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Sodium-dependent binding of L-histidine to a fraction of mucosal brush borders from hamster jejunum

The active transport or "uphill" movement of amino acids from the lumen to the mucosal intracellular region of the small intestine is a Na^+-dependent process[1-4]. It has been postulated that Na^+ may be involved in an initial energy-independent binding step[2,5] as well as in an energy-dependent accumulation step linked to the "downhill" movement of Na^+ (ref. 6). In the initial binding phase, the formation of a ternary complex has been suggested which consists of Na^+, the amino acid, and a component within the brush border membrane of the mucosal cell[2].

A previous study by BURNS AND FAUST[7] had indicated that the preferential binding of actively transported amino acids to isolated mucosal brush borders may be related to the first step in the active transport of amino acids by the small intestine. Although binding in these studies had occurred without the addition of Na^+, it was suggested that sufficient Na^+ was bound to the brush borders to satisfy a possible Na^+ requirement for amino acid binding. Our present experiments were designed to investigate the possible Na^+ dependency for the binding of an actively transported amino acid to a low Na^+ fraction of the intestinal brush border membrane.

Epithelial brush border membranes from the jejunum of 6 hamsters were isolated according to the method that has been previously described[7]. These isolated intact brush borders were disrupted by incubation in 10 ml of distilled water for 30 min at 37°. Tris(hydroxymethyl)aminomethane was not employed for disruption because preferential binding of amino acids does not occur with Tris-disrupted brush borders[7]. Disrupted brush borders were centrifuged at 27000 × *g* for 10 min and resuspended in 2 ml of cold distilled water (4°). This suspension was placed on a 10, 20, 30, 35, and 40% (w/v) Ficoll density gradient and centrifuged in a SW 39L swinging bucket rotor at 112000 × *g* for 110 min. Four bands and a precipitate were obtained under these conditions. The top band, fraction I, floated on the 10% Ficoll layer.

In order to identify the fraction containing sites for binding of actively transported amino acids, isolated intact brush borders were disrupted in the presence of a 17.6 mM sodium phosphate-buffered (pH 7.2) solution containing 1 μM of DL-[3-^{3}H]glutamate, a non-actively transported amino acid, and 0.5 μM of a uniformly ^{14}C-labelled actively transported amino acid. The ^{14}C-labelled amino acid became preferentially bound to the disrupted brush borders[7], and this radioactive suspension was separated by Ficoll density gradient centrifugation. Each fraction was removed and counted with a Mark I Nuclear-Chicago Liquid Scintillation System. In experiments employing either ^{14}C-labelled L-alanine or L-proline or L-histidine with DL-[3-^{3}H]glutamate, it was found that the preferential binding of actively transported ^{14}C-labelled amino acids appeared only in fraction I. No radioactivity was detected in fractions II, III, and IV. The total radioactivity in fraction V, the precipitate, was less than half that observed in fraction I and in all cases, contained more DL-[3-^{3}H]glutamate than the ^{14}C-labelled amino acids.

It is interesting to note that when radioactive disrupted brush borders are separated by glycerol density gradient centrifugation[8], preferentially bound actively

transported amino acids are found in the precipitate fraction (unpublished observation). Furthermore, D-glucose is also preferentially bound to the same fractions obtained by glycerol[9] and Ficoll density gradient centrifugation (unpublished observation). The sugar and amino acid binding sites, however, are not the same[7,9]. The Ficoll gradient is a more useful preparative method because the binding sites are located in a more homogeneous fraction of the disrupted brush borders.

