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Energetics of renal Na^+ and H^+/L-alanine co-transport systems

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The stoichiometric properties of Na^+- and H^+-dependent L-alanine transporters recently identified in luminal-membrane vesicles prepared from proximal convoluted tubules (pars convoluta) and proximal straight tubules (pars recta) of rabbit kidney were studied. We provide indirect evidence suggesting that one Na^+ and one H^+ ion are co-transported with the L-alanine molecule via Na^+-dependent and H^+-dependent transport systems located in vesicles from pars convoluta. Furthermore, our experimental data suggest that both the high-affinity and the low-affinity Na^+-dependent L-alanine transport systems of pars recta vesicles operate with a 1:1 stoichiometry.

INTRODUCTION

Renal transport of a number of important metabolites has been shown to occur via Na^+-coupled co-transport at the luminal membrane of proximal tubules (for a review see Murer & Gmaj, 1986). In previous publications (Jørgensen & Sheikh, 1987; Vorum *et al.*, 1988; Jessen *et al.*, 1988) we have demonstrated the existence of such transport systems for L- and D-alanine in luminal-membrane vesicles isolated from pars convoluta and pars recta of proximal tubules. In these studies we have also reported the presence of a novel proton-dependent transport system for the renal re-absorption of both isomers of this amino acid in membrane vesicles from pars convoluta (Jørgensen & Sheikh, 1987; Vorum *et al.*, 1988; Jessen *et al.*, 1988). However, determinations of the stoichiometric coupling ratio between flows of cations and alanine have not been reported, though they are of considerable interest because this information provides a basis for understanding the energetic cost of the re-absorption process. We now describe the effect of Na^+ and H^+ ions on L-alanine transport under well-defined experimental conditions and report the results of a measurement of the coupling ratio of these cations to the transport of L-alanine.

EXPERIMENTAL

Preparation of luminal-membrane vesicles

Luminal-membrane vesicles were isolated from pars convoluta ('outer cortex') and from pars recta ('outer medulla') of the proximal tubules of rabbit kidney according to the methods already described (Kragh-Hansen *et al.*, 1984, 1985; Sheikh & Møller, 1987) and mentioned here only briefly. The renal tissue was homogenized and luminal-membrane vesicles were prepared by differential centrifugation and Ca^{2+} precipitation. The purity of the membrane-vesicle preparation with regard to the content of luminal vesicles was examined by electron microscopy (Kragh-Hansen *et al.*, 1985) and by measuring specific activities of various enzyme markers (Sheikh *et al.*, 1982). The amount of protein was determined by the method of Lowry *et al.* (1951) modified as described by Peterson (1977), with serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) as standard. All solutions used in this study were sterilized before use. The possible bacterial contamination of membrane-vesicle preparations was examined by incubating samples on blood/agar plates and by electron microscopy. No bacteria were found in these preparations.

Uptake of L-alanine by membrane vesicles

The uptake of L-alanine by luminal-membrane vesicles was studied by Millipore filtration (Hopfer *et al.*, 1973). The details of the individual experiments are given in legends to Figures. Unless otherwise stated the vesicles were suspended in 10 mM-Hepes/Tris buffer, pH 7.4, containing 100 mM-KSCN, 700 mM-mannitol and valinomycin in a concentration of 12.5 μg/mg of protein (added as a stock solution of 25 mg/ml in ethanol). As shown by Turner & Moran (1982*a*), 100 mM-KSCN equilibrium with this concentration of valinomycin is sufficient to short-circuit transmembrane electrical potential differences.

RESULTS AND DISCUSSION

Effect of Na^+ and H^+ on L-alanine transport in vesicles from pars convoluta

We used the 'activation method' (Turner & Moran, 1982*b*) to determine the cation/L-alanine stoichiometry of the pars convoluta L-alanine transporter. In this method one measures the stimulation of substrate (L-alanine) flux by increasing concentrations of activator (Na^+ or H^+). Fig. 1 shows the results of a representative experiment where the initial flux of 0.15 mM-L-alanine was measured as a function of Na^+ concentration over the range 0–200 mM. The experiment was carried out at pH 7.4 ($pH_{in} = pH_{out}$) under zero trans Na^+ and L-alanine concentrations (i.e. initial intravesicular concentrations of Na^+ and L-alanine equal to zero). The values given in Fig. 1(*a*) are the Na^+-dependent component of flux (i.e. L-alanine uptake in the presence of Na^+ minus the flux observed in its absence). It appears from the Figure that L-alanine flux shows a Michaelis–Menten-type dependence on Na^+ concentration. In Fig. 1(*b*) the data have been analysed by using the following equation (Turner & Moran, 1982*c*):

