Stimulatory Effect of Pentobarbital and Some Anesthetics on Gastric Secretion in the Continuously Perfused Stomach in Rats Under Urethane Anesthesia

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Abstract—Effects of some anesthetics (pentobarbital, thiopental, alpha-chloralose and urethane) on gastric acid secretion were studied in the continuously perfused stomach in rats. Under urethane anesthesia, pentobarbital, thiopental and alpha-chloralose showed a definite stimulation on gastric acid secretion. Pretreatment with atropine or hexamethonium abolished the pentobarbital-induced acid secretion. Pentobarbital and thiopental did not elicit acid secretion in bilateral truncal vagotomized rats. In spinal rats, pentobarbital also stimulated acid secretion, but urethane, which was subcutaneously administered, reduced spontaneous acid output. The present results indicated that some anesthetics could stimulate gastric secretion in the anesthetized rat in contrast to the previously described inhibitory effect in the Shay rat.

Materials and Methods

Male Wistar rats weighing 220–270 g were used. The animals were housed under controlled environmental conditions (24±1°C, illuminated between 07:00–19:00) and fed on commercial rat chow (Oriental Yeast Co., Ltd., Japan). Rats were fasted for 24 hr before each experiment, but allowed free access to water.

Experiments were carried out on both anesthetized rats and spinal rats. Animals were anesthetized with 1.35 g/kg urethane, i.p., or transected at the C1–C2 spinal level under ether anesthesia. Cannulation of the femoral vein was used for administration of drugs. Rectal temperature was maintained at 36±1°C by intermittent heating with an infrared lamp. After spinal transection, spinal rats were artificially respired with room air and ether anesthesia was immediately stopped. More than 30 min later, the stomach perfusion was then started. After operation, lidocaine ointment was dermally applied to the injured portions of spinal rats.

Gastric acid secretion assay was performed following the procedure as described by Watanabe and Goto (13) with a slight modification (14). A dual polyethylene gastric cannula was inserted into the gastric lumen after ligation of the pylorus and esophagus, keeping the vagus nerve intact. The inlet and the outlet tubes of the dual cannula were connected to a saline reservoir, and the...
stomach was continuously perfused with a saline solution (pH was adjusted to 5.0) through the gastric cannula by means of a perfusion pump at the rate of 5 ml/min. The perfusate was titrated automatically in the reservoir with 0.01 N NaOH at pH 5.0 using the titrator (HS-2A; TOA Electronics, Ltd., Japan) connected to a DC recorder (Mode 1 EPR-3T; TOA Electronics, Ltd., Japan). The acid amount of the perfusate during a period of 2 min was continuously recorded by using a zero suppression adapter (TOA Electronics, Ltd., Japan). The acid amount of the secreted acid was expressed in terms of $\mu$Eq/10, 30 or 60 min per animal.

Drugs used were pentobarbital sodium (T.C.I.), thiopental sodium (Green Cross), alpha-chloralose (Nakarai), atropine sulfate (Wako), hexamethonium chloride (Wako) and histamine dihydrochloride (Wako). All compounds were dissolved in saline except urethane which was dissolved in distilled water. When the higher dose of pentobarbital was needed, pentobarbital sodium was dissolved in propylene glycol, 40 v/v%, and alcohol, 10.5 v/v% (50 mg in 1 ml). Alpha-chloralose or thiopental sodium was prepared 10 min before administration. Doses of drugs were expressed as the amount of the salt.

**Results**

1. Effect of pentobarbital, thiopental and alpha-chloralose on gastric acid secretion of perfused stomach in rats under urethane anesthesia: Under urethane anesthesia, basal acid secretion was kept rather low during an experimental period of at least 6 hr, and
Table 1. Effect of pentobarbital, thiopental, alpha-chloralose and histamine on gastric acid secretion of perfused stomach in rats under urethane anesthesia

| Drugs            | Dose and Route | Basal level (μEq/30 min) | Acid output (μEq/30 min) | No. of rats |
|------------------|----------------|---------------------------|--------------------------|-------------|
| Pentobarbital    | 30 mg/kg, i.v. | 9.98±3.43                 | 48.80** ±8.44             | 47.92** ±10.73 | 13.59 ±6.79 | 16 |
|                  | 15 mg/kg, i.v. | 7.10±0.41                 | 13.94 ±3.13              | 4.63 ±3.41   | 1.19 ±1.19 | 6  |
| Thiopental       | 30 mg/kg, i.v. | 7.14±0.41                 | 34.20* ±7.57             | 16.62 ±8.37  | 2.29 ±1.28 | 8  |
| Alpha-chloralose | 40 mg/kg, i.v. | 7.24±1.11                 | 48.88* ±14.16            | 81.18* ±15.39| 50.96*±15.11| 6  |
| Histamine        | 10 mg/kg, s.c. | 4.90±2.36                 | 29.12** ±6.60            | 61.26**±9.13 | 35.18*±10.76| 6  |

#Basal level was 3 times the amount of 10-min before drug treatment. *P<0.05, **P<0.01, ***P<0.001, compared with the basal level by the paired t-test.

