Japanese Journal of Physiology, **46**, 53–58, 1996

Increases in Gastric Acidity in Response to Electroacupuncture Stimulation of the Hindlimb of Anesthetized Rats

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Abstract: Acupuncture treatment has been used as physiotherapy to treat gastrointestinal disorders in traditional Oriental medicine. In the present study, we selected anesthetized animals as the subjects of this experiment in order to eliminate emotional factors, and investigated the responses to acupuncture stimulation at only a single point by changing animal conditions.

1) There was marked enhancement of gastric acid secretion by electroacupuncture stimulation of the hindlimb based on pH values of the perfusate. However, the enduring response after the momentary initial decrease suggested the existence of humoral factors.

2) The electroacupuncture-specific response was not observed in rats after sciatic nerve denervation. However, the pH values of the perfusate decreased in response to electroacupuncture stimulation of the hindlimb on the sciatic nerve intact side of the same rat. This suggests that the input pathway of acupuncture stimulation was a somatic nerve efferent pathway from the hindlimb.

3) No response to electroacupuncture stimulation was observed in the rats after vagotomy, but a clear enhancement response was seen after sympathectomy. These findings show that the major efferent path of the responses to acupuncture stimulation is the vagus nerve.

The results of this experiment suggest that electroacupuncture stimulationof the hindlimb of anesthetized rats increases gastric acid secretion with somatic nerves as the afferent pathway and branches of the vagus nerve to the stomach as the efferent pathway. [Japanese Journal of Physiology, 46, 53–58, 1996]

Key words: acupuncture, autonomic nervous system, gastric acid secretion, pH, anesthetized rat.

Acupuncture treatment has been used as physiotherapy to treat gastrointestinal disorders in traditional Oriental medicine [1]. Sodipo *et al.* (1975) reported that acupuncture therapy was effective in treating gastric ulcers [2], and clinical efficacy in several types of gastric dysfunction has been reported [3]. Inhibition of gastric acid secretion by electroacupuncture stimulation has been reported in humans [4, 5] and dogs [6] in medical experiments. Both inhibition and acceleration of gastric motility in response to acupuncture stimulation have been reported in anesthetized rats [7], however, the responses differed according to the segment of the body stimulated. Thus, it is still unclear whether the mechanism of the response to acupuncture stimulation of gastric motility and gastric secretion differs. Since gastric function is easily influenced by emotional factors, it is important to eliminate them when conducting research on changes in gastric acid secretion in response to acupuncture stimulation. Testing the response to stimulation at different loci is also necessary.

In the present study, we selected anesthetized animals as the subjects of this experiment in order to eliminate emotional factors, and investigated responses to acupuncture stimulation at only a single point by changing animal conditions. We chose the

Received on September 5, 1995; accepted on December 16, 1995
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widely used Ghosh and Schild method [8] to assay gastric acid secretion allowing us to easily compare the results with those of other studies.

MATERIALS AND METHODS

Male Wistar rats (180–450 g) were intraperitoneally anesthetized with a mixture of chloralose 50 mg/kg and urethane 500 mg/kg. The corneal reflex was used to check the depth of anesthesia. A cannula was inserted into the trachea, and the animals were artificially ventilated with a respirator (10 ml air/kg × 90 beat/min). The rats were placed in the dorsal position on a thermally insulated stand. Rectal temperature was monitored with a contact thermometer, and the rat's body temperature was maintained at 36°C by heating with an infrared lamp. A cannula was positioned in a jugular vein, and additional anesthetics were injected via the cannula as required.

Recording of gastric acid. The Ghosh and Schild method was used to measure gastric acid secretion. After fasting for 16–24 h, the abdomen was opened by making a midline incision. The duodenal junction was incised, and a polyethylene tube, with an outside diameter of 5 mm, was inserted into the stomach, and ligation was performed with the ligature in placed on the pyloric side. The tube on the pyloric side was drawn out through a small opening in the abdomen, and another polyethylene tube, with an outside diameter of 2 mm, was passed through the esophagus from the oral cavity, inserted into the stomach, and ligated in the cardia, except the vagus nerve. The interior of the stomach was continuously perfused with 0.9% saline heated to 36°C. The perfusate was directed to a fluid-type pH glass electrode (1W-201, IWAKI GLASS), and pH was recorded continuously (Fig. 1).

In a similar system, the amount of gastric acid in gastric juice collected for 2 h was titrated. The correlation between pH changes and HCl changes was approximately linear (Fig. 2).

