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GLUCOSE, GLYCINE AND DIGLYCINE IN TEST MEALS AS STIMULI TO A DUODENAL OSMORECEPTOR SLOWING GASTRIC EMPTYING

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SUMMARY

1. Five subjects took 210 test meals of 750 ml. water containing 30–300 m-molal glucose or glycine, or 15–150 m-molal diglycine, or plain water.

2. The greater the concentration of solute, the greater was the volume of original meal recovered from the stomach after a fixed time.

3. On a molal basis glucose was half as effective as diglycine in slowing gastric emptying. This was consistent with the osmoreceptor being exposed to the diglycine after it had been split by the hydrolase of the cytosol of enterocytes (the absorbing cells of the small intestine).

4. The slowing of gastric emptying (ml./mole.l.) was about 10% greater for glycine than it was for glucose. There was apparently a threshold concentration below which glycine did not slow gastric emptying.

5. It was proposed that the response of the duodenal osmoreceptor might depend upon shrinking and swelling of the lateral intercellular space around the enterocytes.

INTRODUCTION

After an ordinary meal the emptying of the stomach is held in check as a result of stimulation of receptors in the walls of the duodenum. These receptors respond to acid (Hunt & Knox, 1972) to anions of fatty acids (Hunt & Knox, 1968) and to the osmotic pressure of the duodenal contents (Barker, Cochrane, Corbett, Hunt & Roberts, 1974). In the present experiments subjects were given solutions of diglycine as test meals. It was found that the slowing of gastric emptying was greater than could be accounted for by the osmotic pressure of the solutions as given. However, the slowing was appropriate on the basis of osmotic action if it were assumed that each molecule of diglycine had been split to two molecules of glycine. Since this hydrolysis is believed to occur mainly in the cytosol of the absorbing cells, the apparent splitting of the diglycine before it acted allowed some tentative inferences about the site of the osmoreceptor.

METHODS

The methods have been described in detail elsewhere (Hunt & Knox, 1962). After the stomach had been washed out with 250 ml. water at about 8 a.m., test meals of 750 ml. containing various concentrations of single solutes in water were given at 37 °C down a tube into the stomach in about 90 sec. After a time interval that was constant for each subject, but varied from 15 to 25 min for different subjects, the gastric contents were aspirated through the tube. The stomach

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was then washed with 250 ml. distilled water to check the completeness of the recovery. One test meal was given per day. The meals all contained a non-absorbable marker, phenol red (Ivey & Schedl, 1970), which was used to calculate the volume of the instilled meal recovered in the gastric contents. The solutions were given in no particular order, and their compositions were unknown to the subjects. A woman and four men (authors) took fifty-nine meals containing glucose, seventy-four containing glycine, thirty-three containing diglycine and forty-four of plain water. Concentrations varied from 30 to 300 m-mole/l. for glucose or glycine and 15 to 150 m-mole/l. for diglycine. The solutions were not nauseating. The experiments were approved by the local Ethical Committee.

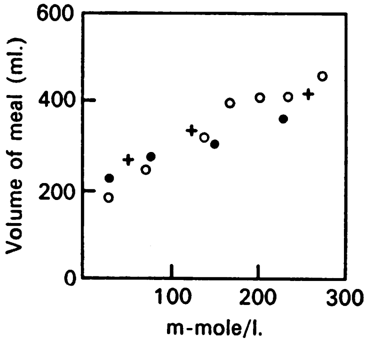

Fig. 1. Mean volumes of recovered test solutions plotted against the concentration of the solute given. Ordinate: volume of test solution recovered after a fixed interval (ml.). Abscissa: concentration of solute in the given solution (m-mole/l.). +, Glucose; ●, diglycine × 2, and ○, glycine.

RESULTS

The general form of the results

The mean volumes of meal recovered after fixed intervals, varying between 15 and 25 min, for the five subjects taking glucose, glycine or diglycine are shown in Fig. 1. The concentrations are expressed as m-mole/l., concentrations for diglycine being shown times 2 as if each mole were completely hydrolysed to 2 moles of glycine. As the concentrations of the given solutes, and thus the osmotic pressures of the solutions, were increased, so were the volumes of meal recovered; the three solutes slowed gastric emptying to almost the same extent. The general result was consistent with the notion that the osmotic pressure of the solution, at some site exposed to the products of the hydrolysis of the diglycine, determined the rate of emptying.

Within-subject analysis

The general form and replicability of the results within-subject can be seen in Fig. 2. Linear regressions within-subject were fitted by least squares to the results for concentration of solute given and volumes of test solution recovered, excluding those for plain water. By analysis of variance it was not possible to distinguish between the results for glucose and those for diglycine ($P > 0{\cdot}2$). However, within-subject regression lines for the pooled results for the three solutes, that is including glycine, had significantly more variance than the regression lines for individual solutes ($P < 0{\cdot}01$). Thus the results for glycine were likely to have been drawn from a population of results different from those for glucose and diglycine.

