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SODIUM DEPENDENCE OF NEUTRAL AMINO ACID UPTAKE INTO RABBIT ILEUM

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Summary

Alanine uses two mediated pathways to enter the rabbit ileal mucosa. Present results suggest that one of them (K_m = 4.1 mM) is fully dependent on sodium in the mucosal medium, while the other (K_m = 91 mM) is sodium-independent. Similar results are obtained for methionine and serine. Reinterpretation of previous alanine/sodium coupling coefficients suggests that two sodium ions per alanine molecule are transported via the high affinity system.

Amino acid transport across the mammalian small intestine is dependent upon sodium. Detailed studies on the mucosal uptake of alanine and other neutral amino acids in rabbit ileum [1—3] led to the following suggestions:

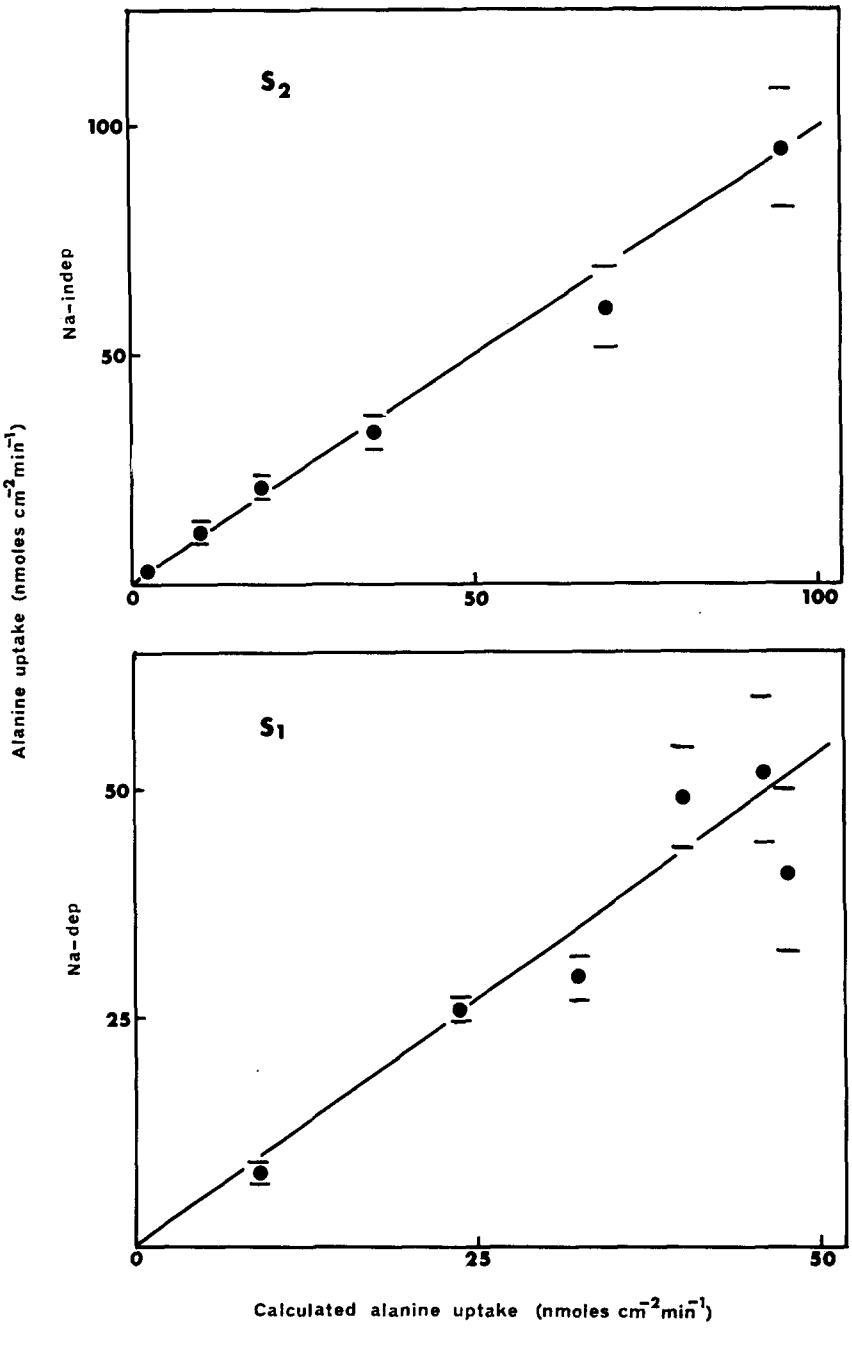
(1) uptake of neutral amino acids is mediated by a single transport system;

(2) this transport system operates in the presence or absence of sodium;

(3) coupling between alanine and sodium influxes ranges from zero, in the absence of sodium, to one for sodium concentrations over 100 mM.

