Relative Effectiveness of Extracellular and Intracellular Sodium in Supporting Leucine Uptake by Isolated Intestinal Epithelial Cells[1] (36459)

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Current concepts on the Na^+ dependence of the active transport of amino acids and sugars across the small intestine favors the Na^+-gradient hypothesis (1–3). One of the major pieces of experimental evidence favoring this hypothesis is the finding that amino acid influx is dependent on the extracellular rather than the intracellular Na^+ concentration (4). However, recent studies measuring methionine influx across intact rat intestine (5) and galactose influx into isolated intestinal epithelial cells from the chicken (6) have indicated that the intracellular Na^+ concentration is more important than the extracellular Na^+ concentration in supporting active transport.

In a previous study of the characteristics of amino acid uptake by isolated intestinal epithelial cells from the rat carried out in this laboratory (7), it was found that Na^+ was required for the active uptake of leucine. However, the nature of the Na^+ dependence was not investigated. The present study describes the effect of changes in the direction of the Na^+ gradient induced by changes in the intracellular and extracellular Na^+ concentrations on the movement of leucine into or out of isolated intestinal epithelial cells from the rat. The purpose of this study was to evaluate the Na^+-gradient hypothesis in the isolated cell preparation by determining whether leucine transport was more dependent on the extracellular or the intracellular Na^+ concentration.

Methods. Wistar strain, male rats weighing 180–260 g were used as a source of the isolated epithelial cells. The animals were fed on a standard diet and watered *ad libitum* but deprived of food 4–8 hr prior to sacrifice. The methodology used to prepare the isolated intestinal cells has been described in detail (7). The only modification employed was the filtering of the cell suspension through a single layer of gauze prior to the final collection step. To measure amino acid uptake, 0.3 ml of the cells representing an average of 6.60 mg protein were added to 5 ml of an oxygenated Krebs–Ringer Tris buffer (pH 7.4) containing 118 m*M* NaCl, 25 m*M* Tris–HCl, 2.5 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4 and 14,000–20,000 dpm/ml of uniformly labeled L-leucine-^{14}C and nonradioactive leucine to a final concentration of 1 m*M*. The reaction mixture was incubated at 37° with shaking for the desired time after which the reaction was terminated by pouring the contents of the reaction mixture into a graduated centrifuge tube in an ice bath, and the cells were centrifuged in the cold at 275*g* for 2 min. The cells were then washed and centrifuged 3 additional times with 5 ml cold Krebs–Ringer Tris buffer. The uptake of the leucine by the cells was determined as previously described (7, 8) and expressed as the concentration of leucine in the cell water.

In order to produce the different gradients of Na^+ across the intestinal epithelial cell, both the extracellular and intracellular Na^+ concentrations were varied. The extracellular medium consisted of either the normal Na^+-containing medium described above (Krebs–Tris–Na^+ medium) or a low Na^+ medium in which the NaCl of the Krebs–Ringer Tris buffer had been isotonically replaced by choline Cl (Krebs–Tris–choline

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medium). The intracellular Na^+ concentration was varied by washing the cells in 20 vol of either the Krebs–Tris–Na^+ medium, the Krebs–Tris–choline medium or the cell collection medium containing 153 m*M* Na^+ (7). Alternately, the intracellular Na^+ concentration was changed by preincubating the cells for 5 min in either the Krebs–Tris–Na^+ medium or the Krebs–Tris–choline medium (5, 9). The Na^+ concentration of the incubation media and the cell pellets was determined by direct analysis in a Coleman flame photometer. The intracellular Na^+ concentration was determined after correction for the Na^+ present in the extracellular portion of the cell pellet.

Results. Table I shows the uptake of 1 m*M* L-leucine after 30 sec and 1 min incubations under conditions where the Na^+ gradient across the intestinal epithelial cell is varied. In the experiments in which the cells were washed with the Krebs–Tris–Na^+ medium, the intracellular Na^+ concentration was initially 121.9 ± 8.4 m*M* (mean ± SEM, $n = 12$). After 1 min incubation in the Krebs–Tris–Na^+ medium and the Krebs–Tris–choline medium, the intracellular Na^+ concentrations fell to 89.9 ± 14.2 m*M* ($n = 14$) and 49.6 ± 2.2 m*M* ($n = 12$), respectively. The cells washed with the Krebs–Tris–choline medium initially had an intracellular Na^+ concentration of 68.3 ± 4.8 m*M* ($n = 12$). This value is in good agreement with the intracellular Na^+ concentration of 74.2 m*M* previously reported for choline washed cells (7). After a 1 min incubation in the Krebs–Tris–choline medium, the intracellular Na^+ concentration of the choline washed cells fell to 18.9 ± 2.6 m*M* ($n = 12$). On the basis of these values and the final extracellular Na^+ concentration of the incubation media shown in Table I, it would be expected that the direction of the Na^+ gradient across the intestinal epithelial cell would be inward during the incubation of the Na^+ and choline washed cells in the Na^+ medium and outward during the incubation of the Na^+ and choline washed cells in the choline medium. The uptake of leucine by the Na^+ washed cells in the Na^+ medium during the first minute of incubation was

