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Modulation of sodium-cotransport systems by other ions

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After a brief review of Na^+-cotransport systems which also accept other ions as co-ions or modifiers, modulation of the Na^+-L-glutamate transport system in rabbit renal brush border membranes by K^+ and H^+ is discussed in more detail. Intravesicular K^+ increases the initial uptake rate and electrogenicity of the cotransport. This effect of K^+ is attributed to the formation of a K^+-carrier complex that moves much more rapidly than do the other complexes. The resulting shift in rate limitancy (relative increase in overall rate over the relative increase in rate of step under consideration) from an electroneutral towards a charge-translocating pathway unmasks the electrogenicity of the initial L-glutamate uptake. A positive correlation between relative rate limitancy of the electrogenic pathway and electrogenicity is demonstrated supporting this model. Protons, in addition to acting as co-ions, modify Na^+-glutamate cotransport by increasing both the initial rate and the electrogenicity of uptake. This phenomenon is assumed to represent a transition of the transport system from a carrier-like to an open channel-like translocation mode. Thus, the intrinsic properties of Na^+-cotransport systems may vary under the influence of other ions. This holds true in particular for the electrogenicity of the initial transport rate which may change independently of alterations in charge stoichiometry.

1. Introduction

During the preceding decades the fundamental role of sodium-linked cotransport systems in maintaining the intracellular milieu, volume, and in transepithelial transport processes has been clearly established [1]. Na^+-cotransport systems, thereby, comprise Na^+-symport and Na^+-antiport systems where Na^+ and the cotransportate are translocated in the same or opposite direction, respectively. It has also been found that a variety of Na^+-cotransport systems are affected by other ions. Some of these other ions for example, K^+ [2], are cotranslocated, i.e., they serve as co-ions whereas others act as modifiers, changing particular properties of the Na^+-cotransport system.

In this contribution we shall deal with the Na^+-glutamate cotransport system in which both phenomena can be observed and sometimes even occur concomitantly. The main property of the transporter we shall be concerned with is its apparent electrogenicity which can be altered by the presence of K^+ or H^+. These changes in electrogenicity are explained by models which emphasize, on the one hand, that the apparent electrogenicity of a system at initial uptake rates depends on the relative rate limitancy of the more electrogenic translocation step compared to the less electrogenic or electroneutral transport step. On the other, a transition from a carrier-like to an open channel-like transport system is postulated to occur, which also increases the electrogenicity of the system.

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2. Basic properties of the Na^+-glutamate cotransport system in rabbit renal brush border

As observed by several authors, the uptake of L-glutamate into isolated renal brush border mem-

brane vesicles is markedly stimulated by the presence of Na^+ on the *cis* side of the membrane [3–6]. Since the brush border membrane vesicles are almost exclusively oriented right-side-out [7], in the following, the term *cis* will here always apply to the extravesicular compartment and the designation *trans* to the intravesicular compartment. The stoichiometry of the transport system is unity for L-glutamate and 2 for Na^+ [6]. In addition, a pH gradient directed from the *cis* to *trans* side drives L-glutamate uphill into the vesicles [5], suggesting H^+ symport together with two Na^+ and one glutamate ion. The charge stoichiometry of this translocation would be two positive charges (assuming that there is no cycloport for protons, in which protons would be associated with the carrier during the translocation from both *cis* to *trans* and *trans* to *cis* [8]).

3. Effect of K^+ on Na^+-glutamate cotransport

K^+ present on the *trans* side of a membrane increases the rate of L-glutamate uptake [3,4] and the electrogenicity of the transport [4]. K^+ exerts its effect through antiport during the translocation of the Na^+-H^+-glutamate carrier complex [9], since a *trans* > *cis* gradient of K^+ can support uphill transport of glutamate into the vesicles [3] and tracer equilibrium exchange of glutamate is inhibited by K^+, suggesting the formation of a carrier-K^+ complex [10] that does not accept glutamate. The most striking effect of K^+ is to increase the electrogenicity of glutamate transport despite a decrease in the charge stoichiometry of transport [4]. The charge stoichiometry should decrease from 2 to 1, since one of the positive charges moving from the *cis* to the *trans* side of the membrane is now compensated by the movement of the cation K^+ in the opposite direction.

A possible explanation for this anomalous behaviour is depicted in fig. 1. Here, a model is shown where in the absence of K^+ the translocation rate of the empty carrier is assumed to be much slower than that of the fully loaded substrate-carrier complex, i.e., translocation of the electroneutral empty carrier is rate-limiting for the whole transport process. In the presence of K^+ a

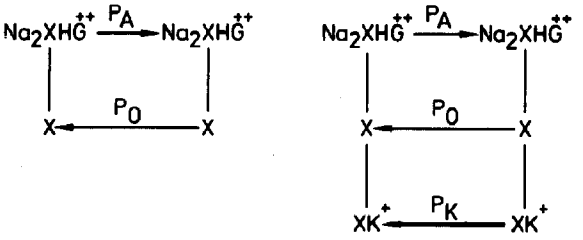

Fig. 1. Simplified model of Na^+-linked glutamate transport without K^+ inside (left) and with K^+ inside (right). P_A, P_O and P_K represent the rate coefficients (probability coefficients) for the fully loaded, empty and K^+-loaded carrier species, respectively. The translocator (X) is assumed to be neutral. (Redrawn from ref. 10.)