It has, however, recently been shown that at least two transport mechanisms for neutral amino acids exist in the rabbit ileum [4]. One of these systems (system one) has a high affinity for several neutral amino acids but a low transport capacity, whereas the second mechanism (system 2) has a low affinity but its transport capacity is high [5]. We have now examined the sodium dependence of these transport systems and re-evaluated previous

Fig. 1. Correlation between experimentally determined and predicted uptakes of alanine in rabbit ileal mucosa. The total uptake of alanine, determined for six different alanine concentrations, was measured in the presence and absence of sodium, as previously described [1, 4]. The mucosal surface was incubated for 45 s with a solution containing L-[^{14}C]alanine and [^{3}H]polyethyleneglycol (PEG 900), after a 10 min preincubation in Krebs-Henseleit buffer (143 mM Na^+ or Na^+-free). Tritiated PEG 900 was used as an extracellular space marker. Incubation was terminated by washing with isotonic ice-



cold mannitol, the tissue being assayed for radioactivity after disruption in 0.1 N HNO_3. The sodium-dependent and sodium-independent uptake values are plotted against the system 1 and 2 uptakes respectively (S_1, S_2), calculated using previously determined values of K_m and J_{max} [5]. Choline was used to substitute for sodium. Concentrations of alanine varied from 1 to 51 mM; mannitol was added to maintain tonicity constant. Values give means ± S.E. of 12 to 16 paired comparisons. The lines are obtained by least squares linear regression analysis.

data on the coupling between alanine and sodium fluxes.

Alanine uptake was measured in the same piece of ileum in the presence and absence of sodium at alanine concentrations ranging from 1 to 51 mM. The apparent affinity constants and maximal uptake rates (K_m and J_{max}) had been determined previously for alanine entry into rabbit ileal mucosa in the presence of 143 mM sodium (K_m values of 4.6 and 91 mM; J_{max} values of 52 and 247 nmol · cm^{-2} · min^{-1} for systems 1 and 2, respectively) [5]. From this one can calculate, for each concentration of alanine used, the amount of amino acid entering the mucosa through each system. These uptakes have been compared with experimentally determined values in Fig. 1.

The sodium-dependent uptake of alanine, calculated by subtraction of alanine uptake measured in the absence of sodium from that measured in the presence of 143 mM sodium, for six different alanine concentrations, showed a one to one correlation with the amount of alanine entry calculated to take place through system 1 (slope 0.99 ± 0.08; $r = 0.99$). There was no such correlation between the sodium-dependent and system 2 uptakes of alanine. The sodium-independent uptake of alanine showed a one to one correlation with the amount of alanine entry calculated to take place through system 2 (slope 1.06 ± 0.20; $r = 0.94$). There was no such correlation between the sodium-independent and the system 1 uptake of alanine. These results suggest that the sodium dependency of alanine uptake is confined to system 1.

An independent assessment of this suggestion can be obtained using the kinetic constants (given in the legend to Fig. 2) describing the interaction between two other neutral amino acids, methionine and serine, with transport system 2 [5]. If the hypothesis is correct these constants should predict the concentration dependence of uptake under sodium-free conditions. Fig. 2 shows the uptake of methionine (Fig. 2a) and that of serine (Fig. 2b) at various amino acid concentrations, in the absence of sodium. The experimental points are compared with predicted system 2 uptake lines. The agreement between prediction and experimental finding is good, thus supporting the conclusion that sodium dependence of system 1 is absolute while system 2 is sodium-independent.