Saline did not influence the basal gastric secretion. Intravenous administration of pentobarbital, thiopental and alpha-chloralose definitely stimulated acid secretion (Fig. 1, Table 1). The stimulating effect of pentobarbital (30 mg/kg), thiopental (30 mg/kg) and alpha-chloralose (40 mg/kg) began about 10 min after administration, and maximal acid output was observed about 20 min after injection. The duration of pentobarbital-induced acid output was about one hour. In these experiments, the variation of the responses had a rather wide range. Intravenous administration of pentobarbital (30 mg/kg) in urethane-anesthetized rats resulted in a decrease in mean blood pressure (about 60 mmHg in maximal fall) and heart rate (about 60 bpm in maximal reduction) followed by return to control values in about 20 min. The respiration was transiently depressed immediately after pentobarbital administration.

2. Effect of atropine, hexamethonium or bilateral truncal vagotomy on pentobarbital-induced acid secretion of perfused stomach in rats under urethane anesthesia: Atropine (1 mg/kg, i.v.) or hexamethonium (5 mg/kg, i.v.) pretreated with atropine (1 mg/kg, i.v.) or hexamethonium (5 mg/kg, i.v.) pretreated with hexamethonium (5 mg/kg, i.v.) did not influence the basal gastric secretion. Intravenous administration of pentobarbital, thiopental and alpha-chloralose definitely stimulated acid secretion (Fig. 1, Table 1). The stimulating effect of pentobarbital (30 mg/kg), thiopental (30 mg/kg) and alpha-chloralose (40 mg/kg) began about 10 min after administration, and maximal acid output was observed about 20 min after injection. The duration of pentobarbital-induced acid output was about one hour. In these experiments, the variation of the responses had a rather wide range. Intravenous administration of pentobarbital (30 mg/kg) in urethane-anesthetized rats resulted in a decrease in mean blood pressure (about 60 mmHg in maximal fall) and heart rate (about 60 bpm in maximal reduction) followed by return to control values in about 20 min. The respiration was transiently depressed immediately after pentobarbital administration.

Fig. 2. Effect of atropine or hexamethonium on pentobarbital-induced acid secretion of perfused stomach in rats under urethane anesthesia. CON: control (pentobarbital 30 mg/kg, i.v., n=16); ATR: pretreated with atropine (1 mg/kg, i.v., n=5); HEX: pretreated with hexamethonium (5 mg/kg, i.v., n=6). Each column represents the mean ± S.E. ***P<0.001, compared with the control group by Student's t-test.

Fig. 3. Effect of bilateral truncal vagotomy on pentobarbital-induced acid secretion of perfused stomach in rats under urethane anesthesia. ■: intact rat, treated with pentobarbital 30 mg/kg, i.v., n=16; ○: vagotomized rat, treated with pentobarbital, 30 mg/kg, i.v., n=6; □: vagotomized rat, treated with saline 0.2 ml/100 g, i.v., n=5; histamine (10 mg/kg, s.c.) was given to vagotomized rat after 30 min of pentobarbital administration, to check peripherally stimulated acid response. Each point represents the mean±S.E. **P<0.01, ***P<0.001, compared with the intact rat group at the same period by Student's t-test.
The basal gastric acid secretion in spinal rats had relatively large variation of secretion rate among individual rats (0 μEq/hr – 415 μEq/hr at the initial one hour after perfusion) and at the 10-minute period of time collection of the same rat (2.14 μEq/10 min – 73.90 μEq/10 min). The rate (around 30 min) of spontaneous gastric acid secretion in the spinal rat was initially low, then gradually increased and became maximal after 1–1.5 hr of perfusion. To avoid the influence of excess water, intravenous administration of pentobarbital, which was dissolved in propylene glycol and alcohol, to spinal rats significantly increased the acid output (Fig. 4). Urethane resulted in inhibition of basal gastric acid secretion at the 60–90 min period after subcutaneous administration (1.35 g/kg) to spinal rats (control group: 59.36±14.73 μEq, urethane group: 15.03±8.85 μEq, *P<0.05). After 90 min, the amount of acid secretion of urethane treated rats tended to be decreased compared with that of the control group.

