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# $K^+$- AND $Na^+$-GRADIENT-DEPENDENT TRANSPORT OF L-PHENYLALANINE BY MOUSE INTESTINAL BRUSH BORDER MEMBRANE VESICLES

A. BERTELOOT *, A.H. KHAN and K. RAMASWAMY **

*University of South Carolina School of Medicine, Department of Medicine, Division of Gastroenterology, Veterans Administration Hospital, Columbia, SC 29208 (U.S.A.)*

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**In the presence of an $Na^+$- or a $K^+$-gradient (outside>inside), L-phenylalanine uptake exhibited an overshoot phenomenon indicating active transport. The amplitudes of the overshoots were increased by increasing either $Na^+$ or $K^+$ concentrations in the incubation media, indicating that binding alone cannot account for the $K^+$ effect. The $K^+$-induced overshoot is not due to the presence of a membrane potential alone, as a gradient of choline chloride failed to produce it. $Li^+$ could also substitute for $Na^+$ though less potent than $Na^+$ in inducing an overshoot. Uptake of L-leucine also showed $Na^+$- and $K^+$-effects and L-leucine and L-alanine could inhibit the $Na^+$- and $K^+$-overshoots obtained with phenylalanine. These results lead us to postulate the presence of a carrier for neutral amino acids dependent on monovalent cation with higher affinity for $Na^+$ in mouse intestine. The $Na^+$- and $K^+$-driven active transport of L-phenylalanine were shown to be dependent on the presence of a membrane potential, as short-circuiting the membrane with FCCP reduced the amplitude of the overshoots seen with both ions. However, substitution of $Cl^-$ by more lipophilic anions ($NO_3^-$, $SCN^-$) produced an inhibition of uptake. A preliminary analysis of the interrelations between $Na^+$ and $K^+$ for L-phenylalanine uptake showed complex interactions which can be best explained by mutual competition for a common carrier at both sides of the membrane. These results suggest the presence of a new transport system or a variant of an ASC-type system for L-phenylalanine (and neutral amino acids) in the mouse intestine. However, our studies do not rule out the possible involvement of more than one system for neutral amino acid uptake.**

## Introduction

Various earlier studies using isolated intestine [1], jejunal sacs [2], intestinal rings [3], short-circuited segments [4], isolated strips [5], and isolated brush border membrane vesicles [6–9], have firmly established that active transport of neutral amino acids by the small intestine occurs in response to an $Na^+$ gradient. In particular, studies using brush border membrane vesicles have unequivocally demonstrated that intestinal transport of amino acids conforms to the predictions of the gradient hypothesis [10–12]. We have recently shown that L-leucine [13], L-phenylalanine [14], and glycine [15] were transported is response to an $Na^+$ gradient by mouse intestinal brush border membrane vesicles. However, it was also shown that in the case of L-phenylalanine [14], a $K^+$ gradient (outside > inside) could elicit active transport of this

* Present address: Department of Physiology, University of Montreal, Montreal, PQ, Canada H3C 3T8.
** To whom all correspondence should be addressed.

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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amino acid. The present study was therefore undertaken in order to fully confirm the presence of this unusual component and to further elucidate some aspects of this new observation.

## Materials and Methods

*(1) Preparation of brush border membrane vesicles*

Control mice (strain C57-BL/Ks J-db/m) were killed by decapitation. The whole intestine was then removed, rinsed with cold NaCl (0.9%), and the mucosa was scraped with a spatula. Brush border membranes were purified by the calcium chloride precipitation method of Schmitz et al. [16] and brush border membrane vesicles were obtained by Hopfer's method [17] as described previously [13].

*(2) Transport studies*

The purified brush border membrane vesicles were resuspended to a final protein concentration of 5–15 mg/ml with 10 mM Tris-Hepes buffer (pH 7.5), 300 mM mannitol and 0.1 mM $MgSO_4$. Incubation media contained, in a 250 $\mu$l final volume: 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 300 mM mannitol or 100 mM mannitol plus 100 mM of the sodium or potassium salts used or choline chloride (150 mM mannitol + 50 mM $Na_2SO_4$ or $K_2SO_4$ when these salts have been used), and 0.01, 0.1 or 1 mM L-phenylalanine with 1.28 $\mu$Ci L-[U-$^{14}$C]phenylalanine (Amersham, spec. act. 513 mCi/mmol). For transport studies with leucine, we used 1.16 $\mu$Ci of L-[U-$^{14}$C]leucine (NEN, spec. act. 344 mCi/mmol) corresponding to a concentration of 0.0135 mM. When salt or substrate concentrations were varied or when the effect of various compounds on the uptake of L-phenylalanine was studied, the osmolarity of the buffer was adjusted to 300 mM by appropriately varying the concentration of mannitol or choline chloride. Transport studies were initiated by the addition of 250–750 $\mu$l of brush border membrane vesicles and were conducted at room temperature. At time intervals, 50 $\mu$g of the reaction mixture, (50–150 $\mu$g protein) were mixed with 1 ml of cold stop solution containing 10 mM Tris-Hepes buffer (pH 7.5), 115 mM mannitol, 0.1 mM $MgSO_4$, 100 mM of either NaCl, KCl or choline chloride depending on the cation present in the incubation medium and 0.66 $\mu$Ci of D-[1(n)-$^3$H]mannitol (NEN, spec. act. 22.4 Ci/mmol). The resulting mixtures were filtered through 0.45 $\mu$m Sartorius filters and washed with 4 ml of nonradioactive stop solution at 4°C. Filters were then processed for counting as previously described [13].

In the experiments using FCCP to shunt the membrane potential [18], the resuspended vesicles were divided in two equal aliquots to which 5 $\mu$l of ethanol (1% final concentration), or FCCP dissolved in ethanol (156 $\mu$M final concentration) were added. After 10 min incubation at room temperature, transport studies were conducted as described above in incubation media containing 1% ethanol (control) or 156 $\mu$M FCCP.

