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HISTIDINE INFLUX ACROSS BRUSH BORDER OF RABBIT ILEUM

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SUMMARY

Histidine influx across the brush border of rabbit ileum is a saturable process that is inhibited by alanine or lysine in the mucosal solution. Replacement of Na^+ in the mucosal solution with choline does not affect the maximal influx but markedly increases the histidine concentration required to elicit a half-maximal influx. Lowering the pH of the mucosal solution from 7.4 to 5 does not significantly affect the kinetics of histidine influx in the presence or absence of Na^+ nor does it affect the degree of inhibition by alanine or lysine. Further, the ability of histidine to inhibit lysine influx does not differ significantly at pH 7.4 and pH 5. Two alternative explanations for these findings are suggested.

INTRODUCTION

The absorption of monoamino-monocarboxylic α-amino acids ("neutral") and diamino-monocarboxylic α-amino acids ("cationic") by mammalian small intestine is often attributed to separate transport mechanisms[1]. This posited duality is supported by two clinical syndromes, in man, resulting from genetic disorders in amino acid transport, cystinuria and Hartnup disease. The former condition is characterized by a deficiency in cationic amino acid absorption and the latter by a deficiency exclusively in neutral amino acid absorption[2]. In recent years, however, interactions between cationic and neutral amino acids during transport across mammalian small intestine have been reported[3–6], indicating that the selectivity of these transport mechanisms is not absolute. Nevertheless, the degree of interaction between cationic and neutral amino acids is generally much less than the interactions between amino acids from within the same "charge" group suggesting that the two postulated transport systems, while not absolutely specific, have strong preferences.

An important, unexplored question is whether the predominant specificity of these transport systems is determined by the charge of the amino acid. For example, does the cationic amino acid transport mechanism strongly prefer lysine because it is a cation at neutral pH or is a more subtle property of this amino acid responsible for the specificity? In addition to its possible role in selectivity, the charge of an amino acid

appears to influence the degree to which the influx of that amino acid is dependent upon Na^+. In the rabbit ileum, the influxes of lysine and arginine are much less dependent upon Na^+ than are the influxes of neutral amino acids, whereas the influxes of glutamate and aspartate are more markedly dependent upon Na^+ than are the influxes of neutral amino acids[7]. These observations suggest that the charge of the transported amino acid influences the requirement for Na^+ such that the binding of a cationic amino acid partially relieves the requirement for Na^+ whereas the binding of an anionic amino acid accentuates the requirement for Na^+ (ref. 7).

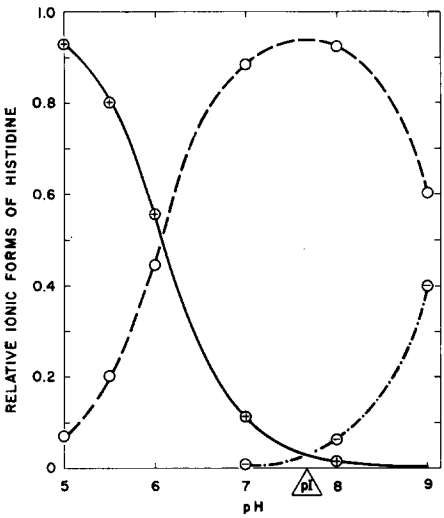


Fig. 1. Relative ionic distribution of L-histidine as a function of pH, calculated from dissociation constants given by EDSALL AND WYMAN[8]. Open circles designate the neutral form, whereas the + and − designate the cationic and anionic forms respectively. p*I* designates the isoelectric pH.

In the present investigation we have examined the role af amino acid charge *per se* in influencing selectivity and Na^+-dependence. L-histidine was selected for this purpose because the net charge of this amino acid varies from predominantly positive at pH 5 to predominantly neutral at pH 7.4. As shown in Fig. 1, the difference between the ionic distribution of L-histidine at these two pH values is quite marked and possible effects of charge on transport parameters should be easily discernable.

