Arteriovenous differences for amino acids across control and acid-secreting rat stomach *in vivo*

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1. A method is described for measuring arteriovenous differences across the rat stomach *in vivo*. 2. Notable results were the uptake of branched-chain amino acids, the uptake of arginine, which was approximately balanced by an output of ornithine, and the output of alanine. 3. The fractional extraction of glutamine from the blood by the stomach wall of pentagastrin-stimulated rats was 4.7%. 4. The arteriovenous differences for ammonia depended upon the blood ammonia concentration. 5. Arteriovenous differences were not affected by the stimulation of acid secretion with pentagastrin. 6. It is concluded that the high activity of branched-chain-amino-acid aminotransferase (EC 2.6.1.42) in the gastric mucosa is associated with metabolism of these amino acids, but that the stomach wall is a less avid user of glutamine than is the small intestine.

Rat gastric mucosa has an activity of branched-chain-amino-acid aminotransferase that is much higher than that found in most other rat tissues, including skeletal muscle (Ichihara *et al.*, 1975). However, a high tissue enzyme activity does not necessarily coincide with a high rate of metabolic flux through the enzyme [e.g. liver glutamine synthetase (EC 6.3.1.2), Lund, 1971]. The first aim of this work was therefore to establish, by measurement of arteriovenous differences, whether the stomach removed branched-chain amino acids from the blood *in vivo*. The second aim was to establish the extent of glutamine metabolism by the stomach wall *in vivo*, for guinea-pig gastric glands *in vitro* appear to oxidize glutamine and glucose at similar rates (Saladino *et al.*, 1981), and glutamine is an important respiratory fuel in the mucosa of the small intestine (Windmueller & Spaeth, 1978). The ultimate intention of this and allied studies is to provide a physiological context for future work on substrate metabolism by isolated parietal cells. No previous measurements of arteriovenous differences across the stomach *in vivo* appear to have been made under either control or acid-secreting conditions.

Materials and methods

Animals

Male Wistar rats (175–225 g body wt.) were obtained from Banting and Kingman, Hull, U.K. Rats were fed on Heygates breeding diet (modified 41 b diet) supplied by Pilsbury, Edgbaston, Birmingham,

U.K. All rats were starved overnight (17 h) before use.

Reagents

Glutaminase (grade V) was obtained from Sigma. All other substrates, enzymes and coenzymes were obtained from Boehringer. Inorganic chemicals and sodium heparin were purchased from BDH.

Measurement of arteriovenous differences

The difficulty of obtaining blood samples from the splenic vein precluded measurement of arteriovenous differences across essentially the whole stomach. Sampling of venous blood from the coronary vein (Greene, 1955), which appears to drain mainly the dorsal fundic region of the stomach was, however, practicable. On a few occasions blood samples were withdrawn from the coronary vein and then the aorta using syringes with the needles inserted into the vessels, but the rate of failure in obtaining a venous sample was so high as to prevent the regular use of this procedure, and an alternative method, described below, which involves simultaneous sampling of venous and arterial blood was adopted as a routine. The results obtained for arteriovenous differences were the same whichever of the two procedures was used.

Rats were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (Sagatal; 60 mg/kg body wt.). The stomach and abdominal aorta were exposed after midline and lateral incisions, and the displacement of other organs. The lower part of

the aorta was cleared of overlying tissue, and the stomach was freed of its connections to the spleen and liver and turned over to expose the coronary vein which was also cleared. Heparin [500 i.u. in 0.2 ml of saline (0.9% NaCl)] was injected into the right common iliac vein. A 25-gauge needle, linked to a plastic collection tube (LP3, Luckham, Burgess Hill, Sussex, U.K.) by polythene tubing (int. diameter 0.58 mm, Portex Ltd., Hythe, Kent, U.K.) was inserted into the aorta. A micro-funnel (max. ext. diameter 3.9 mm) made from the narrow end of a 20–200 μ l disposable pipette tip (GK2, Gordon Keeble, Duxford, Cambridge, U.K.) and also linked by polythene tubing (int. diameter 1.19 mm) to a collection tube, was placed over a small nick made with a pair of fine scissors in the coronary vein. The sealed collection tubes, which were surrounded by ice, were in turn connected to two channels of a peristaltic pump (Gilson HP4, Anachem, Luton, Beds., U.K.) and the pump was then switched on effecting the simultaneous removal of arterial and venous blood and its trapping in the collection tubes. The pump rate was adjusted so that 0.5–0.8 ml of venous and arterial blood was collected in 1 min with little loss of the venous outflow.