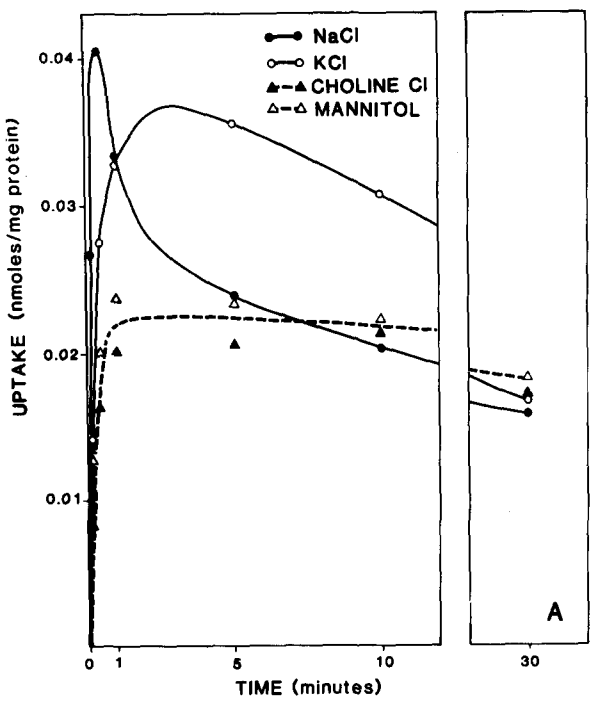
In the experiments describing the interactions between $Na^+$ and $K^+$ on phenylalanine transport, brush border membrane vesicles were resuspended in three equal aliquots with 10 mM Tris-Hepes (pH 7.5), 0.1 mM $MgSO_4$, 300 mM mannitol and either 100 mM choline chloride or KCl or NaCl. After 30 min equilibration at room temperature, transport studies were initiated by the addition of aliquots of each population of vesicles (900 $\mu$g protein) into media containing, in a 250 $\mu$l final volume: 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM mannitol, 0.01 mM phenylalanine (1.28 $\mu$Ci), and either 200 mM choline chloride, or 100 mM choline chloride plus 100 mM NaCl, or 100 mM choline chloride plus 100 mM KCl or 100 mM NaCl plus 100 mM KCl. Transport studies were then conducted as described above.

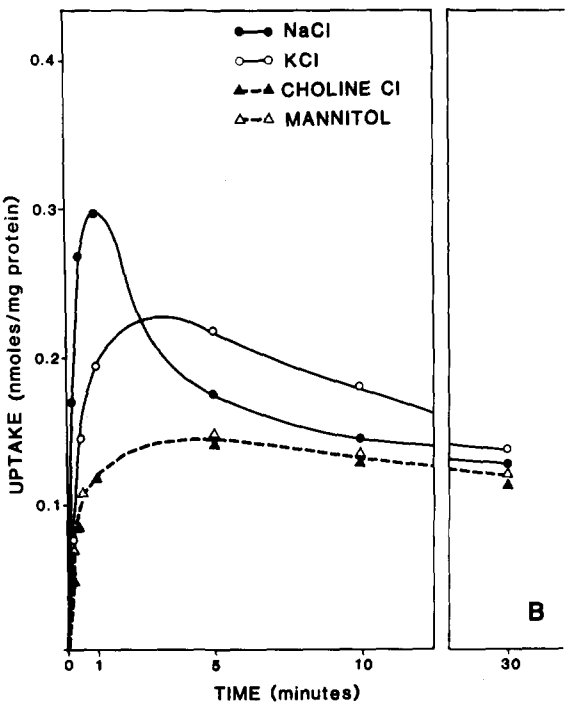
Protein was assayed by the method of Lowry et al. [19] using bovine serum albumin as standard.

## Results

*(1) Time-course of gradient-dependent L-phenylalanine uptake*

The uptake of L-phenylalanine by the membrane vesicles as a function of incubation time is shown in Fig. 1 for 3 different concentrations of the amino acid. At the lowest concentration used, 0.01 mM (Fig. 1A), the presence of an $Na^+$-gradient (outside > inside) stimulated L-phenylalanine uptake as compared to the uptake seen with a choline$^+$ gradient or in the absence of salts. The uptake reached a maximum level at 30 s and



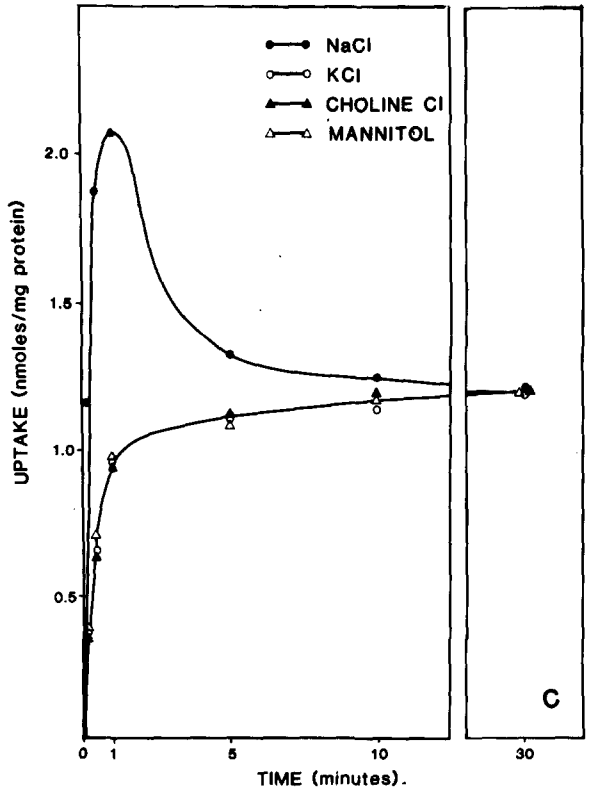




Fig. 1. Time-course of gradient-dependent uptake of L-phenylalanine. Vesicles were resuspended in 10 mM Tris-Hepes buffer (pH 7.5), 300 mM mannitol and 0.1 mM $MgSO_4$ and incubated in media containing 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 0.01 mM (A), 0.1 mM (B) or 1 mM (C) L-phenylalanine, and either 300 mM mannitol (△), 100 mM mannitol + 100 mM choline chloride (▲), 100 mM mannitol + 100 mM NaCl (●) or 100 mM mannitol + 100 mM KCl (○). Values represent the mean for two to five different preparations of vesicles with duplicate assays at 0.15 and 0.45 min.