METHODS

New Zealand white rabbits (male and female), that had been maintained on normal food uptake, were sacrificed by intravenous injection of pentobarbital. A segment of distal ileum was resected, opened along the mesenteric border and mounted mucosal surface up in an apparatus that has been described in detail[9]. In this apparatus, 8 adjacent, defined areas (each 1.13 cm^2) of mucosal surface can be exposed to solutions of desired composition while the serosal surface abuts on a piece of moistened filter paper. The method employed for the determination of the unidirectional influxes of amino acids from the mucosal solution across the brush border into the absorptive epithelium has been described in detail[9]. Briefly, the mucosal surface is exposed to

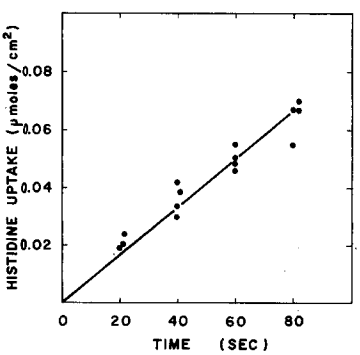
a solution containing [^{14}C]amino acid and [^{3}H]inulin for an accurately measured time in the range of 30–60 sec. The solution is then withdrawn, the exposed surface is washed briefly with ice-cold mannitol solution, and the tissue is punched out and extracted in 0.1 M HNO_3 for 12–24 h. The extract and an aliquot of the mucosal solution are assayed for ^{14}C and ^{3}H simultaneously using a liquid scintillation spectrometer. The ^{14}C content of the extract, after correction for the [^{3}H]inulin space, is a measure of the amino acid influx across the brush border alone.

The mucosal solution contained 140 mM NaCl–10 mM $KHCO_3$–1.2 mM K_2HPO_4–0.2 mM KH_2PO_4–1.2 mM $CaCl_2$–1.2 mM $MgCl_2$. The pH of this solution at 37° when bubbled with a O_2–CO_2 (95:5, by vol.) gas mixture was 7.2–7.4. In order to obtain solutions buffered at pH 5, the $KHCO_3$ and potassium-phosphate salts were replaced with 1.6 mM phthalic acid and 10 mM KCl and the desired pH was obtained by adding small quantities of KOH or HCl. Sodium-free solutions were prepared by replacing NaCl with choline chloride. All amino acids employed were in the L-form. Unless otherwise indicated, the tissues, after mounting, were preincubated for a period of 30 min prior to the influx determination in a buffer having the same composition as that used for the influx determination. Determination of pH after 30-min exposure to the mucosal surface of the tissue indicated that in most instances the pH increased. Solutions that were initially at pH 7.2–7.4 had a final pH between 7.5 and 7.7, whereas solutions that were initially pH 5.0–5.2 had final pH's between 6.0 and 6.4. However, the initial pH of the mucosal solution was not affected by the brief exposure employed during the influx determination.

RESULTS AND DISCUSSION

Histidine influxes at pH 7 and pH 5

The time course of histidine uptake across the brush border of rabbit ileum from a mucosal solution containing 10 mM histidine and 140 mM Na^+ (pH 7.4) is shown in Fig. 2. Uptake is a linear function of time, and the line passes through or



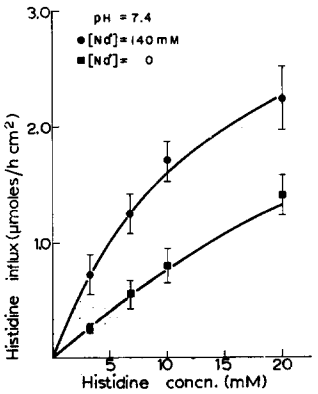


Fig. 2. Histidine uptake across the brush border as a function of time of exposure to a mucosal solution containing 10 mM histidine.

Fig 3. Histidine influx as a function of histidine concentration in the mucosal solution at pH 7.4. Each point represents the mean ± S.E. of 6–8 determinations.

very close to the origin. As discussed previously[9] this observation indicates that the 1-min uptake is a valid measure of the unidirectional influx of histidine across the brush border.

Histidine influx at pH 7.4 in the presence and absence of Na^+ is given as a function of histidine concentration in Fig. 3. Lineweaver–Burk plots of these data were used to estimate the maximal histidine influx (J_H^{im}) and the histidine concentration required to elicit a half-maximal influx (K_t). These values are given in Table I and were used to construct the hyperbolae shown in Fig. 3. The agreement between the experimental data and the curves suggests that histidine influx in the presence and absence of Na^+ is a saturable process that can be described by Michaelis–Menten kinetics. Replacement of Na^+ with choline results in a marked increase in the K_t but does not significantly affect J_H^{im}. Thus, the effect of Na^+ on the kinetics of histidine influx resembles that observed for other neutral[10], cationic[11] and anionic[7] amino acids.