Denervation. 1) The sciatic nerve was exposed in the right thigh, ligated in two places 10 mm apart, and divided in the center. Testing was performed after allowing 2 weeks for recovery. The left sciatic nerve was kept intact as a control.

2) Sympathetic nerve and vagus nerve denervation was performed by applying 80% phenol solution with a cotton swab. More specifically, the vagus nerve trunk was sectioned on the ventral and dorsal side around the esophagus below the diaphragm, and the postganglionic sympathetic nerve fibers were denervated by applying phenol around the visceral gan-

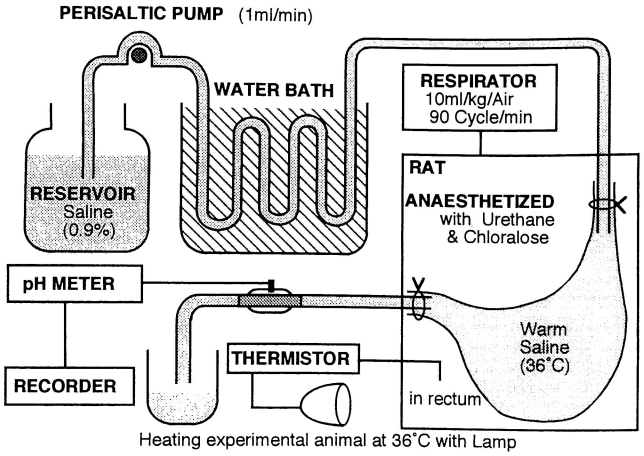

Fig. 1. Schematic diagram of preparation for experiment. Male Wistar rats (180–450 g) were intraperitoneally anesthetized with a mixture of chloralose 50 mg/kg and urethane 500 mg/kg. A cannula was inserted into the trachea, and animals were artificially ventilated with a respirator (10 ml air/kg × 90 beat/min). The rats were placed in the dorsal position on a thermally insulated stand. Rectal temperature was measured with a contact thermometer, the animals' body temperature was maintained at 36°C with an infrared ray lamp. The Ghosh and Schild method was used to measure gastric acid secretion. After fasting for 16–24 h, the abdomen was opened by making a midline incision. A polyethylene tube, with an outside diameter of 5 mm, was inserted into the stomach, and it was ligated on the pylorus side. Another polyethylene tube, with an outside diameter of 2 mm, was passed through the esophagus from the oral cavity and inserted into the stomach, and ligated in cardia, except the vagus nerve. The interior of the stomach was continuously perfused with 0.9% saline heated to 36°C. The perfusate was directed to a fluid-type pH glass electrode, and pH was recorded continuously.

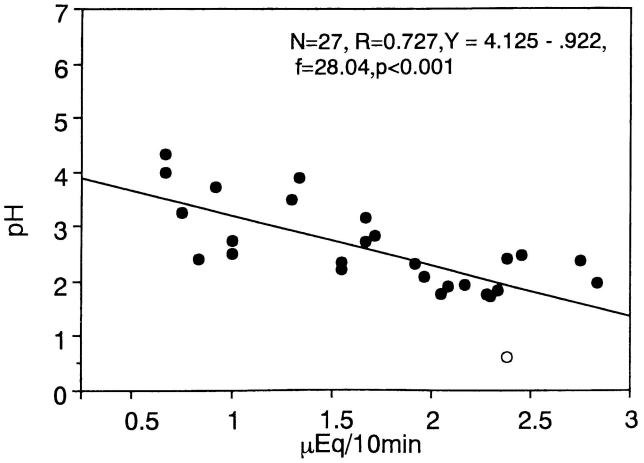

Fig. 2. Correlation between pH values and gastric acid volume. The amount of gastric acid in gastric juice collected using the same apparatus for 2 h was titrated. The gastric acid values and the changes in pH values showed an approximately linear relation, and thus changes of pH value can be used to evaluate gastric acid secretion.

glion. All experiments were performed after waiting for 30 min for the phenol to achieve a denervating effect [9].

Acupuncture stimulation. Two points (meridian points) were used for acupuncture stimulation in the first experiment. The first point (zusanli: S36) was in the hindlimb and is a site related to the stomach in Oriental medicine. The second point (shousanli: Li10) is related to the large intestine. The codes in parentheses are based on World Health Organization criteria [10]. A veterinary acupuncture point chart [11] was used to locate the animal meridian points. Stainless steel needles were used for acupuncture stimulation (diameter: 0.2 mm). The needles were inserted into the skin and muscle to a depth of approximately 5–10 mm. The needle and plate electrode (30 mm×50 mm) attached to back were used to apply electrical stimulation, and 10 Hz, 0.1 ms electric stimuli was delivered for 20 min. Selection of amperage was based on initiation of ankle flexion (Fig. 3).