Measurement and stimulation of acid secretion

Acid secretion was measured by a method similar to that described by Puurunen (1978) and was induced by the subcutaneous injection of pentagastrin (Peptavlon, ICI Pharmaceuticals; 250 mg/kg body wt.). Acid secretion reached a plateau 10–50 min after injection, and arteriovenous difference measurements were made 20 min after administration of pentagastrin. The 'plateau rate' of acid secretion, 2 μ mol of H^+ /min, was 2.3 times that during the 10 min immediately preceding injection of pentagastrin and 10 times that 90–100 min following injection. This latter comparison probably gives a better estimate of the degree of stimulation obtained in separate experiments where only arteriovenous concentration differences were measured, for acid secretion over the period 10–20 min was probably elevated by the manipulation of the stomach during cannulation of the cardiac and pyloric sphincters. Such manipulation did not occur in the experiments where arteriovenous differences and not acid secretion were measured.

Assay of amino acids and glucose

Most amino acids were analysed in plasma rather than whole blood, for, assuming that there is little exchange of amino acids between cells and plasma during their passage through the organ (see below), concentration changes in plasma are likely to be larger and therefore more easily detectable. If such exchanges were to occur then results based solely on measurements of plasma might be underesti-

mates (Felig *et al.*, 1973). Plasma was obtained by centrifuging blood samples for 1 min in a Beckman Microfuge, and it was then deproteinized by mixing with 5 vol. of sulphosalicylic acid (30 g/l). After centrifugation for 15 min at 4°C and 1065 g, the supernatant was stored at –20°C before analysis by automated ion-exchange chromatography using a Locarte amino acid analyser. Assays of glutamine (Lund, 1974), glutamate (Bernt & Bergmeyer, 1974), ammonia (Kun & Kearney, 1974) and glucose (Bergmeyer *et al.*, 1974) were performed on neutralized $HClO_4$ extracts of whole blood (0.5–0.7 ml of blood added to 4 ml of ice-cold 1 M- $HClO_4$, mixed, centrifuged for 15 min at 4°C and 2790 g, and neutralized with 4 M-KOH). No exchange of glutamine between plasma and cells during the passage of blood through the small intestine was found (Windmueller & Spaeth, 1975), and if this is also true for the stomach, results for arteriovenous differences for glutamine expressed per ml of whole blood can be converted to expression per ml of plasma by dividing by 0.53 (0.53 ml of plasma per ml of whole blood).

Results and discussion

Effects of stimulation of acid secretion by pentagastrin

Stimulation of acid secretion by pentagastrin had no effect on the arterial plasma concentration of any of the amino acids that were assayed, and the results (Table 1) have been pooled. With the possible exception of glutamine and glutamate, where no control values for arteriovenous differences were established (see below), the stimulation of acid secretion did not significantly (*t*-test) affect any of the arteriovenous concentration differences (Table 1). Since the blood flow to the mucosa, as indicated by the clearance of [^{14}C]aniline (Main & Whittle, 1973), increases substantially when acid secretion is stimulated, yet the arteriovenous differences were unchanged (Table 1), it is possible that there was a general increase in amino acid metabolism with enhanced acid secretion, but there was no evidence for the metabolism of any particular amino acid being specifically linked with the secretion of acid.

Branched-chain amino acids

There was a significant uptake of all three branched-chain amino acids by the stomach wall in both control and pentagastrin-stimulated rats (Table 1). These amino acids could either be incorporated into tissue protein or be transformed to other metabolites. Since branched-chain amino acids do not make up a particularly large proportion of the amino acids in either gastric mucin or pepsinogen (Kim & Horowitz, 1971; Dayhoff, 1972, respec-

Table 1. *Arteriovenous differences for amino acids across the rat stomach*

Results are expressed as means $\pm$ S.E.M. with the number of animals in parentheses. Measurements were made with plasma except for glutamine and glutamate where whole blood was used (see the Materials and methods section). Arterial blood was sampled from the abdominal aorta, venous blood from the coronary vein (Greene, 1955). No values are shown for tryptophan, which was not stable in the acid extracts, or for asparagine, threonine or serine which were not resolved from glutamine. Glutamine and glutamate were measured enzymically. The probability, P , of differences being significantly different from zero (paired t -test) is denoted as follows: * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, **** $P < 0.001$. Abbreviation used: n.d., not determined.

