Effect of a synthetic gastrin-like pentapeptide upon the intestinal transport of sodium, potassium, and water

J. C. GINGELL, M. W. DAVIES, AND R. SHIELDS

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In addition to its marked stimulation of the stomach to secrete acid, the antral hormone, gastrin, can affect other functions of the alimentary tract. Thus, the highly active preparation of gastrin extracted from hog antral mucosa has been shown to stimulate the motility of the stomach and jejunum, to increase the tone of the gall bladder, and to augment the flow of pancreatic juice and hepatic bile (Gregory and Tracy, 1964).

The entire range of physiological activity displayed by natural gastrin is possessed by the C-terminal tetrapeptide-amide, tryptophan, methionine, aspartic acid, and phenylalanine NH₂ (Tracy and Gregory, 1964). The synthetic analogue, which has been produced by the Imperial Chemical Industries (I.C.I. 50,123) and has t-butylxycarbonyl-β-alanine added to the functional tetrapeptide sequence, seems to have the properties of natural gastrin although it is probably less potent on a molar basis (Konturek and Grossman, 1966; Wormsley, Mahoney, and Ng, 1966; Logan and Connell, 1966). Because one of the important functions of the gastrointestinal tract is the absorption of water and electrolytes, we decided to study the effect of the gastrin-like pentapeptide upon the intestinal handling of sodium, potassium, and water.

METHODS

The experiments were performed in trained, placid mongrels, weighing 10-12 kg., in whom segments of jejunum (two dogs), ileum (two dogs), and colon (one dog) were isolated as Thiry-Vella fistulas. The serosal surface area of the intestinal segments measured approximately 100 cm.². The details of the animal preparation and of the technique of measuring the rates of net transport and unidirectional fluxes of sodium, potassium, and water have been given elsewhere (Shields, Mulholland, and Elmslie, 1966).

Briefly, the isolated intestinal segments were first rinsed with modified Tyrode’s solution (Code and McIntire, 1956) at pH 7 and 37°C, until the returning fluid was clear. After 30 minutes, exactly 25 ml. test solution was instilled into the lumen of the fistula. At 10 minutes as much as possible of the luminal solution was withdrawn and the segment immediately irrigated with 100 ml. non-radioactive Tyrode’s solution. From the radioactivity acquired by this solution, the volume of test solution, which could not be aspirated at 10 minutes, was calculated.

The instillation of solutions into, and their withdrawal from, the isolated intestinal segments were facilitated by modified Foley catheters (Code, Bass, McClary, Newnum, and Orvis, 1960) which were inserted into each end of the fistula, leakage being prevented by inflation of the balloons of the catheters.

Two further 10-minute absorption tests were carried out in a similar manner at 1½ and 2½ hours after the end of the first test. The three absorption tests constituted a single experiment.

During all experiments an isotonic solution of sodium chloride was infused intravenously at a rate of 10 ml. per hour by a Palmer constant-rate infusion pump. In some experiments, the pentapeptide (I.C.I. 50,123) was added to the infusion solution to supply a dose of 4 µg. or 8 µg. per kilogram body weight per hour. In control experiments the saline solution was infused alone.

TEST SOLUTION The test solution was modified Tyrode’s solution (Code and McIntire, 1956) containing the radioactive isotopes of sodium (⁴Na—2 µc./litre solution) and of potassium (⁴K—4 µc./litre solution), and the stable isotope of water, deuterium oxide (D₂O—1% v/v). The reaction of the solution, the temperature of which was maintained at 37°C, was brought to pH 7 with 0.1N hydrochloric acid.

ESTIMATIONS The concentrations of sodium and potassium in the test solution were estimated by flame photometry. The separate activities of ⁴Na and ⁴K in mixtures were determined by differential counting in a well-type scintillation counter and in a Geiger-Muller M6 tube, with a wall 1 mm. thick (Veall and Vetter, 1958). The concentration of D₂O was determined by infrared spectrometry using a modification (Shields et al., 1966) of the method of Berglund-Larsson (1956).
CALCULATIONS The rates of movement of sodium, potassium, and water into and out of the intestinal lumen were determined from the formulae of Visscher, Fetcher, Carr, Gregor, Bushey, and Barker (1944a) and Visscher, Varco, Carr, Dean, and Erickson (1944b).

The errors of the method and the assumptions inherent in the calculations have been discussed in detail (Shields et al., 1966).