Statistical analysis. Continuously recorded pH values were sampled at 2-min intervals, and means and standard errors were calculated. Statistical analysis was performed by analysis of variance (ANOVA) of mean pH values every 10 min. Values before acupuncture stimulation and at 10-min intervals after stimulation were compared by using Fisher's least significant difference (LSD) and the Tukey method. Statistical significance at the 5% level is indicated in the figure.

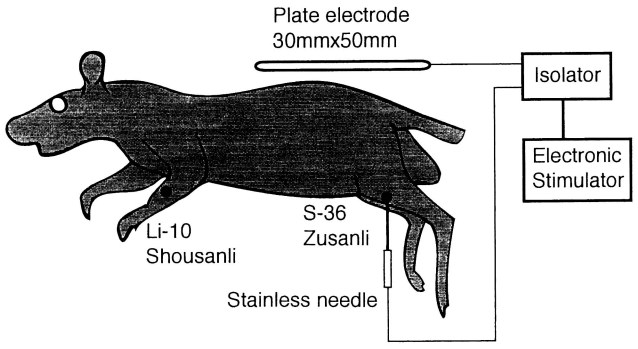

Fig. 3. Acupuncture points in the rat and the electrical stimulation system. Acupuncture stimulation loci in the rat and the electrical stimulation system. Two meridian points (S-36, "zusanli" on the hindlimb and Li-10, "shousanli" on the forelimb) were stimulated. Stainless steel needles were used for acupuncture stimulation (diameter: 0.2 mm). The needles were inserted into the skin and muscle to a depth of approximately 5–10 mm. The needle and plate electrode (30 mm×50 mm) attached to the back were used to apply electrical stimulation and 10 Hz, 0.1 ms electric stimuli was delivered for 20 min. (Figure illustrates acupuncture stimulation of Hindlimb.)

RESULTS

1. Effects of electroacupuncture simulation of the forelimb and hindlimb

The effects of electroacupuncture stimulation were compared at the points in the forelimb and hindlimb. The pH value of the perfusate decreased slightly the moment the needle was inserted. The decrease continued gradually after electrical simulation as well, and did not stop when electrical stimulation was discontinued (Fig. 4A). The hindlimb response was significant (ANOVA: $p<0.01$), but the forelimb response was not (ANOVA: N.S.). The hindlimb response began to significantly decrease 10 min after the start of electrical stimulation (Fig. 4B).

