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Preferential binding of amino acids to isolated mucosal brush borders from hamster jejunum

Previous experiments in this laboratory have indicated that there is a correlation between some of the properties of D-glucose binding to isolated intact and Tris-disrupted brush borders prepared from hamster jejunum and the first step in the active transport of this sugar by the small intestine[1,2]. Since the brush border or microvillous membrane of the mucosal cell is also responsible for the active transport of certain amino acids by the small intestine[3], it was of interest to determine if actively transported amino acids were preferentially bound to isolated brush borders.

Epithelial brush-border membranes from the jejunum of 6 hamsters were isolated according to the method that has been previously described[1] and were suspended, without disruption, in 4.2 ml of cold (4°) distilled water. 1-ml aliquots of this suspension were placed in test tubes. In control tubes, 1 ml of distilled water was substituted for the brush-border suspension. Then to all the tubes were added 2.2 ml of a 0.02 M phosphate-buffered (pH 7.2) solution, containing unlabeled experimental compounds and 0.1 ml of a radioactive amino acid mixture composed of DL-[3-^{3}H] glutamate, a nonactively transported amino acid and a ^{14}C-labeled actively or nonactively transported amino acid. The concentration of DL-[3-^{3}H]glutamate in the total 3.3 ml was 1 μM, and for the uniformly ^{14}C-labeled amino acid it was 0.5 μM. The experimental and control tubes were incubated for 30 min at 37°. After incubation, the experimental tubes containing the brush borders were centrifuged, and the ^{3}H/^{14}C

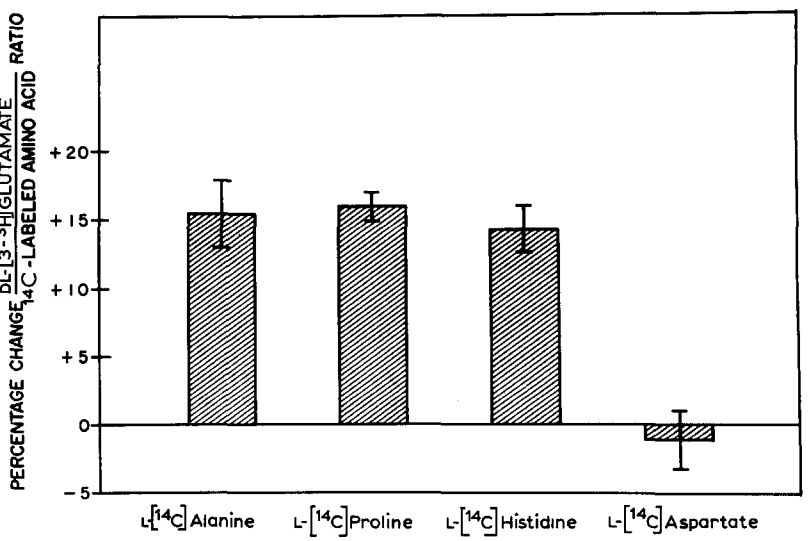

Fig. 1. Preferential binding of uniformly ^{14}C-labeled amino acids to isolated mucosal brush borders from hamster jejunum. The isolated brush borders were incubated for 30 min at 37° (pH 7.2) in the presence of 1 μM DL-[3-^{3}H]glutamate and 0.5 μM of a uniformly ^{14}C-labeled amino acid. Each bar point represents the mean of at least 7 experiments. The vertical lines represent 1 S.E. above and below the bar points.

disint./min ratios were obtained from the supernatant fluid and were compared with the ratios in the control tubes without brush borders. An increase in the initial $^{3}H/^{14}C$ disint./min ratio indicated preferential binding of the [^{14}C]amino acid to the brush borders.

In Fig. 1 it is shown that the actively transported amino acids, L-alanine, L-proline and L-histidine, are preferentially bound to brush borders. In all of these cases, there is approximately a 15 % increase in the $^{3}H/^{14}C$ disint./min ratios which indicates that each actively transported amino acid has a similar affinity for binding to isolated brush borders. When the nonactively transported amino acid, L-aspartate, is substituted for one of the actively transported [^{14}C]amino acids, no preferential binding is observed and the $^{3}H/^{14}C$ disint./min ratio is unchanged. If Tris-disrupted brush borders are substituted for the initially intact brush borders, then no preferential binding occurs with the actively transported amino acids. It is interesting to note that this loss of amino acid binding is not due to the disruption of the brush borders but is caused by the presence of Tris ions. This conclusion is based on the observation that initially intact brush borders become disrupted after the 30-min incubation period at 37° in the osmotically low suspension medium employed in these experiments.