then decreased with time to the steady-state level observed at 1 min in the presence of choline or mannitol. The presence of a $K^+$-gradient (outside > inside) also elicited an overshoot of L-phenylalanine uptake that occurred later in time (between 1 and 5 min of incubation) but with an amplitude close to that seen with the $Na^+$ gradient (1.97 as compared to 2.25). At the intermediate concentration of 0.1 mM (Fig. 1B), essentially similar results were observed with maximum overshoot values at 1 min for $Na^+$-gradient and between 1 and 5 min for $K^+$-gradient conditions. However, the relative amplitude of the maximum uptake value obtained in the presence of $K^+$ was significantly lower as compared to $Na^+$ (1.68 versus 2.37, respectively). Finally, at the higher concentration of 1 mM, the overshoot phenome-

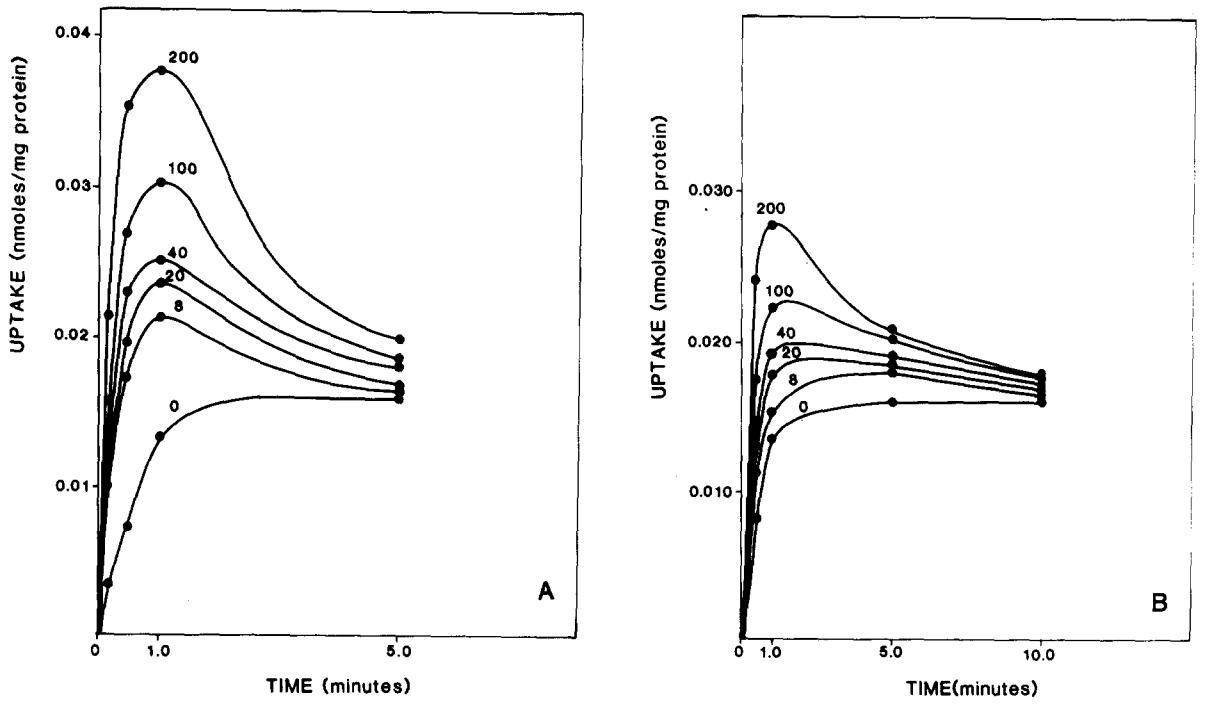



Fig. 2. Effect of salt concentrations on $Na^+$-dependent (A) and $K^+$-dependent (B) uptake of L-phenylalanine. Vesicles were resuspended in 10 mM Tris-Hepes buffer (pH 7.5), 300 mM mannitol and 0.1 mM $MgSO_4$ and incubated in media containing 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 0.01 mM L-phenylalanine and 200 mM total salt concentrations. Variable $Na^+$ and $K^+$ concentrations from 0 to 200 mM were obtained by substitution with choline chloride. Final $Na^+$ and $K^+$ concentrations are shown on the top of each curve.

non was observed only in the presence of $Na^+$ with a relative amplitude of 1.73 after 1 min incubation. We have already shown that uptake occurred into an osmotically reactive intravesicular space [14,15], so that the overshoots seen in the presence of $Na^+$ or $K^+$ represent active transport of L-phenylalanine by the vesicles resulting from the presence of transient electrochemical gradients formed by the NaCl and KCl gradients. Also, the superpositions of the choline and mannitol curves rule out the effect of $Cl^-$-generated membrane potential alone as an explanation for the overshoots seen in the presence of $K^+$. Further, increasing $Na^+$ and $K^+$ concentrations (Fig. 2) in the incubation media showed that the amplitude of the overshoots was increased with both $Na^+$ (Fig. 2A) and $K^+$ (Fig. 2B). The relations between intial rates of uptake (0.15 min for $Na^+$ and 0.5 min for $K^+$) and salt concentrations are depicted in Fig. 3. For both $Na^+$ and $K^+$, there was a rapid increase in initial rates of uptake for salt con-

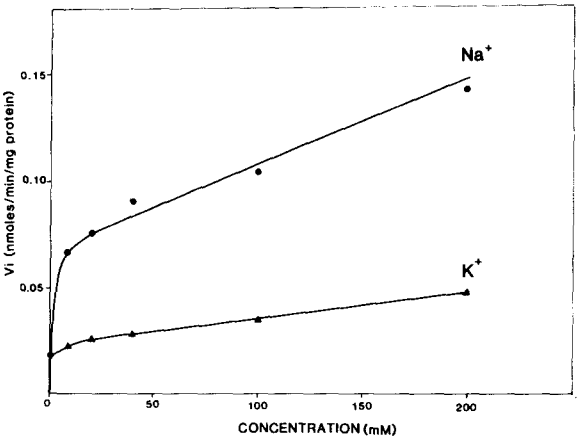



Fig. 3. Effect of $Na^+$ and $K^+$ concentrations on initial rates of L-phenylalanine uptake. Experimental conditions were as described in the legend of Fig. 2. Initial rates of uptake were taken at 0.15 min and 0.5 min for $Na^+$ and $K^+$, respectively, and transformed to nmol/min per mg protein for purpose of comparison.

