Role of Vagus Nerve in Secretion of Gastric Inhibitory Polypeptide in Dogs

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OHNEDA, A., KOBAYASHI, T., NIHEI, J., IMAMURA, M., NAITO, H. and TSUCHIYA, T. Role of Vagus Nerve in Secretion of Gastric Inhibitory Polypeptide in Dogs. Tohoku J. exp. Med., 1985, 147 (2), 183-190 —— In order to clarify the role of the vagus nerve in the secretion of gastric inhibitory polypeptide (GIP), experiments were performed on dogs. Response of plasma GIP to intraduodenal instillation of glucose was slightly lower in a group which received atropine, than in a group of normal dogs. The response of plasma GIP to intraduodenal glucose load was not different between vagotomized dogs and normal dogs. Electric stimulation of the vagus nerve did not produce any significant changes in plasma GIP in anesthetized dogs. In conclusion, the present study indicates that the role of the vagus nerve on GIP secretion is tiny, if any, and that the nervous influence does not overcome the effect of intraluminal administration of glucose.

Since development of the radioimmunoassay for gastric inhibitory polypeptide (GIP) (Kuzio et al. 1974), a lot of knowledges have been accumulated concerning secretion of GIP (Brown et al. 1974; Cataland 1978; Williams et al. 1981). Among various factors, which affect the secretion of GIP, nutrients administered into the gastrointestinal tract are considered to be most powerful (Cataland 1978; Williams et al. 1981). In contrast, only a few reports have been published concerning influence of the vagus nerve on GIP secretion and the results of the reports are controversial (Thomford et al. 1974; Larrimer et al. 1978; Baumert et al. 1978). The clinical study from our laboratory suggested that the response of plasma GIP to oral glucose is greater in the patients with selective vagotomy than in those with truncal vagotomy (Ohneda et al. 1984). Therefore, in order to elucidate the role of the vagus nerve in GIP secretion, the present study was performed using dogs.

MATERIALS AND METHODS

Seventeen healthy mongrel dogs, weighing approximately 15 kg, were used. To avoid the influence of gastric juice and different passage time, two Thomas cannulae were attached. 

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in a group of 5 normal dogs to the anterior wall of the gastric body and the opposite side of the duodenal papilla after the abdomen was opened by a midline incision under anesthesia with pentobarbital sodium. In other 5 dogs, both the ventral and dorsal trunks of the vagus nerve were transected below the diaphragm and pyloroplasty was performed. Furthermore, the Thomas cannulae were implanted at the stomach and the duodenum, as described above. These animals were subjected to experiments in a fully conscious state approximately 4 weeks after the operation. After an overnight fast, both the ventricular and duodenal cannulae were opened and 20% solution of glucose (2 g/kg) was administered into the duodenum through a catheter with a balloon within 5 min in a group of 5 normal dogs. Blood samples were drawn from the jugular vein before and 15, 30, 45, 60, 90 and 120 min after glucose ingestion. One week after the first experiment, the same animals underwent intraduodenal glucose load with atropine after an overnight fast. The fasting sample was drawn and an intravenous atropine injection (7.5 µg/kg) was followed by the intraduodenal glucose administration, as mentioned above. In this experiment, the second injection of atropine was given 10 min after the glucose load. In a group of 5 vagotomized dogs, intraduodenal instillation of glucose was performed after an overnight fast and blood samples were drawn from the jugular vein at 30 min intervals for 120 min. Furthermore, in order to investigate the direct effect of the vagus nerve upon the secretion of GIP, another experiment was carried out in a group of 7 healthy dogs. After an overnight fast, the abdomen was opened by a midline incision under anesthesia with pentobarbital sodium and both the dorsal and ventral trunks of the vagus nerve were dissected below the diaphragm. A polyethylene catheter was inserted through the mesenteric vein into the portal vein, and another plastic needle was indwelt into the femoral artery. After an approximately 60-min rest, bipolar electrodes were attached to the peripheral stump of the cut dorsal trunk of the vagus nerve and electric stimulation was given for 10 min using an electric stimulator (Nihon Koden, SEW-3201). Blood samples were collected from the portal vein and the femoral artery for hormone assay and blood glucose determination, respectively. Forty minutes after the start of the first electric stimulation, 1 mg of atropine was given intravenously and 10 min later the second electric stimulation was given. Blood samples were obtained for 100 min. Electric stimulation was given for 10 min with rectangular wave pulses of 5 mA and 2 msec at a frequency of 20 Hz.

