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The stimulating effect of low pH on the amino acid transferring systems of the small intestine

We have previously shown that while high pH (7.8) has little effect on the intestinal transfer of glycine, low pH (6.3) increases its transfer and the transfer potential differences generated by the addition of the amino acid to the mucosal fluid bathing the everted jejunum of the rat incubated *in vitro*[1]. Other amino acids (leucine and proline) also showed enhanced transfer at low pH. Recent studies have shown that there are at least two separate mechanisms for neutral amino acid transfer in rat intestine. One can be called the 'sarcosine system', as this amino acid appears to be exclusively transferred by this system[2], while the other has the greatest affinity for methionine[3]. Both mechanisms can handle other amino acids like proline and glycine but leucine appears to prefer the methionine mechanism[3]. This present communication deals with the effects of low pH on these various transfer mechanisms. The results indicate that transfer by the 'sarcosine system' is stimulated more by low pH than that by the methionine system.

The experiments were conducted using mid-jejunal intestine removed from fed, female rats. Everted sacs were prepared and incubated *in vitro* for 60 min at 37° in buffered salines of varying composition gassed with O_2–CO_2 (95:5, v/v) or 100% O_2 (phosphate salines). The various amino acids were initially present in the mucosal fluid only at a concentration of 7.5 mM. The parameter chosen to express transport was 'mucosal transfer per g initial wet weight intestine' obtained by measuring the amount of amino acid (or sugar) present in the gut wall and serosal fluid after incubation. It is identical with the net amount of solute disappearing from the mucosal fluid[4]. The ^{14}C-labelled amino acids were obtained from the Radiochemical Centre, Amersham, England. Radioactivity was counted in a Packard Tricarb scintillation counter in a standard scintillation fluid[5] after deproteinisation. The use of radioactive amino acids for intestinal transfer experiments has been previously investigated and found satisfactory[6]. The buffers used for incubations were a standard pH 7.3 bicarbonate saline buffer[7] and its modification to a phosphate saline buffer by replacing the bicarbonate with a sodium phosphate buffer (0.1 M) at pH 7.3. A phosphate saline buffer of low pH was prepared by bringing the sodium phosphate to pH 6.3 by addition of HCl, while the bicarbonate saline buffer was modified to pH 6.3 by lowering the sodium bicarbonate concentration from 25 to 5 mM but maintaining its osmolarity and Na^+ concentration by increasing the NaCl content. No $CaCl_2$ was present in the phosphate buffers in order to avoid precipitation of calcium phosphate; control experiments showed that absence of Ca^{2+} had no effect on intestinal transfer.

The results for the transfers of various amino acids and of galactose are shown in Table I. Apart from methionine, all the amino acids tested had their transfer significantly increased by low pH when compared to the control transfers at pH 7.3. Because the effect is observed in phosphate and bicarbonate saline buffers, the stimulation is most likely caused by the low pH rather than by the changes in HCO_3^- or PO_4^{3-}. In both buffers the transfers of proline, sarcosine and β-alanine are stimulated more by low pH than those of glycine and leucine. Methionine transfer is apparently unchanged by low pH. Glycine transfer, however, in the presence of 15 mM

TABLE I

THE EFFECTS OF LOW pH ON THE MUCOSAL TRANSFER OF VARIOUS AMINO ACIDS AND OF GALACTOSE BY EVERTED JEJUNUM OF RAT SMALL INTESTINE INCUBATED *in vitro*

See text for details. Initial concentration in mucosal fluid, 7.5 mM. The results are given as the mean ± S.E. The figures in parentheses indicate the number of animals used.

