± 4.7 (13)	3.6
79.8	1.5	34.7 ± 12.0 (14)	2.9
114.0	0.88	35.8 ± 12.6 (14)	2.8

different Na^+ concentrations. The control Ringer's solution was the same as described before and the s.c.c. obtained under these conditions was taken as 100 %. The experimental values of the s.c.c. were again expressed as percentages of the control values Under these experimental conditions Na^+ transport can be described by simple Michaelis-Menten kinetics. $(\Phi\,\%)_{max}$ is only 45.5 % of the total Na^+ transport and K_m is greatly inceased compared with the values obtained in the presence of Cl^- (namely 25.5 as opposed to 4.45 mM). This implies a decrease in the affinity for Na^+ of the carrier mechanism responsible of this part of the Na^+ transport. As the diffusional component disappears in the absence of Cl^- we may assume that this process is probably totally dependent on Cl^-.

DISCUSSION

The model we have chosen to describe our results consists of two kinetically distinct components for the entry of Na^+ into the frog skin and only one pump system. The Na^+ entering the frog skin by any of the two ways, will reach the same transportable pool. The reason why we postulated only one pump system is because we are not aware of any evidence which might force us to accept a more complex model.

The two kinetic components of Na^+ uptake described by the equation mentioned before are not equally dependent of the presence of Cl^- in the Ringer's solution bathing the frog skin preparation. The Michaelis–Menten component is affected by a considerable increase in the K_m in the absence of Cl^- implying that the affinity of a probable carrier for Na^+ is diminished. On other hand, the diffusional component disappears completely in the absence of Cl^- suggesting a straight Cl^- dependence. The relation between these two components and the intercellular or intracellular pathways or any type of parallel intracellular pathway can not yet be defined.

Cereijido *et al.*[6] using similar techniques studied the influence of Na^+ concentration of the Ringer's solution on the s.c.c. and got a saturation component only. They used choline instead of Mg^{2+} for replacing Na^+. As not only the Cl^- Ringer's solution but also those containing SO_4^{2-} used in our experiments contain Mg^{2+}, the disappearance of the linear component can probably be attributed to the absence of Cl^-.

It is not the first time that more than one mechanism has been postulated for the entry of Na^+ into frog skin. Sharp and Leaf[9] described one aldosterone-sensitive and one pitressin-sensitive pathway for the entry of Na^+ in the toad bladder. Herrera

and Curran[3] mentioned two different sites or channels for the action of antidiuretic hormone and Ca^{2+} at the mucosal barrier of the frog skin. One of us[10], by studying the interference of Cu^{2+} and antidiuretic hormone, concluded that these act independently, stimulating the s.c.c. in an additive manner. Finally, Biber and Curran[4], using initial uptake rates of Na^+ across the outer barrier of the frog skin, showed two distinct kinetic components, a saturation component and a linear one. Doubts were raised by the same authors[11] and by Erlij and Smith[12] about the meaning of the linear component because it decreases substantially when mannitol is used as an extracellular marker instead of inulin.

It must be emphasized here that although several authors have already mentioned different entry mechanisms of Na^+ at the outer barrier, they are by no means identical with each other. At the moment we do not have enough evidence to compare our results with any of those described above. The only comparison we can make is with the results of Biber and Curran[4] saying that at least the linear component is different as it also contributes to the s.c.c.

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