$$\text{Flux} = V_{max.}[Na^+]^n/(K_{0.5}{}^n + [Na^+]^n)$$

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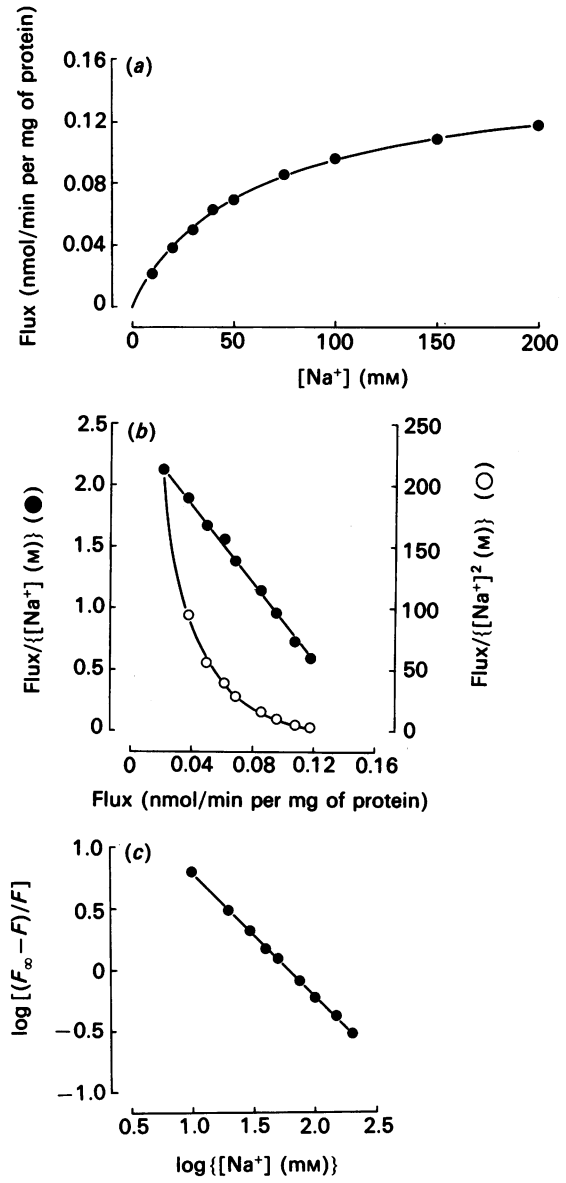


Fig. 1. Effect of Na^+ concentration on the uptake of L-alanine by luminal-membrane vesicles from pars convoluta

The incubation media contained 10 mM-Hepes/Tris buffer, pH 7.4, 300 mM-mannitol, 0.1 mM-L-[^{14}C]alanine, 100 mM-KSCN and various concentrations of NaCl ranging from 0 to 200 mM (final concentration). Choline replaced Na^+ iso-osmotically to obtain the various Na^+ concentrations studied. (*a*) Plot of flux versus Na^+ concentration. (*b*) Plots of flux/$[Na^+]^n$ versus flux for $n = 1$ (●) and $n = 2$ (○). The units of $[Na^+]$ are M. (*c*) Double-logarithmic (Hill-type) plot. F is the uptake at a given Na^+ concentration and F_∞ is the maximal uptake at saturating Na^+ concentration [the intercept of the regression line with the abscissa in (*b*)].

