Pharmacological Regulation of Postprandial Gastrointestinal Motility by Glucagon in Conscious Dogs

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Abstract

The physiological or pharmacological role of glucagon in the postprandial regulation of gastrointestinal motility has not yet been clarified. To clarify it, the following experiments were performed on conscious dogs. Antral, duodenal, jejunal and ileal contractile activities were monitored by chronically implanted strain gauge force transducers without restraint. The serum gastrin concentration in response to ingestion was measured by radioimmunoassay. 1) When glucagon (5~50 μg/kg, drip infusion for 5 minutes) was administered before ingestion of meal or 2 hours after ingestion, it inhibited postprandial motility dose-dependently in the antrum, while enhancing it in the duodenum, jejunum and ileum. 2) At the same time, glucagon inhibited the meal-induced elevation of the serum gastrin concentration. 3) On the other hand, glucagon did not inhibit the contractions induced by pentagastrin (1 μg/kg s.c.) or those induced by acetylcholine chloride (0.5 mg/kg, drip infusion for 10 minutes) in any region. 4) These glucagon-induced inhibitory effects in postprandial antral motility were not affected by phentolamine (0.5 mg/kg, i.v.) or nitro-L-arginine-methyl ester (L-NAME) (3 mg/kg/hr, drip infusion for 30 minutes). These results suggest that: 1) Glucagon inhibits the postprandial elevation of the serum gastrin concentration and thus inhibits postprandial antral motility. 2) On the other hand, in the intestine, glucagon-induced inhibitory responses might be reversed by glucagon-induced excitatory responses through preganglionic cholinergic motor neurons. 3) The mechanism of inhibition of gastrin release was not definite in my experiments, but one of the candidates may be activation of somatostatin release from the D cells by glucagon.

Key words: glucagon, postprandial gastrointestinal motility, gastrin, somatostatin, conscious dogs

Introduction

Among the many kinds of hormones released by meal, gastrin, cholecystokinin (Wingate et al., 1978a), secretin, glucagon (Wingate et al., 1978b), insulin (Bueno et al., 1977) and neurotensin (Al Saffar et al., 1981) disrupt the interdigestive pattern of gastrointestinal motility when infused intravenously. Accordingly, these hormones may play a role in the change of motility
to the postprandial pattern. However, no information is available to confirm a physiological role for these hormones.

Pancreatic glucagon is secreted from the A cells of the pancreatic islets. It is endogenously released by ingestion of meal, and plays a role in the change in the motility pattern after feeding (Wingate et al., 1978b). Furthermore, exogenously administered glucagon causes inhibition of gastrointestinal motility (Whalen et al., 1974) and inhibition of gastric acid secretion (Christiansen et al., 1976). However, none of these effects is likely to occur in a physiological concentration. Pancreatic glucagon also has been demonstrated in the A cells in the oxyntic gland mucosa of the dog (Larsson et al., 1975). Extracts of canine gastric mucosa contain large amounts of pancreatic glucagon but none can be demonstrated in humans (Leduque et al., 1982).

In a pharmacological concentration glucagon has a hypomotility and hypotonicity action on gastrointestinal motility (Stunkard et al., 1955; Sudsaneh et al., 1959; Deteval et al., 1963; Necheles et al., 1966). In a previous report I demonstrated that glucagon inhibits cholinergic motor activities not directly, by binding to either receptor on the smooth muscle cells, but through postganglionic cholinergic neurons in the antrum during the interdigestive state (Shimatani, 1997).

The purpose of the present study is to investigate the pharmacological effects of glucagon and the exact mechanism of its action on postprandial motility of the gastrointestinal tract in conscious dogs.