	Mucosal transfer (μmoles/g initial wet wt. per h)		*Stimulation (%)*	*Significance of difference between means*
	pH 6.3	*pH 7.3*		
Phosphate salines				
Glycine	55.0 ± 1.5 (14)	43.4 ± 2.2 (10)	27	<0.001
Proline	60.3 ± 2.8 (10)	33.9 ± 5.9	78	<0.001
Sarcosine	32.7 ± 2.3 (5)	21.4 ± 1.9	53	<0.01 >0.001
β-Alanine	44.5 ± 2.5 (5)	21.0 ± 1.5	112	<0.001
Leucine	37.1 ± 2.6 (5)	27.2 ± 2.1	36	<0.02 >0.01
Methionine	37.6 ± 2.1 (5)	33.1 ± 3.2 (4)	14	>0.3
Bicarbonate salines				
Glycine	57.8 ± 2.4 (14)	44.3 ± 1.7 (10)	31	<0.001
Proline	76.4 ± 2.5 (9)	46.5 ± 1.9	64	<0.001
Sarcosine	43.5 ± 0.4 (5)	24.2 ± 1.2	80	<0.001
β-Alanine	43.4 ± 1.5 (5)	26.7 ± 1.8	63	<0.001
Leucine	49.7 ± 2.9 (5)	36.8 ± 2.5	35	<0.01 >0.001
Methionine	47.4 ± 3.6 (5)	40.9 ± 2.3	16	>0.1
Glycine (+15 mM methionine)	31.6 ± 3.3* (5)	11.6 ± 0.2*	172	<0.001
Galactose (28 mM)	109.7 ±14.3 (10)	91.6 ± 13.8	20	>0.3

* Glycine transfers in the presence of 15 mM mucosal methionine.

methionine, is dramatically stimulated at low pH by 172%. Methionine, at this concentration, is known to completely block the carrier for glycine that is methionine sensitive[3], thus the stimulated glycine transfer must have been by the methionine-insensitive 'sarcosine–glycine–proline' system. Hence this carrier or pathway appears to be exceptionally sensitive to the stimulating effects of low pH. The fact that there is a very large stimulation of β-alanine transfer at low pH confirms this conclusion, as this amino acid appears to be transferred mainly by the sarcosine system[8]. Preliminary experiments measuring the transfer potential differences generated by the electrogenic active transfer mechanisms for amino acids[9,10] have shown that the transfer potentials produced by addition of glycine, leucine, proline, β-alanine and valine to the mucosal solution bathing isolated rat jejunum were always greater at pH 6.3 than at pH 7.3, yet the intestinal resistance was not altered by low pH. These results indicate that it is the electrogenic active transfer mechanism for amino acids that is stimulated by low pH and add support to the chemical transfer data of Table I. It is worth mentioning that even methionine transfer potentials were increased at pH 6.3, despite the absence of a statistically significant increase in transfer. Electrical measurements, however, are much more sensitive than absorption experiments so that it is likely that there is even a small stimulation of methionine transfer by low pH.

One unanswered question is how the increased hydrogen ion concentration stimulates amino acid transfer? It could act by modifying the ionisation and thus the affinity of either the carrier site for the amino acids or of the amino acids for the site,

by increasing the supply of energy to the active transfer mechanisms or by affecting the ion binding site for Na^+ on the postulated ternary 'amino acid–Na^+–carrier' complex[11]. The stimulating effect of low pH is not observed in rat ileum or in hamster mid-jejunum (E. THOMPSON AND R. J. LEVIN, unpublished results) despite the fact that identical changes must occur in the ionisation of amino acids. We think this indicates that it is unlikely that changes in the ionisation of amino acids play a major role in enhancing transfer and strongly suggests that low pH has a specific action on some aspect of the jejunal mucosal cell transfer systems. If this action is to increase their energy supply, it must be specific to certain amino acid transfer mechanisms as galactose transfer is unaltered by low pH. Further experiments are necessary before the other postulated mechanisms can be profitably discussed.

A previous study[12] on the effects of pH on intestinal valine transfer led the authors to report that 'there appeared to be no change in transfer over the pH range 5–8' and that the intestinal carrier site for amino acids had 'no functional constituents with pK changes in the pH range 5–8'. Our data show that the latter conclusion is unwarranted and that low pH, in the physiological range for the jejunum *in vivo*[1], stimulates the transfer of a variety of amino acids.

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