TABLE I. Effect of the Na^+ Gradient on the Uptake of 1 m*M* L-Leucine by Isolated Intestinal Epithelial Cells After 0.5 and 1 Min Incubation.[a]

Time (min)	Na^+ cells (washed)				Choline cells (washed)			
	Krebs–Tris–Na^+ medium		Krebs–Tris–choline medium		Krebs–Tris–Na^+ medium		Krebs–Tris–choline medium	
	Leucine (m*M*)	Na^+ (m*M*)	Leucine (m*M*)	Na^+ (m*M*)	Leucine (m*M*)	Na^+ (m*M*)	Leucine (m*M*)	Na^+ (m*M*)
0.5	0.68 ± 0.05	122.7 ± 3.9	0.41 ± 0.03	8.1 ± 0.2	0.72 ± 0.03	116.0 ± 4.6	0.33 ± 0.02	1.6 ± 0.3
1.0	1.14 ± 0.11	121.0 ± 4.0	0.62 ± 0.06	8.4 ± 0.3	1.11 ± 0.10	115.2 ± 3.3	0.50 ± 0.03	1.5 ± 0.4
	Na^+ cells (preincubated)				Choline cells (preincubated)			
0.5	0.82 ± 0.03	117.9 ± 1.1	0.52 ± 0.04	9.6 ± 0.4	0.77 ± 0.03	113.4 ± 1.2	0.27 ± 0.02	0.7 ± 0.1

[a] Values expressed as mean ± SEM. Each value is derived from 14 individual experiments with the washed cells and 18 individual experiments with the preincubated cells. The cells were washed in 20 vol of either Krebs–Tris–Na^+ (Na^+ cells) or Krebs–Tris–choline (choline cells). In the preincubation experiments, the cells were preincubated for 5 min in either the Krebs–Tris–Na^+ or the Krebs–Tris–choline media prior to incubation.

TABLE II. Effect of the Na^+ Gradient on the Movement of Leucine Into or Out of Leucine-Loaded Cells.[a]

Incubation time (min)	Na^+ cells			Choline cells		
	1 m*M* Leucine incubation medium	Extracellular Na^+ (m*M*)	Cellular leucine (m*M*)	1 m*M* Leucine incubation medium	Extracellular Na^+ (m*M*)	Cellular leucine (m*M*)
0			1.37 ± 0.05			1.22 ± 0.07
1	Krebs–Tris–Na^+	114.9 ± 1.7	1.62 ± 0.05	Krebs–Tris–Na^+	106.5 ± 2.4	1.47 ± 0.05
1	Krebs–Tris–choline	11.1 ± 0.4	1.07 ± 0.04	Krebs–Tris–choline	3.4 ± 0.2	0.91 ± 0.06

[a] Values expressed as mean ± SEM from 18 individual experiments. Cells were first incubated in Krebs–Tris–Na^+ containing 1 m*M* leucine for 1.5 min and then washed in either Krebs–Tris–Na^+ (Na^+ cells) or Krebs–Tris–choline (choline cells). The cells were then either washed (0 time) or reincubated for 1 min in either Krebs–Tris–Na^+ or Krebs–Tris–choline both containing 1 m*M* leucine.

similar both with regard to magnitude and linearity (*i.e*, initial velocity) to that previously reported for unwashed cells in the Na^+ medium (8, 10). It can therefore be assumed that leucine uptake at 1 min and below, approximates leucine influx. Incubation of the Na^+ washed cells in the choline medium reduced leucine uptake by about 40%. Leucine uptake by the choline washed cells in the Na^+ medium was statistically identical to that of the Na^+ washed cells in the Na^+ medium. Incubation of the choline cells in the choline medium reduced leucine uptake by 55%. The uptake of leucine by the Na^+ washed cells in the choline medium was about 20% greater ($p < .01$) than that of the choline washed cells in the choline medium. Washing the cells with the collection medium (153 m*M* Na^+) instead of the Krebs–Tris–Na^+ medium resulted in the same pattern of leucine uptake when the cells were incubated in the Na^+ and choline media.