**Materials and methods**

**Preparation of animals**

Seven healthy adult mongrel dogs of either sex weighing 8.8-14.6 kg were used in these experiments. The procedures were approved by the Review Committee on Laboratory Animal Science of Hiroshima University, Japan. Under pentobarbital sodium anesthesia (25 mg/kg body weight, i.v.), the abdominal cavity was opened and strain gauge force transducers (Star Medical, Japan, F-121S) were sutured onto the serosal side of various regions of the gastrointestinal tract (the gastric antrum 5 cm proximal to the pyloric ring, the duodenum at the level of the main pancreatic duct, the jejunum 15 cm distal to the ligament of Treitz, and the ileum 15 cm proximal to the ileo-cecal junction) so that the contractile activities of the circular muscle could be recorded. Transducer lead wires were taken out of the abdominal cavity through the subcutaneous tunnel and brought out through a skin incision at the middle region of the superior end of the bilateral shoulder blade. After closure of the abdominal cavity, Silastic tubes (Argyle, Japan, 1216-27-P) were inserted into the right and left femoral vein for intravenous administration of glucagon or other agents and for blood sampling. The tubes were brought out through another skin incision on the back and their outer ends were fixed to the skin with nylon sutures. After surgery, jacket-type protectors (Star Medical, Japan, FPJ-12) were put on the dogs to protect the lead wires and the tubes from scratching and biting. The dogs were housed in individual air-conditioned (26°C) cages and administered intravenously 200 ml of physiological saline with 0.5 g of cefminox sodium (CMNX) for 3 days after the operation.
Pelleted dog diet (150 g, Nihon Clea, Japan, CD-5) was mixed with bread gruel (bread 500 g boiled with 200 ml of water, and 25 g of powdered skim milk added), and given regularly at noon (approximately 600 kcal/day).

**Monitoring of gastrointestinal contractions**

The lead wires of the strain gauge force transducers were connected to an amplifier (Star Medical, Japan, FS-02) and a recorder (Graph-tec, Japan, WR-3701) during the experiments, and the gastrointestinal contractile activities were recorded continuously in a conscious state without restraint. Additionally, contractile signals from the gastric antrum and duodenum were integrated (Nihon Koden, Japan, EI-601G) and also recorded. The motility index was calculated as in a previous report (Okajima, 1988).

**Monitoring of serum gastrin concentration**

Blood samples were drawn painlessly from the left femoral vein through the Silastic tube in a conscious state. The serum gastrin concentration was measured by radioimmunoassay.

**Materials**

The following agents were used in these experiments: glucagon (Novo-Nordisk, Japan), acetylcholine chloride (Daiichi Seiyaku, Japan), pentagastrin (ICI Pharma, Japan), nitro-L-arginine-methyl ester (L-NAME) (Wako, Japan), phentolamine mesylate (Ciba-Geigy, Japan), hexamethonium bromide (C6) (Sigma Chemical, USA), pentobarbital sodium (Abbott Laboratories, USA), cefminox sodium (CMNX) (Meiji Bristol-Myers Squibb, Japan), physiological saline (Otsuka, Japan) and 20% glucose solution (Otsuka, Japan).

**Experimental procedures**

Experiments were started after regular occurrence of gastric phase III contractions. After an overnight fasting, the first spontaneous phase III contractions of the day were recorded and agents were administered 15 minutes after the end of these contractions. Glucagon was diluted with 20 ml of physiological saline after being dissolved with 1 ml of pure water and administered from the right femoral vein through the Silastic tube by drip infusion for 5 minutes. Administration of hexamethonium bromide was begun 5 minutes before the administration of glucagon. Administration of L-NAME was started 15 minutes before the administration of glucagon. The other agents were administered by bolus injection or drip infusion before the administration of glucagon.

**Statistics**

The results are expressed as the means±S.D. of the values obtained from 3~5 dogs. The data for each group were compared using Student's t test. P values of <0.05 were considered statistically significant.
Results

1. Inhibitory and excitatory effects of glucagon on postprandial gastrointestinal motility when administered before feeding.

After ingestion of meal during the quiescent phase of interdigestive migrating contractions (IMC), the patterns of gastrointestinal motility were changed to steady low-amplitude contractions, called postprandial motility (Fig. 1-A). Intravenous administration of glucagon (5～50 μg/kg body weight, drip infusion for 5 minutes) before feeding inhibited postprandial motility in the antrum, but in the duodenum, jejunum and ileum somewhat enhanced it (Fig. 1-B). These results are shown in Fig. 2 by means of a motility index. In the antrum glucagon inhibited postprandial motility for 30 minutes (Fig. 2-A), while in the duodenum it enhanced it for 5 minutes (Fig. 2-B).

2. Influences of glucagon on serum gastrin concentration.

The serum gastrin concentration was measured simultaneously before and after feeding (Fig. 3). After feeding the serum gastrin concentration rose immediately to approximately 170

![Effects of glucagon on postprandial gastrointestinal motility when administered before feeding.](#)

A: After ingestion of meal during the quiescent phase (phase I) of IMC, patterns of motility were changed to steady low-amplitude patterns, called postprandial motility.