A coupling coefficient between alanine and sodium entry into rabbit ileal mucosa has been determined previously from the slope of a line relating sodium uptake to total alanine uptake at 140 mM sodium and varying concentrations of alanine [1]. In this analysis it was assumed that all of alanine uptake was sodium dependent. A coupling coefficient of 0.96 was calculated over a limited range of alanine concentrations. We can now recalculate this coupling ratio using our values for system 1 entry rather than the values for total uptake. The results obtained are shown in Fig. 3. The points can be considered to lie on a straight line of slope 1.84 ± 0.11 ($r = 0.99$). This ratio can be further approximated to a value of 2.

We would like to suggest on the basis of these and earlier results: (1) that alanine entry into rabbit ileal mucosa takes place through two mechanisms [4, 5]; (2) that both mechanisms operate in the presence of sodium but only system 2 operates in the absence of sodium; (3) that the stoichiometry of this transport system is such that two sodium ions enter for each alanine molecule, at a sodium concentration of 143 mM.

The strongest argument against such a model is the previous finding that the maximum velocity (V) for alanine uptake remained constant, whether or not sodium was present [1]. The answer to this apparent paradox probably lies in the low capacity of this transport system. The V of system 1 represents only about 20% of the capacity of system 2 and both estimates are associated with a standard error of about ± 18%. This means that the V for system 1 lies within one standard error of the V for system 2. It would not be surprising if the