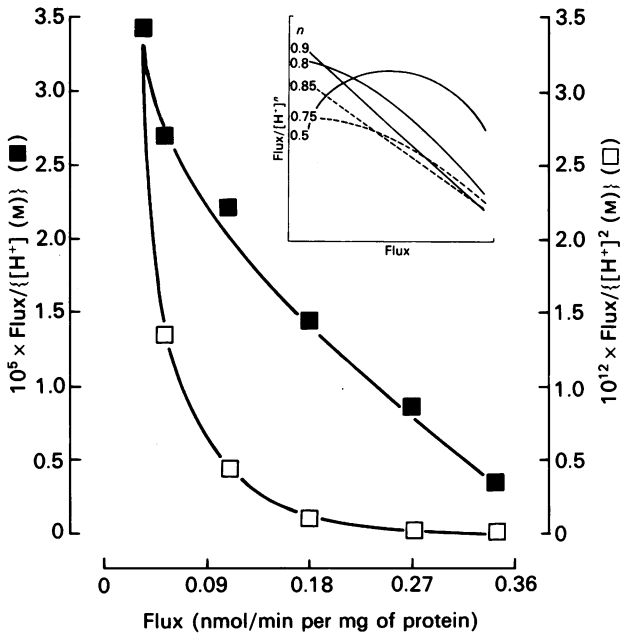


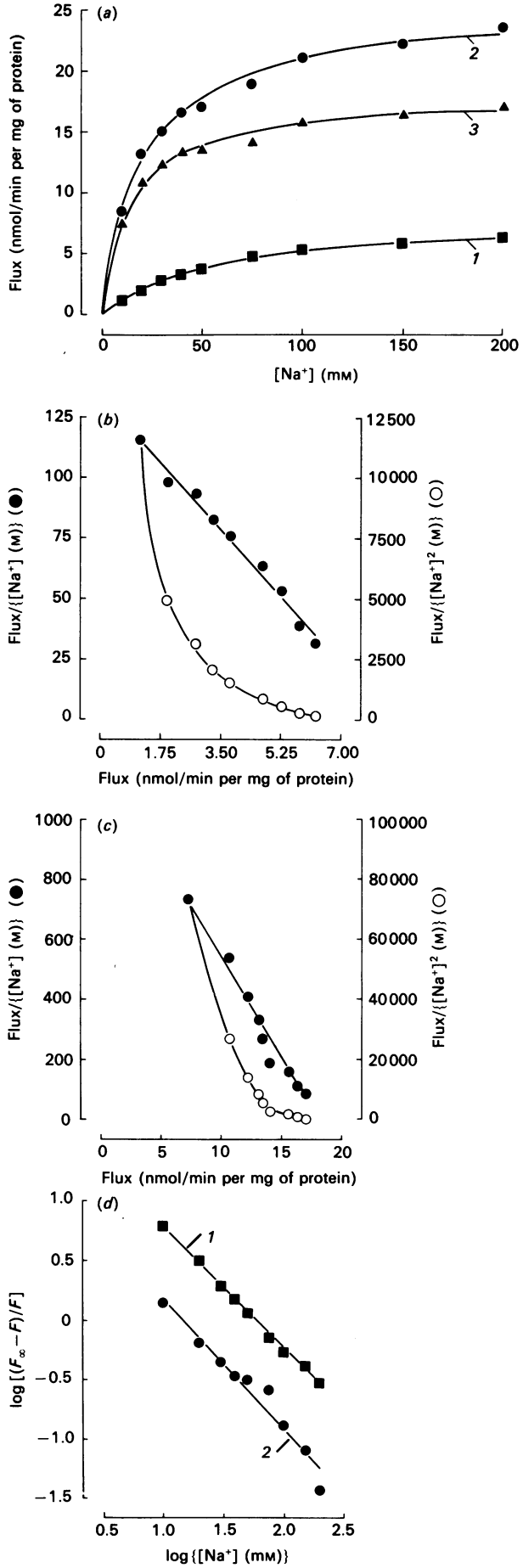
Fig. 2. Effect of H^+ gradient (extravesicular > intravesicular) on the uptake of L-alanine by luminal-membrane vesicles from pars convoluta

The vesicles were preincubated with 155 mM-KCl in 15 mM-Hepes/Tris buffer, pH 7.5. The incubation media contained 0.15 mM-L-[^{14}C]alanine and 155 mM-KCl in various Hepes/Tris or Mes/Tris buffer solutions with pH ranging from 7.5 to 5.0. The quantities plotted in the Figure are the H^+-gradient-dependent component of flux, i.e. all uptake values were corrected for the uptake in the absence of H^+ gradient ($pH_{out} = pH_{in} = 7.5$). Plots of flux/$[H^+]$ versus flux for $n = 1$ (■) and $n = 2$ (□). The inset shows a series of plots of flux/$[H^+]^n$ against flux for different values of n ranging from 0.5 to 0.9.

The equation assumes the existence of n essential co-operative Na^+ sites per L-alanine site. According to this equation a plot of flux/$[Na^+]^n$ against flux for the correct value of n will yield a straight line with slope $1/K_{0.5}{}^n$. Fig. 1(*b*) shows two such plots for the data of Fig. 1(*a*), assuming n values of 1 and 2. Visual inspection of Fig. 1(*b*) indicates that an n value of 1 provides the best fit to these data. The experimental data are re-analysed in Fig. 1(*c*) on a double-logarithmic (Hill-type) plot. The numerical value of the slope of the least-squares fit to these points that gives the Na^+/L-alanine stoichiometry is 1.01 ± 0.07, again indicating a 1:1 interaction.