TABLE I

KINETIC PARAMETERS OF HISTIDINE INFLUX

K_t has units of mM and J_H^{im} is in μmoles/h·cm².

pH	$[Na^+] = 140$ mM		$[Na^+] = 0$	
	K_t	J_H^{im}	K_t	J_H^{im}
7.4	15	4	70	6
5.0	20	5	70	5

Because of the high K_t for histidine influx in the absence of Na^+ it is difficult to statistically exclude a linear relation between influx and concentration. In order to obtain additional evidence for carrier-mediated influx, the effect of alanine on histidine influx in the absence of Na^+ was investigated. Histidine influx from a solution containing 5 mM histidine and 20 mM mannitol* averaged 0.47 ± 0.04 μmoles/h·cm² whereas influx in paired tissues from the same animal from a mucosal solution containing 5 mM histidine and 20 mM alanine averaged 0.27 ± 0.03 μmoles/h·cm² (8 determinations). These data indicate a significant inhibition of histidine influx by alanine in the absence of Na^+ and support the conclusion that influx in the absence of Na^+ is a carrier-mediated process.

Histidine influx at pH 5 in the presence and absence of Na^+ is given as a function of the histidine concentration in the mucosal solution in Fig. 4. The J_H^{im} and K_t estimated from Lineweaver–Burk plots of these data are given in Table I and the curves shown in Fig. 4 were constructed from these values. It is clear that the kinetic parameters of histidine influx in the presence and absence of Na^+ do not differ significantly at pH 7.4 and 5.0.

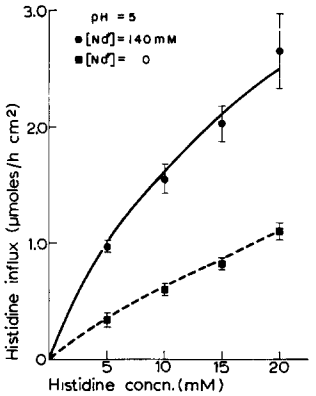
The data illustrated in Figs. 3 and 4 were obtained in 4 series of experiments using tissue from different animals. In order to confirm the absence of an effect of pH on

* In these and subsequent experiments examining the effect of one amino acid on the influx of another, mannitol was added to the control solution to exclude effects that could arise from differences in osmolarity.

histidine influx, paired experiments were performed in which influxes in the presence and absence of Na^+ at pH 5 and pH 7 were determined on tissue from the same animals. These results are given in Table II and are consistent with the conclusions drawn from the kinetic studies.

Effects of other amino acids on histidine influx

The effects of alanine and lysine on histidine influx at pH 7.4 and pH 5.0 are illustrated in Fig. 5. In these experiments, histidine influx was determined from a solution containing 3 mM histidine and 15 mM mannitol (control) and compared with influx determined on adjacent segments of tissue from the same animal from



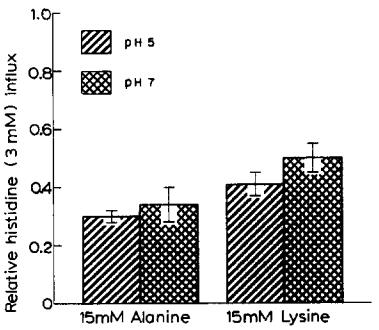


Fig. 4. Histidine influx as a function of histidine concentration in the mucosal solution at pH 5. Each point represents the mean ± S.E. of 8 determinations.

Fig. 5. Effect of 15 mM alanine and 15 mM lysine on histidine influx at pH 5 and 7. Data represent mean ± S.E. of 6–8 determinations.

TABLE II

HISTIDINE INFLUX AT PH 7.4 AND PH 5.0

Influxes from solutions containing 1 mM histidine. Values are μmoles/h·cm² ± S.E. of 8 determinations.

pH	$[Na^+] = 140$ *mM*	$[Na^+] = 0$
7.4	0.50 ± 0.02	0.16 ± 0.02
5.0	0.46 ± 0.03	0.16 ± 0.03

TABLE III

EFFECT OF HISTIDINE AND PH ON LYSINE INFLUX

Influxes in the presence of 140 mM Na^+. All values are in μmoles/h·cm² ± S.E. Number of determinations given in parentheses.

