The antral hormone, gastrin, is a linear polypeptide with seventeen amino-acid residues. Since Tracy and Gregory (1) elucidated that the C-terminal tetrapeptide amide, Trp.Met. Asp.Phe.NH₂ produces all the physiological effects of the entire molecule, C-terminal tetrapeptide and pentapeptide amides instead of gastrin have been used for investigation of gastric acid secretion and diagnosis of gastric function. There have been numerous reports concerning pharmacological effects of gastrin and these peptides, very little, however, is known about their metabolism.

The present study is an attempt to investigate the difference in distribution, absorption and excretion of exogenous gastrin and gastrin-like tetrapeptide using iodine-125 labeled gastrin and tritiated tetrapeptide while making a comparison of their biological activities.

METHODS AND MATERIALS

Preparation of the labeled compounds

Synthetic human gastrin I purchased from Imperial Chemical Industries, Ltd. (England) was labeled with ¹²⁵I by a conventional method, but this labeled compound showed several spots in radioscanograms using various solvent systems. This commercial synthetic gastrin was then purified using the AE cellulose column. The purified synthetic human gastrin I (SHG-I) was labeled with ¹²¹I in the benzene ring of the tyrosine residue by the method of Stagg et al. (2).

Labeled SHG-I was separated from iodine-125 using Sephadex G-10. Radiochemical purity was established by thinlayer chromatography employing a n-butanol-acetic acid-water-pyridine (15 : 3 : 12 : 10) solvent system. The purified material was diluted with carrier drug to yield a product with a specific activity of 32.2 μCi/mg. As a gastrin-like tetrapeptide amide, t-amyloxycarbonyl Trp.Met.Asp.Phe.NH₂ was used. This had been synthesized in our laboratory and was tritiated by the method of Wilzbach (3). Lavile ³H was removed by repeated recrystallization. Final recrystallization yielded a single spot of ³H-AOC-TP with a specific activity of 0.226 mCi/mg on a radioscanogram of a thinlayer developed by a n-butanol-acetic acid-water (4 : 2 : 1) solvent system. Radio

scans of ³H-AOC-TP and ¹²⁵I-SHG-I are shown in Fig. 1.
Distribution studies

Male Donryu rats weighing approx. 200 g were placed in individual metabolism cages designed for separate collection of urine and feces. $^{125}$I-SHG-I and $^3$H-AOC-TP were injected intramuscularly at 50 µg/kg and 500 µg/kg, respectively. The animals were sacrificed by exsanguination 1 or 2 hr after injection, as maximum responses of the peptides were obtained 1 hr after injection in rats with acute fistula and, at 2 hr, the responses were hardly detectable as shown in Figs. 5 and 6. Tissues were procured, rinsed with water and weighed. Urine, gastric contents and small and large intestinal contents were preserved.

Absorption and excretion studies

Male Donryu rats weighing approx 200 g were kept fasting overnight except for free access to water. They were anesthetized with urethane (1.2 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 fore-stomach to collect gastric juice. A dual polyethylene cannula was also set at the urinary bladder and polyethylene tubes were cannulated into the common bile duct and the carotid artery. $^{125}$I-SHG-I and $^3$H-AOC-TP were injected intramuscularly at 50 µg/kg and 500 µg/kg, respectively. Urine, blood, gastric juice and bile were collected at 0.5, 1, 2, 3, 4 and 5 hr after injection.

Measurement of radioactivity

For measurement of $^{125}$I activity, tissues were homogenized in distilled water by an Ultra-Turax homogenizer and radioactivity was determined by a Packard autogammer spectrometer. Blood, urine, bile and gastric juice were diluted with distilled water before use. For measurement of tritium activity, samples were firstly subjected to combustion by a sample oxidizer, Packard model 300, and radioactivity was measured in a Packard liquid scintillation counter, model 3380.

