PHARMACOLOGICAL ACTIVITIES OF t-AMYLOXYCARBONYL TRYPTOPHANYL-METHIONYL-ASPARATYL-PHENYLALANINE AMIDE

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The antral hormones, gastrin I and II were isolated in pure form from hog antral mucosa by Gregory and Tracy (1). Gregory et al. (2) showed that the gastrins were polypeptides containing 17 amino acids and their structures were subsequently confirmed by total synthesis by Anderson et al. (3). During the studies on synthetic polypeptides structurally related to gastrin, Tracy and Gregory (4) discovered that the C-terminal tetrapeptide, Try. Met. Asp. Phe. NH₂, could show the full range of physiological actions of natural porcine gastrin, even though it was quantitatively less active.

The gastric secretory actions of gastrin-like peptides have been investigated in anesthetized rats by Barrett (5), and Barrett et al. (6), in conscious or anesthetized dogs by Morley et al. (7), Konturek and Grossman (8), on the isolated gastric mucosa of the bullfrog by Davidson et al. (9), and in clinical investigations by Wormsley et al. (10), Makhlouf et al. (11), Konturek (12) and Fitzgerald (13). They described that gastrin-like peptides were the specific gastric secretory stimulants which possessed the properties necessary to replace histamine in assessing the maximal acid response of patients and were useful as the new tool for research in gastric secretion.

Recently, we gained a new acylated derivative of tetrapeptide amide, t-amyloxycarbonyl Try. Met. Asp. Phe. NH₂ (14). The present study is concerned in the comparison of this tetrapeptide with other secretagogues such as other gastrin-like peptides, histamine, bethanechol etc. using various preparations of rats and in other pharmacological studies of several gastrin-like peptides.

MATERIALS AND METHODS

Gastrin-like tetra or pentapeptides used in this study are as follows. All of them were supplied by Dr. Sakakibara of the Protein Research Institute, Osaka University.

AOC-TP: t-Amyloxycarbonyl Try. Met. Asp. Phe. NH₂
Z-TP: Benzyloxycarbonyl Try. Met. Asp. Phe. NH₂
BOC-PP: t-Butyloxycarbonyl Ala. Try. Met. Asp. Phe. NH₂

Crude gastrin was a gift from Prof. M. I. Grossman of Veterans Administration Center, which was prepared by the method of Gregory and Tracy (1) carrying the procedure...
only through the isoproterenol stage. Dose of crude gastrin is expressed as the wet weight of mucosa from which the extract was derived.

Histamine dihydrochloride, bethanechol chloride and methacholine chloride were used as secretagogues and the doses are expressed as weight of these salts.

**Acute fistula rats**

Male *Donryu* rats weighing between 160 and 200 g were fasted overnight before use but were allowed free access to water. They were anesthetized with urethane (1 g/kg, i.m.). The stomach was exposed through a mid-line incision and the pylorus was ligated. A dual polyethylene cannula was set at the forestomach and the stomach was lavaged with physiological saline until the fluid was clear. The gastric juice was washed out with 10 ml of saline every hour and the washing solution was titrated with 0.1 N NaOH using phenolphthalein as indicator. The acid output was expressed in mEq of HCl per one hour or two hours.

**Pylorus ligated rats (Shay rats)**

Male *Donryu* rats weighing between 160 and 200 g were fasted overnight before use but were allowed free access to water. Surgical procedures were carried out according to the routine method described by Shay et al. (15). The drugs were administered intramuscularly immediately after the operation. After 5 hours, animals were sacrificed by exanguination and the stomach was removed. The gastric content was emptied into calibrated centrifuge tubes and was centrifuged at 3,000 c.p.m. for 5 minutes. Free and total acidity were determined by titrating the samples with 0.1 N NaOH using Topfer’s reagent and phenolphthalein as indicators. Pepsin activity was determined by the method of Anson using hemoglobin as substrate.

**Chronic fistula rats**

The preparation of chronic denervated gastric pouch in the rat was performed according to our modified surgical technique of the Alphin and Lin method (16). Male Wistar rats weighing over 300 g were used in this experiment. Under light pentobarbital anesthesia, the stomach was exposed through a mid-line incision and was separated gently with two small clamps. An incision was made between them, and the mucosa and the muscular wall were sutured. The stainless steel cannula which was 3 cm in length with an inside diameter of 3 mm was set at the denervated pouch. After closing the mid-line incision of the abdominal wall, the rat was treated with procaine penicillin. Besides cubed diet, they were administered glucose, amino acids and vitamin B complex in order to supplement the lack of nutrition; moreover, they were given the choice of tap water and 0.4% saline solution considering the loss of gastric juice from denervated pouch. They were used for the study of gastric secretion about a week after operation.