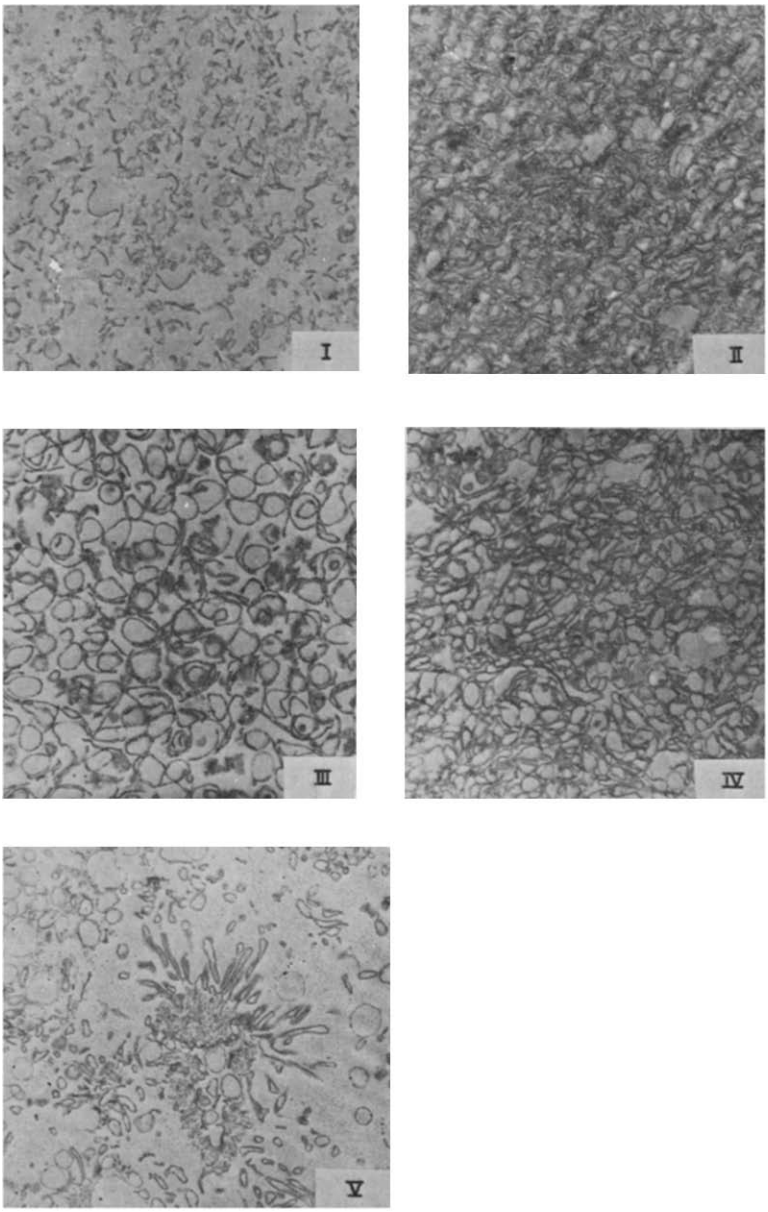

Fig. 1. Electron photomicrographs of fractions I–V obtained from the Ficoll density gradient centrifugation of disrupted brush borders. Glutaraldehyde and osmium. Approx. × 7500.

Fig. 1 shows electron photomicrographs of sections from each of the Ficoll density gradient fractions which were prepared by the glutaraldehyde, osmium tetroxide method reported by OVERTON *et al.*[10]. Fraction I contains filaments resembling microvillus core material[10–12], as well as vesicles. The filamentous material appears to be similar to that observed in the precipitate of the glycerol density gradient[10] which also preferentially binds actively transported amino acids. No filamentous material exists in the heterogeneous debris found in fraction V.

Since fraction I was to be employed for binding experiments, it was prepared from nonradioactive disrupted brush borders. After the initial Ficoll density gradient centrifugation, fraction I was washed with cold distilled water and purified by recentrifugation on a Ficoll density gradient for 30 min. Fraction I was washed again in cold distilled water and resuspended in 2 ml of water. A 0.5-ml aliquot of this suspension was employed for binding studies and was approx. 10 μM in Na^+, as determined by flame photometry. This concentration of Na^+ was much lower than the 270 μM present in the brush borders of our previous studies[7] which showed no effect of added Na^+ on amino acid binding.

To the 0.5-ml aliquots of fraction I were added 2.4 ml of a 17.6 mM sodium or sodium-free buffer (pH 7.2) and 0.1 ml of a radioactive amino acid mixture composed of DL-[3-^{3}H]glutamate and actively transported L-[^{14}C]histidine. The concentration of DL-[3-^{3}H]glutamate in the total 3.0 ml was 1 μM and for the L-[^{14}C]histidine it was 0.5 μM. Control tubes contained 0.5 ml of water instead of the fraction I suspension. Both the experimental and control tubes were incubated for 30 min at 37°. After incubation, the experimental tubes were centrifuged, and the ^{3}H/^{14}C disint./min ratios were obtained from the supernate and compared with the ratios in the control tubes. An increase in the initial ^{3}H/^{14}C disint./min ratio indicated preferential binding of L-[^{14}C]histidine to fraction I.

The effect of replacing the Na^+ in bicarbonate and phosphate buffers on the preferential binding of L-[^{14}C]histidine to fraction I is illustrated in Fig. 2. No preferential binding of the actively transported L-[^{14}C]histidine to fraction I was observed when NH_4^+ and Li^+ were substituted for Na^+ in the bicarbonate buffer. The substitution of K^+ for Na^+ in the phosphate buffer produced approximately an 81 % reduction in the preferential binding of L-[^{14}C]histidine. The increase in binding ($P < 0.01$) observed when sodium phosphate is substituted for the sodium bicarbonate buffer can be accounted for by the increase in the Na^+ concentration under these conditions. The Na^+ concentrations in the sodium bicarbonate and sodium phosphate buffers are 17.6 and 32.3 mM, respectively. A similar increase in the ^{3}H/^{14}C disint./min ratio caused by an increase in the Na^+ concentration is illustrated with the phosphate buffer in Fig. 3. It can be seen that no binding occurs at a 1 mM Na^+ concentration, and maximum amino acid binding is observed at 32.3 mM of Na^+. However, there is no increase in this binding even though the Na^+ concentration is elevated to 100 mM.