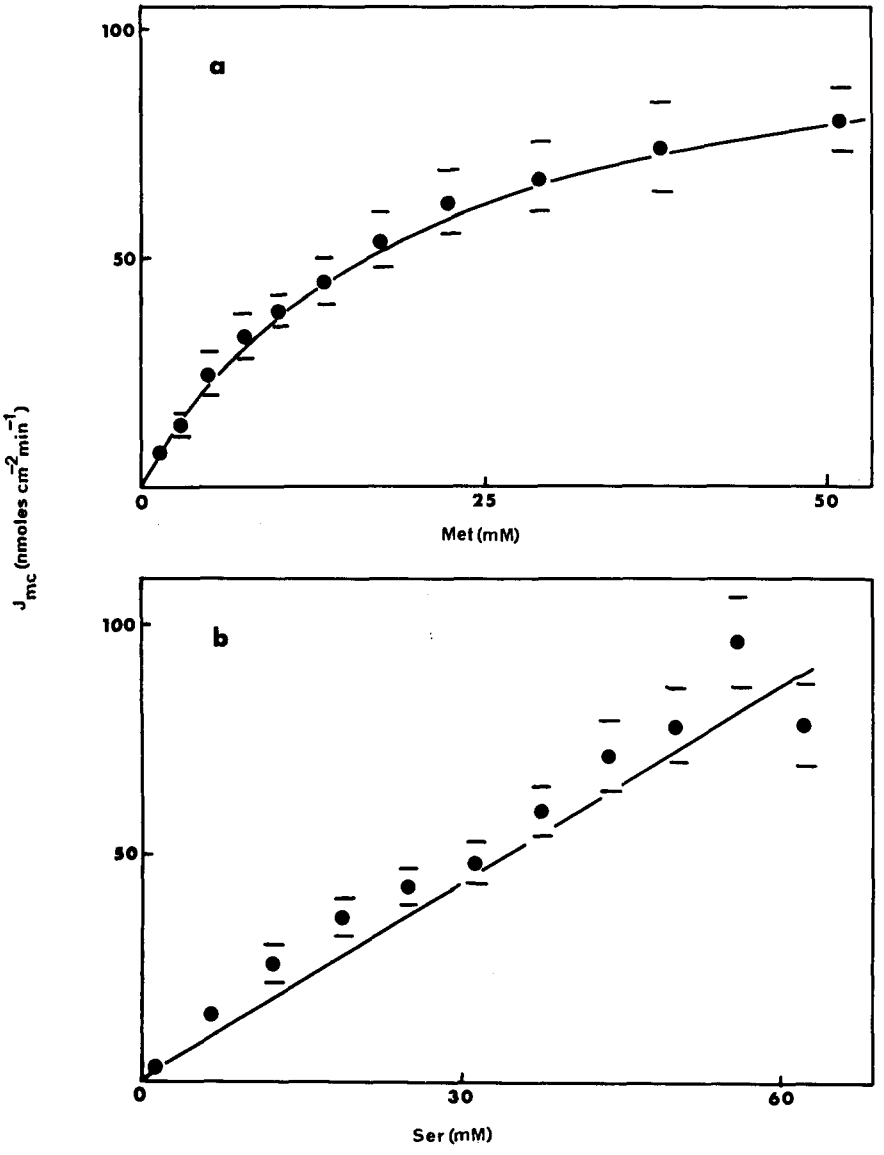


Fig. 2. Concentration dependence of methionine (a) and serine (b) uptake by rabbit ileal mucosa in the absence of sodium. Uptake (J_{mc}) of amino acids was measured over a 45 s period as described in the legend to Fig. 1, from solutions in which all sodium had been replaced by choline. Each point gives the mean ± S.E. of 8 (Met) and 15 (Ser) estimations. The lines give transport system 2 uptake calculated from previously derived constants: (K_m values 22 and 2931 mM; J_{max} values 115 and 4090 nmol · cm^{-2} · min^{-1}, for methionine and serine, respectively [5].

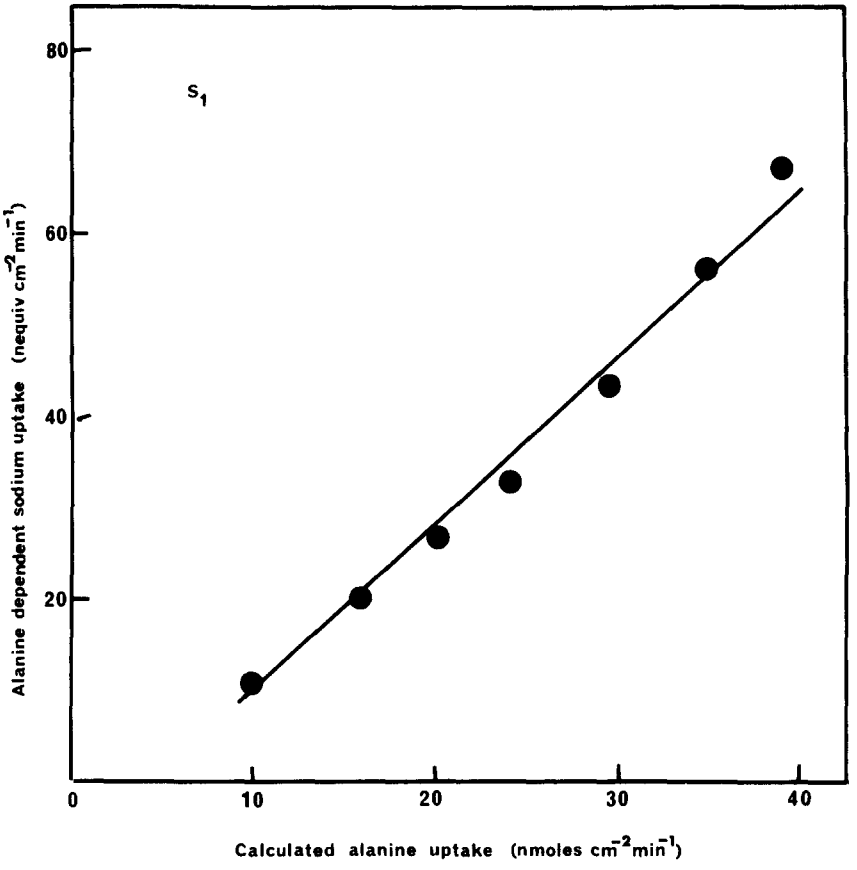


Fig. 3. Relation between alanine-dependent sodium influx and the system 1 uptake of alanine. Values of alanine-dependent sodium flux have been taken from previously published results [1] and the amount of alanine entry by system 1 calculated using constants described in the text. The line of best fit has a slope of 1.84 ± 0.11. This value is only approximate because of the error involved in measuring alanine-dependent sodium influx in the presence of a large alanine-independent uptake of sodium.

V for system 1 remained undetected under these circumstances.

The present model is very similar to that proposed previously to describe the sodium-dependent entry of glycine into pigeon red blood cells [6, 7]. It remains to be seen whether similar coupling coefficients are found for other neutral amino acids in rabbit ileum.

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