Measurement of gastric secretory activity

Anesthetized rats with acute gastric fistula and continuous perfusion technique of rat stomach were used. Operative techniques have been described in a previous paper (4). In anesthetized rats with acute gastric fistula, gastric juice was washed out with 10 ml of saline every 30 min and the washing solution was titrated with 0.1 N NaOH using phenolphthalein as indicator. Acid output was expressed in ml of 0.1 N HCl. In continuous perfusion of rat stomach, 1/4,000 N NaOH was perfused through the stomach and secretory activity was expressed in the fall of perfusate pH.
RESULTS

Distribution of radioactivity

| Sample          | Percent of administered radioactivity* in 1 g of sample | 1 hr | 2 hr | 1 hr | 2 hr |
|-----------------|--------------------------------------------------------|------|------|------|------|
| Brain           | 0.14                                                   | 0.35 | 0.05 | 0.04 |
| Heart           | 0.39                                                   | 0.33 | 0.21 | 0.16 |
| Lung            | 0.75                                                   | 0.43 | 0.38 | 0.28 |
| Liver           | 3.24                                                   | 1.80 | 0.25 | 0.15 |
| Kidney          | 2.79                                                   | 1.95 | 0.45 | 0.37 |
| Pancreas        | 0.52                                                   | 0.63 | 0.38 | 0.20 |
| Spleen          | 0.66                                                   | 0.52 | 0.42 | 0.44 |
| Forestomach     | 0.33                                                   | 0.31 | 2.39 | 0.65 |
| Corpus          | 0.47                                                   | 0.54 | 2.68 | 1.13 |
| Antrum          | 0.40                                                   | 0.48 | 5.45 | 0.12 |
| Duodenum        | 1.88                                                   | 1.47 | 1.12 | 0.72 |
| Small intestine | 3.54                                                   | 3.45 | 0.30 | 0.23 |
| Caecum          | 0.52                                                   | 0.53 | 0.16 | 0.10 |
| Large intestine | 0.43                                                   | 0.35 | 0.26 | 0.19 |
| Thyroid         | 0.63                                                   | 1.18 | 187.86 | 352.35 |

* Average data of 2 animals

| Sample          | Percent of administered radioactivity* in total sample | 1 hr | 2 hr | 1 hr | 2 hr |
|-----------------|--------------------------------------------------------|------|------|------|------|
| Brain           | 0.18                                                   | 0.53 | 0.07 | 0.05 |
| Heart           | 0.39                                                   | 0.25 | 0.17 | 0.12 |
| Lung            | 1.33                                                   | 0.36 | 0.47 | 0.30 |
| Liver           | 20.35                                                  | 10.24 | 2.19 | 1.34 |
| Kidney          | 4.34                                                   | 2.75 | 0.74 | 0.57 |
| Pancreas        | 0.13                                                   | 0.11 | 0.07 | 0.04 |
| Spleen          | 0.30                                                   | 0.18 | 0.16 | 0.12 |
| Forestomach     | 0.10                                                   | 0.08 | 0.53 | 0.18 |
| Corpus          | 0.29                                                   | 0.31 | 1.38 | 0.55 |
| Antrum          | 0.06                                                   | 0.07 | 0.63 | 0.25 |
| Duodenum        | 0.61                                                   | 0.42 | 0.23 | 0.16 |
| Small intestine | 18.16                                                  | 18.03 | 1.94 | 1.76 |
| Caecum          | 0.44                                                   | 0.49 | 0.15 | 0.16 |
| Large intestine | 0.49                                                   | 0.33 | 0.33 | 0.25 |
| Thyroid         | 0.02                                                   | 0.03 | 2.63 | 5.99 |
| Gastric contents| 0.56                                                   | 0.32 | 3.64 | 5.99 |
| Intestinal contents | 17.32                                               | 20.11 | 1.04 | 1.25 |
| Urine           | 5.73                                                   | 8.70 | 11.66 | 11.96 |