Blood glucose was determined with the glucose oxidase method (Teller 1956). For the hormone assay, 4 ml of blood were collected with a heparinized syringe and poured into a glass tube containing 1,000 KIU aprotinin (Trasylol®, Bayer Company, West Germany) in an ice. Immediately after the completion of the experiment, plasma was separated by centrifugation and was stored at –20°C until the assay began. Plasma insulin (IRI) was measured by the immunoassay using the two antibody system (Morgan and Lazarow 1963). Plasma immunoreactive glucagon (IRG) was determined with antiserum G21, specific to C-terminal of glucagon (Ohneda et al. 1975). Plasma GIP was assayed by the two antibody method, as described previously (Ohneda et al. 1983).

In the present study, values are given in terms of mean ± S.E. and differences between mean values were analyzed with Student’s t-test.

**Results**

*Glucose load in normal dogs*

The changes in blood glucose and plasma IRI, IRG and GIP during glucose administration in a group of 5 normal dogs are presented in Fig. 1. Blood glucose rose from the base line of 91 ± 1.4 mg/100 ml to a peak of 152 ± 15 mg/100 ml at 60 min (p < 0.02), declining thereafter. Plasma IRI was 2 µU/ml at fasting and slightly but significantly increased at 90 and 120 min (p < 0.01). Plasma IRG
was 21 ± 3 pg/ml at fasting and increased slightly 90 and 120 min after glucose administration. Plasma GIP was 105 ± 38 pg/ml at fasting and increased abruptly to a peak of 1,188 ± 249 pg/ml at 15 min (p < 0.01), declining slowly thereafter.

Glucose load after atropine injection in normal dogs

Fig. 2 shows the changes of blood glucose, plasma IRI, IRG and GIP following intraduodenal instillation of glucose (2 g/kg) in a group of 5 normal dogs. Values are expressed in terms of mean ± S.E.
Glucose administration in vagotomized dogs

The findings observed in a group of 5 vagotomized dogs are presented in Fig. 3. Blood glucose rose from the fasting level of 82 ± 1.0 mg/100 ml to a peak of 122 ± 7.3 mg/100 ml at 30 min (p < 0.01), returning to the initial level by 120 min. Plasma IRI increased from the basal level of 6 ± 0.8 μU/ml to 23 ± 3.5 μU/ml at 15 min (p < 0.01) and then fell to the initial level at 120 min. The level of plasma IRG was 34 ± 11 pg/ml at fasting and revealed a tendency to increase after glucose load, although not significantly. Plasma GIP increased from the fasting level of 202 ± 63 pg/ml to a peak of 1,328 ± 173 pg/ml at 30 min (p < 0.01) and remained elevated till 90 min following glucose administration (p < 0.01 or less).

Comparison of GIP responses

In order to compare the response of plasma GIP to the intraduodenal administration of glucose, maximum increments and integrated increment area of plasma GIP for 120 min were calculated (Table 1). The maximal increment was slightly reduced in the atropine group, whereas it rather increased in the vagotomized group. However, these differences were not significant. The increment area was reduced in both the groups of atropine-injected dogs and vagotomized dogs.
compared with the normal group, although the difference was not significant.

