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THE EFFECTS OF HYPOTHYROIDISM AND FASTING ON ELECTROGENIC AMINO ACID TRANSFER: POSSIBLE EVIDENCE FOR MULTIPLE NEUTRAL AMINO ACID CARRIER SYSTEMS IN RAT JEJUNUM

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Summary

The jejunal mechanisms for the electrogenic transfer of four neutral amino acids (alanine, leucine, methionine, valine) and for sarcosine were characterised by an electrical method in vitro. The values for apparent K_m obtained electrically agree well with those assessed by conventional chemical techniques. Hypothyroidism and/or fasting rats for 3 days induced differential changes in the apparent K_m and $p.d._{max}$ for the various amino acids. These alterations were interpreted as indicating the presence of at least three mechanisms for neutral amino acid transfer and one for sarcosine.

In euthyroid rats, only alanine showed changes in apparent K_m (decrease) and $p.d._{max}$ (decrease) after fasting for 3 days. With hypothyroidism the kinetic parameters of electrogenic transfer for alanine, valine and sarcosine were significantly altered while those for leucine and methionine were unaffected.

Introduction

The number of separate mechanisms for the transfer of amino acids across the small intestine is still a contentious and confusing issue. It is generally agreed that at least one active transfer system exists for the neutral (monoamino-monocarboxylic-isoionic), the basic (diamino-monocarboxylic-cationic) and the acidic (monoamino-dicarboxylic-anionic) amino acids in many species [1—3]. However, a more recent review [4] describes at least five distinct pathways for L-amino acid transfer. With the neutral amino acids there is further controversy with evidence that various neutral amino acids can use each of the five pathways described by Wiseman [4]. On the other hand Lerner [5] suggests three systems and Boorman [6] two. An alternative explanation to this con-

fusion was suggested by Alvarado [7], that is, there is a single polyfunctional carrier containing multiple binding sites with allosteric interaction.

Practically all the evidence for delineating the pathways stems from in vitro studies on the mutual inhibition of transfer by various amino acids. Unfortunately such experiments do not distinguish between competition by two amino acids for a common carrier or a common energy source [2,8]. This is especially so when the experiments are undertaken in vitro with intestinal preparations that are not supplied with an exogenous metabolisable substrate.

In order to avoid such difficulties we have investigated the transfer of various neutral amino acids and of sarcosine across intestine taken from rats exposed to hormonal and dietary changes. These changes have previously revealed the presence of multiple hexose transfer systems [9—11], which has been independently confirmed [12—14].

Rats were made hypothyroid, or were fasted, or were fasted in the hypothyroid state. The jejuna from rats in these different states were removed and the electrogenic transfer of four neutral amino acids (valine, leucine, methionine, alanine) and of sarcosine were characterised in vitro by an electrical method which allows the operational kinetic parameters of apparent K_m and the $p.d._{max}$ (maximum potential generated) to be obtained. If the neutral amino acids were transferred by the same electrogenic active transfer mechanism the qualitative changes induced in their operational kinetic parameters by dietary and hormonal stress should be similar. Analysis of the induced changes in electrogenic transfer, however, showed clear differences between some of the neutral amino acids. One possible interpretation of these results is that at least three electrogenic transfer mechanisms exist for neutral amino acids and a separate one for sarcosine. The data, moreover, are of additional interest in that they characterise the effects of hypothyroidism and of fasting on the electrogenic transfer mechanisms for the various amino acids.

Materials and Methods

Animals. White male rats of the Sheffield strain weighing 230—250 g were used. Before experiments the animals were allowed free access to food (Diet 86 supplied by Burnhill, Cleckheaton) and water. When animals were fasted the food was removed for 3 days but they were allowed free access to water.

Production of hypothyroid rats. Rats were made hypothyroid by adding 0.5 mM 6-*n*-propyl-2-thiouracil to their drinking water for approximately 28 days. Details of the various measurements confirming that this treatment induced hypothyroidism have been published previously [15].