**Continuous perfusion of rat stomach**

Male *Donryu* rats weighing between 160 and 180 g were used. The method used in this study was described by Gosch and Schild (17). The animals were not starved in this experiment, and they were anesthetized with urethane (1 g/kg, i.m.). The trachea was exposed and cannulated, and a polyethylene tube was passed into the lower esophagus
at the neck and tied there. The stomach was exposed through a mid-line incision and pylorus was ligated. A polyethylene cannula was introduced into the stomach and secured firmly by a ligature. The stomach was then perfused continuously with a dilute solution of warm 0.00025 N NaOH. The fluid emerging from the stomach passed over a glass electrode which records pH continuously. A femoral vein was cannulated and through it drugs were injected.

_Gastric motility in rats_

Male _Donryu_ rats weighing about 200 g were anesthetized with urethane (1 g/kg, i.m.). A small rubber balloon filled with water was introduced into the glandular stomach through the fore-stomach. The pressure of intragastric balloon was recorded with an electric manometer on pen-writing oscillogram.

_Isolated guinea pig ileum_

Spasmodic activity was examined by using isolated guinea-pig ileum suspended in Tyrode's bath at 27°C. The effects of AOC-TP on the spasmodic actions of histamine and acetylcholine were investigated using same preparations. The application of stimulants was carried out by the Rossum's cummulative method (18).

_Blood pressure in dogs_

Male and female mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg, i.v.) were used for recording of femoral arterial blood pressure using the electric manometer. Drugs were injected intravenously via a cannula placed in the brachial vein. Simultaneously, the electrocardiogram recording was performed in the first lead.

**RESULTS**

_The quantitative comparison of AOC-TP, Z-TP and BOC-PP_

The quantitative comparison of AOC-TP, Z-TP and BOC-PP in secretory activities were carried out in anesthetized rat with acute fistula. Drugs were injected intramuscularly at doses of 100 and 500 µg/kg, and acid output was expressed by mEq of HCl per 2 hours after injection. As shown in Fig. 1, there was no significant difference

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**Fig. 1.** Gastric secretory activities of AOC-TP, Z-TP and BOC-PP administered intramuscularly 100 and 500 µg/kg in anesthetized rats with acute fistula. Each point is the mean with standard error of 10 animals.
among them. Accordingly, AOC-TP, Z-TP and BOC-PP have the same secretory activities.

_Dose-response curves of AOC-TP, bethanechol and histamine_

Dose-response curves of AOC-TP, bethanechol and histamine were obtained by using anesthetized rats with acute fistula. All of them were administered intramuscularly and acid output was expressed in mEq of HCl per hour immediately after injection. The results are shown in Fig. 2. The secretory activity of AOC-TP was about ten times of that of histamine, and the dose-response curve of AOC-TP was superimposed on that of bethanechol but the maximum response attained with bethanechol was distinctly lower than that of AOC-TP or histamine.

Fig. 2. Dose-response curves of AOG-TP (a), bethanechol (b) and histamine (c) administered intramuscularly on gastric secretion in anesthetized rats with acute fistula. Each point is the mean of 8 animals.

Fig. 3. Secretory patterns of various secretagogues administered intramuscularly in anesthetized rats with acute fistula. Each point is the mean of 8 animals. a : Crude gastrin 5 g/kg, b : AOC-TP 1 mg/kg, c : bethanechol 500 μg/kg, d : histamine 4.5 mg/kg, e : methacholine 1 mg/kg.
Secretory patterns of various secretagogues

In order to compare the duration of secretion of various secretagogues, doses which showed about same secretory activities were injected intramuscularly to acute fistula rats and secretory patterns were compared. As shown in Fig. 3, secretory patterns were not similar. Gastrin or AOC-TP produced a more sustained secretion, while responses to parasympathomimetic drugs such as bethanechol and methacholine were of short duration.

Effect on gastric secretion in pylorus ligated rats (Shay rats)

The effect of AOC-TP (500 µg/kg, i.m.) on gastric acid secretion and pepsin activity in pylorus ligated rats are shown in Table 1. AOC-TP increased gastric volume, free and total acidities and acid output compared with controls, while it did not affect the pepsin activity at this dose level.

