PRO EXPERIMENTIS

Gastric Acid Secretory Responses of Acute Gastric Fistula Preparation in Anesthetized Young Chickens[1]

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Summary. A reliable technique which permits quantitative determination of the gastric acid secretory responses to drugs was developed in anesthetized young chickens.

Although various species of animals have been used for pharmacological studies on gastric acid secretion, no one species or method can fulfill all of the requirements for pharmacological research. Some authors[2-4] have presented fundamental data on the physiological properties of the gastric secretion of mature chickens with chronic gastric fistula. They have pointed out two important properties of chicken gastric secretion; a high rate of basal gastric secretion and a high sensitivity to secretagogues. We found that young chickens were also endowed with these properties, especially with the high rate of basal acid secretion even under deep anesthesia. Young chikens are very convenient to treat and the anatomy of the digestive tract of this animal provides various advantages for surgical operation. On the basis of these findings, we started our study to examine the usefulness of acute gastric fistula preparation of young chickens for pharmacological studies on gastric secretion.

The present paper reports the standard technique for obtaining a quantitative acid secretory response to secretagogues in anesthetized young chickens with acute gastric fistula, and presents an example to show its usefulness in the investigation of the mode of action of drugs.

Materials and methods. Male young chickens (white leghorn, 6–10 days old after hatching) were used. Their weights ranged from 50 to 95 g. After fasting for at least 5 h, the birds were anesthetized with urethane (1.2 g/kg, i.p.). Body temperature of the animals was kept at about 27–32 °C by means of an electric heating mat.

Operative technique. The trachea was exposed and cannulated. Then the lower esophagus was ligated just below the crop. The abdomen was opened through a small incision, taking care to avoid injuring the peritoneal air-sacs. The proventriculus-gizzard junction was exposed and a small polyethylene cannula inserted through a cut in the junction into the glandular stomach and secured by ligating around the junction. When the esophagus and the cannula were ligated, blood vessels and vagus nerves were carefully excluded. Moist cotton-wool was placed on the cut edges to avoid tissue drying. The gross anatomy of the upper digestive tract of the chicken is schematically shown in Figure 1.

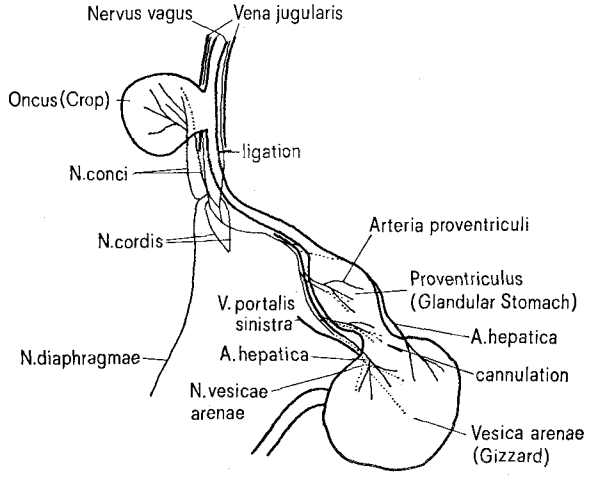

Fig. 1. Schematic representation of the upper digestive tract in the young chicken and the positions of operative treatments.

Determination of acid output. The stomach was perfused with 2 ml warm saline solution through the gastric cannula at intervals of 15 min. The perfusate was titrated for acid content using phenolphthalein as the indicator with N/50 NaOH solution. The acid secretory rate was expressed in terms of μeq.H^+/15 min/50 g of body weight (b.w.). The basal secretory responses were measured for 60 min, and then the inhibitor or saline was administrated s.c. 15 min before the injection of each secretagogue.

Materials. The drugs used in this experiment were as follows: tetragastrin (Nissui, Japan), methacholine chloride (Nakarai, Japan), histamine dihydrochloride (Wako Pure Chem., Japan), atropine sulphate (Wako Pure Chem., Japan), burimamide (Smith Kline & French Lab., England, kindly supplied by Dr. J. W. BLACK). All drugs were administrated subcutaneously.