As pointed out by Turner & Moran (1982*b*), the activation method, however, does not distinguish between 'energetic' and 'catalytic' coupling mechanisms. In other words, the results presented in Fig. 1 suggest that one Na^+ ion is involved per L-alanine transport event, but it does not indicate whether the stimulation produced by Na^+ is a result of its being co-transported with L-alanine (energetic coupling) or its interacting with the carrier in some other way which leads to a enhancement of L-alanine flux without concomitant Na^+ transport (catalytic coupling). However, our previous studies on the characteristics of L-alanine transport in vesicles from pars convoluta showed that both the Na^+- and H^+-gradient-dependent transport of this amino acid are electrogenic processes (Jørgensen & Sheikh, 1987). Therefore it is reasonable to conclude that one Na^+ and one H^+ ion are co-transported with the L-alanine molecule via Na^+-dependent and H^+-dependent transport systems respectively.

Fig. 2 shows the results of representative experiments in which the effect of H^+ gradient alone on the uptake of



L-alanine was examined. These experiments were performed in the absence of Na^+ ions (Na^+ is replaced by K^+), and the initial flux of 0.15 mM-L-alanine was measured in the presence of different magnitudes of H^+ gradient (extravesicular > intravesicular). The quantity plotted in the Figure is the H^+-gradient-dependent component of flux. It is seen that assuming $n = 1$ a slightly curved line relationship between flux/$[H^+]^n$ against flux is obtained. To examine whether more than one molecule of L-alanine is transported together with one H^+ ion, a detailed analysis of the experimental data is performed. The inset in Fig. 2 shows a series of plots assuming values of n ranging from 0.5 to 0.9. A closer examination of these curves indicates that a value of n of 0.9 provided the best fit to these data, suggesting that one H^+ ion is involved in the translocation of L-alanine in these vesicle preparations. The distinction between $n = 1$ and $n = 0.9$ may simply reflect uncertainty in the basal L-alanine uptake.

Stoichiometry of Na^+-dependent L-alanine transport in vesicles from pars recta

In previous papers (Jørgensen & Sheikh, 1987; Vorum *et al.*, 1988) we indicated the presence of two electrogenic and Na^+-requiring systems for the transport of L-alanine in luminal-membrane vesicles from pars recta. In this section we determine the stoichiometric coupling ratio of Na^+ to the high-affinity (half-saturation approx. 0.14 mM) and low-affinity (half-saturation approx. 9.6 mM) L-alanine-transport systems located in this region of the nephron. Fig. 3 shows the results of a representative experiment designed on the same principle as the one shown in Fig. 1. To study the coupling ratio of Na^+ to both the high-affinity and low-affinity L-alanine transporters, we measured the initial flux of 0.15 mM- (curve *1* of Fig. 3*a*) and 5 mM-L-alanine (curve *2* of Fig. 3*a*) at various Na^+ concentrations in the medium. It appears from the Figure that the apparent K_m for the overall transport of L-alanine with respect to Na^+ depends on the L-alanine concentration. However, it is reasonable to assume that the initial-flux measurements of radioactive L-alanine carried out at 0.15 mM concentration in the medium represent the transport of this amino acid mediated predominantly by a high-affinity system, whereas the influx values obtained at 5 mM-L-alanine concentration may represent both the high-affinity and low-affinity transport of this compound. An attempt is made to assess the low-affinity component of L-alanine trans-

Fig. 3. Effect of Na^+ concentration on uptake of L-alanine by luminal-membrane vesicles from pars recta

The composition of the incubation media was the same as in the legend to Fig. 1 except that the uptake of L-alanine was studied at two different concentrations of L-alanine. (*a*) Plots of flux versus Na^+ concentration for 0.15 mM-L-alanine [curve *1* (■)] or for 5 mM-L-alanine [curve *2* (●)]. Curve *3* (▲) shows the difference between flux at 5 mM- and 0.15 mM-L-alanine (i.e. curve *2* minus curve *1*). (*b*) Plots of flux/$[Na^+]^n$ versus flux for $n = 1$ (●) and $n = 2$ (○) for the high-affinity system (0.15 mM). (*c*) Plots of flux/$[Na^+]^n$ versus flux for $n = 1$ (●) and $n = 2$ (○) for the low-affinity system (5 mM minus 0.15 mM). (*d*) Double-logarithmic (Hill-type) plots. Curve *1* (■), the high-affinity system; curve *2* (●), the low-affinity system.