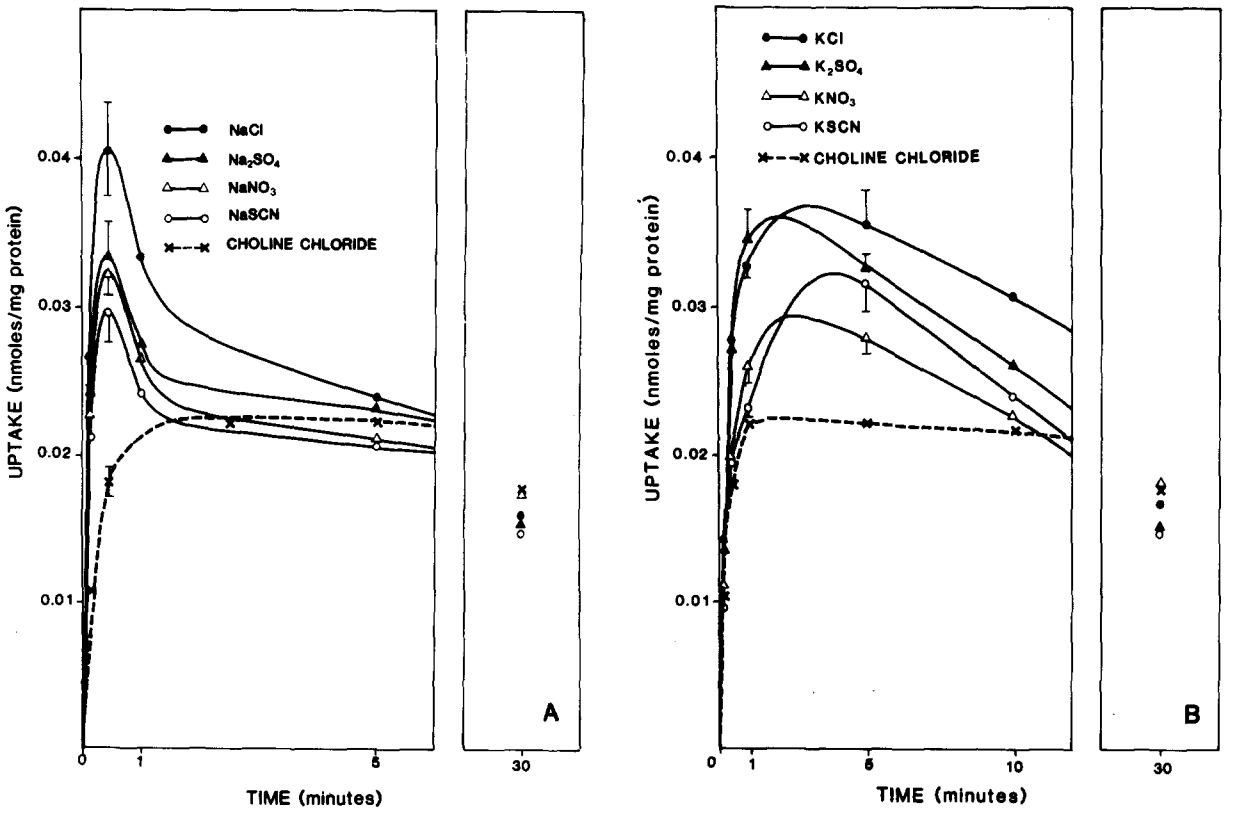



Fig. 4. Effect of anions on $Na^+$-dependent (A) and $K^+$-dependent (B) L-phenylalanine uptake. Experimental conditions were as described in the legend of Fig. 1 at 0.01 mM phenylalanine concentration with 100 mM NaCl or KCl (●), 100 mM $NaNO_3$ or $KNO_3$ (△), 100 mM NaSCN or KSCN (○), 100 mM choline chloride (×) and 50 mM $Na_2SO_4$ or $K_2SO_4$ + 150 mM mannitol (▲). Values represent the mean ± 1 S.E. for two to four different preparations of vesicles with duplicate assays at 0.15 and 0.45 min.

centrations varying between 0 and 20 mM. The initial rates then become linearly related to salt concentrations up to 200 mM.

*(2) Effect of anions on gradient-dependent L-phenylalanine uptake*

The effect of various anions on gradient-dependent uptake of 0.01 mM L-phenylalanine is shown in Fig. 4. In the presence of a sodium-gradient (Fig. 4A), the anions increased the amplitude of the overshoots and the following order was observed: $SCN^- \leq NO_3^- \leq SO_4^{2-} < Cl^-$. A similar effect was also found in the presence of a $K^+$-gradient (Fig. 4B), the order now being $NO_3^- \leq SCN^- \leq SO_4^{2-} \leq Cl^-$. It should be pointed out that this anion effect is different from what would be predicted from the order of diffusibility of these ions and the resultant amplitude of the membrane potential ($SO_4^{2-} < Cl^- < NO_3^- < SCN^-$) [20]. The above results, therefore, do not lead to any definite conclusion on the sensitivity of L-phenylalanine uptake to membrane potential.

*(3) Membrane potential-dependence of L-phenylalanine uptake*

The membrane potential dependence of L-phenylalanine uptake was tested by measuring the effect of short-circuiting the membrane potential with FCCP [18,37] and the results are shown in Fig. 5. In the presence of both $Na^+$- and $K^+$-gradients, the addition of FCCP decreased the amplitude of the overshoots seen in its absence. As FCCP minimizes the influence of anion diffusion potentials on voltage-sensitive transport, these results show that L-phenylalanine uptake is sensitive to the membrane potential.