TERMINOLOGY Sodium, potassium, and water move simultaneously in both directions across the intestinal mucosa. 'Absorption' of a substance is applied to the situation where more of the substance leaves the intestinal lumen and enters the body than moves in the opposite direction into the intestinal lumen. 'Secretion' represents the converse situation, where the substance enters the intestinal lumen more rapidly than it leaves. The terminology describing the directions of movement of water and electrolytes across the intestinal mucosa has been reviewed (Shields, 1964).

RESULTS

NET TRANSPORT (TABLE I) In control experiments sodium and water were absorbed by the isolated segments of jejunum, ileum, and colon. The rates of net transport of these substances were greater in the ileum and colon than in the jejunum. Potassium was secreted by the small intestine but was absorbed by the colon.

When the synthetic gastrin-like pentapeptide was infused at rates of 4 and 8 μg. per kilogram per hour, the rates of absorption of sodium and water were significantly reduced in the ileum. The pentapeptide had no effect upon the net transport of these substances in the jejunum or in the colon.

In the jejunum the secretion of potassium was significantly increased when the pentapeptide was infused intravenously at rates of 4 and 8 μg. per kilogram per hour. In the ileum, the rate of potassium secretion was increased during the infusion of 4 μg. per kilogram per hour, but not with the larger dose of pentapeptide. The net transport rate of potassium in the colon was not affected by the intravenous infusion of the pentapeptide.

**TABLE I**

<table>
<thead>
<tr>
<th>Site</th>
<th>Experiment</th>
<th>Sodium Transport (μ-equiv./10 min)</th>
<th>Potassium Transport (μ-equiv./10 min.)</th>
<th>Water Transport (ml./10 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Net¹ Out of Lumen Into Lumen</td>
<td>Net¹ Out of Lumen Into Lumen</td>
<td>Net¹ Out of Lumen Into Lumen</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Control (9)</td>
<td>+2 ± 26 417 ± 31 415 ± 32</td>
<td>−3 ± 2 14 ± 1 17 ± 2</td>
<td>+0.2 ± 0.1 7.0 ± 0.4 6.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Pentapeptide-4μg./kg./hr. (12)</td>
<td>−64 ± 44 504 ± 43 628 ± 58</td>
<td>−11.3 ± 1 17 ± 1 28 ± 3</td>
<td>−0.1 ± 0.2 7.5 ± 0.8 7.6 ± 0.9</td>
</tr>
<tr>
<td>Difference between 1 and 2</td>
<td>Mean ± S.E.</td>
<td>66 ± 55 147 ± 56 213 ± 73</td>
<td>8.2 ± 4 3 ± 1 11.4 ± 2</td>
<td>0.3 ± 0.3 5 ± 1 0.8 ± 1.2</td>
</tr>
<tr>
<td>t</td>
<td>1.2 2.6 2.9</td>
<td>2.6 3.0 2.7</td>
<td>1.0 0.5 0.7</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.02 &lt;0.01 &lt;0.01</td>
<td>&lt;0.02 &lt;0.01 &lt;0.02</td>
<td>&lt;0.02 &gt;0.5 &gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>Control (20)</td>
<td>+190 ± 25 671 ± 36 481 ± 34</td>
<td>−3 ± 1 20 ± 1 23 ± 1</td>
<td>+1.2 ± 0.1 10.6 ± 0.6 9.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Pentapeptide-4μg./kg./hr. (6)</td>
<td>+31 ± 48 619 ± 16 588 ± 53</td>
<td>−15 ± 4 19 ± 2 34 ± 6</td>
<td>+0.9 ± 0.3 9.0 ± 0.5 8.5 ± 0.6</td>
</tr>
<tr>
<td>Difference between 1 and 2</td>
<td>Mean ± S.E.</td>
<td>159 ± 53 52 ± 67 107 ± 69</td>
<td>12 ± 3 1 ± 2 10 ± 4</td>
<td>0.7 ± 0.3 16.1 ± 0.9 9.1 ± 1.2</td>
</tr>
<tr>
<td>t</td>
<td>3.0 0.8 1.5</td>
<td>3.4 0.5 2.5</td>
<td>2.3 1.3 0.7</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.01 &gt;0.4 &gt;0.1</td>
<td>&lt;0.001 &gt;0.6 &lt;0.02</td>
<td>&lt;0.05 &gt;0.2 &gt;0.4</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>Control (3)</td>
<td>+158 ± 115 389 ± 97 231 ± 211</td>
<td>1 ± 10 0 ± 1 1 ± 10</td>
<td>0.9 ± 0.3 4.4 ± 1.7 3.8 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Pentapeptide-4μg./kg./hr. (3)</td>
<td>+120 ± 80 381 ± 180 261 ± 105</td>
<td>+4 ± 1 12 ± 1 8 ± 2</td>
<td>+1.2 ± 0.3 5.4 ± 0.4 4.2 ± 0.7</td>
</tr>
<tr>
<td>Difference between 1 and 2</td>
<td>Mean ± S.E.</td>
<td>38 ± 134 8 ± 243 30 ± 207</td>
<td>6.5 ± 3 4 ± 2 9 ± 3</td>
<td>0.5 ± 0.8 0.6 ± 0.8 0.0 ± 0.5</td>
</tr>
<tr>
<td>t</td>
<td>0.3 0.03 0.1</td>
<td>1.2 0.8 3.0</td>
<td>0.5 0.6 N.S.</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.7 &gt;0.9 &gt;0.8</td>
<td>&gt;0.3 &gt;0.4 &lt;0.05</td>
<td>0.6 &gt;0.5 N.S.</td>
<td></td>
</tr>
</tbody>
</table>