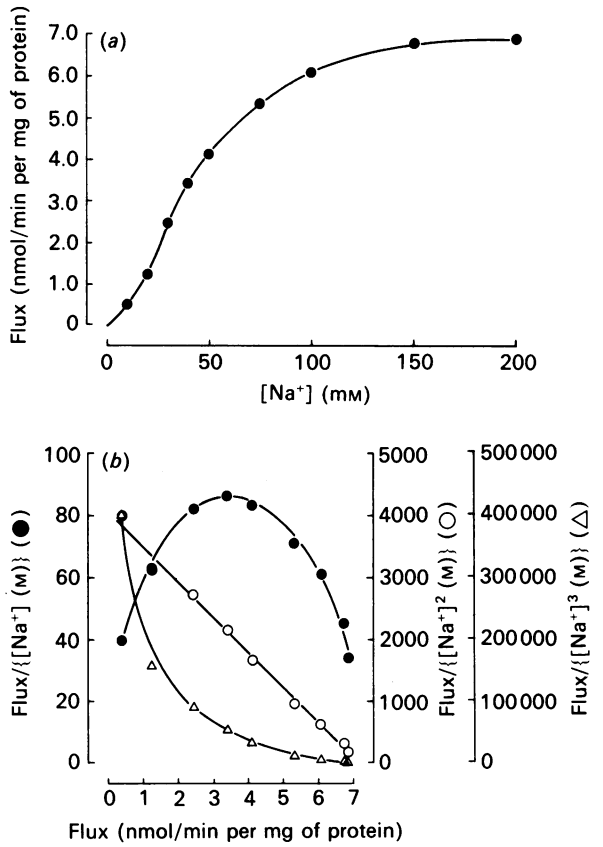


Fig. 4. Na^+-dependent D-glucose flux as a function of Na^+ concentration in luminal-membrane vesicles from pars recta

The incubation media contained 10 mM-Hepes/Tris buffer, pH 7.4, 0.3 mM-D-[^{14}C]glucose, 100 mM-KSCN and various concentrations of NaCl ranging from 0 to 200 mM (final concentration). Choline replaced Na^+ iso-osmotically to obtain the various Na^+ concentrations studied. (*a*) Plot of flux versus Na^+ concentration. (*b*) Plots of flux/$[Na^+]^n$ versus flux for $n = 1$ (●), $n = 2$ (○) and $n = 3$ (△). The units of $[Na^+]$ are M.

port by subtracting the activity of the high-affinity system from the total Na^+-dependent transport activity (curve *3* = curve *2* minus curve *1*) at different concentrations of Na^+. It is apparent that L-alanine flux shows a Michaelis–Menten-type dependence on Na^+ concentration. The values plotted in the Figure are the Na^+-dependent component of flux. It appears from Figs. 3(*b*) and 3(*c*) that assuming $n = 1$ a straight-line relationship between flux/$(Na^+]^n$ against flux is obtained at both low and high L-alanine concentration, suggesting that the Na^+/L-alanine ratio probably is 1:1 for both the high-affinity and low-affinity transport systems. The data are re-analysed in Fig. 3(*d*) on a double logarithmic (Hill-type) plot, which also indicates 1:1 Na^+/L-alanine interaction.

For the sake of comparison initial flux of D-glucose as a function of Na^+ concentration was also measured in luminal-membrane vesicles from pars recta and from pars convoluta. Fig. 4(*a*) shows that increasing the concentration of NaCl in the extravesicular medium produced a sigmoid stimulation in the rate of D-glucose transport in vesicles from pars recta. This sigmoid relationship suggests that more than one Na^+ ion is associated with the transport process. Fig. 4(*b*) shows a series of plots for the data of Fig. 4(*a*) assuming *n* values of 1, 2 and 3. A closer examination of Fig. 4(*b*) indicates that an *n* value of 2 provides the best fit to these data, strongly suggesting that the Na^+/D-glucose coupling ratio is 2:1 in vesicles from pars recta. Similar experimental results (not shown) with vesicles from pars convoluta showed Na^+/D-glucose stoichiometry 1:1. These observations are in agreement with the findings obtained by Turner & Moran (1982*c*).

In conclusion, the results presented in this paper show that one Na^+ and one H^+ ion are involved in the electrogenic transport of L-alanine in vesicles from pars convoluta. In vesicles from pars recta both the high-affinity and low-affinity Na^+-dependent L-alanine transport systems operate with 1:1 stoichiometry. The experimental data on D-glucose transport in these vesicle preparations indicate that these two vesicle preparations are not significantly cross-contaminated and further support the findings described in this paper.

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