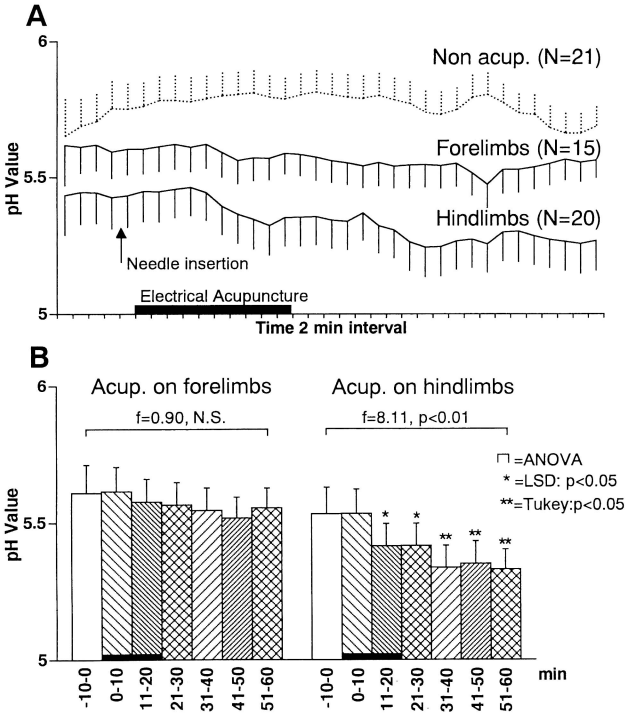

Fig. 4. Effects of electroacupuncture stimulation of the forelimb and hindlimb. A: Continuously recorded pH values were sampled at 2-min intervals, and means and standard errors were calculated. The pH of the perfusate decreased slightly the moment the needle was inserted. After electrical stimulation, the decrease gradually continued and persisted after electrical stimulation was discontinued. Vertical bar: 1/2 (SEM), arrow: insertion of the needle, horizontal bar: electrical stimulation period. **B:** Statistical analysis was performed by analysis of variance (ANOVA) on the mean of pH value every 10 min. The pH values at 10-min intervals were compared with the pH before acupuncture stimulation using Fisher's least significant difference (LSD) and the Tukey method. ⌐¬: ANOVA, *=LSD: $p<0.05$, **=Tukey: $p<0.05$, N.S.: not significant. The hindlimb response was significant (ANOVA: $p<0.01$), but the forelimb response was not (ANOVA: N.S.). The pH values decreased significantly starting with the initial pH measurement 10 min after initiation of electrical stimulation.

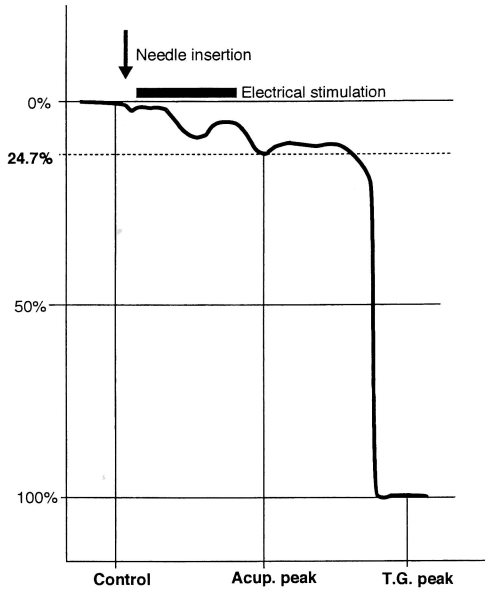

Fig. 5. Comparison of the effect on acupuncture acid output and maximal acid output. Maximal acid output in response to a gastrin dose (MAO) was examined after acupuncture stimulation in 12 rats. Gastrin was administered through a cannula in a jugular vein. The enhancement of acid secretion by acupuncture reached 24.7% of maximal acid secretion in response to gastrin infusion.

Comparison of the effect on acupuncture acid output and maximal acid output

Maximal acid output (MAO) in response to a gastrin dose was examined after acupuncture stimulation in 12 rats. Gastrin was administered through the cannula in a jugular vein. The enhancement of acid secretion by acupuncture reached 24.7% of the maximal acid secretion due to gastrin infusion (Fig. 5).

2. Effect of electroacupuncture stimulation on sciatic nerve sectioned and intact hindlimbs

The experiment was performed 2 weeks after sciatic nerve section. Atrophy of the tibialis anterior muscle was observed in sciatic nerve-sectioned hindlimbs. Electrical stimulation of both hindlimbs was performed with the voltage set at 10 V. However, the sciatic nerve-sectioned side did not show any response to electrical stimulation. The response to electroacupuncture stimulation on the sciatic nerve-sectioned side was compared with the response on the intact side, with stimulation performed on the sectioned side first and then on the intact side. No effect of acupuncture stimulation was observed on the sciatic nerve sectioned side (Fig. 6A). However, a statistically significant decrease was observed on the intact side ($p<0.05$) (Fig. 6B).