Mucosal solution	*pH 7.4*	*pH 5.0*
3 mM lysine + 20 mM mannitol	0.94 ± 0.21 (5)	0.91 ± 0.13 (6)
3 mM lysine + 20 mM histidine	0.61 ± 0.08 (5)	0.59 ± 0.12 (6)

solutions containing 3 mM histidine and either 15 mM alanine or 15 mM lysine; the relative influxes at pH 7 and pH 5 are plotted in Fig. 5. Alanine and lysine each significantly inhibit histidine influx at pH 5 and pH 7 but in each instance the degree of inhibition is not significantly affected by the pH of the mucosal solution.

The effect of histidine (20 mM) on lysine (3 mM) influx at pH 7 and pH 5 is given in Table III. Neither lysine influx nor the degree of inhibition by histidine is significantly affected by lowering the pH of the mucosal solution from 7 to 5.

CONCLUSIONS

These results indicate that the kinetics of histidine influx across the brush border of rabbit ileum and the interactions between histidine and alanine or lysine are not affected by the charge distribution of histidine in the bulk mucosal solution. Although we cannot exclude small differences between the behavior at pH 7.4 and that at pH 5.0, because of statistical variance, these differences can at most be minimal and cannot parallel the difference in charge distribution illustrated in Fig. 1.

Two explanations for these observations are possible. The first is that protonation or deprotonation of the imidazole nitrogen does not affect the interaction between histidine and its influx mechanism or the dependence of this process on Na^+. Converting histidine from the neutral zwitterionic form to a monovalent cation neither decreases its interaction with alanine nor increases its interaction with lysine suggesting that charge alone cannot be the determining factor in selectivity. This is certainly the most straight-forward conclusion that can be drawn from these results. Since the purpose of this study was to evaluate the effect of net charge on interactions among amino acids the choice of alanine and lysine as prototypes of neutral and cationic amino acids seems reasonable. Histidine interacts with the transport of both of these amino acids and the degree of interaction is not affected by the net charge on the histidine molecule. It is quite possible that different results might have been obtained using other neutral and cationic amino acids. However, were this the case factors other than net charge alone would have to be invoked.

However, an alternative conclusion, that cannot be excluded, is that the ionic form in which histidine interacts with the influx mechanism is not determined by the charge distribution of histidine in the bulk solution. If the microclimate of the brush border, or in the region of the histidine influx mechanism, were characterized by a pH 1–2 units lower than that in the bulk solution, histidine in this microclimate would be predominantly in the cationic form when the mucosal solution is at pH 7.4 or pH 5. This could result if the surface of the brush border and/or the region of the histidine influx mechanism possesses a high density of fixed negative charges, such as carboxylate or phosphoryl residues with pK values of 3–5. Indeed, studies on the electrophoretic mobilities of a variety of nonepithelial[12–15] and epithelial[16–18] cells indicate that the isoelectric pH is equal to or lower than 3.5, that is, the surface of shear bears a net negative potential ("zeta" potential) above pH 3.5. Studies on human colonic mucosal cells indicate a negative surface charge density at pH 7.0 of approx. $2 \cdot 10^3$ esu/cm^2, a value that is in fair agreement with that reported for several other cells[17].

Although we cannot at present evaluate this second alternative two considerations suggest that it cannot be simply dismissed. First, HOGBEN *et al.*[19] concluded

from their studies on the absorption of weak acids and bases by perfused *in vivo* rat small intestine that there is a zone immediately adjacent to the brush border that is characterized by a pH between 5 and 6 and that this value is only slightly influenced by large changes in the pH of the perfusate. Studies using *in vitro* rabbit ileum could determine whether a similar microclimate characterizes this preparation. Second, previous studies have suggested a kinetic model for the interaction between Na and amino acid influx in which