From inspection of Fig. 2 it appeared that the volumes recovered with low concentrations of glycine were less than those with glucose and diglycine. At high concentrations the volumes recovered with glycine appeared to be more than with the two other solutes. The paragraphs below are concerned with tests of these impressions.

The intercepts on the ordinate of the five regression lines for glycine were consistently less than those for glucose by a mean of 73 ml. s.e. ± 26 ($P < 0\cdot05$). The mean of the five slopes for the regression lines for glycine was greater than that for glucose by 0·45 ml./m-mole.l., s.e. ± 0·16 ($P < 0\cdot05$). The lines for glycine crossed those for glucose. The mean of the five slopes of the regression lines for glycine was also greater than the mean of the five slopes for diglycine by 0·52 ml./m-mole.l., s.e. ± 0·17 ($P < 0\cdot05$). The mean of slopes for glucose and for diglycine differed by only 0·07 ml./m-mole.l., s.e. ± 0·38 ($P > 0\cdot8$).

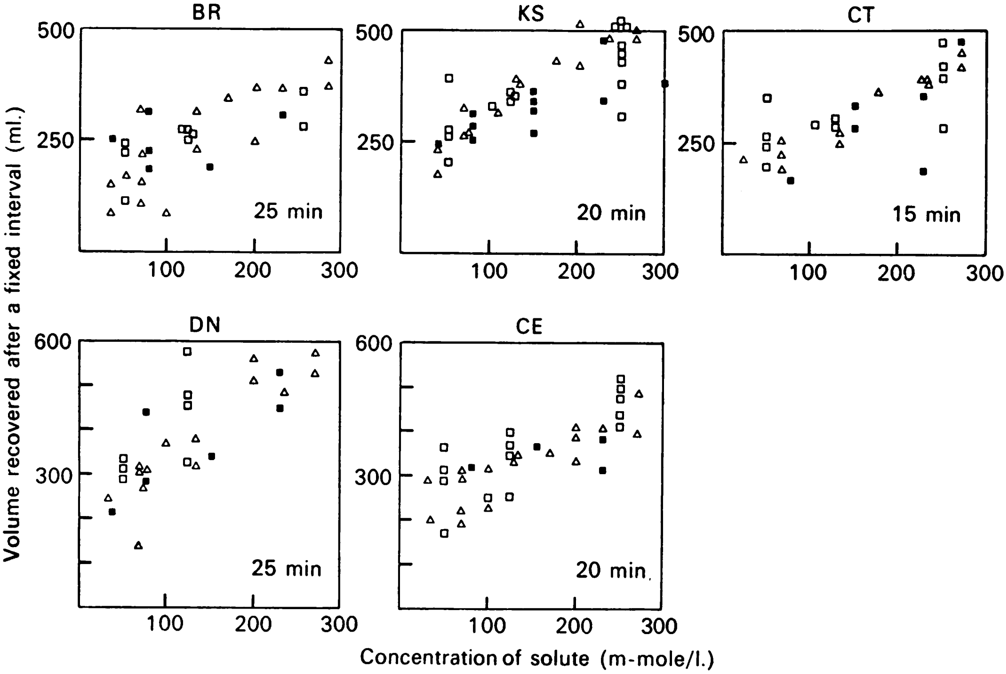

Fig. 2. Volumes of given solutions recovered from five subjects after time intervals, fixed for each subject, but varying from 15 to 25 min between subjects. Ordinate: volume of test solution recovered (ml.). Abscissa: concentration of given solute (m-mole/l.). □, Glucose; ■, diglycine × 2, and △, glycine. Values on the face of the Figures are duration of the tests in that subject.

Although results for test meals of water were available, they were not used to fit regressions to the results of Fig. 2, since the values for water tend to make the regression lines for all solutes run through a common origin. The regression lines for glycine project to the ordinate at a mean volume of 89 ml. s.e. ± 17·4 ($P < 0\cdot01$) less than the mean volumes recovered with water, 253 ml. The mean of intercepts on the ordinate for glucose was 237 ml. and for diglycine was 219 ml. They did not differ, by within-subject testing, from the mean recovered volume of water measured experimentally ($P > 0\cdot3$).

DISCUSSION

If complete hydrolysis of diglycine were assumed, the results for this solute and for glucose were consistent with the slowing of gastric emptying being caused by action on an osmoreceptor. Glycine at high concentrations was about 10% more effective in slowing gastric emptying than would be predicted on the basis of its osmolal concentration, as judged from the results for glucose.