B: Glucagon inhibited postprandial motility in the antrum, while enhancing it in the duodenum, jejunum and ileum.
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Fig. 2. Effects of glucagon on postprandial antroduodenal motility by means of a motility index.
A: In the antrum, glucagon significantly inhibited postprandial motility for 30 minutes.
B: In the duodenum, glucagon somewhat enhanced motility for a short time.
[●●: Feeding (n=13), ○○: Glucagon+Feeding (n=9)]

Fig. 3. Effects of glucagon on serum gastrin concentration before and after feeding.
After ingestion of meal, the serum gastrin concentration rose immediately to approximately 170 pg/ml. Glucagon significantly inhibited the postprandial elevation of the serum gastrin concentration.
[●●: Feeding (n=9), ○○: Glucagon+Feeding (n=9)]

pg/ml. On the other hand, administration of glucagon completely inhibited the postprandial elevation of the serum gastrin concentration.

3. Inhibitory and excitatory effects of glucagon on postprandial motility when administered 2 hours after feeding.

Administration of glucagon (5~50 μg/kg body weight, drip infusion for 5 minutes) 2 hours after feeding also inhibited postprandial motility in the antrum, but somewhat enhanced it in the duodenum, jejunum and ileum (Fig. 4-A and B). These results are shown in Fig. 5 by means
of a motility index-30, an average of motility index for 30 minutes after the administration of glucagon. In the antrum these inhibitory responses of glucagon were observed at doses of 5, 10, 20, 30 and 50 \(\mu\)g/kg body weight and seemed to be dose-dependent. The maximal effects were observed at a dose of 30 \(\mu\)g/kg or more. On the other hand, in the duodenum postprandial motility was dose-dependently enhanced by glucagon. The maximal glucose concentration after administration of glucagon (30 \(\mu\)g/kg body weight, drip infusion for 5 minutes) was compatible with that of an administration of glucose at a dose of 0.3 g/kg body weight) (Shimatani, 1997). In order to examine the indirect effects of glucagon through hyperglycemia, glucose was used in place of glucagon, but did not affect postprandial motility in either region (Fig. 5).

4. Effects of glucagon on pentagastrin-induced postprandial motility-like contractions and acetylcholine chloride-induced contractions.

Pentagastrin is about as potent as endogenous gastrin-17 and gastrin-34 on the canine antral muscle (McDonald et al., 1979). During phase 3 contractile activities, administration of pentagastrin (4 \(\mu\)g/kg body weight, subcutaneous injection) induced postprandial motility-like
contractions in every region (Fig. 6-A). Glucagon did not affect these contractions in the antrum at all, but in the duodenum, jejenum and ileum somewhat enhanced them (Fig. 6-B). These results are shown in Fig. 7 by means of a motility index after injection of pentagastrin. Intravenous administration of acetylcholine chloride (0.5 mg/kg body weight, drip infusion for 10 minutes), exogenous acetylcholine, induced irregular phasic contractions for a short time after pre-treatment with hexamethonium bromide (10 mg/kg body weight, bolus injection and 10 mg/kg body weight, drip infusion for 30 minutes) (Fig. 8-A). Glucagon also did not inhibit these contractions in any region (Fig. 8-B).

5. Influences of phentolamine and L-NAME on glucagon-induced inhibitory effects on postprandial motility.

Administration of glucagon inhibited antral postprandial motility while enhancing intestinal motility (Fig. 4). Phentolamine (0.5 mg/kg body weight, intravenous injection), an α-adrenergic receptor antagonist, did not affect glucagon-induced inhibitory responses in the antrum (Fig. 9-A). Furthermore, L-NAME (3 mg/kg/hr, drip infusion for 30 minutes), a nitric oxide synthase inhibitor, also did not affect it (Fig. 9-B).

Discussion

It is well known that many gut hormones regulate gastrointestinal secretion and motility. Several hormones, released in response to food intake, have significant inhibitory effects on secretion and motility. Glucagon, secreted by the pancreatic A cells, also has a hypomotility and hypotonicity action on gastrointestinal motility (Stunkard et al., 1955; Sudsaneh et al., 1959; Detevall et al., 1963; Necheles et al., 1966). It is thus now commonly used as a pre-
Fig. 6. Effects of glucagon on pentagastrin-induced postprandial motility-like gastrointestinal contractions.
A: During phase I, pentagastrin induced continuous contractions similar to postprandial motility in every region.
B: Glucagon did not inhibit the contractions induced by pentagastrin in the antrum, but in the duodenum, jejunum and ileum enhanced it slightly for a short time.