$$K_t = K_1K_2/(K_2 + [Na^+]_m)$$

where K_1 is the dissocation constant of the amino acid-carrier complex, K_2 is the dissociation constant that characterizes the binding of Na^+ to the amino acid-carrier compex, and $[Na^+]_m$ is the Na^+ concentration in the mucosal solution[10]. This model has been shown to be consistent with findings on a variety of neutral amino acids[10] as well as anionic[7] and cationic amino acids[11]. From the data given in Table I, the K_1 for histidine at pH 7 and pH 5 is 70 mM. At pH 7.4, K_2 is 38 mM and at pH 5.0 K_2 is 56 mM; it is not likely that these values are significantly different, and an average K_2 of 47 mM is probably adequate for the present discussion. Previous studies (summarized in refs. 7 and 20) have indicated that the K_2 values for 5 neutral amino acids do not differ significantly and are approx. 20 mM. On the other hand, the K_2 for lysine is 56 mM whereas the K_2 values for glutamate and aspartate are each approx. 10 mM. The possible significance of these differences has been discussed in detail[7,20]. K_2 for histidine more closely agrees with the K_2 of lysine (indeed, at pH 5 they are identical) than with those of the neutral amino acids. Indeed, if the difference between K_2 at pH 7 and that at pH 5 is real, the direction of change is precisely what one would expect for an increase in the proportion of histidine present in the cationic form. If our previous interpretation of the relation between amino acid charge and K_2 is correct, these data suggest that histidine interacts with the influx mechanism predominantly in the cationic form even when the pH of the mucosal solution is 7.4.

These results compare with those obtained by CHRISTENSEN[21] for Ehrlich ascites cells. In this system, as in rabbit ileum, histidine inhibits the uptake of lysine and the degree of inhibition is essentially the same from pH 5 to pH 7.4. Furthermore, lysine inhibits histidine uptake and lowering the pH from 7.4 to 5.0 increases the lysine sensitive component from approx. 30% to approx. 40% of the total rate of uptake. Our data do not reveal a statistically significant increase in sensitivity to lysine at pH 5. However, it should be noted that the effect observed by CHRISTENSEN[21] is rather small considering that at pH 7.4 more than 90% of histidine is in the neutral zwitterionic form whereas at pH 5.0 more than 90% is in the form of a monovalent cation. Thus, the incease in sensitivity to lysine does not parallel the more then 10-fold decrease in neutral zwitterion concentration and the more then 10-fold increase in cationic form in the bulk solution. In contrast, in the rabbit reticulocyte[21–23] histidine is a much more effective competitive inhibitor of lysine uptake at pH 6.2 than at pH 7.4. As in the present study, the uptake of histidine was pH independent and inhibited to the same extent by lysine at pH 6.2 and pH 7.4.

Finally, histidine uptake by rabbit ileum is subject to inhibtion by both neutral and cationic amino acids as has also been reported for ascites cells[21] rabbit reticulocytes[22] pigeon erythrocytes[21] and rat intestine[24]. However the kinetics of influx in

rabbit ileum are consistent with the presence of a single saturable influx process. It is possible that kinetic studies employing higher or lower concentrations than those employed in the present studies would disclose another process. However, the present results are sufficient to exclude the possibility that histidine influx is mediated by two nonoverlapping influx processes, one subject exclusively to inhibition by neutral amino acids and the other subject exlcusively to inhibition by cationic amino acids. As shown in Fig. 5, in experiments on paired tissues from the same animals alanine and lysine each inhibit more than 50% of histidine influx. Indeed, the total inhibitory effect of lysine and alanine on histdine influx amounts to 110% at pH 7.4 and 130% at pH 5.0, and it is unlikely that each of these amino acids, at a concentration of only 15 mM, is exerting its maximal inhibition. Thus, regardless of the number of agencies responsible for histidine influx, some fraction must be subject to inhibition by both alanine and lysine. Further study is necessary to define the extent of overlap and the number of distinct agencies involved in histidine influx. However, as pointed our previously[4,22], the observation that one amino acid inhibits the transport of another does not imply that the inhibitor is actually transported by the same mechanism. Thus, the present results do not imply that there is more than one mechanism responsible for histidine influx or that alanine and/or lysine are transported by this mechanism. Further study is clearly necessary to clarify the nature of competitive interactions in amino acid transport, particularly those involving amino acids belonging to different charge groups, and to define the possible role of "abortive" or "nonproductive" complex formation[25] in transport processes.

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