Preparation of everted sacs and measurement of amino acid transfer potential differences. The techniques used to prepare the everted sacs and measure their transfer potential differences in vitro were similar to those published previously [9,15]. A full description is given elsewhere [16]. The serial additions of sarcosine, methionine, alanine, leucine or valine were made to the bicarbonate saline bathing the mucosal surface of the jejunal sac (mucosal solution). The actual range of serial concentrations used (0.5, 1, 2, 4, 8 mM; 1, 2, 4, 8, 16 mM; 2, 4, 8, 16, 32 mM) depended on the amino acid and the hormonal and/or dietary status of the rat. Correction of the amino acid transfer p.d.

values for the osmotic effects of the added amino acids was done by making serial additions of like concentrations of mannitol to the mucosal fluid of a second everted sac taken from the same animal incubated at the same time. The osmotic p.d. values produced were then added to the transfer p.d. values. Addition of the amino acids to the mucosal fluid did not significantly alter its pH.

Expression of results. Results are expressed as the mean ± standard error (S.E.) of the mean with the number of animals in brackets. The data were treated by an analysis of variance and the differences between groups located by the least significant range test (LSR) as described by Sokal and Rohlf [17].

$$\text{LSR} = Q \sqrt{MS_{\text{within}}\left(\sqrt{\frac{n_1 + n_2}{2n_1n_2}}\right)}$$

Q is obtained from the tables of ref. 18 and MS_{within} is taken from the analysis of variance table while n_1 and n_2 are the number of observations in each of the two groups compared. The results are taken as significant when the observed difference between two means was greater than the computed difference. The test was applied at the $P = 0.05$ level.

Results

The operational kinetic parameters for electrogenic amino acid transfer of the amino acids studied are listed in Table I. The values for valine in euthyroid and hypothyroid rats have been reported previously [16]. In Table II the effects of fasting and hypothyroidism on the operational kinetic parameters for the electrogenic transfer of the amino acids are given allowing comparisons to be made. Because the parameters for leucine and methionine were unaffected by changes in either the hormonal or the dietary status of the rats they are not mentioned specifically in the following sections.

Effects of hypothyroidism on amino acid electrogenic transfer

(a) Fed rats. (Comparison 1, Table II). The kinetic parameters for valine and alanine show strikingly different behaviour in the fed hypothyroid rat. In the case of valine the p.d_{max} was significantly stimulated by 61% but the apparent K_m was unaffected. With alanine the opposite sequence occurred, its apparent K_m was significantly depressed by 26% but its p.d._{max} was unaffected. In contrast both the apparent K_m and p.d._{max} for sarcosine were significantly reduced. The depression of its apparent K_m was so large (56%) that it was necessary to use a much lower serial range of concentrations than that used for euthyroid intestine to elicit the transfer kinetics.

(b) Fasted rats. (Comparison 4, Table II). In a number of cases hypothyroidism had different effects on kinetic parameters in the fasted animal compared to that induced in the fed state (Tables I and II). The apparent K_m for valine was significantly reduced by 24% but the p.d._{max} was unaffected. The p.d._{max} for alanine showed a small (13%) but significant increase while the apparent K_m was unaffected. With sarcosine its apparent K_m decreased by 41.5% but the p.d._{max} was unaffected.

TABLE I

THE OPERATIONAL KINETIC PARAMETERS OF APPARENT K_m (mM) AND p.d.$_{max}$ (mV) FOR VALINE, LEUCINE, METHIONINE, ALANINE AND SARCOSINE IN FED AND FASTED EUTHYROID AND FED AND FASTED HYPOTHYROID RATS

The results are given as the mean ± S.E. The figure in brackets represents the number of animals used. The concentration range of amino acids used was 2, 4, 8, 16 and 32 mM unless otherwise indicated.

	Valine		Leucine		Methionine		Alanine		Sarcosine	
	Apparent K_m (mM)	P.D.$_{max}$ (mV)	Apparent K_m (mM)	P.D.$_{max}$ (mV)	Apparent K_m (mM)	P.D.$_{max}$ (mV)	Apparent K_m (mM)	P.D.$_{max}$ (mV)	Apparent K_m (mM)	P.D.$_{max}$ (mV)
Euthyroid fed	2.75 ± 0.21 (14)	4.57 ± 0.19 (14)	1.63 ± 0.32 * (9)	3.02 ± 0.21 (9)	1.17 ± 0.09 ** (14)	3.24 ± 0.23 (14)	6.34 ± 0.55 (9)	9.43 ± 0.60 (9)	10.85 ± 0.94 (10)	3.08 ± 0.25 (10)
Hypothyroid fed	2.83 ± 0.24 (15)	7.25 ± 0.27 (15)	0.99 ± 0.12 * (9)	3.09 ± 0.18 (9)	1.07 ± 0.13 ** (11)	4.06 ± 0.21 (11)	4.72 ± 0.50 (11)	9.04 ± 0.50 (11)	4.83 ± 0.82 ** (6)	1.36 ± 0.11 (6)
Euthyroid fasted	2.59 ± 0.19 (16)	4.86 ± 0.28 (16)	1.67 ± 0.38 * (7)	4.00 ± 0.42 (7)	0.96 ± 0.06 ** (12)	3.36 ± 0.27 (12)	2.98 ± 0.46 (9)	6.65 ± 0.59 (9)	8.78 ± 0.74 (11)	2.05 ± 0.22 (11)
Hypothyroid fasted	1.86 ± 0.20 (10)	5.62 ± 0.52 (10)	1.18 ± 0.28 * (10)	4.29 ± 0.61 (10)	1.11 ± 0.15 ** (9)	3.97 ± 0.30 (9)	3.41 ± 0.26 (9)	7.49 ± 0.56 (9)	5.03 ± 0.63 ** (20)	2.41 ± 0.28 (20)