Results. 1. Basal acid secretory response. Young chickens showed acid secretory rates of 15 to 40 μeq.H^+/15 min/50 g of b.w. in 210 experiments. The rates in chickens with a higher secretory rate than 40 μeq.H^+/15 min/50 g showed a tendency to decline rapidly. It was also proved that a constant secretory rate was not kept in preparations with a lower rate than 15 μeq.H^+/15 min/50 g. Therefore, the preparations with 15 to 40 μeq.H^+/15 min/50 g of acid secretory rate were selected in our experiments. The acid secretory rate was almost constant for over 135 min in the control groups with average secretory rate of about 20 μeq.H^+/15 min/50 g. The rate was maintained at the level of 83.6% of the initial rate even 4 h after operation.

2. Effect of some secretagogues. The effects of 3 representative secretagogues were examined on gastric acid secretion in the anesthetized chicken preparations. The stimulatory effect of tetragastrin is shown in Figure 2. Tetragasrin at the dose of 6.25 μg/kg s.c. significantly stimulated acid output, and at the dose of 12.5 to 100 μg/kg augmented acid output dose-dependently. Figure 2 also shows the stimulatory effects of histamine and

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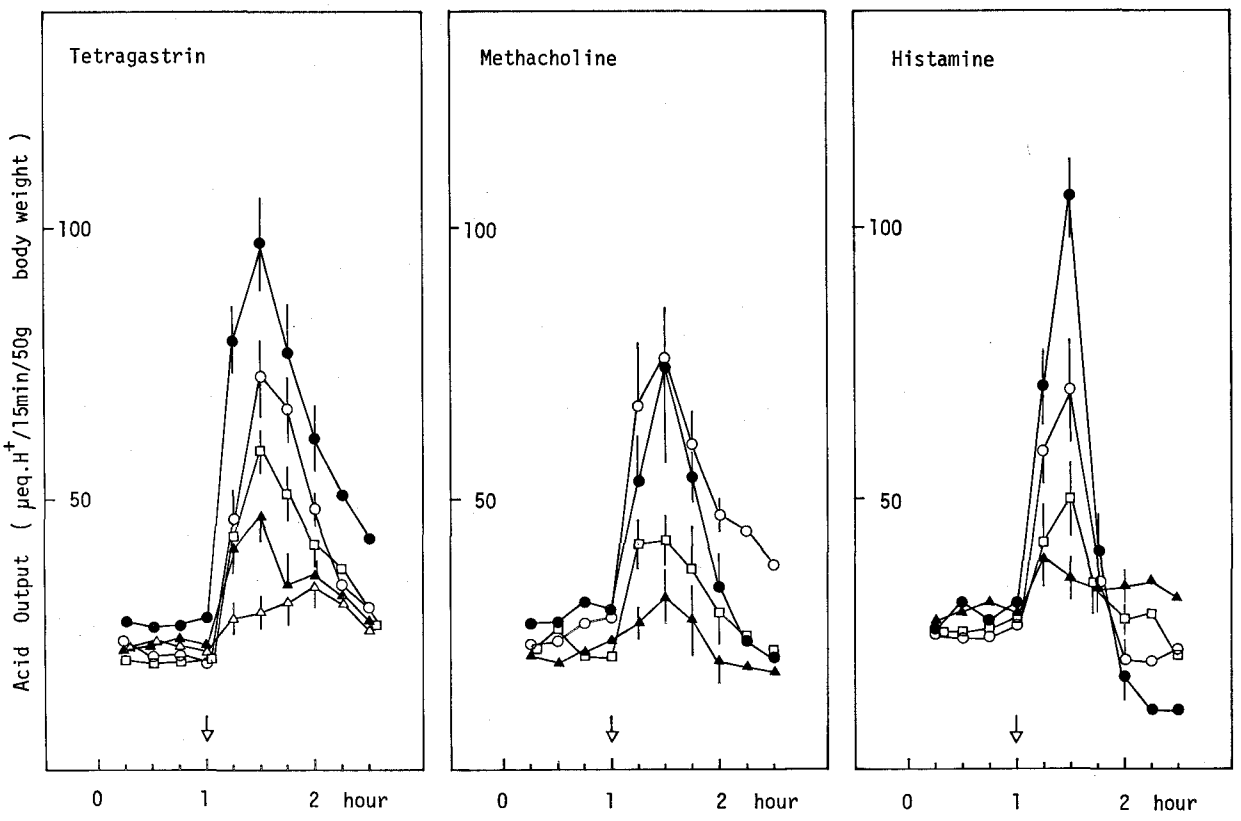