In order to establish if L-alanine, L-proline and L-histidine are being bound to the same site, the possible competitive effects of these amino acids for a common binding site within the brush border membrane was explored. The effects of unlabeled L-alanine, L-proline, L-histidine and L-aspartate, at the relatively high concentration of 1 mM, on preferential amino acid binding to brush borders are presented in Table I. Unlabeled L-alanine inhibits the preferential binding of each actively transported [^{14}C]amino acid. Unlabeled L-proline, however, is only inhibitory to the preferential binding of L-alanine ($P<0.01$) and L-proline, and no inhibition of L-histidine binding ($P>0.3$) is observed in the presence of this amino acid. In the presence of unlabeled L-histidine, the preferential binding of L-alanine is unaffected ($P>0.3$), but L-proline and L-histidine binding to the brush borders is inhibited. The nonactively transported, unlabeled L-aspartate, has no significant effect on the preferential binding of any of the actively transported amino acids that have been employed ($P>0.2$). These results suggest that the binding location within the brush border membrane for L-alanine,

TABLE I

EFFECTS OF UNLABELED AMINO ACIDS ON THE PREFERENTIAL BINDING OF UNIFORMLY ^{14}C-LABELED L-ALANINE, L-PROLINE AND L-HISTIDINE TO ISOLATED BRUSH BORDERS

The concentration of the ^{14}C-labeled amino acids is 0.5 μM. The number of experiments is given in parentheses. The S.E. is given for each mean. All amino acids employed were of the highest purity obtainable and it has been concluded from analytical data that these effects could not be attributed to the presence of contaminating amino acids.

Unlabeled amino acid (1 mM)	*Percentage inhibition of binding of:*		
	L-[^{14}C]*Alanine*	L-[^{14}C]*Proline*	L-[^{14}C]*Histidine*
L-Alanine	50.7 ± 6.0 (6)	99.5 ± 0.5 (3)	22.6 ± 3.4 (5)
L-Proline	21.2 ± 1.7 (5)	83.0 ± 9.8 (4)	−14.4 ± 8.4 (3)
L-Histidine	−21.8 ± 7.5 (3)	91.4 ± 8.2 (4)	63.3 ± 5.3 (4)
L-Aspartate	−14.9 ±12.0 (7)	−14.4 ± 8.0 (3)	− 1.1 ± 0.6 (3)

TABLE II

PREFERENTIAL BINDING OF AMINO ACIDS IN THE PRESENCE OF VARIOUS COMPOUNDS

The number of experiments is given in parentheses. The S.E. is given for each mean.

Uniformly ^{14}C-labeled amino acid (0.5 μM)	*Percentage change in $^{3}H/^{14}C$ disint./min ratio in presence of:*					
	No addition	D-*Glucose* (*1 mM*)	D-*Glucosamine* (*1 mM*)	*NaCl* (*100 mM*)	*KCl* (*100 mM*)	*p-Hydroxy-mercuribenzoate* (*1 mM*)
L-Alanine	15.5 ± 2.4 (17)	14.4 ± 3.2 (4)	19.9 ± 1.0 (3)	13.3 ± 1.2 (3)	10.4 ± 1.7 (3)	0.4 ± 2.6 (3)
L-Proline	15.9 ± 1.0 (12)	16.9 ± 4.5 (4)	23.0 ± 3.4 (6)	18.5 ± 1.8 (5)	14.1 ± 2.1 (3)	−0.2 ± 1.8 (3)
L-Histidine	14.4 ± 1.7 (18)	9.5 ± 2.6 (4)	16.5 ± 1.3 (3)	19.2 ± 3.2 (3)	17.7 ± 4.1 (3)	−1.4 ± 3.4 (3)

L-proline and L-histidine may not be precisely the same. If these amino acids were sharing the same binding site, then unlabeled L-histidine should have inhibited L-alanine binding, unlabeled L-proline should have inhibited the preferential binding of L-histidine and unlabeled L-proline should have been more inhibitory to the binding of L-alanine. It is possible, however, that the L-alanine, L-proline and L-histidine binding sites may be in close proximity to each other or even be overlapping. Therefore, the position of these binding sites may account for the inhibition of L-[^{14}C]proline binding by unlabeled L-histidine and L-alanine and for the inhibition of L-[^{14}C]histidine binding by unlabeled L-alanine. These observations could be considered to be in accordance with the results of experiments by others with the intact small intestine which demonstrate that a separate transport system exists for L-alanine, L-proline and L-histidine[4–6] instead of a common amino acid transport system[7,8].

Additional information concerning the properties of amino acid binding to isolated hamster brush borders is shown in Table II. Neither unlabeled 1 mM D-glucose nor 1 mM D-glucosamine, a potent inhibitor of preferential D-glucose binding to isolated brush borders[2], affect amino acid binding ($P > 0.05$). Consequently, it seems that amino acids and sugars do not share the same binding site within isolated hamster brush-border membranes. A similar conclusion was reached by FAUST *et al.*[2] from the results of studies which demonstrated that amino acids did not inhibit preferential D-glucose binding to isolated hamster brush borders. Furthermore, our results are compatable with the findings of others who have interpreted their data obtained with the intact small intestine as indicating that sugars and amino acids do not share a similar binding site[9–11]. This is in contrast with the results of ALVARADO[12] which suggest that sugars and amino acids share a common transport site.

It can also be seen in Table II that neither 100 mM NaCl nor KCl has an effect upon the binding of amino acids. As it has been indicated for preferential sugar binding to brush borders[1], there are sufficient Na^+ in our preparation to satisfy a Na^+-dependent binding requirement for these amino acids[13]. It is obvious, however, that sulfhydryl groups are involved in some manner in the binding of amino acids to isolated brush borders. A sulfhydryl-reacting compound, *p*-hydroxymercuribenzoate, inhibits the preferential binding of L-alanine, L-proline and L-histidine. A similar inhibitory effect on D-glucose binding to isolated hamster brush borders has been previously demonstrated with this compound[2].

The data presented demonstrate that actively transported amino acids are

preferentially bound to isolated mucosal brush borders. In addition, this process may be related to the initial step in the active transport of amino acids by the small intestine.

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