Table 1. Effect of AOC-TP administered intramuscularly 500 µg/kg on gastric secretion in 2 hours pylorus ligated rats. Each point is the mean of 12 animals.

<table>
<thead>
<tr>
<th></th>
<th>Volume ml</th>
<th>Free acid mEq/l</th>
<th>Total acid mEq/l</th>
<th>Acid output mEq</th>
<th>Pepsin activity mg/ml as tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.8 ± 0.7</td>
<td>62.0 ± 6.8</td>
<td>96.9 ± 3.2</td>
<td>0.38 ± 0.04</td>
<td>4.83 ± 0.24</td>
</tr>
<tr>
<td>AOC-TP</td>
<td>4.7 ± 0.7</td>
<td>82.7 ± 6.0</td>
<td>108.5 ± 2.4*</td>
<td>0.51 ± 0.03*</td>
<td>4.48 ± 0.21</td>
</tr>
</tbody>
</table>

* Statistically significant at p = 0.01

Dose response curve of AOC-TP and histamine on continuous perfusion of rat stomach

The dose response curves for intravenous injection of AOC-TP and histamine on continuous perfusion of rat stomach are shown in Fig. 4. The linear relationship between

![Fig. 4. Dose-response curves of AOC-TP and histamine administered intravenously on the change of pH in continuous perfusion of rat stomach. Each point is the mean of 10 animals.](image-url)
dose and fall of pH on AOC-TP was observed in a broad range of 0.1 and 100 μg/kg, while that of histamine was in a relatively shorter range of 0.1 and 5 mg/kg. In this experiment, the slopes of the curves for AOC-TP and histamine were not parallel.

**Effect of gastric secretion in chronic fistula rats**

Effect of AOC-TP (500 μg/mg, i.m.) on gastric acid secretion in conscious rats with denervated pouch was shown in Table 2. In this experiment, the rats were not fasted and normal cubed diet and tap water were given *ad libitum* before and during the experiment. Gastric juice was collected for successive 2 hours after injection. AOC-TP (500 μg/kg, i.m.) increased the volume of gastric juice by 67% and the acid output by 72% which were statistically significant at p=0.01 compared with controls.

**Table 2. Effect of AOC-TP administered intramuscularly 500 μg/kg on gastric acid secretion in chronic fistula rats with denervated pouch.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Control (Feeding)</th>
<th>AOC-TP + Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume ml</td>
<td>Acid output mEq</td>
</tr>
<tr>
<td>1</td>
<td>1.8</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>1.6</td>
<td>0.41</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.92</td>
</tr>
<tr>
<td>5</td>
<td>2.2</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
<td>0.30</td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>0.80</td>
</tr>
<tr>
<td>9</td>
<td>0.7</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>0.7</td>
<td>0.07</td>
</tr>
<tr>
<td>11</td>
<td>1.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Mean (±S.E.)</td>
<td>1.2 (±0.14)</td>
<td>0.47 (±0.08)</td>
</tr>
</tbody>
</table>

* Statistically significant at p=0.01

**Combination effect of AOC-TP plus histamine or bethanechol on gastric secretion**

Anesthetized rats with acute fistula were used in this study; AOC-TP, bethanechol and histamine were given separately or in combination. The results are shown in Fig. 5. Potentiation was said to exist if the response to the doses of the two agents given together exceeded the responses to each agent alone used at twice dose; and if the both responses were nearly similar, this effect was said to be addition. Judging from this criterion, it was concluded that effect of AOC-TP plus histamine was addition, while that of AOC-TP plus bethanechol was potentiation.

**Gastric motility in rats**

The records of gastric motility in rats are shown Fig. 6. The spontaneous movement was inhibited in one or two minutes after the intravenous injection of AOC-TP (100 μg/kg). Such inhibition was also found in the case of subcutaneous injection (500
Considering the absorption of drug, it is hard to consider that these inhibition is due to the pharmacological action of AOC-TP, and it may be due to the other action indifferent to AOC-TP.

**Isolated guinea-pig ileum**

The high concentration of AOC-TP ($10^{-3}$–$10^{-4}$ g/ml) slightly contracted the isolated ileum of guinea-pig. The effect of AOC-TP ($10^{-9}$ g/ml) on the dose-response curve of histamine was shown in Fig. 7. AOC-TP strengthened the spasmodic action of histamine, while this phenomenon was not found in the contraction stimulated by acetylcholine.
Effects on blood pressure

As shown in Fig. 8, AOC-TP, Z-TP and BOC-PP lowered the blood pressure of anesthetized dog, but in some dogs slight hypertension was observed after hypotension.
The hypotensive action was observed at dose level between 1 and 100 $\mu$g/kg but the dose-dependency was relatively small. As shown in Fig. 9, tachyphylaxis was observed after the administration of as few as two or three successive doses of them.