Fig. 2. Acid secretory patterns induced by the secretagogue actions of tetragastrin, methacholine and histamine in anesthetized young chickens. The dose of each drug tested in this experiment is as follows: tetragastrin (△—△ 3.125, ▲—▲ 6.25, □—□ 25, ○—○ 50, ●—● 100 μg/kg, s.c.), methacholine (▲—▲ 400, □—□ 800, ○—○ 1,000, ●—● 1,600 μg/kg, s.c.), histamine (▲—▲ 25, □—□ 50, ○—○ 100, ●—● 200 μg/kg, s.c.). Ordinate indicates the acid secretory rate (μeq.H^+/15 min/50 g of b.w.). All points and bars represent the mean of experiments and the standard errors of the mean.

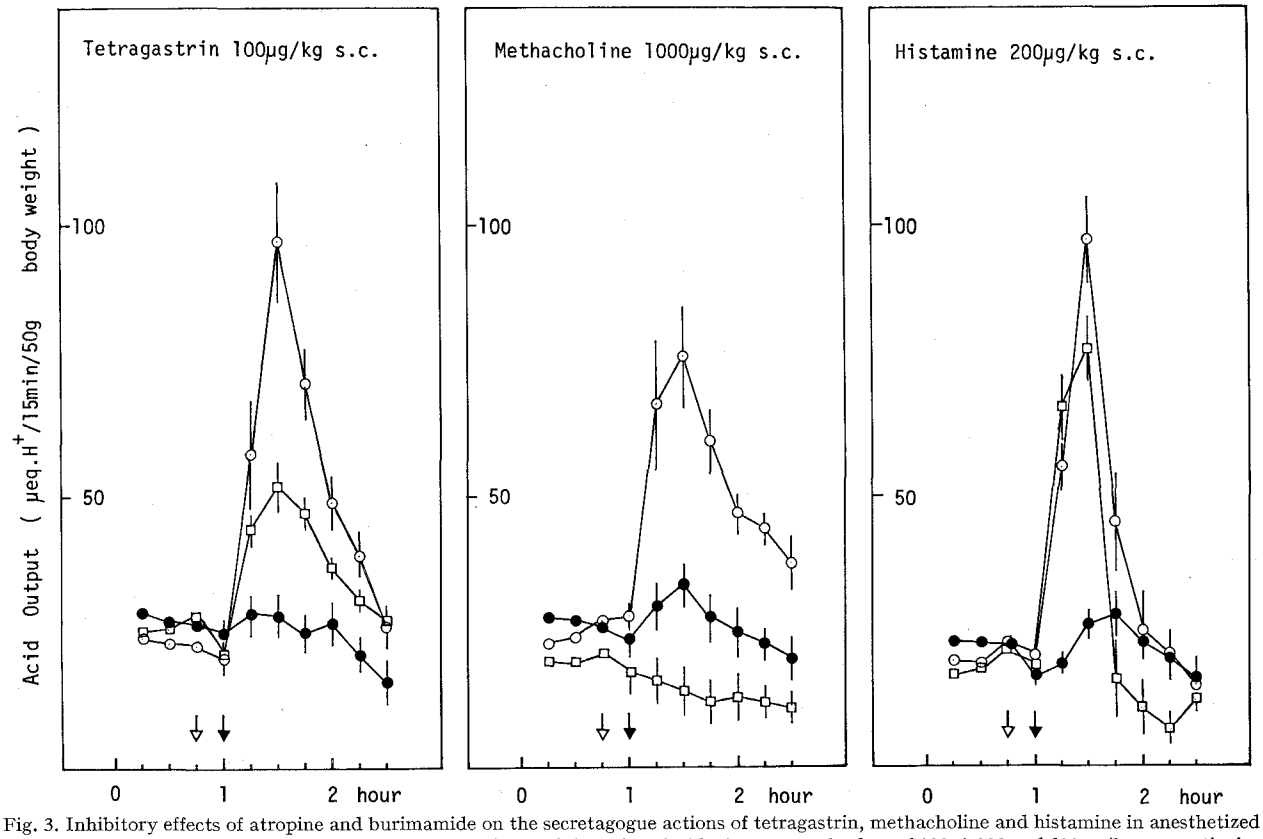

Fig. 3. Inhibitory effects of atropine and burimamide on the secretagogue actions of tetragastrin, methacholine and histamine in anesthetized young chickens. Tetragastrin, methacholine and histamine are injected at the black arrow at the dose of 100, 1,000 and 200 μg/kg, respectively. At the white arrow, saline (○—○), 5 mg/kg of atropine (□—□) and 1 mg/kg of burimamide (●—●) are premedicated s.c. All points are the mean of 6–10 experiments and the bars are the standard errors of the mean.