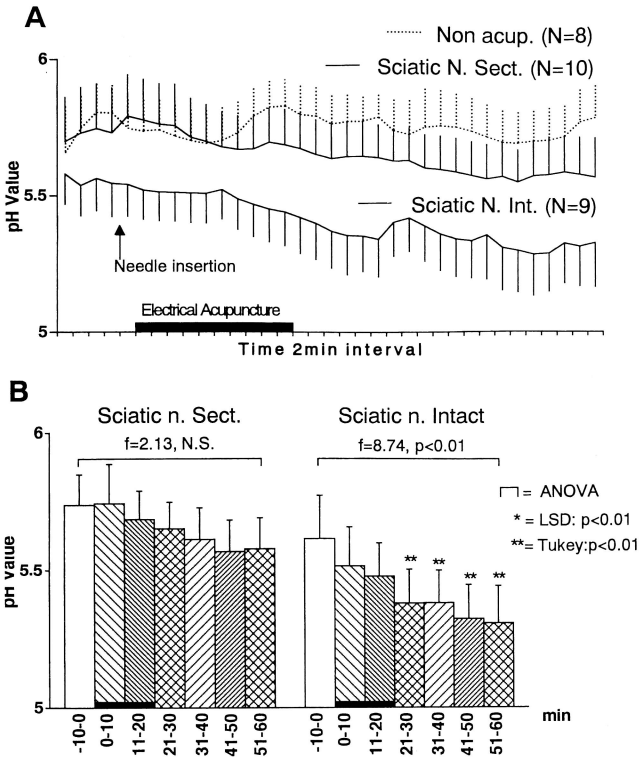

Fig. 6. Effect of electroacupuncture stimulation of sciatic nerve sectioned and intact hindlimb. A: Electrical stimulation of both hindlimbs was performed with the voltage set at 10 V. The sciatic nerve-sectioned side did not show any response to electrical stimulation. The response to electroacupuncture stimulation on the sciatic nerve-sectioned side was compared with the response on the intact-side, with stimulation performed on the sectioned side first and then onthe intact side. No effect of acupuncture stimulation was observed on the sciatic nerve sectioned side. . . . : no acupuncture stimulation, ____: sciatic nerve section and sciatic nerve intact, vertical bar: 1/2 (SEM), arrow: insertion of the needle, horizontal bar: electrical stimulation period. **B:** A statistically significant decrease was observed on the intact side ($p<0.05$). ⌐: ANOVA, *=LSD: $p<0.01$, **=Tukey: $p<0.01$, N.S.: not significant.

3. Effect of electroacupuncture stimulation after sympathetic and vagus nerve denervation

The experiment was performed 30 min after phenol application. After sympathetic nerve denervation, the pH of the gastric perfusate decreased markedly the moment the needle was inserted, and the decrease continued gradually in response to electrical stimulation ($p<0.05$) (Fig. 7A). However, there was hardly any response in vagotomized rats. Statistical analysis of sympathectomized rats showed significance at the 0.05% level,but the response in the vagotomized rats was not significant (NS). pH values decreased significantly in the sympathectomized rats beginning 10 min after the start of electric stimulation.

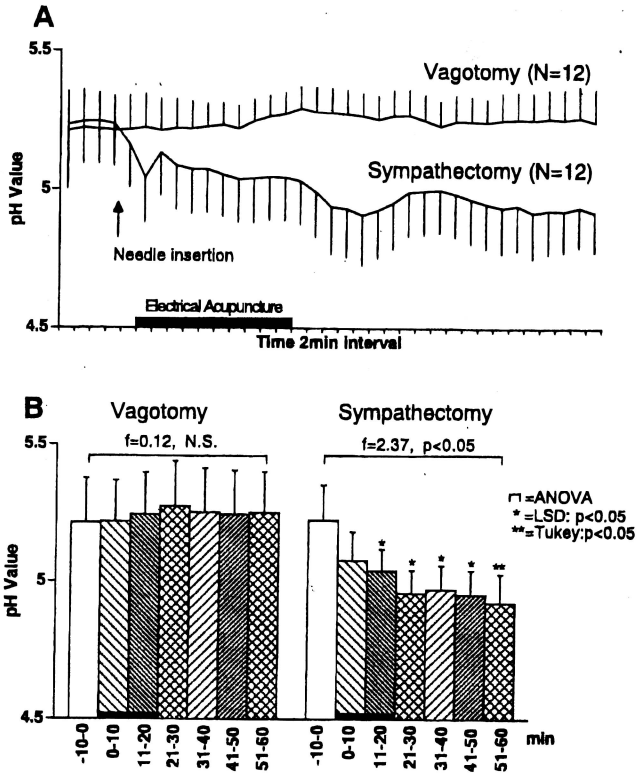

Fig. 7. Effects of electroacupuncture stimulation after sympathetic and vagus nerve denervation. A: The experiments were performed 30 min after phenol application. In sympathetic nerve-denervated rats, the pH of gastric perfusate decreased markedly at the moment of needle insertion, and the decrease gradually continued upon electrical stimulation. There was hardly any response in vagotomized rats. Vertical bar: 1/2 (SEM), Arrow: insertion of the needle, horizontal bar: electrical stimulation period. **B:** The results of statistical analysis in sympathectomized rats revealed a significant difference at the 0.05% level, but the response in the vagotomized rats was not significant. The pH values decreased significantly together with the pH values measured 10 min after the startof electrical stimulation. ⊓: ANOVA, *=LSD: $p<0.01$, **=Tukey: $p<0.01$, N.S.: not significant.