¹The plus and minus signs preceding the mean rates of net transport indicate absorption and secretion respectively.
²The figures in parenthesis indicate the number of experiments.
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per hour, potassium entered the lumen of the jejunum, ileum, and colon more rapidly than in the control experiments. When the rate of infusion of pentapeptide was doubled, the increase in the rate of potassium movement into the lumen was maintained in the jejunum. Also, in the jejunum, at both rates of pentapeptide infusion, potassium moved more rapidly out of the lumen than in control experiments. In the ileum at the higher rate of infusion the rates of unidirectional movement of potassium did not differ significantly from those in control experiments.

The pentapeptide had no constant effect upon the unidirectional fluxes of sodium and water.

CONCENTRATION OF ELECTROLYTES IN THE LUMINAL SOLUTION (TABLE II) In control experiments the concentration of sodium in the jejunal and ileal solutions did not change significantly during 10 minutes. Also, in the jejunum, no change was observed in the concentration of potassium. There was a slight but significant increase in the ileal concentration of potassium during an absorption test.

During the infusion of the synthetic pentapeptide (at a rate of 4 μg/kg/hr), the concentration of sodium in the ileum rose slightly. The concentration of potassium in both jejunum and ileum increased significantly when the pentapeptide was infused at this dose rate. During the intravenous infusion of the pentapeptide the concentrations of potassium in the jejunal and ileal lumen after 10 minutes were significantly greater (P < 0.02 and P < 0.05, respectively) than the corresponding final concentrations of potassium in the control experiments.

An increase of similar magnitude was observed in the concentration of potassium in the jejunum when the pentapeptide was infused at 8 μg per kg per hour.

DISCUSSION

These results show that, when the synthetic gastrin-like pentapeptide was infused intravenously into dogs, the rates of movement of sodium, potassium, and water into and out of isolated segments of small intestine were markedly altered. The intestinal action of the pentapeptide seemed to be selective. Thus in the ileum, but not in the jejunum, the rates of absorption of sodium and water were greatly reduced. In both the ileum and jejunum, when pentapeptide was infused at 4 μg per kilogram per hour, potassium was secreted more rapidly than in control studies. At the higher rate of infusion of pentapeptide (8 μg/kg/hr), the increase in potassium secretion was maintained in the jejunum but, in the ileum, the rate of secretion returned to control levels. There is no obvious explanation for the failure of the larger dose of pentapeptide to stimulate the ileal secretion of potassium. The colonic handling of water and electrolytes was not affected by the infusion of the gastrin-like pentapeptide.

Recently Gardner, Peskin, Cerda, and Brooks (1967) studied the effect of several gastrointestinal hormones upon the transport of water and electrolytes by everted sacs of hamster intestine in vitro. The introduction of a crude porcine extract of gastrin into the solution bathing the mucosal surface of sacs formed from the distal third of small intestine was followed by a reduction in the net absorption of sodium, chloride, and water. Potassium transport was not affected nor did the remainder of the small intestine show any alteration in the handling of water and electrolytes. The unidirectional flux rates of sodium were studied: the mucosal-to-serosal movement of sodium was reduced when gastrin was instilled into the mucosal solution. The selective action of the gastrin extract upon the ileal absorption of sodium and water is similar to that found in the present study. However, close comparison of the results must be avoided in view of the differences in the nature of the gastrin, in the type of experimental preparation, and in the species of animal.