Response to electric stimulation of the vagus nerve

Fig. 4 shows the mean values of blood glucose, plasma IRI, IRG and GIP in a group of 7 anesthetized dogs. Blood glucose in the femoral artery was $91 \pm 3.7$ mg/100 ml at fasting and did not change significantly after the first electric stimulation. Plasma IRI in the portal vein was $37 \pm 6.4$ \( \mu \)U/ml at the base line and slightly increased after the first electric stimulation, although not significantly. Plasma IRG in the portal vein was $232 \pm 63$ pg/ml at the initial
level and slightly increased after the first electric stimulation. Plasma GIP in the portal vein was $356 \pm 39$ pg/ml and did not change after the first electric stimulation at all. When the second electric stimulation was given 10 min after atropine injection, blood glucose did not change throughout the experiment. Plasma IRI was $65 \pm 44$ μU/ml and slightly decreased after atropine injection. The second electric stimulation did not elicit any discernible change in plasma IRI. Plasma IRG was $210 \pm 67$ pg/ml at 40 min slightly decreased after atropine injection. Plasma IRG did not change after the second electric stimulation but revealed a tendency to increase at the end of the experiment. Plasma GIP was $346 \pm 45$ pg/ml at 40 min and decreased slightly 10 min after atropine injection ($276 \pm 28$ pg/ml. $p < 0.05$). The second electric stimulation did not elicit any marked change in plasma GIP.

**DISCUSSION**

In the present study with conscious dogs, two Thomas cannulae were attached previously to the body of the stomach and the descending portion of the duodenum to avoid the influence of gastric juice and to administer glucose into the

![Figure 4](image-url)
Vagus Nerve and GIP Secretion

In such animal preparations, glucose can be given into the duodenum without any different emptying time.

At first, the effect of atropine upon GIP secretion was investigated. The response of plasma GIP after glucose administration was not altered significantly by atropine. An effect of atropine on meal-induced GIP release was reported by Baumert and his associates (1978). According to their results, plasma GIP following meal ingestion was completely abolished in animals which were administered atropine. However, they gave a meal to the dogs by mouth and gastric emptying was markedly delayed by atropine, as suggested from time course of change in plasma glucose. Therefore, it is difficult to indicate dependence of GIP release on vagal nerve, as they stated. In contrast, the present finding is in part consistent with that reported by Larrimer and his co-workers (1978), who observed a significantly smaller response of plasma GIP to glucose in human subjects injected with atropine. The difference in GIP response between dogs and humans might be explained by several factors: species difference, the amount of atropine (7.5 μg/kg twice vs 15 μg/kg plus 17 μg/min for 60 min), duration of glucose instillation (5 min vs 60 min). Furthermore, in the human experiment glucose was aspirated beyond the ligament of Treitz. Therefore, glucose did not reach the jejunum in the human study, while it reached the small intestine in the dog experiment.

However, intraduodenal instillation of glucose did not reveal any discernible difference in the GIP response between normal and vagotomized dogs, although the increment area of plasma GIP was slightly reduced in the vagotomized group. In this context, an exaggerated GIP response to oral glucose was observed in vagotomized patients in our previous study (Ohneda et al. 1984) and in the clinical experiment reported by Thomford and his co-workers (1974). However, these patients underwent pyloroplasty in addition to vagotomy and glucose administered reached rapidly the duodenum, as demonstrated by the glucose curve. Consequently the conclusion that GIP response to glucose is exaggerated in vagotomized patients is not reasonable. It is necessary to compare the GIP response to intraduodenal instillation of glucose in the normal subjects and vagotomized patients.

In order to investigate the role of the vagus nerve in GIP secretion, electric stimulation of the vagus nerve was given in anesthetized animals. Even under anesthesia, the electric stimulation produced increases of plasma IRI and IRG although not significant because of wide deviations. In contrast, plasma GIP did not change following the electric stimulation at all. The failure of GIP response seems unlikely to be due to anesthesia because of positive responses of insulin and glucagon. The fact that neither plasma IRI nor IRG was altered by the electric stimulation following atropine injection, suggests an important role of muscarinic action of the vagus nerve for the endocrine function of the pancreas. In this context, participation of the muscarinic action in GIP secretion is presumed from
the present study in which plasma GIP was slightly reduced following the atropine injection. However, according to Williams and associates (1980), acetylcholine infused for 30 min did not alter the fasting level of plasma GIP but reduced glucose-induced GIP response in dogs. The discrepancy in the response of GIP to agonist or antagonist of parasympathetic nerve cannot be fully interpreted at present. Therefore, the role of the parasympathetic nerve in GIP secretion remains to be investigated furthermore.

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References