* Concentration range = 0.5, 1.0, 2.0, 4.0, 8.0 mM

** Concentration range = 1, 2, 4, 8, 16 mM

TABLE II

DATA PRESENTED IN TABLE I ANALYSED BY THE LEAST SIGNIFICANT RANGE TEST

The test [17] compared the computed difference between actual difference between two particular means. The results significant at $p = 0.05$ are marked by an asterisk (*).

Comparison	Valine		Leucine		Methionine		Alanine		Sarcosine	
	Δ Apparent K_m (mM)	Δ P.D.$_{max}$ (mV)	Δ Apparent K_m (mM)	Δ P.D.$_{max}$ (mV)	Δ Apparent K_m (mM)	Δ P.D.$_{max}$ (mV)	Δ Apparent K_m (mM)	Δ P.D.$_{max}$ (mV)	Δ Apparent K_m (mM)	Δ P.D.$_{max}$ (mV)
1. Euthyroid fed vs. hypothyroid fed	0.08	2.68 *	0.64	0.07	0.10	0.82	1.62 *	0.39	6.02 *	1.72 *
2. Euthyroid fed vs. euthyroid fasted	0.16	0.29	0.04	0.98	0.21	0.12	3.36 *	2.78 *	2.07	1.03
3. Hypothyroid fed vs. hypothyroid fasted	0.97 *	1.63 *	0.19	1.20	0.04	0.09	1.31	2.55 *	1.15	1.05
4. Euthyroid fasted vs. hypothyroid fasted	0.73 *	0.76	0.49	0.29	0.15	0.61	0.43	0.84 *	2.80 *	0.36

The effect of fasting on amino acid electrogenic transfer

(a) Euthyroid rats. (Comparison 2, Table II). Remarkably only the electrogenic transfer of alanine appeared to be significantly affected by the 3-day fast. Compared to fed euthyroid rats, the apparent K_m was reduced by 53% while the $p.d._{max}$ fell by 30%.

(b) Hypothyroid rats. (Comparison 3, Table II). Comparison of the electrogenic parameters in fed hypothyroid animals to those in the fasted condition indicates that only valine and alanine show significant changes. The apparent K_m for valine was depressed by 34% and its $p.d._{max}$ by 22% whereas with alanine only a modest (but significant) decrease in $p.d._{max}$ was observed, the change in apparent K_m being insignificant.

Discussion

The data will be discussed firstly for investigating the effects of diet and hypothyroidism per se on amino acid transfer mechanisms and second for assessing the various transfer processes for amino acids.

(1) The effects of hypothyroidism and fasting on electrogenic amino acid transfer

It has been previously shown that in hypothyroidism the V and $p.d._{max}$ for valine transfer are greatly enhanced [9,19]. The present study extends the observations to four other amino acids and has shown that the electrogenic transfer of alanine and sarcosine are affected by hypothyroidism whereas leucine and methionine are unaffected. Thus valine appears unique in exhibiting an enhanced $p.d._{max}$ with no significant change in apparent K_m.

Fasting causes a dramatic reduction in the apparent K_m values for actively transferred sugars in euthyroid rats both in vitro and vivo [9,11,20,21]. In the case of the amino acids studied it is remarkable that alanine electrogenic transfer alone shows significant changes in the apparent K_m (decreases) and the $p.d._{max}$ (decreases) upon fasting euthyroid rats.