The major purpose of the preincubation study was to lower the intracellular Na^+ concentration from the 68.3 m*M* present in the choline washed cells in order to determine whether leucine uptake would be reduced under conditions where the intracellular Na^+ concentration would be too low to directly energize amino acid uptake by the operation of the (Na^+ + K^+)-activated ATPase as proposed by Kimmich (6). The intracellular Na^+ concentrations of the Na^+ and choline preincubated cells were 56.7 ± 11.3 m*M* (n = 14) and 8.6 ± 2.1 m*M* (n = 12), respectively. Despite their low intracellular Na^+ concentration, the choline preincubated cells accumulated leucine as well as the Na^+ preincubated cells when placed in the Na^+ medium (Table I). In general, the uptake results obtained with the preincubated cells were analogous to those obtained with the washed cells. The one major difference noted was that leucine uptake in the choline medium was 50% greater in the Na^+ preincubated cells than in the choline preincubated cells (compared to the 20% difference noted using washed cells).

Table II shows the effect of changes in the extracellular and intracellular Na^+ concen-

trations on the movement of leucine into or out of leucine-preloaded cells. In these studies the cells were first incubated for 1.5 min in the Krebs–Tris–Na^+ medium containing 1 m*M* leucine and then washed in either the Na^+ or choline media. The leucine-loaded cells were then either counted in order to obtain the cellular leucine concentration (0 time) or reincubated in either the Na^+ or the choline media containing 1 m*M* leucine for 1 min after which the cellular leucine concentration was determined. When the extracellular Na^+ concentration was initially 118 m*M*, both the Na^+ and choline washed cells continued to accumulate leucine against a concentration difference. When the extracellular Na^+ was replaced by choline, the cellular concentration of leucine in both the Na^+ and choline washed cells fell to levels not significantly greater than that of the extracellular medium.

Discussion. In order to describe the direction of the Na^+ movements in these experiments, it was necessary to obtain accurate estimates of the extracellular and intracellular Na^+ concentration during the period of leucine transport. Reasonably precise measurements of the extracellular Na^+ concentrations as well as the intracellular Na^+ concentrations of the choline washed and choline preincubated cells were normally obtained. However, precise measurements of the intracellular Na^+ concentration of the cells after washing or preincubation in the Na^+ medium was difficult because of the small volume of cell water and the presence of high concentrations of Na^+ in the comparatively large volume comprising the 82.5% extracellular space of the cell pellet (7). Despite this difficulty, only in the case of the Na^+ washed cells incubated in the Na^+ medium was there any question as to the direction of the Na^+ gradient during the transport study. The findings that preincubation of the Na^+ washed cells in the Na^+ medium for 5 min decreased the intracellular Na^+ concentration from 121 to 57 m*M* indicates that the intracellular Na^+ concentration of 90 m*M* found in these cells after a 1 min incubation in the Na^+ medium represents a meaningful decrease in the intracellular Na^+. Therefore, the conclusion that the Na^+ gradient under these conditions is from the extracellular medium to the cell is justified.

On the basis of these results it must be concluded that the extracellular rather than the intracellular Na^+ concentration is the primary factor in the Na^+ dependence of leucine transport by the cells. The main experimental evidence in favor of this conclusion can be outlined as follows:

a. When the extracellular Na^+ concentration was initially 118 m*M*, leucine influx was optimal and not influenced by changes in the initial intracellular Na^+ concentration over the range 8–122 m*M*.

b. Lowering the initial extracellular Na^+ concentration to zero resulted in a 40% decrease in leucine influx and the magnitude of this decrease was not influenced by initial intracellular Na^+ concentrations over the range 57–122 m*M*.

c. Leucine moved into leucine-loaded cells against a concentration gradient when the extracellular Na^+ concentration was initially 118 m*M* but moved out of the cells when the extracellular Na^+ concentration was initially 0 even though the initial intracellular Na^+ concentration was as high as 122 m*M*. The only evidence for a leucine transport process dependent on the intracellular Na^+ concentration came from studies using the preincubated cells. The residual leucine uptake in the absence of extracellular Na^+ was increased twofold when the initial intracellular Na^+ concentration was raised from 8 to 57 m*M*. This finding may be interpreted as evidence for the existence of an intracellular as well as an extracellular site of Na^+ action in the transport of leucine. While these results give no direct indication of the mechanism by which extracellular Na^+ is required for active leucine transport, they are generally consistent with the Na^+-gradient hypothesis for amino acid transport (1, 2).

Summary. The effects of changes in the extracellular as well as the intracellular Na concentrations on the movement of leucine into or out of isolated intestinal epithelial cells was determined. The influx of leucine was optimal at an initial extracellular Na^+ concentration of 118 m*M* despite variations

in the intracellular Na^+ concentration over the range 8–122 m*M*. The influx of leucine was decreased 40% when the initial extracellular Na^+ concentration was zero and the magnitude of the reduced influx was not affected by initial intracellular Na^+ concentrations over the range 57–122 m*M*. Leucine movement into or out of leucine-loaded cells followed the direction of the Na^+-gradient across the cell membrane. These results indicate that extracellular rather than intracellular Na^+ is the determining factor in the Na^+ dependence of leucine transport, a finding consistent with the Na^+-gradient hypothesis for amino acid transport.

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