The changes in the intestinal transport of water

### TABLE II

<table>
<thead>
<tr>
<th>Segment</th>
<th>Solution</th>
<th>Control Mean (s.e.)</th>
<th>Pentapeptide Mean (s.e.)</th>
<th>Control Mean (s.e.)</th>
<th>Pentapeptide Mean (s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>Concentration at t&lt;sub&gt;0&lt;/sub&gt;</td>
<td>150±0.4 (9)</td>
<td>152±0.8 (12)</td>
<td>0.87</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td>Concentration at t&lt;sub&gt;10&lt;/sub&gt;</td>
<td>151±1.0 (9)</td>
<td>154±1.0 (12)</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td></td>
<td>Mean difference ± S.E. mean</td>
<td>1.0±1.0</td>
<td>2±1.3</td>
<td>N.S.</td>
<td>2±3</td>
</tr>
<tr>
<td>Ileum</td>
<td>Concentration at t&lt;sub&gt;0&lt;/sub&gt;</td>
<td>152±0.7 (20)</td>
<td>149±1.1 (6)</td>
<td>N.S.</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Concentration at t&lt;sub&gt;10&lt;/sub&gt;</td>
<td>152±0.6 (20)</td>
<td>151±0.7 (6)</td>
<td>N.S.</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Mean difference ± S.E. mean</td>
<td>0±0.9</td>
<td>2±0.8</td>
<td>N.S.</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

| Potassium | Jejunum | Concentration at t<sub>0</sub> | 3.84±0.09 (9) | 3.98±0.14 (12) | 0.15±0.10 | 0.43±0.19 | 1:5 | 2:3 |
|           |         | Concentration at t<sub>10</sub> | 3.99±0.06/9 | 4.4±0.12 (12) | 0.15±0.10 | 0.43±0.19 | >0.1 | >0.05 |
|           | Mean difference ± S.E. mean | 0.16±0.09 | 0.42±0.19 | N.S. | <0.05 |

| Ileum | Concentration at t<sub>0</sub> | 3.92±0.10 (20) | 4.00±0.13 (6) | 0.33±0.14 | 0.90±0.35 | 2.3 | 2.9 |
|       | Concentration at t<sub>10</sub> | 4.25±0.10 (20) | 4.90±0.30 (6) | 0.33±0.14 | 0.90±0.35 | P <0.05 | <0.05 |

*Figures in parenthesis indicate number of experiments.*
and electrolytes may have been brought about by the direct action of the pentapeptide upon the mucosal cells of the small intestine. On the other hand, the observed effects may have been both secondary and non-specific. First, the reduction in the rates of absorption of sodium and water and the increase in potassium secretion, following the infusion of the pentapeptide, may be secondary to changes in, for example, intestinal motility. However, not only is the influence of motility on intestinal absorption far from clear (Shields and water, 1964) but also the alterations in the motility of the small intestine produced by the pentapeptide are not marked (Logan and Connell, 1966; Neely, 1967; Bennett, Misiewicz, and Waller, 1967).

Second, the observed alterations in absorption and secretion may have been secondary to the stimulation of gastric secretion of acid. Indeed the doses of pentapeptide used in this study were those which Thomas and Forrest (1967), working in this laboratory, have found to produce the highest output of acid from denervated Heidenhain gastric pouches. However, there is no evidence to suggest that such hypersecretion of acid by the intact stomach and, particularly, the entry of considerable quantities of the acid into the small intestine would per se affect the absorptive and secretory behaviour of isolated segments of intestine (Miles, Davies, and Shields, 1965).

Finally the effect of the pentapeptide on the intestinal transport of water and electrolytes may be the non-specific action of a foreign peptide. For this reason the influence of pure antral gastrins isolated by Gregory and Tracy (1964) upon intestinal absorption should be studied.

These changes in the handling of water and electrolytes by the small intestine were observed over three hours, in isolated segments of bowel with a serosal surface area of 100 cm.². From the data given by Davenport (1961) it can be calculated that the mucosal area of these segments would probably represent one-seventh of the mucosal surface area of the small bowel. If the entire intestine responded to the pentapeptide in a manner similar to that of the isolated fistulas the losses of fluid and electrolytes would be considerable. However, certain additional points must be taken into consideration. First, the absorptive ability of the colon was quite unaffected by the pentapeptide. Because the colon has a reserve capacity to absorb five to seven times as much water and salt as it is normally called upon to do (Shields and Miles, 1965), increased absorption of sodium and water by the colon may very well compensate for the observed reduction in absorption by the ileum. Second, the artificial nature of these experiments must be borne in mind. Before any definitive conclusions can be reached upon the effect of the natural antral hormone upon the intestinal handling of water and electrolytes, the intestine will have to be studied with its normal anatomical relationships preserved so that intestinal mucosa is in contact with food and alimentary secretions.