*(4) Effect of lithium on L-phenylalanine transport*

The effect of substitution of NaCl by the same

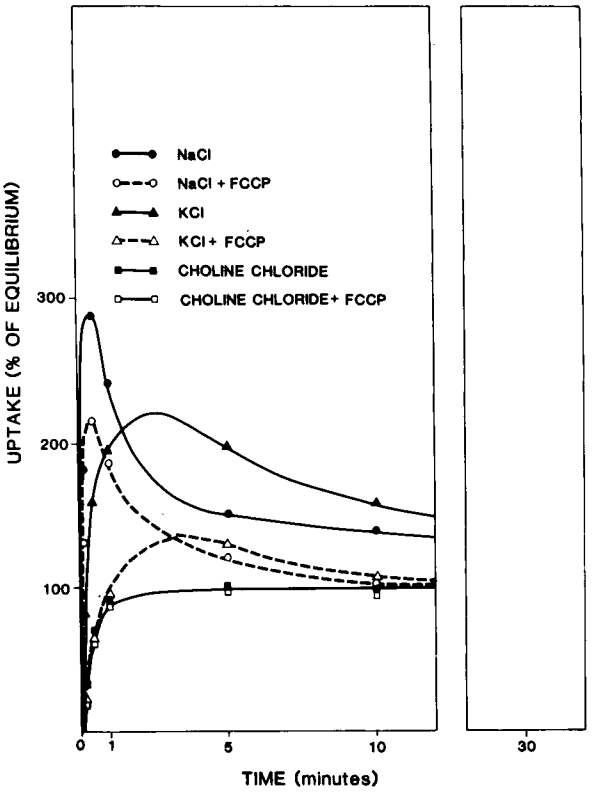



Fig. 5. Effect of short-circuiting the membrane potential by FCCP on L-phenylalanine uptake. Vesicles were resuspended in 50 mM Tris-Hepes buffer (pH 7.5), 300 mM mannitol and 0.1 mM $MgSO_4$ and incubated for 10 min at room temperature with 1% ethanol (full lines) or 156 $\mu$M FCCP dissolved in ethanol (dashed lines). Transport studies were initiated by the addition of vesicles to media containing 50 mM Tris-Hepes buffer (pH 7.5), 100 mM mannitol, 0.1 mM $MgSO_4$, 0.01 mM L-phenylalanine, 1% ethanol (closed symbols) or 156 $\mu$M FCCP (open symbols) and either 100 mM NaCl (●,○), 100 mM KCl (▲,△) or 100 mM choline chloride (■,□).

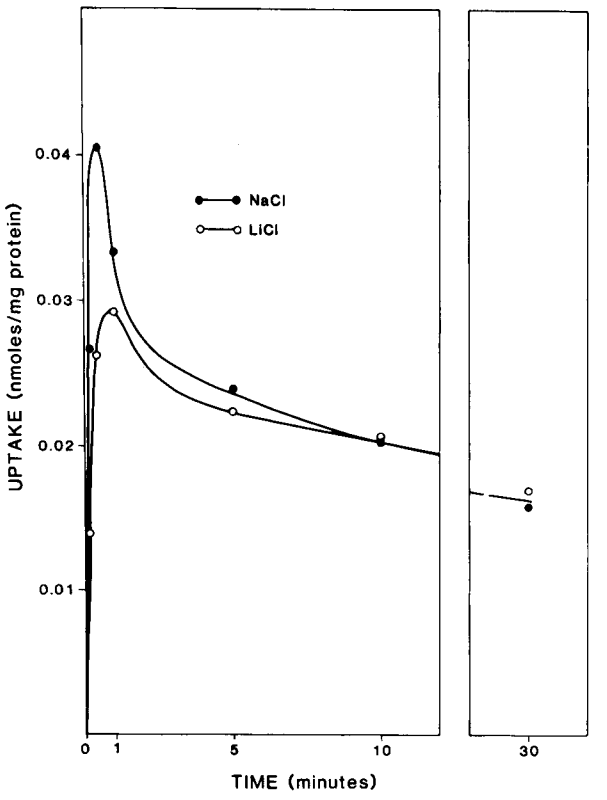
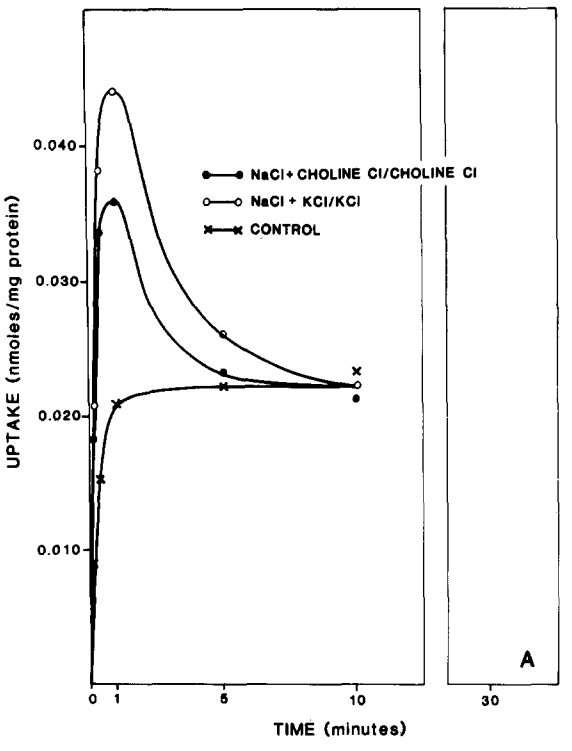


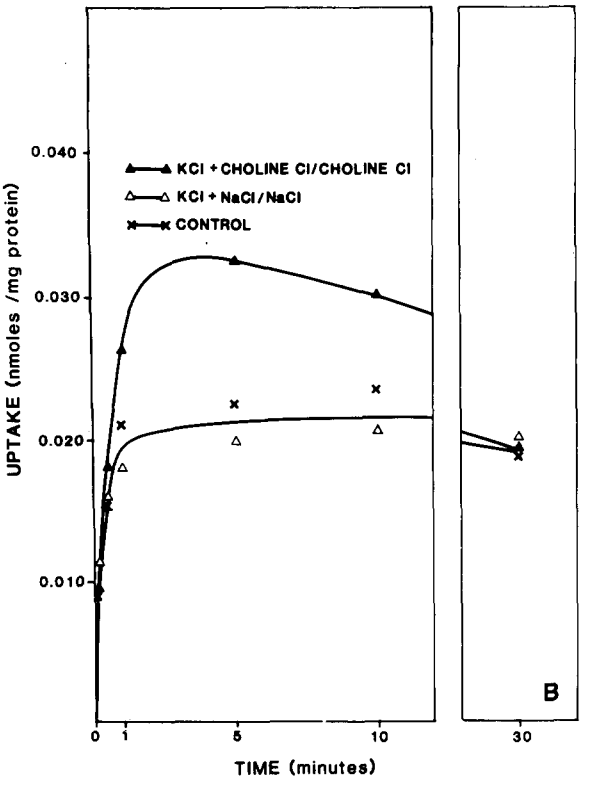

Fig. 6. Effect of sodium replacement by lithium on L-phenylalanine uptake. Experimental conditions were as described in the legend of Fig. 1 with an incubation medium containing 0.01 mM L-phenylalanine and either 100 mM NaCl (●) or 100 mM LiCl (○).

concentration of LiCl showed that $Li^+$ was also able to induce active transport of L-phenylalanine but that this cation was less potent than $Na^+$ in producing an overshoot (Fig. 6).