Where is the membrane which is impermeable to glucose?

A number of authors have come to the conclusion that the osmoreceptors mediating the slowing of gastric emptying are distal to the pylorus (Hunt, 1960; Mallinson, 1968; Bell & Mostaghni, 1975; Burn-Murdoch, Fisher & Hunt, 1978) but that they do not respond to the contents of the jejunum in man (Meeroff, Go & Phillips, 1975).

In the present experiments the stimulus which slowed gastric emptying was specified as the concentration of the solute in the test solution as given. However, by the time the solution had reached the duodenum there had been considerable dilution (Meeroff *et al.* 1975). Presumably, the duodenal receptor responded to some concentration which was proportional to that of the given solution, since there was a reliable increase in volume of solution recovered as the concentration of the solute was increased. We assume that the solute, say glucose, reduced the activity of the water in the lumen of the duodenum and thus reduced the flux of water into some structure, as for example occurs with erythrocytes (Ponder, 1940). A change in the tension in the wall of this structure, or of a nerve filament applied to it, could be transduced into a signal which slowed gastric emptying. However, there is some difficulty in finding a structure with the requisite properties.

Solutes which are confined to the lumen of the intestine, say raffinose, mol. wt. 594 (Wheeler, Menzies & Creamer, 1976) are as effective, mole for mole, as glucose (Hunt & Knox, 1971) which is absorbed into the enterocytes. If for the sake of the argument, it is assumed that at the high luminal concentrations used in the present experiments the intracellular concentration of glucose can be treated as zero (Esposito, Faelli & Capraro, 1973) raffinose and glucose could reduce the flux of water from the lumen to cell equally, which could reduce the volume of the enterocyte. But the results of the present experiments sink this argument, since the products of hydrolysis of the diglycine are in all probability formed in the enterocyte (Kim, Birtwhistle & Kim, 1972; Wiseman, 1977) and should swell it, yet gastric emptying was slowed to an extent appropriate to complete hydrolysis of the diglycine. It thus becomes necessary to seek a structure which could be influenced in the same direction by raffinose in the duodenal lumen and the products of hydrolysis of diglycine in the enterocyte. Given some assumptions, the lateral intercellular space around the enterocyte seems a candidate.

Is the lateral intercellular space the osmoreceptive element?

Test meals of saline, 125 m-equiv/l. empty through the pylorus more quickly than plain water (Hunt & Pathak, 1960). Tomasini & Dobbins (1970) found in rats that during the perfusion of the lumen of the jejunum with saline, the lateral intercellular spaces were widened, presumably by water moving secondary to actively transported sodium. ATPase was shown to be localized in the walls of the intercellular spaces. It is suggested here that minimal inhibition of gastric emptying by duodenal osmoreceptors occurs when the lateral intercellular spaces are maximally distended. From the studies of McElligort, Beck, Dinda & Thompson (1975) it appears that in the jejunum of the hampster the enlargement of the lateral intercellular spaces, during absorption of sodium, is confined to the cells at the tip of the villus. These are the cells which are mature, in the sense that they have their full complement of disaccharidases, and presumably of their intracellular hydrolase which splits diglycine.

Mole for mole, mannitol was as effective as raffinose or glucose in slowing gastric emptying (Hunt & Knox, 1971). McElligott *et al.* (1975) found that mannitol when added to saline prevented the widening of the lateral intercellular spaces at the tip of the villus. Thus expansion of the lateral intercellular space occurred under the conditions when gastric emptying could be considered as uninhibited and collapse of the space occurred when the gastric emptying was slowed.

The anomalous effects of glycine

In the present experiments, glycine, at low concentrations, did not slow gastric emptying as effectively as glucose. This follows from the extrapolation of the regression line to the ordinate at zero concentration giving a value for volume recovered less than that for water directly determined.

There are a number of solutes which give similar results. Besides sodium chloride, glycerol and urea solutions empty faster than pure water (Hunt & Pathak, 1960). Urea (Fordtran, Rector, Ewton, Sotev & Kinney, 1965) and presumably glycerol (Buckell & Walsh, 1964) are readily abosorbed from the lumen of the intestine. They should entrain water and carry sodium into the enterocytes, thus promoting its transport into the lateral intercellular space. Since the molecular weight of glycine lies about half way between that of urea and glycerol, it seems plausible that glycine should operate in the same way as the other two solutes.which hasten emptying relative to that of water. The proposed explanation of the action of all three solutes is only tentative.

The notion that osmoreception occurs at the level of the lateral intercellular space is put forward because this is the structure nearest to the lumen which could serve. The lacteal is presumably the next candidate.

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