Effect of Acetylcholine on The Secretion of Gut Glucagon Immunoreactivity and Gut Glucagon-Like Immunoreactivity in Pancreatectomized Dogs

TOSHIHIDE YOSHIDA AND MOTOHARU KONDO

First Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kamikyo-ku, Kyoto 602, Japan

Synopsis

Effect of the infusion of acetylcholine on the secretion of gut glucagon immunoreactivity (gut GI) that was measured using C-terminal specific glucagon antiserum after pancreatectomy, and gut glucagon-like immunoreactivity (gut GLI) that was obtained by subtracting GI from total glucagon-like immunoreactivity (total GLI) which was measured using non-specific glucagon antiserum, was investigated in sixteen pancreatectomized dogs untreated with insulin, in order to demonstrate whether the secretion of gut GI and gut GLI is influenced by the parasympathetic nervous system.

During the infusion of acetylcholine at a rate of $10 \mu\text{M/kg/min}$, gut GI in the femoral venous blood showed a significant increase from the basal value of $181 \pm 22 \text{ pg/ml}$ to a maximum of $569 \pm 107 \text{ pg/ml}$ at 30 min ($p < 0.01$), and "true gut GI secretion increment" in the portal venous blood showed a maximum significant increase of $916 \pm 144\%$ at 30 min from the basal value ($p < 0.001$). However, gut GLI showed no significant change. One shot administration of atropine at a rate of $15 \mu\text{g/kg}$ could significantly inhibit the stimulatory effect of acetylcholine on gut GI ($p < 0.05-0.001$).

It is concluded that the parasympathetic nervous system might play an important role in the control mechanism of the release of gut GI, but not of gut GLI in pancreatectomized dogs untreated with insulin.

Although the high levels of circulating gut glucagon immunoreactivity (gut GI) that was measured using C-terminal specific glucagon antiserum after pancreatectomy, and gut glucagon-like immunoreactivity (gut GLI) that was obtained by subtracting GI from total glucagon-like immunoreactivity (total GLI) which was measured using non-specific glucagon antiserum, have been demonstrated in pancreatectomized dogs untreated with insulin (Matsuyama and Foa, 1974; Vranic *et al.*, 1974; Mashiter *et al.*, 1975; Yoshida, 1977), the regulation mechanism of the secretion of extrapancreatic

glucagon has not been clearly explained.

In order to demonstrate whether the secretion of gut GI and gut GLI is influenced by the parasympathetic nervous system, the present investigation was designed to find the effect of acetylcholine on the secretion of gut GI and gut GLI in pancreatectomized dogs.

Materials and Methods

Sixteen healthy mongrel dogs weighing 9.0-11.2 kg, which had fasted for 24 hr, were used. Under ketamine hydrochloride (Ketalar®) and thiopental sodium (Ravonal®) anesthesia, total pancreatectomy was performed. Polyethylene catheters were then

inserted into the hilar region of the portal vein for blood sampling and the measurement of outflow of blood and hematocrit, into the femoral vein for blood sampling, and into the axillar vein for the infusion of drugs. Thereafter, these dogs were maintained without insulin therapy for three days. On the fourth day, six dogs were again fasted for 24 hr and given the infusion of acetylcholine chloride (Sigma chemical Co. U.S.A.) at a rate of $10 \mu\text{M}/\text{kg}/\text{min}$ for 30 min under Ketalar anesthesia. Another five dogs were fasted for 24 hr and were infused with the same amount of acetylcholine for 30 min, and atropine (Merk Co. Darmstadt) was intravenously injected at a rate of $15 \mu\text{g}/\text{kg}$ for 30 sec at the same time with acetylcholine. The rest fasted for 24 hr were given only one shot of atropine for 30 sec. Blood was simultaneously withdrawn at appropriate intervals from the portal and femoral vein. Outflow of blood in the portal vein was measured directly by blood volume per sec during one sampling through the polyethylene catheter, was calculated by percent difference from the baseline volume and was expressed as "outflow increment of blood". The blood sample drawn was immediately transferred into a chilled tube containing 1.2 mg of EDTA and 1000 KIU of aprotinin (Trasylol®, Bayer Co.) per ml of blood. The sample was centrifuged as soon as possible and the plasma was stored at -20°C until used. Glucagon was measured by radioimmunoassay using the talc method (Sakurai *et al.*, 1973b). Two kinds of antiglucagon sera were used: the C-terminal specific antiserum for GI, 30K (obtained from Dr. R. H. Unger, Dallas, Texas), and the non-specific antiserum, K4023 (obtained from Novo Industri, Copenhagen, Denmark) for the assay of total glucagon-like immunoreactivity (total GLI). Gut glucagon-like immunoreactivity (gut GLI) was calculated by subtracting GI from total GLI. The calculated plasma glucagon value in the portal vein was corrected from the following formulae: Calculated plasma glucagon = plasma glucagon concentration $\times$ outflow increment of blood $\times \left(\frac{100 - \text{hematocrit}}{100} \right)$. The value was further calculated and expressed as "true glucagon secretion increment" in percent difference from the baseline value. Insulin was measured by radioimmunoassay using the double-antibody method (Hales and Randle, 1963) and glucose by the glucose-oxidase method. Blood pressure was monitored during these experiments.