*(5) Interactions between $Na^+$ and $K^+$ on L-phenylalanine uptake*

From the above results, it appears that both $Na^+$- and $K^+$-gradients may elicit active transport of L-phenylalanine. Further studies were carried out to define the interactions between these two ions when they are both present in the incubation medium on the same or opposite sides of the membrane. Vesicles from the same preparation were loaded with either choline chloride, NaCl or KCl and then incubated with different combinations of these ions as described under Materials and Methods. The three controls (200 mM choline chloride outside/100 mM choline chloride inside, 100 mM KCl + 100 mM choline chloride outside/100 mM KCl inside, and 100 mM NaCl + 100 mM choline chloride outside/100 mM NaCl inside) gave uptake curves which were not significantly different from each other and the results were, therefore, pooled together (control curve in Figs. 7A, 7B, and 7C). However, the initial velocities of uptake were slightly different in these three conditions with values of $0.0078 \pm 0.0007$ nmol/mg protein per 0.15 min (200 mM choline chloride outside/100 mM choline chloride inside), $0.0087 \pm 0.0018$ nmol/mg protein per 0.15 min



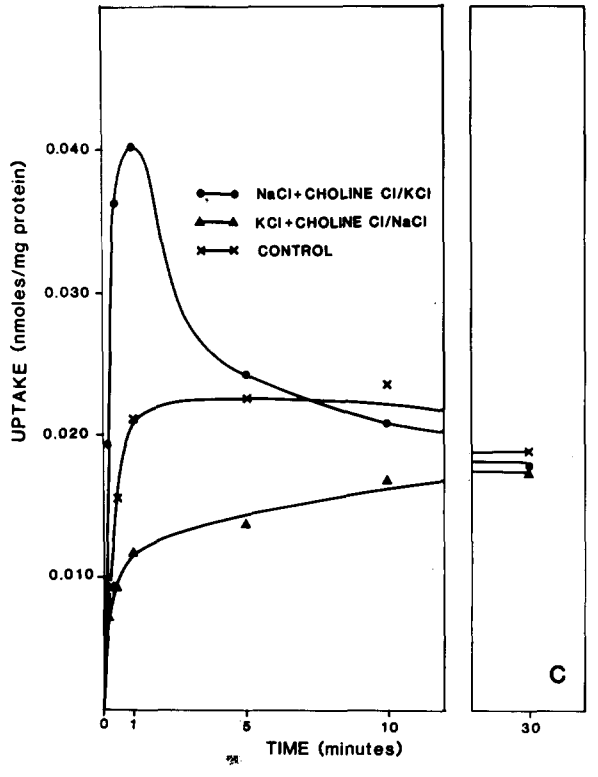




Fig. 7. Interactions between $Na^+$ and $K^+$ for L-phenylalanine uptake. Experimental conditions were as described under Materials and Methods. (A) Effect of $K^+$ on $Na^+$-gradient-dependent phenylalanine transport. (B) Effect of $Na^+$ on $K^+$-gradient-dependent phenylalanine transport. (C) Effect of opposite $Na^+$ and $K^+$ gradients on L-phenylalanine transport. The salt concentrations inside and outside the vesicles are respectively shown on the right and left-hand sides of the figures. The control curves are as discussed in the text.

(100 mM KCl + 100 mM choline chloride outside/100 mM KCl inside) and $0.0109 \pm 0.0010$ nmol/mg protein per 0.15 min (100 mM NaCl + 100 mM choline chloride outside/100 mM NaCl inside). The above increasing order was identical with what has been observed in gradient conditions. Moreover, the absence of an overshoot when $Na^+$ and $K^+$ were equilibrated on both sides of the membrane clearly showed that the effects of $Na^+$ and $K^+$ are abolished when the driving force was shunted. Fig. 7A shows the effect of $K^+$ on $Na^+$-gradient transport of L-phenylalanine. The previously observed overshoot in the presence of an $Na^+$-gradient was still obtained in these new conditions (100 mM NaCl + 100 mM choline chlo-

ride outside/100 mM choline chloride inside in Fig. 7A). However, when $K^+$ is present on both sides of the membrane, there was an increase in the amplitude of this overshoot (100 mM NaCl + 100 mM KCl outside/100 mM KCl inside in Fig. 7A). Fig. 7B shows the effect of $Na^+$ on $K^+$-gradient dependent transport of L-phenylalanine. In contrast to the above result, the presence of $Na^+$ (100 mM NaCl + 100 mM KCl outside/100 mM NaCl insdie in Fig. 7B) suppressed the overshoot seen in the presence of a $K^+$-gradient alone (100 mM KCl + 100 mM choline chloride outside/100 mM choline chloride inside in Fig. 7B) and the uptake curve was identical with the control curve. Finally, Fig. 7C shows the effect of opposite $Na^+$- and $K^+$ gradients on L-phenylalanine uptake. With $Na^+$ outside and $K^+$ inside (100 mM NaCl + 100 mM choline chloride outside/100 mM KCl in Fig. 7C), the overshoot seen in the presence of $Na^+$ alone was increased (compare with 100 mM NaCl + 100 mM choline chloride outside/100 mM choline chloride inside in Fig. 7A). However, with $K^+$ outside and $Na^+$ inside (100 mM KCl + 100 mM choline chloride outside/100 mM NaCl inside in Fig. 7C), not only the overshoot seen in the presence of $K^+$ alone was abolished (100 mM KCl + 100 mM choline chloride outside/100 mM choline chloride inside in Fig. 7A) but, also, the equilibrium value was reached more slowly than with the controls.