methacholine. Dose-dependent augmentation of acid secretion response was obtained by histamine within the dose range of 50 to 200 μg/kg, and by methacholine 1,000 to 1,600 μg/kg. The maximal responses were obtained 30 min after the injection of each stimulant. The stimulatory effect of histamine was exerted without a latent period, and then it returned to the basal level of acid output within 45 min, although that of tetragastrin or methacholine remained within 60 min after the medication.

3. Inhibitory effect of atropine and burimamide. The interactions between these secretagogues and some inhibitors were inspected in the fowl preparations. Atropine and burimamide were used in this experiment. The latter is reported to have a specific histamine H_2-receptor antagonistic effect[5]. As shown in Figure 3, atropine completely abolished the stimulatory effect of methacholine. It was confirmed that this inhibitor also depressed the action of tetragastrin significantly but showed no influence on the action of histamine. Burimamide was shown to suppress the effects of all 3 secretagogues.

Discussion. Many investigators[6–8] have used rat stomach preparations with acute fistula for the study of gastric acid secretion. The rat seems, however, less sensitive to histamine than other animals. Therefore, it seems inadequate to employ only the rat as the experimental model in the study of histaminergic mechanisms of acid secretion. Since our preliminary experiments on the effect of histamine on gastric acid secretion have revealed that anesthetized young chicken preparations were more sensitive to histamine than were rats, it was decided to develop a standard technique which permits quantitative determinations of the gastric secretory responses to several drugs.

We first checked the durability of the basal acid secretory responses. The mean of basal acid secretory rates of 210 chickens was 96.7 ± 2.0 μeq.H^+/60 min/50 g of b.w. for the first 1 h. This value remained almost constant for the next 2 h. This acid output seemed to be considerably higher than that of other authors[3,4,9], who examined unanesthetized adult chickens.

In the next experiment, dose-response relationships for histamine, tetragastrin and methacholine were examined for the quantitative estimation of secretagogue actions. In the anesthetized young chickens, it was shown that histamine, tetragastrin and methacholine produced maximal stimulation at the dose of 200, 100 and 1,000 μg/kg, respectively. It was shown that tetragastrin was 2–4 times as potent as histamine and 10–16 times as potent as methacholine. RUOFF and SEWING[4] have already reported that histamine, pentagastrin and carbachol stimulated acid output maximally at the dose of 400, 200 and 160 μg/kg, respectively, in the unanesthetized chickens. Conspicuous differences in the sensitivity to cholinergic agents were observed between their preparations and ours. It was obvious that the dose of tetragastrin and histamine required to elicite the stimulation of acid secretion was lower in the young chickens than in the rats. In regard to the sensitivity to histamine, the young chicken seems to be 10–40 times as susceptible as the rat. In addition, the effects of 2 types of antagonists, atropine and burimamide, were tested on the secretagogue-stimulated acid secretion. The anticholinergic drug showed complete antagonism to methacholine and significant depression of tetragastrin. However, the facilitating action of histamine was not affected by atropine. On the other hand, burimamide showed an inhibitory effect on all types of secretagogues.

The anesthetized young chicken preparations appear preferable for the investigation of acid secretion in the following respects: 1. the secretory rate was fairly high under anesthesia and constant for 2–3 h; 2. the acid secretion was dose-dependently augmented with 3 representative secretagogues; 3. the pattern of secretory responses is essentially similar to those known to occur in the mammals; 4. the antagonistic interactions between secretagogues and inhibitors were clearly demonstrated; 5. large numbers of chickens from a homogeneous colony are easily obtained; 6. a large number of birds can be studied simultaneously; and 7. only a small amount of test drugs are required for screening experiments. For these reasons, we concluded that the acute gastric fistula preparation of fowls provides a convenient experimetal method for the study of acid secretion.

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