INTERACTIONS BETWEEN LEUCINE AND ARGININE TRANSPORT IN CHICKEN SMALL INTESTINE

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The apparent initial reactions of neutral and basic amino acids with the brush-border epithelium in chicken small intestine, as studied by an *in vitro* tissue-accumulation procedure in 5 sec experiments take place by separate, nonoverlapping processes. The previously observed one-way interaction in which neutral amino acids diminish the uptakes of basic amino acids in 5 min experiments can now be attributed to a process that is separate from the apparent initial event in transport.

We previously suggested that the 5 min absorptions of neutral and cationic amino acids by the small intestine of the chicken occur by distinct mechanisms[1]. In partial support of this contention, we observed that while cationic amino acids were good inhibitors of each other in transport, this class of amino acids was poor or insignificant in effect on the uptakes of methionine and leucine. The absorption routes for these classes, however, cannot be judged in 5 min experiments as completely separate since other data in the same report showed arginine and lysine to be strongly inhibited by several long-chain neutral amino acids. Similar observations, especially with regard to this one-way interaction, have been noted in a number of other animal tissues and are reviewed by Herzberg, *et al.*[1].

To explore the one-way effect further, we developed a technique to measure amino acid fluxes in very short incubation periods. The present data indicate that the apparent initial reactions of leucine and arginine with the absorptive epithelium do occur by separate processes as adduced by the absence of cross inhibitions, whereas, the previously observed interaction becomes manifested beyond the 5-sec incubation time.

MATERIALS AND METHODS

Black sex-linked female chickens, 8-13 weeks old, were fasted 24 hr prior to sacrifice and then killed by cervical dislocation. A modified tissue-accumulation method was used[1]. In essence, 100 mg sections of tissue on either side of the yolk stalk were incubated for designated periods (15 sec through 5 min) at 37°C in Krebs-Henseleit buffer (5 ml) containing [^{14}C] amino acid, alone, or in the presence of inhibitory amino acid, and in an atmosphere of 95% O_2:5% CO_2 (v/v). Tissues were homogenized in 2.5% trichloroacetic acid using 5 ml of this solution per g of tissue; the extracts were clarified by centrifugation and radioactivity was determined by liquid scintillation techniques.

The short incubation method developed by Schultz and Curran[2] was applied in modified form for 5 sec experiments. Briefly, tissues were incubated on the surface of wet Whatman No. 1 filter paper, which was seated on a Hirsch funnel. The latter was mounted on a vacuum flask. The reaction was monitored with a timer-timeswitch equipped with automatic buzzer alarm (Fisher Scientific). Incubation was initiated by pouring the preheated (37°C) and pregassed 5 ml portion of amino acid solution onto the filter and was terminated as the buzzer sounded by opening a clamp which allowed a vacuum to evacuate substrate solution from the filter. In the final step, tissues were washed with Krebs-Henseleit buffer.

Uniformly labeled L-[^{14}C]leucine, L-[^{14}C]arginine and carboxy-[^{14}C] inulin were obtained from New England Nuclear. All compounds were of the highest purity offered. Inulin was further purified by column chromatography on Sephadex G-25 (fine) using distilled water as eluent. Total tissue water was 77 percent of the total wet tissue weight. Values for extracellular space, determined by inulin penetration, were one percent (5sec incubation), 4.6 percent (30 sec), 4.8 percent (one min), 5.5 percent (3 min) and 5.6 percent (5 min), respectively, of the total wet tissue weight according to the method of Kipnis and Parrish[3]. With values of this magnitude, it was not necessary

to correct amino acid accumulation for nonspecific entry into extracellular space.

RESULTS

Table 1 shows the accumulations of leucine and arginine from 0.1 mM solutions over incubation periods from 5 sec through 5 min. With progressing intervals of time, the observed uptakes diminish relative to their respective calculated values, which are based upon the assumption that substrate entry is linearly related to time through 5 sec. The arginine uptake value at 5 min is 57 percent of the calculated value, while that for leucine is 38 percent.

TABLE 1--EFFECT OF TIME ON AMINO ACID UPTAKE

Experimental conditions are given in the text. The number of animals used to obtain each mean is given in parentheses. Values given in brackets have been calculated on the basis of the assumption that uptake rate is linearly related to time through 5 sec of incubation. Amino acid concentration 0.1 mM.