*(6) Specificity of $K^+$ effect*

In view of the above results, it would be interesting to test if active transport elicited by $K^+$ gradient is restricted to L-phenylalanine or of more

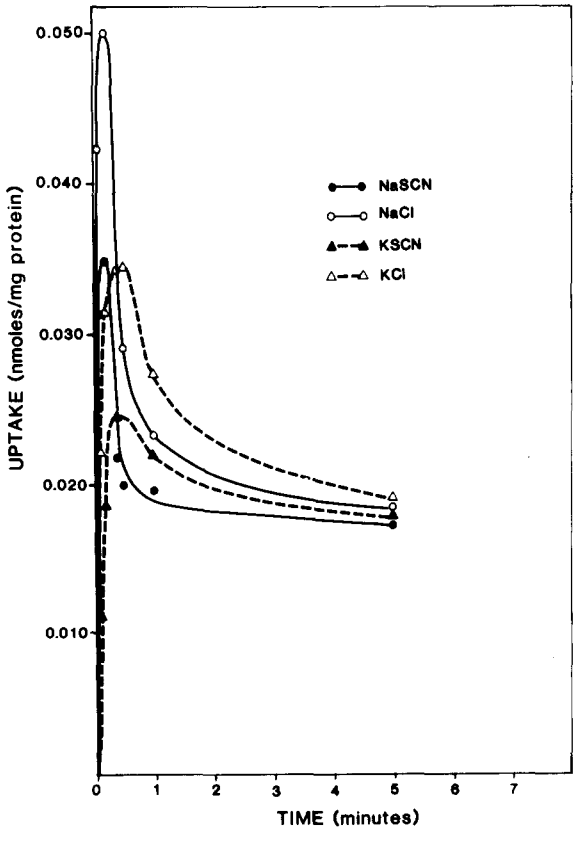



Fig. 8. Effect of salt gradients on L-leucine uptake. Experimental conditions were as described in the legend of Fig. 1 with an incubation medium containing 0.0135 mM L-leucine and either 100 mM NaSCN (●), NaCl (○), KSCN (▲), or KCl (△).

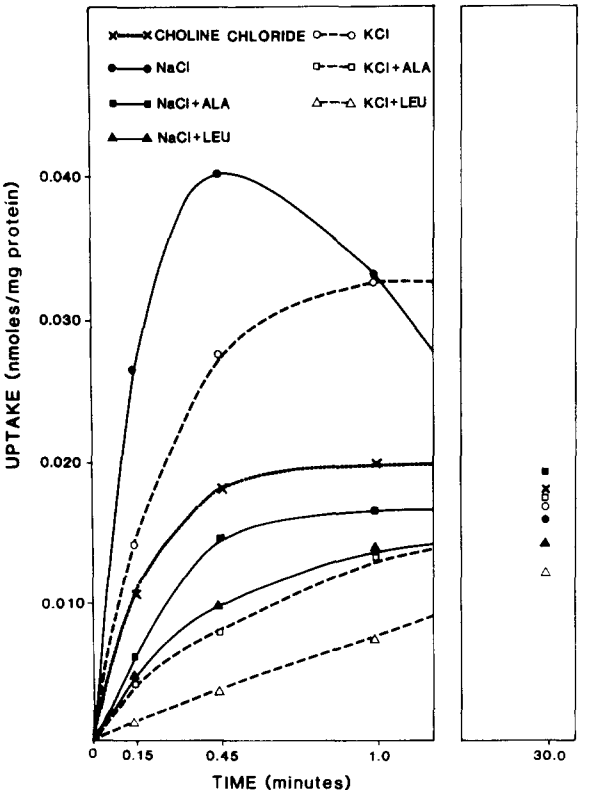



Fig. 9. Inhibition of $Na^+$ and $K^+$ gradient-dependent active transport of L-phenylalanine by L-leucine and L-alanine. Experimental conditions were as described in the legend of Fig. 1 with incubation media containing 0.01 mM L-phenylalanine and 100 mM NaCl (○)+either 10 mM alanine (■) or leucine (▲), 100 mM KCl (○)+either 10 mM alanine (□) or leucine (△) and 100 mM choline chloride (×).

general significance. In a previous report [13], we have shown that L-leucine and D-glucose were not sensitive to a $K^+$ gradient at the concentration of 1 mM chosen for these experiments, as it is the case with L-phenylalanine (Fig. 1C). We then tested uptake of L-leucine and D-glucose at very low concentrations (0.0135 mM and 0.041 mM, respectively). It can be seen from Fig. 8 that uptake of L-leucine was sensitive to both $Na^+$- and $K^+$-gradients in these conditions and that $SCN^-$ inhibited both $Na^+$- and $K^+$-induced overshoot to some extent, similar to its effect on L-phenylalanine. However, glucose transport was insensitive to $K^+$-gradient and the replacement of $Cl^-$ by $SCN^-$ increased the amplitude of the $Na^+$-overshoot (results not shown). Finally, both L-leucine and L-alanine at a concentration of 10 mM inhibited the overshoots obtained with phenylalanine in the presence of NaCl and KCl gradients and L-leucine inhibited uptake more than L-alanine (Fig. 9). It also has to be noted that L-leucine and L-alanine inhibited L-phenylalanine uptake observed in the absence of salts and that inhibitions were stronger in the presence of $K^+$ than in the presence of $Na^+$.

## Discussion

This paper describes some properties of L-phenylalanine transport in brush border membrane vesicles of the mouse intestine. In the presence of an $Na^+$- or a $K^+$-gradient (outside > inside), L-phenylalanine uptake showed an overshoot phenomenon indicating active $Na^+$ and $K^+$ gradient-dependent transport of L-phenylalanine (Fig. 1). This conclusion is strengthened by the observation that shunting of the driving forces by equilibration of $Na^+$ and $K^+$ on both sides of the membrane abolished the overshoot phenomenon (Fig. 7, control curves). Though evidence for $Na^+$-dependent uptake of amino acids by intestinal brush border membrane vesicles has been well documented [6–8,13,14], this is the first time that evidence for $K^+$-dependent uptake of neutral amino acids is presented for mammalian intestine. However, $K^+$-dependent L-phenylalanine uptake has recently been reported in membrane vesicles isolated from the midgut of *Chilosamia cynthia* larvae [21] and a new, previously uncharacterized monovalent cation-dependent amino acid carrier was described in murine L1210 leukemia cells [22], which showed high-affinity for amino acids such as leucine and phenylalanine and tolerated substitution of lithium and potassium for sodium ions. It is noteworthy that the same characteristics apply in mouse enterocytes, as we showed that both leucine (Fig. 8) and phenylalanine (Fig. 1) were actively transported in the presence of $Na^+$ or $K^+$ gradients and that $Li^+$ could also elicit active transport of L-phenylalanine (Fig. 6). Our results also showed that the $K^+$-dependent transport of L-phenylalanine cannot be explained due to the action of a membrane potential alone as choline chloride failed to elicit increased uptake as compared to mannitol (Fig. 1). Also, the effect of a $K^+$ gradient on L-phenylalanine transport cannot be an artefact of the vesicle preparation as D-glucose uptake at tracer concentrations failed to show the $K^+$ effect (results not shown). The presence of a monovalent cation-dependent carrier specific for neutral amino acids with higher affinity for $Na^+$ must therefore be postulated. This system appears to be shared at least by L-phenylalanine, L-leucine and L-alanine, as L-phenylalanine and L-leucine showed similar $Na^+$- and $K^+$-gradient dependent transport (Figs. 1 and 8) and as both $Na^+$- and $K^+$-gradient driven uptake of L-phenylalanine were inhibited by L-leucine and L-alanine (Fig. 9). The amplitude of the overshoots seen with phenylalanine uptake in the presence of $Na^+$- and $K^+$-gradient was also increased by increasing the concentrations of $Na^+$ and $K^+$ outside (Fig. 2), therefore ruling out binding as an alternative explanation. This interpretation is strengthened by our previous demonstrations of an osmotically reactive intravesicular space available for uptake in our vesicles [14,15]. The relationship between initial rates of uptake and $Na^+$ and $K^+$ concentrations showed similar biphasic patterns for both ions (Fig. 3), thus indicating that $Na^+$ and $K^+$ are effective on the same carrier-protein. However, this biphasic pattern may also suggest that more than one carrier is involved in phenylalanine transport. Our results also showed that both $Na^+$- and $K^+$-driven active transport are electrogenic, being sensitive to the membrane potential as shown by the inhibitory effect of short-circuiting the membrane potential with FCCP on the amplitude of the overshoot (Fig. 5).