**DISCUSSION**

A large number of peptides related to the C-terminal tetrapeptide sequence of gastrin have been synthesized and examined for biological activity by Tracy and Gregory (4) and by Morley et al. (7). Morley et al. reported that among these active peptides, the pentapeptide (BOC-PP) which was most commonly referred to by its code number, I.C.I. 50123 was more potent than tetrapeptides. In the studies on dog with gastric fistula, Konturek and Grossman (8) found that the dose required for halfmaximal response was about the same for tetrapeptides and the pentapeptide, but the maximal response to the pentapeptide was relatively high. In our present investigation, the tetrapeptides, AOC-TP and Z-TP, and the pentapeptide, BOC-PP, have the same activity in anesthetized rats with acute fistula at dose levels of 100 and 500 $\mu$g/kg, i.m. AOC-TP stimulated gastric acid secretion in pylorus ligated rats (Shay rats) and stomach perfused rats. But these acute experiments have several faults such as anesthesia, local trauma, pyloric ligation, shock and so on. Therefore, chronic fistula rats with denervated pouch were prepared and after the recovery of surgical trauma they were used for the study of gastric secretion. AOC-TP stimulated gastric acid secretion in all of these preparations by intravenous or intramuscular administration.

In the experiments of anesthetized rats with acute fistula AOC-TP showed the potentiative effect with bethanechol and the additive effect with histamine. Grossman et al. reported that the maximal response to combination of two agents was greater than the maximal responses to either agent alone in dogs with Heidenhain pouch for the combination of bethanechol plus gastrin (19) and gastrin plus histamine (20). Although, as Makhlouf et al. (11) pointed out, the few attempts to demonstrate such potentiation in human subjects have not succeeded.
AOC-TP slightly contracted the isolated ileum of guinea-pig at high concentration. In this experiment, a synergism was found in the combination with histamine but not with acetylcholine. From the studies on guinea-pig ileum, Bennett (21) suggested that stimulation of gastric secretion by gastrin might be affected by the hormone acting on nerves to release acetylcholine. Mikos and Vane (22) concluded from their results on isolated smooth muscle that the relative potencies of gastrin and its analogues on smooth muscle were similar to their potencies on gastric secretion. However, the concentration of gastrin and its analogue in a bath required to stimulate the isolated smooth muscle was considerably higher than that to stimulate gastric secretion and the modes of action to stimulate gastric acid secretion and smooth muscle may be not similar. It may be difficult to analyse the mechanism of gastrin and its analogues on gastric acid secretion from the results of contraction of smooth muscle.

AOC-TP is a specific stimulant of gastric acid secretion and it has little or no effect on other pharmacological actions. However, AOC-TP, Z-TP and BOC-PP have slight hypotensive effects on arterial blood pressure in anesthetized dog. Barrett (5) reported that BOC-PP exerted slight pressor effects in anesthetized rats, but in our present study BOC-PP as well as Z-TP and AOC-TP had the hypotensive action. Logan et al. (23) reported that BOC-PP slightly increased the arterial blood pressure and heart rate in human subjects. Grossman (24) described in his recent review that they found that both gastrin and pentapeptide produced a small and inconstant depression of blood pressure in conscious dogs. This discrepancy may be due to the difference of species of animals and route of administration.

From these experiments it may be concluded that AOC-TP is an important new tool for research in gastric acid secretion and is useful as a diagnostic drug for the assessment of gastric secretion.

SUMMARY

Gastrin-like tetrapeptide, t-amyloxy carbonyl Try. Met. Asp. Phe. NH₃, is the specific gastric secretory stimulant and has the same activity as benzyl oxy carbonyl Try. Met. Asp. Phe. NH₃ or t-butyloxy carbonyl Ala. Try. Met. Asp. Phe. NH₃ which were described by Gregory et al. AOC-TP stimulated gastric secretion in anesthetized rats with acute fistula, pylorus ligated rats, perfused stomach preparation and conscious rats with denervated pouch but not stimulate pepsin secretion. It has little or no effect on gastric motility in anesthetized rats in situ, but slightly causes the contraction of isolated ileum of guinea-pig. AOC-TP and other related peptides have hypotensive action in anesthetized dog and tachyphylaxis was observed.

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REFERENCES