	Amino acid accumulation (nmoles/ml extract ± S. E.)	
Time (sec)	Arginine	Leucine
5	0.42 ± 0.02 (10)	1.56 ± 0.08 (23)
30	2.56 ± 0.08 (10)	6.82 ± 0.45 (15)
	[2.52]	[9.36]
60	3.65 ± 0.30 (10)	10.6 ± 0.90 (14)
	[5.04]	[18.7]
300	14.4 ± 0.70 (5)	35.1 ± 1.5 (14)
	[25.2]	[93.6]

Lineweaver-Burk plots for the uptakes of arginine and leucine in 5 sec are presented in Fig. 1; the apparent Michaelis constants are 7.1 mM and 2.6 mM, respectively; v_{max} values are 400 nmoles arginine/ml extract per min and 500 nmoles leucine/ml extract per min.

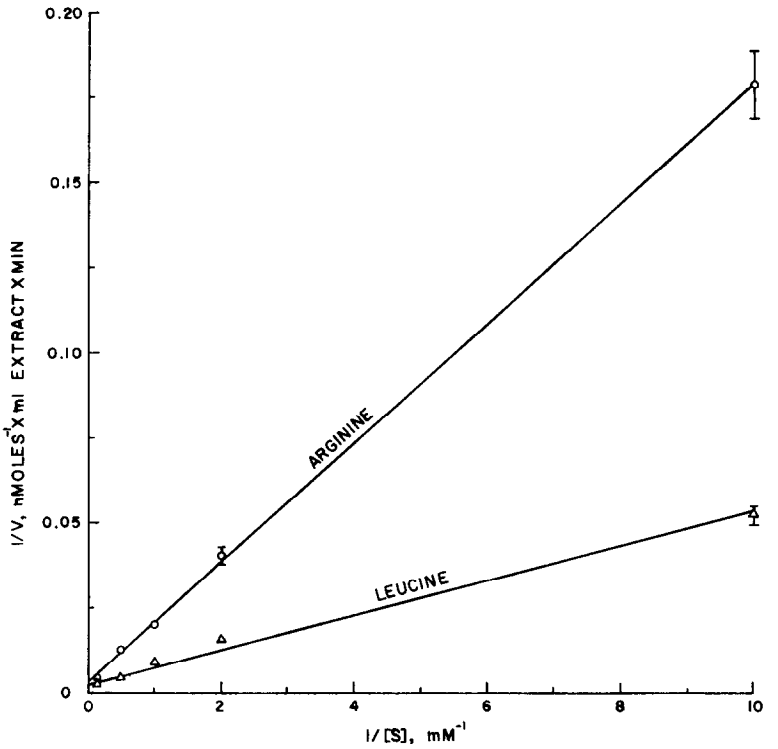

Fig. 1. Lineweaver Burk plots for the 5 sec uptakes of arginine and lysine. Incubation of labeled amino acids was in Krebs-Henseleit buffer as described in the text. Each graphed value represents the mean of ten determinations on a total of 40 intestinal segments. Ten animals were used to obtain each point. Variability is represented by the standard error of the mean.

The inhibition of 0.1 mM leucine transport by 2.5 mM isoleucine (Fig. 2) is a constant in incubation experiments ranging in time from 15 sec through 5 min, though a somewhat smaller effect is seen in 5 sec. Leucine uptake under the same conditions is not reduced by homoarginine in trials performed at 5 sec or at 5 min, where the percentage inhibitions were found to be 3.4 ± 4.6 ($n = 10$) and 0.4 ± 7.5 ($n = 5$), respectively. The inhibition of arginine flux by its analog homoarginine increases in a constant manner with time, the 5 min value being approximately two-fold higher than that obtained in 5 sec (Fig. 2). The reduction of arginine accumulation as caused by leucine parallels the effects of homoarginine between 30 sec and 5 min, although leucine is ineffective in the 5 sec experiment.