Amino acid	Arterial plasma concn. (nmol/ml)	Venous — arterial concn. (nmol/ml)	
		Control	Pentagastrin-stimulated
Valine	117 $\pm$ 6 (15)	-11.7 $\pm$ 2.3 (8)***	-7.4 $\pm$ 1.6 (7)***
Leucine	95 $\pm$ 5 (15)	-10.5 $\pm$ 1.5 (8)****	-7.4 $\pm$ 1.3 (7)***
Isoleucine	68 $\pm$ 3 (15)	-7.1 $\pm$ 1.6 (8)***	-6.5 $\pm$ 1.2 (7)***
Cysteine	43 $\pm$ 4 (14)	-5.9 $\pm$ 0.8 (7)****	-9.3 $\pm$ 2.3 (7)***
Arginine	119 $\pm$ 4 (13)	-8.8 $\pm$ 2.8 (7)***	-5.7 $\pm$ 1.3 (6)***
Tyrosine	41 $\pm$ 2 (14)	-4.7 $\pm$ 1.0 (7)***	-2.5 $\pm$ 0.6 (7)***
Phenylalanine	43 $\pm$ 2 (15)	-4.4 $\pm$ 0.8 (8)***	-2.9 $\pm$ 0.3 (7)****
Proline	83 $\pm$ 2 (15)	-0.8 $\pm$ 2.4 (8)	3.1 $\pm$ 2.9 (7)
Methionine	25 $\pm$ 1 (15)	-1.9 $\pm$ 0.8 (8)*	-1.2 $\pm$ 0.8 (7)
Histidine	55 $\pm$ 2 (15)	-0.5 $\pm$ 1.7 (8)	-0.7 $\pm$ 0.5 (7)
Lysine	216 $\pm$ 12 (15)	6.2 $\pm$ 3.7 (8)	7.2 $\pm$ 3.7 (7)
Glycine	216 $\pm$ 7 (15)	7.6 $\pm$ 4.2 (8)	10.4 $\pm$ 6.4 (7)
Aspartate	7 $\pm$ 1 (14)	2.7 $\pm$ 0.6 (7)***	4.2 $\pm$ 0.7 (7)***
Ornithine	31 $\pm$ 1 (15)	11.5 $\pm$ 3.7 (8)**	6.7 $\pm$ 2.0 (7)**
Alanine	215 $\pm$ 13 (14)	15.9 $\pm$ 4.9 (7)**	19.7 $\pm$ 5.3 (7)***
Arterial blood concn. (nmol/ml)			
Glutamine	475 $\pm$ 17 (20)	n.d.	-22.4 $\pm$ 9.5 (20)*
Glutamate	165 $\pm$ 7 (20)	n.d.	8.1 $\pm$ 2.3 (20)***

tively), and since relatively few other amino acids are taken up to a similar extent, and several are produced by the tissue, it is unlikely that the uptake can be accounted for by protein synthesis. The high activity of branched-chain-amino-acid aminotransferase in the stomach glandular mucosa suggests that these amino acids may be transaminated, producing the corresponding 2-oxo acid and glutamate. The output of glutamate, and the amino acids aspartate and alanine, which can receive nitrogen from glutamate by transamination, supports the occurrence of transamination of branched-chain amino acids, but an output of glutamate, aspartate and alanine could also result from the metabolism of glutamine (see below). Whether the 2-oxo acids produced by the branched-chain-amino-acid aminotransferase are further metabolized, or as in skeletal muscle released to a considerable extent into the blood (Livesey & Lund, 1980) is uncertain, but Ichihara *et al.* (1975) demonstrated oxidation of leucine by the gastric mucosa *in vitro*, so it is possible that the branched-chain amino acids could be oxidized to provide energy.