treatment drug for radiodiagnostics (Miller et al., 1974; Kreeel et al., 1975; Carsen et al., 1976; Ishii et al., 1978) or endoscopic examinations (Qvigstad et al., 1979) of gastrointestinal tract in patients with various complications such as heart diseases (Giesen, 1978; Harada et al., 1997), glaucoma (Sissons et al., 1991; Fink et al., 1995) and hypertrophy of the prostate (Chernish et al., 1972). Enteroglucagons are products of the same gene that is processed differentially to form pancreatic glucagon. The intestinal L cell produces two elongated forms of glucagon, glicentin and oxyntomodulin, from the amino-terminal region and another biologically active peptide, glucagon-like-peptide-1 (GLP-1), from the carboxyl-terminal portion of preproglucagon. All of the intestinal gene products are released together when the L cell is exposed luminally to the products of mixed meal including carbohydrates and long-chain fatty acids. The most important circulating intestinal glucagon gene product appears to be GLP-1, and it may be one of the candidates of the "ileal-brake", which inhibits upper gastrointestinal functions elicited by the presence of unabsorbed nutrients in the ileum (Holst, 1997). Glucagon gene products react with at least two different high-affinity receptors, one specific for glucagon
Fig. 7. Effects of glucagon on pentagastrin-induced postprandial motility-like antroduodenal contractions by means of a motility index.
A: In the antrum, glucagon did not affect pentagastrin-induced contractions at all.
B: In the duodenum, glucagon somewhat enhanced them for 15 minutes.
[●-●: Pentagastrin (n=13), ○-○: Glucagon+Pentagastrin (n=9)]

and one specific for GLP-1. But glucagon and GLP-1 interact with a common receptor in a somatostatin-secreting cell line (Gros, et al., 1993). In a physiological concentration GLP-1 inhibited gastric emptying (Wettergren et al., 1993), but it is not definite how it affects gastrointestinal motility or how it interacts with glucagon.

Previous reports have focused on the mechanism of glucagon's inhibitory effects on interdigestive contractions. It was shown to have a direct effect not on the receptors on smooth muscle but on the myenteric nervous system in rabbits (Takenaka et al., 1975), and to interfere with intramural cholinergic neuronal transmission in rat esophagus (Lin et al., 1989). In a previous report I showed that glucagon inhibits cholinergic motor activities through postganglionic cholinergic neurons in the antrum during the interdigestive state in dogs (Shimatani, 1997). Thus it is now thought that the enteric nervous system might be a target of glucagon. However, the exact mechanism of the effects of glucagon on postprandial motility has not yet been clarified. In my study the exact mechanism of glucagon on postprandial gastrointestinal motility was examined in conscious dogs.

After ingestion of food, the periodic migrating motor complexes were immediately replaced by continuous phasic contractions, called postprandial motility, simultaneously throughout the antrum and ileum (Fig. 1-A). Intravenous administration of glucagon before ingestion partially inhibited postprandial motility in the antrum for 30 minutes, whereas it enhanced motility in the duodenum, jejunum and ileum for 5 minutes (Fig. 1-B, Fig. 2-A and B). When administered 2 hours after feeding, glucagon also inhibited antral contractions while enhancing intestinal contractions (Fig. 4). These inhibitory responses in the antrum and excitatory responses in the duodenum seemed to be dose-dependent (Fig. 5). To clarify the mechanism of these inhibitory effects, endogenous gastrin, which is released by ingestion of food containing peptides, amino acids and calcium, and which may also contribute to the regulation of postprandial antral motor activity (Stunz et al., 1979), was measured before and after feeding.
Fig. 8. Effects of glucagon on acetylcholine chloride (ACh)-induced gastrointestinal contractions after pre-treatment with hexamethonium bromide (C6). 
A: After pre-treatment with hexamethonium bromide, acetylcholine chloride induced irregular phasic contractions in every region.
B: Glucagon did not affect these contractions in any region.

The serum gastrin concentration rose immediately after feeding, whereas administration of glucagon before feeding strongly inhibited postprandial elevation of the serum gastrin concentration (Fig. 3). On the other hand, administration of pentagastrin, exogenous gastrin, also induced continuous phasic contractions similar to postprandial motility (Fig. 6-A), and glucagon did not inhibit these pentagastrin-induced contractions in the antrum at all (Fig. 6-B). These facts show that glucagon inhibits antral postprandial motility by inhibition of endogenous gastrin release.

Endogenous and exogenous gastrin both humorally stimulate release of endogenous acetylcholine from cholinergic nerve endings, and endogenous acetylcholine stimulates smooth muscle contractions (Lipshutz et al., 1972, Vizi et al., 1973). Thus acetylcholine may be the main final transmitter in the regulation of postprandial motility. To examine the inhibitory effects of glucagon directly on the smooth muscle cells, acetylcholine was administered exogenously. Acetylcholine chloride induced phasic contractions (Fig. 8-A) and glucagon did not inhibit these
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Phentolamine 0.5mg/kg, i.v.
↓ Glucagon 30μg/kg, i.v.