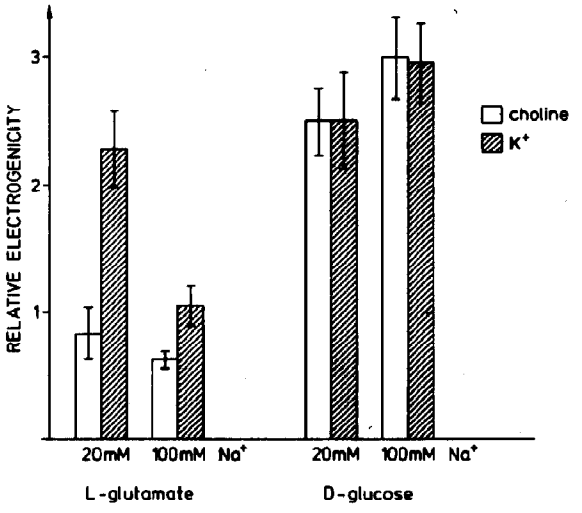

Fig. 2. Effect of changes in extravesicular Na^+ concentration on the relative electrogenicity of glutamate and glucose transport. The relative electrogenicity is defined as

$$\frac{J_2 - J_1}{\epsilon_1 - \epsilon_2} \cdot \frac{1}{J_1}$$

where J_2 denotes the flux in the presence of an $NaNO_3$ gradient and J_1 that in the presence of an Na_2SO_4 gradient. ϵ is a function of the electrical potential difference across the membrane. $\epsilon_1 - \epsilon_2$ was assumed to be unity. In the experiments performed at 20 mM Na^+, Na^+ was replaced by choline. Each column represents the average of 10 independent determinations, with the standard error being indicated by bar. At low outside Na^+ the relative electrogenicity of L-glutamate transport rises, especially in the presence of intravesicular K^+, where the difference is highly significant ($P < 0.01$). (Reprinted from ref. 10 with kind permission.)

new pathway is opened up by which the carrier returns as the K^+-carrier complex to the *cis* side of the membrane. The translocation of this complex is assumed to be much faster than that of the empty carrier and even more rapid than that of the electrogenic fully loaded substrate-carrier complex. Thus, the electrogenicity of a cotransport system is revealed only to the extent that the electrosensitive step becomes predominantly rate-limiting. In our system, such a change in rate limitancy was brought about in two ways: either by accelerating the substrate-free step (K^+ experiment), or by retarding the substrate-bearing one (see below). It can be seen that the rate limitancy of any step is not constant, but may vary according to the experimental conditions.

In order to test the hypothesis that the electrogenicity of Na^+-glutamate cotransport is positively correlated to the relative rate limitancy of the translocation of the fully loaded substrate-carrier complex, we reduced the rate of translocation by decreasing the Na^+ concentration at the *cis* side. As depicted in fig. 2 at a lower Na^+ concentration – which leads to a decrease in the initial uptake rate by 75% – the relative electrogenicity of Na^+-glutamate cotransport in the presence of K^+ increases dramatically. It should be noted that the effect is much smaller in the absence of K^+, since under these conditions, according to our model, the relative rate limitancy of the fully loaded substrate-carrier complex is much lower. Fig. 2 also demonstrates that the Na^+-glucose cotransport system, which is not affected by K^+, behaves differently. There is no increase in relative electrogenicity, indicating that the effects observed with glutamate require the interaction between K^+ and the Na^+-glutamate cotransport system. In fig. 3 the individual experiments in the investigation have been plotted. A positive correlation between rate limitancy and electrogenicity is evident. In other words, at low values of transport rates the electrogenicity of Na^+-glutamate cotransport is much more pronounced than at high values. This phenomenon can explain some of the discrepancies found in the literature concerning the electrogenicity of Na^+-glutamate transport in the presence of K^+ at the *trans* side [3,4].

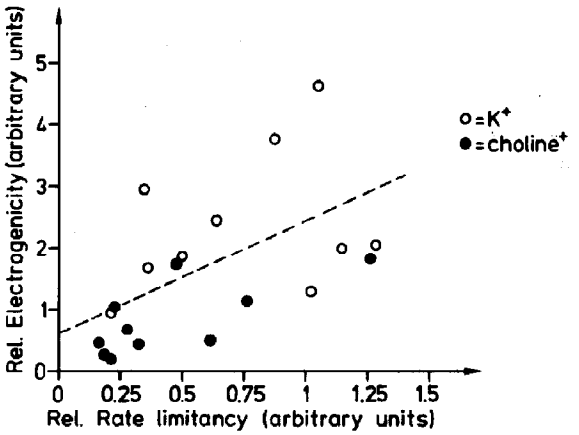

Fig. 3. Correlation between relative electrogenicity of transport and relative rate limitancy of the electrogenic $2Na^+$-H^+-glutamate translocation. The relative electrogenicity is plotted vs. the corresponding rate limitancy for a total of 20 independent experimental determinations. Relative rate limitancy is defined as

$$\frac{J_2 - J_1}{A_2 - A_1} \cdot \frac{A_1}{J_1}$$

where J_2 represents the flux at substrate concentration A_2 and J_1 that at substrate concentration A_1. The broken line was calculated by the method of least squares. Its slope is significantly different from 0 ($P = 0.01$). (Reprinted from ref. 10 with kind permission.)