Glutamine, glutamate and ammonia

In both control and pentagastrin-stimulated-rats the arteriovenous differences for glutamine varied

quite considerably between animals, and were not related to variation in the concentration of glutamine in blood [cf. the small intestine (Windmueller & Spaeth, 1974; Hanson & Parsons, 1977)]. A large number of measurements were performed with rats injected with pentagastrin so as to obtain a satisfactory estimate of glutamine metabolism under these circumstances. Statistical analysis (paired t -test) of the results of 20 experiments, 15 of which showed an uptake of glutamine and five an output, demonstrated that on average in a population of rats injected with pentagastrin there was a significant uptake of glutamine by the stomach wall (Table 1). There was also an output of glutamate. The linear regression of the arteriovenous difference for glutamate on the arteriovenous difference for glutamine was:

$$(\text{Venous} - \text{arterial}) \text{ glutamate concn.} = 0.004 - 0.174 \\ \times (\text{venous} - \text{arterial}) \text{ glutamine concn.}$$

(correlation coefficient = -0.746, $P < 0.01$), which suggests that about half of the total glutamate (Table 1) was derived from glutamine. The outputs of alanine and aspartate (Table 1) can also be ascribed to transamination of glutamate derived from glutamine.

Fig. 1. Relationship between arteriovenous difference for ammonia and arterial blood ammonia concentration. Results are from 23 rats injected with pentagastrin (250 mg/kg body wt.). The line was drawn by linear regression analysis of the data, the regression equation being $y = 0.08 - 0.560x$, correlation coefficient -0.504 ($P < 0.05$). A comparison of the deviations from linear regression with the deviations from the second degree polynomial $y = a + bx + cx^2$ (Snedecor & Cochran, 1967) did not suggest any significant curvilinear relationship between the arteriovenous difference for ammonia and the blood ammonia concentration.

Metabolism of glutamine by the gastric mucosal glutaminase (EC 3.5.1.2; Pinkus & Windmueller, 1977) should produce ammonia. Analysis of all the experiments in which the arteriovenous difference for ammonia across the stomach was determined in rats injected with pentagastrin demonstrated a linear correlation between the arteriovenous concentration difference for ammonia and the blood ammonia concentration (Fig. 1). Thus when the blood ammonia concentration was low there was an output by the stomach wall but at higher blood ammonia concentrations there was an uptake (Fig. 1). High concentrations of ammonia might have been a result of trauma associated with surgical manipulation prior to sampling. The mean value for the arteriovenous concentration difference for ammonia was 17.4 ± 11.1 nmol/ml ($n = 23$) which was not sig-

nificantly different from zero. However, a series of experiments in which both glutamine and ammonia were measured showed a significant relationship between the arteriovenous difference for ammonia and that for glutamine; linear regression:

$$(\text{Venous} - \text{arterial}) \text{ ammonia concn.} = 0.003 - 0.709 \\ \times (\text{venous} - \text{arterial}) \text{ glutamine concn.}$$

(correlation coefficient -0.851 ; $P < 0.01$). Thus it seems that an uptake of glutamine (arteriovenous difference < -0.004 nmol/ml) will result in an output of ammonia. The relationships between the arteriovenous differences for ammonia and glutamine, and between the arteriovenous difference for ammonia and the blood ammonia concentration, suggested that there might be a significant relationship between blood ammonia concentration and the arteriovenous difference for glutamine, but we were unable to show this.

The mean fractional extraction of glutamine (4.7%) was much lower than that found in the small intestine *in vivo* (35%; Windmueller & Spaeth, 1975), yet the blood flow (ml/min per g wet wt.) to the two tissues is similar (Malik *et al.*, 1976). Furthermore the mean (venous - arterial) concentration difference for glutamine (Table 1) was much smaller than that for glucose [-109 ± 26 ($n = 8$) nmol/ml], whereas in the jejunum of post-absorptive rats the two substrates are metabolized at similar rates (Windmueller & Spaeth, 1978). The stomach wall, therefore, appears a much less avid user of glutamine than is the small intestine, which accords with the substantially lower activity of glutaminase in the gastric mucosa than in the mucosa of the small intestine (Pinkus & Windmueller, 1977).

Other amino acids

No obvious explanation is available for the uptake of cysteine which is quite large and significantly different from zero (Table 1). There was a significant uptake of arginine which was approximately balanced by an output of ornithine. It would appear that the stomach wall possesses a cell type containing arginase (EC 3.5.3.1) to which plasma arginine has access.

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