* Average data of 2 animals
Distribution of radioactivity in rats necropsied 1 or 2 hr after intramuscular injection of 500 μg/kg of ³H-AOC-TP or 50 μg/kg of ¹²⁵I-SHG-I is summarized in Tables 1 and 2. Table 1 shows the percentage of dose recovered in 1 g of sample, i.e. concentration of radioactivity. Table 2 shows the percentage of dose recovered in the sample, i.e. the total amount of radioactivity. In rats treated with ³H-AOC-TP, radioactivity was relatively high in liver, kidney, duodenum and small intestine with a large amount recovered in the liver, small intestine and intestinal contents. On the other hand, in rats treated with ¹²⁵I-SHG-I, relatively high radioactivity was found in the gastrointestinal tracts such as the forestomach, corpus, antrum and duodenum except for extremely high activity in the thyroid. A great amount of activity was recovered in liver, corpus, small intestine, thyroid and gastric and intestinal contents. In another experiment, the thyroid was blocked by a conventional method using KI. Characteristic distribution to gastrointestinal tracts was not observed and specific incorporation into the thyroid was not seen. Of particular interest is the difference in radioactivity between ³H and ¹²⁵I found in the gastric and intestinal contents; ³H was recovered 20% in intestinal contents and only 0.3% in gastric contents at 2 hr, whereas ¹²⁵I was recovered 1.25% and 6%, respectively.

Absorption and excretion studies

Blood levels of radioactivity of ³H and ¹²⁵I in anesthetized rats treated with intramuscular injection of 500 μg/kg of ³H-AOC-TP and 50 μg/kg of ¹²⁵I-SHG-I are shown in Fig. 2. Maximum activity of ³H was obtained 1 hr after injection and that of ¹²⁵I was obtained 30 min after injection. Although a difference in secretory pattern between two drugs was seen, nearly the same response was obtained in an experiment of gastric secretory activity using anesthetized rats with acute fistula (Figs. 5 and 6).

Excretion of radioactivity in the urine, bile and gastric juice is shown in Figs. 3 and 4. As expected from the result of distribution study, there was a difference in recovery of radioactivity between ³H-AOC-TP and ¹²⁵I-SHG-I. In rats treated with ³H-AOC-TP, about 98% of the dose was excreted in the bile during the 6 hr following injection. Less than 1% was excreted in the urine and gastric juice (Fig. 3). On the other hand, as for ¹²⁵I-SHG-I, radioactivity was recovered 23.3% in the gastric juice, 12.1% in the urine and 8.4% in the bile (Fig. 4).

Measurement of gastric secretory activity

In order to clarify the relative importance of the biliary excretory pathway of the peptides, the following experiments using anesthetized rats were carried out. Effects of injecting AOC-PT and SHG-I into the portal vein and femoral vein were compared. AOC-TP and SGH-I were injected at a constant rate for 30 min using
FIG. 3. Recovery of radioactivity in bile, urine and gastric juice in anesthetized rats after intramuscular injection of 500 μg/kg of $^3$H-AOC-TP. Data average of 2 animals.

FIG. 4. Recovery of radioactivity in bile, urine and gastric juice in anesthetized rats after intramuscular injection of 50 μg/kg of $^{131}$I-SHG-I. Data average of 2 animals.

FIG. 5. Comparison of gastric secretory activity of SHG-I between intrafemoral vein injection and intraportal vein injection in anesthetized rats with acute fistula. Data average of 10 animals.

FIG. 6. Comparison of gastric secretory activity of AOC-TP between intrafemoral vein injection and intraportal vein injection in anesthetized rats with acute fistula. Data average of 10 animals.

an infusion pump. As Fig. 5 demonstrates, in rats treated with 50 μg/kg of SHG-I no significant difference in acid secretion induced by infusion into the femoral vein and the portal vein was seen. By contrast, as shown in Fig. 6, response to AOC-TP injected into the portal vein was significantly lower than that obtained by systemic venous injection. This experiment suggests that the liver plays an important role in inactivation of AOC-TP and in order to clarify whether or not AOC-TP is decomposed in the liver or excreted into the bile without decomposition, the following experiment was performed. Anesthetized rats with acute fistula into the bile duct were given an intravenous injection of AOC-TP or SHG-I, dosage 100 μg/kg. During the first 1 hr following injection, the bile was collected and diluted 10 times with saline, then injected into the rats used for gastric perfusion experiment at a dosage of 1 ml/kg, i.v. The rats were given various doses of SHG-I and AOC-TP alternately and dose-response curves for the peptides were prepared.
Results are shown in Figs. 7 and 8. The bile obtained from the rats treated with AOC-TP showed a response corresponding to activity of 2 \mu g/kg of AOC-TP, namely the concentration of the bile was equivalent to 20 \mu g/ml of AOC-TP. Volume of the bile during the first 1 hr after the injection was 2.11 \pm 0.08 ml/kg and the total output of AOC-TP was 42 \mu g/kg. Consequently, about 42\% of the dose was excreted into the bile without inactivation. On the contrary, secretory activity of bile obtained from rats treated with SHG-I was below that of 0.01 \mu g/kg of SHG-I. This indicates that less than 0.2\% of administered SHG-I was excreted into the bile. No secretory activity in bile of non-treated rats was seen and as shown in Fig. 9, the same bile had no effect on the secretory activity of SHG-I and AOC-TP.