However, an unexpected phenomenon has been found when the membrane potential was varied using different anions (Fig. 4), the reactive order $SCN^- \leqslant NO_3^- \leqslant SO_4^{2-} \leqslant Cl^-$ being different from what would be predicted from the lipophilicity of these anions and the consequent artificial diffusion potentials created [20]. It must then be concluded that the more lipophilic anions may partially inhibit uptake of phenylalanine (Fig. 4) and of leucine (Fig. 8). Such inhibitions of carrier-mediated transport by lipophilic anions have already been reported for ketone bodies [23], sulfate [24], and glycine [25,26]. From the studies on glycine, Imler and Vidaver [26] concluded that there was an anion-combining site on the glycine carrier that is filled prior to glycine binding. However, a direct demonstration of cotransport of anion with glycine and $Na^+$ could not be made [25,26]. The similar sensitivities of both $Na^+$- and $K^+$-gradient-dependent phenylalanine transport to membrane potential and to inhibition by lipophilic anions strengthen the above conclusion that $Na^+$ and $K^+$ must interact on the same carrier proteins [5].

The effect of external $K^+$ on $Na^+$-dependent transport processes presents a complex problem that is as yet incompletely resolved [11] and our results again point out the complexity of these interactions. The previously observed inhibitory effect of high external $K^+$ concentrations [11] may reflect competition between $K^+$ and $Na^+$ for the $Na^+$-dependent transport system, as suggested by the data of Nathans et al. [27], Kipnis and Parrish [28], Eddy et al. [29], and Crane et al. [30]. Such a competition seems also possible in our system and we present direct evidence that the resulting $K^+$ complex is capable of translocation by showing a stimulatory effect of $K^+$ in the complete absence of $Na^+$. A direct evaluation for competition between $Na^+$ and $K^+$ for binding to amino acid transport sites has been attempted in our studies (Fig. 7). With the membrane potential held constant by adjusting $Cl^-$ concentrations, it appeared that $Na^+$ at the same concentration on both sides of the membrane could effectively inhibit the $K^+$-driven accumulation of amino acid, in accordance with the competition postulated earlier (Fig. 7B). However, when $K^+$ was equilibrated on both sides of the membrane, an unexpected stimulation of $Na^+$-driven accumulation occurred (Fig. 7A). These results can be reconciled by postulating (1) the presence of two cation-binding sites on the carrier with one of them acting as a regulatory site, or (2) a velocity-type system in which carrier-sequestration by $K^+$ at the inner face of the membrane leads to a decrease in maximal efflux velocity but not influx velocity as discussed by Crane [12]. These hypotheses are also compatible with the effect of opposite $Na^+$ and $K^+$ gradients on phenylalanine uptake (Fig. 7C) as the presence of a $K^+$ concentration difference ($K_i^+ > K_o^+$) failed to stimulate $Na^+$-gradient driven transport of the amino acid over values obtained when $K^+$ has been equilibrated ($K_i^+ = K_o^+$) (Fig. 7A). The second hypothesis may, however, be more compatible with the reduction of $K^+$-gradient-dependent uptake to lower than control values observed with an outwardly directed $Na^+$-gradient (Fig. 7C). In this case, we have to assume that $Na^+$ sequestration at the inner face leads to increased maximal rate of efflux that cannot be matched by the slow rate of influx in presence of $K^+$. It has to be noted that $K^+$-acceleration of $Na^+$-dependent transport has been reported recently for L-glutamate in kidney brush border membranes of rabbit [31] and rat [32,33] but many differences exist between the interrelations of $Na^+$ and $K^+$ for the glutamate [31–33] and phenylalanine carrier (this paper). Finally, the observation that L-phenylalanine uptake was reduced to lower than control values with an outwardly directed $Na^+$-gradient (Fig. 7C) or by the inhibitory effects of alanine and leucine under $Na^+$- and $K^+$-gradient conditions (Fig. 9) seems to indicate that mediated transport of L-phenylalanine (probably via facilitated diffusion) may occur in the complete absence of $Na^+$ or $K^+$.

In conclusion, our results on L-phenylalanine uptake by mouse intestinal brush border membrane vesicles are in accordance with the suggestions of Christensen [34], Christensen and Handlogten [35], and Sepulveda and Smith [36] that the $Na^+$-dependent absorption of neutral amino acids in the small intestine presents a greater similarity with system ASC (alanine, serine and cysteine preferring) than with the alanine preferring system A (broad substrate specificity, possible substitution of $Na^+$ by $Li^+$). However, the mouse system exhibits a very unusual feature in comparison with the other systems in that $K^+$ may substitute for

$Na^+$. A preliminary analysis of the interrelations of $Na^+$ and $K^+$ with the neutral amino acid carrier revealed complex interactions which are best explained so far by a competition between these ions for a carrier site at both sides of the membrane. However, our studies do not rule out the possible involvement of more than one system for neutral amino acid uptake. Clearly, detailed kinetic analysis should be performed in the future to gain a more comprehensive view on this complex system.

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