The results of this investigation have demonstrated that the preferential binding of an actively transported amino acid to a low Na^+ fraction of mucosal brush borders is dependent upon the Na^+ concentration in the incubation medium. This observation lends further support to the hypothesis[7] that preferential binding of actively transported amino acids to a component within mucosal brush borders may be related to the initial step in the mechanism of active amino acid transport by the small intestine.

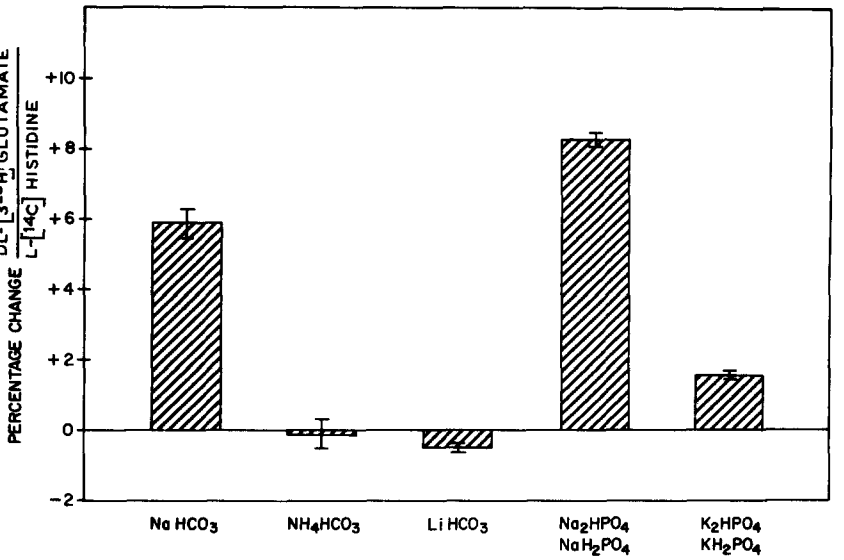

Fig. 2. Preferential binding of L-[^{14}C]histidine to brush border fraction I in Na^+ and Na^+-free buffers. Fraction I was incubated for 30 min at 37° (pH 7.2) in the presence of 1 μM DL-[3-^{3}H]-glutamate and 0.5 μM of L-[^{14}C]histidine. The specific activities of DL-[3-^{3}H]glutamate and L-[^{14}C]histidine were 27.0 C/mmole and 250 mC/mmole, respectively. All buffers were at a concentration of 17.6 mM. The Na^+ concentration in Na_2HPO_4–NaH_2PO_4 was 32.3 mM, but only 17.6 mM in the $NaHCO_3$ buffer. All of the bicarbonate buffers were gassed (carbon dioxide: oxygen, 5:95) during the incubation period. Each bar point represents the mean of at least 4 experiments. The vertical lines represent 1 S.E. above and below the bar points.

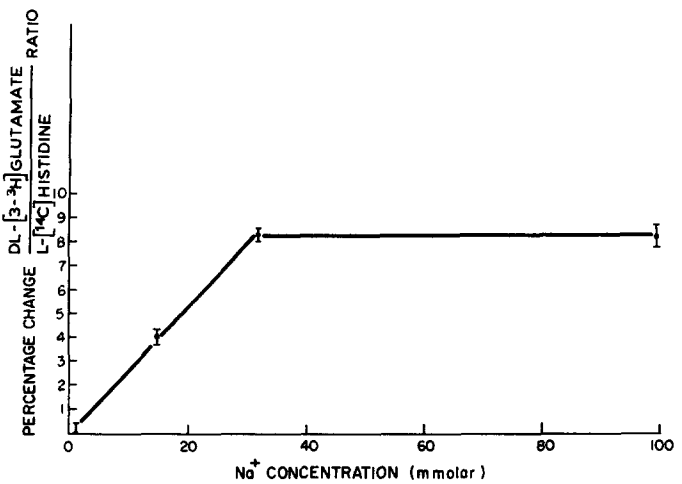

Fig. 3. Effect of various Na^+ concentrations on the preferential binding of L-[^{14}C]histidine to brush border fraction I. Sodium phosphate buffer (pH 7.2) was employed. Each point is the average of at least 3 determinations and the vertical lines indicate $\pm$ 1 S.E. of the mean.

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