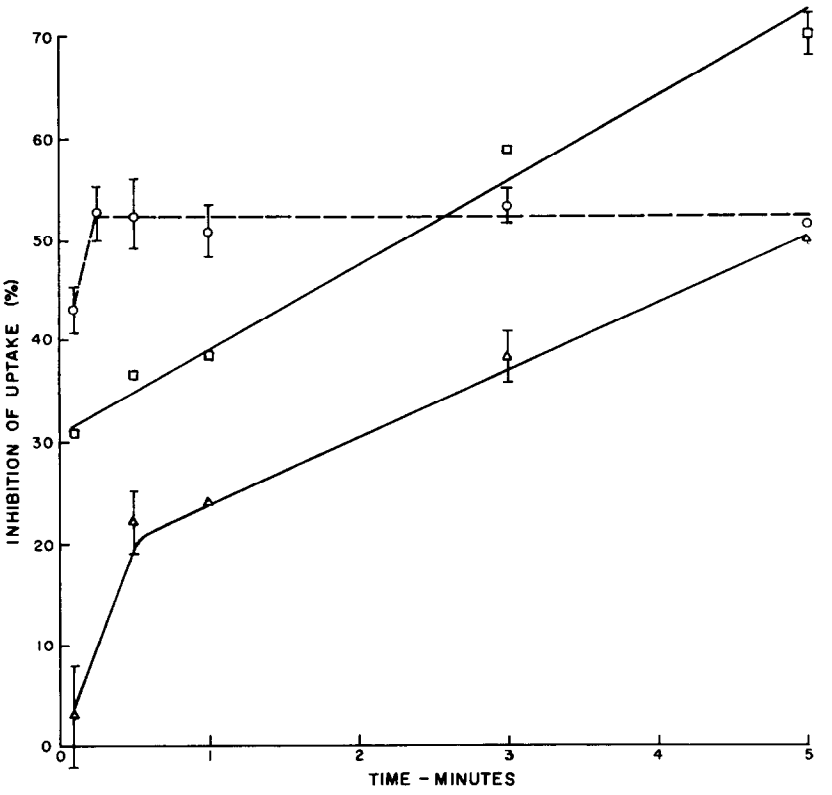

Fig. 2. Time course of amino acid inhibition. ---o---, percent inhibition of leucine uptake in the presence of isoleucine; —□— ,percent inhibition of arginine uptake in the presence of homoarginine; —Δ—, percent inhibition of arginine uptake in the presence of leucine. Each graphed value represents the mean of approximately 5 or more determinations. Substrate concentration 0.1 mM, inhibitor concentration 2.5 mM. Sample variability is given by the standard error of the mean.

DISCUSSION

Munck and Schultz[4] have postulated that leucine (in low medium concentration relative to lysine) stimulated the efflux of lysine across the lateral and/or serosal borders of rabbit ileum by interacting at an agency which does not appear to be involved in leucine influx. (At high ratios of leucine to lysine, leucine causes inhibition of lysine transport.) They have noted the former process to be characteristically Na^+-independent. Likewise, we found in previous work in chicken intestine that, contrary to the uptakes of arginine and leucine, the inhibitory effect of leucine on arginine transport is relatively Na^+-independent (Ref. 1 , Fig. 9). According to Munck and Schultz,

the stimulatory phenomenon cannot be explained in terms of known carrier models; they suggest, instead, that leucine acts in a direct noncompetitive manner with a carrier responsible for lysine movement across the serosal boundary of the epithelium. We are reminded that our former work as well as that of Munck and Schultz pertains to experiments in which the effects of leucine occur in incubation periods no shorter than 5 min. Our current results, on the contrary, show no significant cross interactions between these representative neutral and basic amino acids in 5 sec experiments. Thus, the apparent initial reactions for these classes take place by separate processes. Moreover, the fact that the one-way reaction between leucine and arginine is time dependent may indicate some degree of spacial separation between their site of interaction (serosal boundary of the epithelial cells?) and the sites involved in their entries into the epithelial cells. The spacial orientation hypothesis may also provide an explanation for the progressive inhibitory action of homoarginine on arginine uptake in that these effects may represent interactions, with respect to time, at multiple sites.

Consistent with the present findings, inhibition of basic amino acid transport (5 min experiments) brought about by neutral amino acids, such as leucine, appears to occur at a site other than the one involved in the transport of the neutral compound[1]. Thus, at least two neutral amino acids, 2-amino-bicyclo[2.2.1]heptane-2-carboxylic acid and serine, which modify methionine transport strongly in chicken intestine, do not significantly inhibit lysine entry. Furthermore the strong reduction of lysine flux caused by phenylalanine, we found, could not be altered in the presence of excess serine, which blocks the reaction of phenylalanine with the site of transport for leucine and methionine.

The composite of these results serves to emphasize the viewpoint that subtle regulatory mechanisms exist on the substrate level which modulate the time-dependent aspects of amino acid absorption.

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