The relationship between the results of the present study and certain aspects of the Zollinger-Ellison syndrome remains to be discussed. The cause of the diarrhoea and hypokalaemia which are additional, occasionally dominating, features of this syndrome (Zollinger and Grant, 1964) remains obscure. The pancreatic adenoma is considered to produce a gastrin-like hormone which stimulates the hypersecretion of acid by the stomach (Gregory and Tracy, 1964). Some have attributed the accompanying diarrhoea to irritation of the small intestine by large volumes of acid (Donaldson, Vom Eigen, and Dwight, 1957; Summerskill, 1959; Rawson, England, Gillam, French, and Stammers, 1960; Parker, Soergel, and Ellison, 1963; Miles et al., 1965). However, it may be that the hormone liberated by the pancreatic adenoma has a dual effect upon the intestinal handling of water and electrolytes. In addition to the secondary irritation of the small intestine with gastric acid, the gastrin-like hormone may, as suggested by the present study, act directly upon the small intestine to augment the secretion of potassium by the jejunum and ileum, and to reduce the absorption of sodium and water in the ileum. The critical experiment remains to study the effect upon intestinal absorption of a potent extract of pancreatic adenoma from a patient with the Zollinger-Ellison syndrome.

A less common form of the syndrome consists of a non-beta islet cell tumour of the pancreas associated with profuse watery diarrhoea and severe hypokalaemia with hyperchlohydria, or even achlorhydria, and without peptic ulceration (Verner and Morrison, 1958; Matsumoto, Peter, Schultz, Hakim, and Franck, 1966). In one patient, presenting such features, the increased secretion of potassium seemed to be caused by an increase in the movement of potassium ions into the intestinal lumen (Espiner and Beaven, 1962)—a response similar in character to the one observed in the present study. In this context, it is of interest to note that increased movement of potassium into the intestinal lumen has also been observed in other situations in which hormones seem to be implicated, for example, following the administration of aldosterone (Shields et al., 1966), in primary hyperaldosteronism (Shields, 1966), and during salt depletion (Clarke, Miller, and Shields, 1967).

In two patients with diarrhoea and hypokalaemia
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but without gastric hypersecretion and peptic ulceration, assay of the pancreatic adenoma did not reveal any gastrin-like activity (Matsumoto et al., 1966). The mechanism of production of the diarrhoea remains obscure in these cases and the existence of another hormone, originally suggested by Telling and Smiddy (1961), seems most likely. Attempts by experiments in vitro to demonstrate that extracts from these tumours can affect intestinal absorption and secretion of fluid and electrolytes have not met with success (Matsumoto et al., 1966). However, in vitro preparations are not entirely suitable for the study of intestinal transport of water and electrolytes (Shields, 1964).

SUMMARY

The movement of sodium, potassium, and water into and out of isolated segments of jejunum, ileum, and colon of dogs was studied in vivo during the intravenous infusion of a synthetic gastrin-like pentapeptide (I.C.I. 50,123) in doses of 4 μg. and 8 μg. per kilogram body weight per hour. There was a marked reduction in the rates of absorption of sodium and water in the ileum. In the jejunum and ileum the rates of secretion of potassium were increased when the pentapeptide was infused at a rate of 4 μg. per kilogram per hour and, in the jejunum alone, with the higher dose of pentapeptide. The rates of transport of these substances in the colon were not affected by the pentapeptide.

There was a significant increase in the concentration of potassium in the lumen of the small intestine during the infusion of pentapeptide at 4 μg. per kilogram per hour.

The action of the pentapeptide is considered to be a direct and specific one on the small intestinal mucosa.

The relationship between the results of this study and the variants of the Zollinger-Ellison syndrome is discussed.

We thank Professor A. P. M. Forrest for his advice on the dosage of the pentapeptide. We are grateful to Dr J. D. Fitzgerald of Imperial Chemical Industries Limited, for the supplies of I.C.I. (50,123). This work was supported by a grant from the Medical Research Council to Mr. R. Shields.

We are grateful to Professor C. A. Taylor of the Department of Physics, University College, Cardiff, for granting access to a Stantec Zebra computer on which the rates of net transport and of unidirectional fluxes were calculated.

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