**DISCUSSION**

There have been many reports concerning pharmacological and clinical studies on gastrin-like tetra or pentapeptide amide comparing natural and synthetic gastrin. Little information concerning differences in metabolism of gastrin and gastrin-like peptides however is available in scientific literature. Gillespie and Grossman (5) demonstrated that gastrin injected into the portal vein resulted in equal a secretory response as when injected into a systemic vein in dogs with Heidenhain pouches. Trueblood (6) did not implicate the kidney as the major organ for inactivation of gastrin. Laster et al. (7) detected amidase activity in rat liver homogenate by which gastrin-like tetrapeptide amide is transformed to hormonally inactive tetrapeptide. Ishimori et al. (8) showed that experimental liver damage induced by carbon tetrachloride reduced inactivation of AOC-TP in the liver.
Jaffe et al. (9) demonstrated the lack of hepatic sequestration of $^{125}$I gastrin and significant renal cortical sequestration.

In the present investigation, $^{125}$I showed high activity in the gastrointestinal tracts such as the forestomach, corpus, antrum and duodenum, while $^3$H showed high activity in the liver, kidney, duodenum and small intestine. These was the significant difference in recovery of activity between $^3$H and $^{125}$I found in the gastric and intestinal contents; recovery of $^3$H was 20% in the intestinal contents and 0.3% in the gastric contents, while that of $^{125}$I was 1.25% and 6%, respectively. It suggests that a large amount of AOC-TP is excreted into the intestinal tract, probably through the bile duct.

This assumption was supported by experiment with absorption and excretion studies. Radioactivity of $^3$H-AOC-TP was approx. 98% recovered in the bile during 6 hr after injection but less than 1% was recovered in the urine and gastric juice, while the activity of $^{125}$I-SHG-I was about 23% recovered in the urine and only 8% in the bile. Difference of hepatic inactivation of AOC-TP and SHG-I was also demonstrated in rats with acute gastric fistula. Contrary to SHG-I, the response to AOC-TP injected into the portal vein was significantly lower than that obtained by systemic venous injection. It was ascertained that AOC-TP was excreted into the bile without hepatic decomposition, namely about 42% of the secretory activity of administered AOC-TP was recovered from rat bile during 1 hr following intravenous injection of AOC-TP. By contrast, activity of SHG-I was hardly detectable in the bile. These experiments suggest that AOC-TP is excreted into the bile without decomposition and SHG-I is mainly excreted into the gastric juice and urine. The difference of inactivation between AOC-TP and SHG-I may be due to the higher lipophylic property of AOC-TP as a result of belonging to the t-amlyloxy carbonyl group.

Gastrin-like tetra and pentapeptide amides have been used as diagnostics for gastric function tests and also as tools for pharmacological studies replacing gastrin. AOC-TP was also found to be useful for analysis of gastric conditions following a multicenter pilot study included 401 patients (10). As indicated in this study, differences in metabolism of these peptides exist and speculation of the metabolism of endogenous gastrin from results obtained using gastrin-like tetra and pentapeptide amides appears complicated.

SUMMARY

Absorption, distribution and excretion of $^{125}$I labeled synthetic human gastrin I (SHG-I) and tritiated t-amlyloxy carbonyl tetrapeptide amide (AOC-TP) were studied in rats. Significant differences were found among these peptides. Activity of $^3$H was highly recovered in the bile and that of $^{125}$I was recovered mainly in gastric juice and urine. AOC-TP was excreted into the bile without hepatic decomposition. This could be attributed to the high lipophylic property of the protecting group.

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