It is of interest to note that the apparent K_m values for the various amino acids obtained by our electrical technique agree well with those obtained by conventional chemical studies of transfer in vitro (Table III). A similar agreement can also be observed between the electrical measurement, $p.d._{max}$, and the V (maximum transfer capacity) obtained chemically [9,22].

(2) The assessment of multiple transfer mechanisms for neutral amino acid transfer

It is clear from our results that the experimental hormonal and dietary states induced differential changes in the apparent K_m values and $p.d._{max}$ values for the electrogenic transfer of sarcosine and the four neutral amino acids. While the kinetic parameters for methionine and leucine were unaffected by the various experimental conditions those for valine, alanine and sarcosine were changed. Initially this suggests the presence of at least two mechanisms for neutral amino acid transfer. More detailed analysis of the changes in the kinetic parameters in Table II, however, reveals the necessity for postulating more than two mechanisms. If the fed euthyroid group is compared with the fed hypo-

TABLE III

Comparison of estimates of apparent K_m values obtained from euthyroid rats for leucine, valine, alanine, methionine and sarcosine from chemical measurements of transfer undertaken in vitro and from electrical measurements of transfer p.d. values obtained in this present study and by other authors.

	Apparent K_m (mM) from chemical measurements	Apparent K_m (mM) from electrical measurements
L-Leucine	0.7 [27]	2.2 [36]
	2.2 [28]	1.6 (this study)
	1.7 [26]	
L-Valine	2.1 [27]	2.8 (this study)
	2.9 [29]	
	6.8 [30]	
	2.0 [19]	
	3.3 [28]	
	1.8 [26]	
	4.4 [24]	
L-Alanine	5.0 [27]	5.0 [22] *
	6.3 [28]	6.3 (this study)
	7.5 [31] *	
L-Methionine	5.3 [28]	0.8 [22] *
	2.2 [32] *	0.7 [36]
	0.9 [27]	1.2 (this study)
	0.6 [33]	
	1.1 [34]	
	1.0 [24]	
Sarcosine	1.9 [28]	10.9 (this study)
	10.0 [35]	

* Estimated from data

thyroid rats (comparison 1, Table II) it is clear from the differential changes in the kinetic parameters for each amino acid that some four mechanisms are needed to accommodate such changes. One is needed for valine where the $p.d._{max}$ is significantly enhanced by the hypothyroid state, one for alanine where the apparent K_m is decreased but the $p.d._{max}$ is unaltered, one for leucine and methionine [23] which remain unaltered and lastly one for sarcosine [23] whose apparent K_m and $p.d._{max}$ fall. As described in the introduction, previous studies on the mutual competition of neutral amino acids for transfer have led to a confused picture but our present studies would indicate the presence of three transfer systems with a separate mechanisms for sarcosine. Confirmation for the suggestion that the transfer systems for valine and methionine respond differently has been obtained by Jones and Smith [24]. They found that the toxic polyphenol, gossypol, differentially affected the transfer of valine and methionine despite the fact that they are supposed to be transported by one system.

In our analysis we have assumed that the differential changes in the operational parameters of apparent K_m and $p.d._{max}$ for the various amino acids indicates that they traverse the membrane by different mechanisms. It is important, however, to assess whether other explanations can account for the

observed changes in the kinetic parameters. Alterations either in intestinal resistance or unstirred layers can be excluded as these are unaffected by the conditions employed [16]. Alterations in the pH of the microclimate at the luminal surface of the enterocytes could affect the ionization of individual amino acids and influence their transfer. While previous studies have shown that electrogenic amino acid transfer can be influenced differentially by modest changes in mucosal fluid pH [25], it is unlikely that this mechanism is the cause of the changes observed in present experiments. For example, while valine and leucine have very similar pI values (isoelectric points) they behave differently (Table II) moreover leucine and methionine have very different pI values but show identical behaviour. Another possibility is that altered levels of cyclic AMP affects the amino acid uptake mechanisms. Kinzie et al. [26] have claimed that uptake of lysine and leucine by jejunal mucosa was stimulated by increased levels of tissue cyclic AMP concommitant with a decrease in their apparent K_m values. As we know of no data about cyclic AMP levels in fed and fasting hypothyroid intestine it is impossible to say whether this mechanism could be involved in our changes. It is very unlikely, however, as the stimulation of uptake of leucine by the nucleotide was associated with a decrease in its apparent K_m. In all of our experimental conditions the apparent K_m for leucine remained unaltered.

In conclusion we feel that the best interpretation of our data at present is that there are three carriers for neutral amino acids.

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