4. Effect of pH on Na^+-glutamate cotransport

In addition to acting as a cotransportate, H^+ also exerts a modifying effect on Na^+-glutamate cotransport system when present at the *cis* side. As shown in fig. 4, acidification of the extravesicular medium leads to a marked increase in initial uptake rate. This effect cannot be attributed to an increase in the driving force for the transport due to the establishment of an H^+ gradient across the membrane, since it is also observed, although to a slightly lesser extent, when the pH at the *cis* and *trans* sides is set to 5.4. It should be noted that, quite contrary to the relationship stated above, acidification also leads to an increase rather than a decrease in relative electrogenicity of the Na^+-glutamate cotransport system. Furthermore,

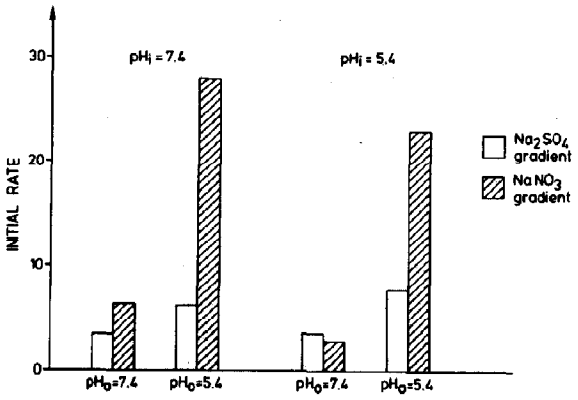

Fig. 4. Effect of extravesicular and intravesicular pH on initial uptake rate and electrogenicity of Na^+-glutamate cotransport. Data were obtained essentially as described in ref. 10 and are representative experiments.

acidification at the *cis* side markedly reduces the stimulatory action of *trans* K^+ on the transport system (data not shown).

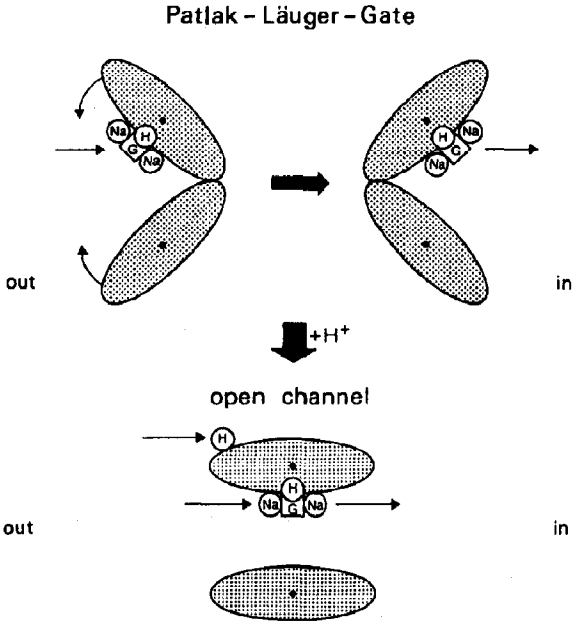

Fig. 5. Simplified model for the modification of the translocation mode by extravesicular H^+. Occupancy of a modifier site by H^+ changes the Na^+-glutamate cotransport system from a more carrier-like system (upper panel) to an open channel-like system (lower panel).

A model which could explain all these phenomena is illustrated in fig. 5. It assumes that the Na^+-glutamate cotransport system possesses at the *cis* side of the membrane a modifier site which, when occupied by a proton, alters the mode of operation of the transport system. In the absence of the proton the system behaves more like a carrier represented in fig. 5 as a Patlak-Läuger gate, whereas in its presence, it behaves like an open channel. Open channel properties for Na^+-glutamate cotransport have also been postulated by Berteloot [11] for rabbit intestinal brush borders. Such a transition would explain the increased initial transport rate, increased electrogenicity and decreased sensitivity towards K^+. Indeed, computer simulation of such a phenomenon has yielded results very similar to those obtained experimentally (E. Heinz et al., manuscript in preparation).

5. Conclusion

The above serves to demonstrate that Na^+-cotransport systems are not rigid, but may vary their intrinsic properties under the influence of other ions. For instance, the electrogenicity of Na^+-glutamate cotransport is strongly increased by *trans* K^+ or *cis* H^+, in the first case in spite of a decrease in charge stoichiometry, in the second presumably by changing the mode of translocation. As electrogenicity may thus change independently of the charge stoichiometry, conclusions concerning charge stoichiometry drawn from measurements on electrogenicity alone are of doubtful value (see also ref. 12).

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