DISCUSSION

Acupuncture has been used as physiotherapy to treat gastrointestinal disorders in traditional Oriental medicine. The effects of acupuncture on gastric function have already been reported in some acupuncture studies. Sodipo *et al.* (1975) [2] reported that acupuncture was effective in thetreatment of gastric ulcers, and that the mechanism was inhibition of gastric acid secretion. Tougas *et al.* (1992) [4] and Lax *et al.* (1994) [5] reported an inhibitory effect of acupuncture stimulation on gastric acid secretion in humans, and Zhou *et al.* (1984) [6] reported similar results in conscious dogs. Sato *et al.* (1993) [7], however, reported both inhibition and enhancement of gastric motility by acupuncture stimulation in studies on anesthetized rats. The inhibition occurred as a result of stimulation of the trunk and this response represented an inhibitory reflex mediated by sympathetic nerves in response to stimulation of the trunk region. The enhancement occurred as a result of stimulation of the limbs, and this response was a vagal reflex via the central nervous system. Kametani *et al.* (1979) [12] reported that when the trunk and hindlimb were pinched simultaneously, the gastric motility inhibition response had priority. Acupuncture of the trunk region was also included in experiments by Sodipo *et al.*, Lux *et al.*, and Zhou *et al.* [2, 5, 6]. Their results showing inhibition of gastric acid secretion suggested the possibility of an inhibitory reaction to trunk stimulation. The previous report of gastric acid secretion inhibition was based on the results of a study performed in conscious subjects. Electroacupuncture stimulation of zusanli decreased human basal acid output (BAO) in a previous study of ours [14], and gastrin stimulation increased MAO. Conflicting results such as these have sometimes been encountered. Gastric function is readily influenced by emotional factors in experiments on conscious subjects. Human experiments were performed on conscious subjects, and thus gastric function was readily influenced by emotional factors. For example, the pain of the cannula for gastric juice collection is very stressful, and this stress is thought to influence the experimental results obtained in conscious subjects. Accordingly, the effects of acupuncture stimulation in experiments on conscious subjects and anesthetized subjects may differ. This is a problem which has not been resolved in acupuncture studies on humans. Thus, emotional factors need to be eliminated from studies to determine the true response mechanism to acupuncture. Testing various individual acupuncture sites is also needed. In the present study, changes in pH values in the stomach were investigated by acupuncture stimulation at just one site. The changes in gastric acid and changes in pH values showed an approximately linear correlation, and thus gastric acid secretion can be evaluated on the basis of changes in gastric pH [13]. Continuous changes could also be monitored by the titration method.

1) Compared to electroacupuncture stimulation of the forelimb, there was marked enhancement of gastric acid secretion by electroacupuncture stimulation of the hindlimb based on pH values of the perfusate. The results of our experiment showed that the effect of acupuncture stimulation on gastric pH is similar to its effect on gastric motility. However, the enduring response after the momentary initial decrease suggested the existence of humoral factors. The responses to

acupuncture stimulation of the forelimb and hindlimb were compared, and both enhanced gastric acid secretion compared to the absence of stimulation. However, it was interesting to find that there was a difference in response at sites with different meanings in Oriental medicine.

2) The electroacupuncture-specific response was not observed in rats after sciatic nerve denervation. However, the pH values of the perfusate decreased in response to electroacupuncture stimulation of the hindlimb on the sciatic nerve intact side of the same rat. This suggests that the input pathway of acupuncture stimulation was a somatic nerve efferent pathway from the hindlimb.

3) No response to electroacupuncture stimulation was observed in the rats after vagotomy, but a clear enhancement response was seen after sympathectomy. This response was clearer than the response in intact rats. This suggests that the response to acupuncture stimulation of intact rats is due to sympathetic inhibition. Berthoud *et al.* [15] reported that gastric acid secretion by anesthetized rats increases upon stimulation of the vagus nerve, and Kametani *et al.* [12] have reported a response in which somatic nerve stimulation in the hindlimb enhanced gastric motility efferently via the vagus nerve. These findings show that the major efferent path of the responses to acupuncture stimulation is the vagus nerve. They also suggested an enhancement response via a similar pathway for the enhanced gastric acid secretion response to acupuncture stimulation.

The results of this experiment suggest that electroacupuncture stimulation of the hindlimb of anesthetized rats increases gastric acid secretion with somatic nerves as the afferent pathway and branches of the vagus nerve to the stomach as the efferent pathway.

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