### Discussion

The results presented in this report indicated that pentobarbital, thiopental and alpha-chloralose stimulated gastric acid secretion in the continuously perfused rat stomach, while urethane decreased acid output.

In 1967, Lee and Thompson (1) reported that maximal histamine-induced gastric acid secretion in Shay rats was depressed by general anesthetics such as pentobarbital, alpha-chloralose and urethane when compared to the control rats (they were operated under local anesthesia with 2% procaine). However, in 1973, Öztürkcan (11) reported that under similar experimental conditions, ether, urethane or pentobarbital altered neither volume nor acidity of gastric juice, but mucosal blood flow was highest in pentobarbital anesthetized rats. In our experiments, the behavior of pentobarbital stimulated gastric acid secretion was contradictory to Lee's observation. In our study, the animals were anesthetized with urethane, and the time course of acid secretory response to drugs was precisely recorded. This might explain the discrepancy between the present results and the previous reports.

In order to determine whether pentobarbital-induced acid stimulation depends on urethane, the effect of pentobarbital in the spinal rats was examined, and it was found that pentobarbital also provoked the acid stimulatory response. These data strongly support that our method is more suitable for the detection of the stimulatory effect of
anesthetics on gastric acid secretion than the previously reported methods.

It was reported that variation in the basal gastric secretion was found to be rather large in unanesthetized fistula rats (4). In studies on spinal rats, we also found that there were relatively large variations among individual rats and among individual juice collections in a rat. We agreed with Borella (4) in that this variation was not an artifact but rather reflected the physiology of gastric secretion in the rat. An apparent disadvantage of the Shay rat method, as compared with the urethane anesthetized perfused preparation, was the large variation in the rate of gastric secretion. Low basal secretion can be maintained for a longer period in the urethane-anesthetized condition, so that it was easy to detect the change in gastric acid stimulation.

In the spinal rats, gastric acid secretion gradually increased in the initial phase, then gradually decreased. Pentobarbital was administered at 2.5 hr after perfusion, when the gastric secretory rate became low. The drug produced a marked increase in acid secretion, and then the maximal response appeared 30 min later.

Pentobarbital-induced gastric acid secretion was inhibited by atropine, hexamethonium or acute vagal nerve section. This suggests that pentobarbital-induced acid secretion is mediated via the central nervous system and depends on the integrity of the vagus nerve.

The results of our experiments on alphachloralose were not in accordance with those conducted by the Shay rat method. Alphachloralose must have been dissolved in a large amount of saline, and the excess water load might have affected the extent of acid output (in the Shay rat study, alphachloralose was dissolved in propylene glycol). Furthermore, Schachter et al. (8, 10) and Stokkilde-Jørgensen et al. (9) had demonstrated that alphachloralose elicited a brisk gastric secretion in dogs and in pigs, respectively. This response was considered to largely depend on the integrity of the vagus nerve (8, 10).

Pentobarbital-induced gastric acid secretion may be explained by three possibilities. Firstly, it is generally accepted that cytoglucopenia in the brain stimulates vagal neurons controlling gastric acid secretion (15). Pentobarbital (thiopental) has been reported to reduce glucose utilization in the brain (16–20). Secondly, several reports have recently indicated the significance of involvement of the GABA system in the central regulation of gastric acid secretion by evaluation of actions of GABA (21), muscimol (22), baclofen (21, 23) and gamma-butyrolactone (24) which all stimulate gastric acid secretion. Much evidence shows that barbiturates enhance GABA response by an increase in receptor affinity and also exert direct transmitter-like effects in opening chloride ionophores (25). Finally, as regards to the higher central control, there are numerous reports on pentobarbital action increasing food intake in the ventromedial hypothalamus (26, 27). The ventromedial hypothalamus is considered to be an inhibitory center of gastric acid secretion (28). It is possible that depression of the inhibitory center by pentobarbital evokes gastric acid secretion.

In our previous studies, we found that centrally acting agents such as ergotamine (14), baclofen (21) and gamma-butyrolactone (24) markedly stimulate acid secretion, in a similar fashion to insulin, 2-deoxyglucose and thyrotropin-releasing hormone (29), in urethane anesthetized rats. In the present study, we found the stimulatory effect of general anesthetics on gastric acid secretion via the central nervous system. At the present, we are extending our research to elucidate the mechanism of action of the anesthetics on gastric secretion with special reference to the involvement of the central GABAergic system.

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