Antrum  I 50g
Duodenum  I 50g
Jejunum  I 50g
Ileum  I 50g

L-NAME 3mg/kg/hr, i.v.
Glucagon 30μg/kg, i.v.

Antrum  I 50g
Duodenum  I 50g
Jejunum  I 50g
Ileum  I 50g

Fig. 9. Influence of phentolamine and L-NAME on glucagon-induced inhibitory responses on postprandial gastrointestinal motility.
A: Phentolamine, an α-adrenergic receptor antagonist, did not affect glucagon-induced inhibitory responses in any region.
B: L-NAME, a nitric oxide synthase inhibitor, somewhat enhanced postprandial motility, but did not affect the glucagon-induced inhibitory responses.

Acetylcholine chloride-induced contractions (Fig. 8-B). Thus the inhibitory effects of glucagon are not directly, by binding to either receptor on the smooth muscle cells, but through inhibition of endogenous acetylcholine release.

It is well known that inhibitory neurons modulate gastrointestinal motility, including adrenergic neurons (Nishi et al., 1973, Vizi et al., 1973) and nitronergic neurons (Sarna et al., 1993). The adrenergic nerves inhibit gastrointestinal postprandial motility via α2-adrenergic receptors on postganglionic cholinergic motor neurons (Fujii et al., 1989). Phentolamine, an α-adrenergic receptor antagonist, was administered to block the adrenergic inhibitory neurons but did not affect the inhibitory effects of glucagon on postprandial antral motility (Fig. 9-A).

On the other hand, the nitronergic nerves, one of the non-adrenergic, non-cholinergic (NANC) inhibitory nerves, also inhibit gastrointestinal postprandial motility through postganglionic cholinergic motor neurons (Ojima, 1997). L-NAME, a nitric oxide synthase inhibitor, somewhat enhanced postprandial motility but did not affect the inhibitory effects of glucagon on postprandial motility (Fig. 9-B). These facts indicate that the inhibitory effects of glucagon mediate neither via the adrenergic neurons nor the nitronergic neurons.
Several hormones and paracrine substances, including somatostatin, influence endogenous gastrin release from the G cells. The D cells, which produce somatostatin, are located near the G cells. Somatostatin indeed inhibits gastrin release (Harty et al., 1985), and SMS 201–995, an exogenous somatostatin analogue, also inhibits it (Fujii, 1990). Glucagon stimulates somatostatin release in dog pancreas (Kawai et al., 1989). In fact, glucagon receptors are demonstrated in the somatostatin-secreting cell line RIN T3 (Gros et al., 1993) and on rat pancreatic D cells (Kieffer et al., 1996). Furthermore, pancreatic glucagon is produced in the A cells in the dog stomach in vivo (Larsson et al., 1975). These facts indicate that inhibition of gastrin release may result from activation of somatostatin release from the D cells by glucagon, probably through glucagon receptors on the D cells.

Administration of glucagon inhibited postprandial motility in the antrum whereas it enhanced motility in the duodenum, jejunum and ileum. The source of these paradoxical effects is very important but difficult to ascertain. Gastrin induced continuous contractions in the antrum and upper small intestine (Fig.6–A). Glucagon inhibited postprandial serum gastrin release (Fig. 3). Thus glucagon should inhibit postprandial motility in the intestine as well as in the antrum. In a previous report, I showed that glucagon enhanced cholinergic motor activities not in the antrum, but in the intestine, through preganglionic cholinergic neurons involving nicotinic and muscarinic receptors (Shimatani, 1997). Consequently, in the antrum glucagon inhibited postprandial motility through inhibition of gastrin release, but in the intestine glucagon-induced inhibitory responses might be reversed by glucagon-induced preganglionic activation. These differences between the antrum and intestine may result from the physiological roles of endogenous glucagon or enteroglucagon.

In conclusion, glucagon inhibits postprandial elevation of the serum gastrin concentration and thus inhibits postprandial antral motility. Thus, it is effective and safe as a pre-treatment drug for X-ray studies and endoscopic examinations of the upper gastrointestinal tract in patients with various complications which are incompatible with anticholinergic drugs even in a postprandial state. The mechanism of inhibition of gastrin release was not definite in my study, but one of the candidates may be activation of somatostatin release from the D cells by glucagon.

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References


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