All data are shown as mean $\pm$ SEM. Student's *t*-test was used for statistical analysis: measured values were compared with the basal ones or between the two groups.

Results

1) Effect of the infusion of acetylcholine on the secretion of GI, gut GLI, insulin and plasma glucose level.

During the infusion of acetylcholine at a rate of $10 \mu\text{M}/\text{kg}/\text{min}$ for 30 min, GI level in the femoral venous blood showed a significant increase from the basal value of $181 \pm 22 \text{ pg/ml}$ to a maximum of $569 \pm 107 \text{ pg/ml}$ at 30 min ($p < 0.01$), while gut GLI showed no significant change. In the portal venous blood, outflow increment of blood rose significantly up to $63 \pm 16 \%$ at 30 min from the baseline value ($p < 0.005$), but hematocrit showed no significant change from the basal value. "True GI secretion increment" showed a significant increase to a maximum of $916 \pm 144 \%$ at 30 min from the baseline value ($p < 0.001$). However, "true gut GLI secretion increment" revealed no significant increase from the basal value. Plasma glucose level and insulin were not changed. (Fig. 1)

Average systolic blood pressure slightly decreased from 128 mmHg to 116 mmHg during the infusion of acetylcholine.

2) Effect of the infusion of acetylcholine with one shot administration of atropine on the secretion of GI, gut GLI, insulin and plasma glucose level.

When atropine was intravenously injected at the same time with the infusion of acetylcholine, GI level in the femoral venous blood, although showed no significant change in the initial 15 min, revealed gradual rise after 20 min and reached to a maximum value of $350 \pm 62 \text{ pg/ml}$ at 30 min ($p < 0.05$), while gut GLI showed no significant change. In the portal venous blood, outflow increment of blood and hematocrit showed no significant change from the basal value. "True GI secretion increment" showed a significant increase of $117 \pm 29 \%$ at 30 min from the baseline value ($p < 0.01$), while "true gut GLI secretion

Fig. 1. Effect of acetylcholine on the secretion of GI, gut GLI, insulin and plasma glucose level in six pancreatectomized dogs.

Fig. 2. Effect of acetylcholine with one shot administration of atropine on the secretion of GI, gut GLI, insulin and plasma glucose level in five pancreatectomized dogs.

Fig. 3. Effect of one shot administration of atropine on the secretion of GI, gut GLI, insulin and plasma glucose level in five pancreatectomized dogs.

increment" showed no significant increase. Insulin and plasma glucose level were not changed. (Fig. 2)

Average systolic blood pressure showed little change during the infusion of acetylcholine with one shot administration of atropine.

These findings clearly demonstrated that atropinization showed significant suppression of GI responses in the femoral vein ($p < 0.05$), and in the portal vein ($p < 0.02-0.001$).

3) Effect of one shot administration of atropine on the secretion of GI, gut GLI, insulin and plasma glucose level in pancreatectomized dog.

When atropine was injected at a rate of $15 \mu\text{g/kg}$ for 30 sec, no significant change was observed in plasma glucose level, insulin, GI and gut GLI in both femoral and portal venous blood. (Fig. 3)

Discussion

It has been demonstrated that gut glucagon immunoreactivity (gut GI) as measured with C-terminal specific glucagon antiserum, and gut glucagon-like immunoreactivity (gut GLI) obtained by subtracting GI from total GLI, increased in pancreatectomized dogs untreated with insulin (Matsuyama and Foa, 1974; Vranic *et al.*, 1974; Mashiter *et al.*, 1975; Yoshida, 1977).

Concerning the regulation mechanism of extrapancreatic glucagon, it has been known that gut GI is inhibited by insulin injection (Matsuyama and Foa, 1974; Vranic *et al.*, 1974) or by somatostatin infusion (Sakurai *et al.*, 1975), but gut GI is promoted by the withdrawal of insulin therapy (Matsuyama and Foa, 1974; Vranic *et al.*, 1974), by the administration of arginine (Mashiter *et al.*, 1975; Matsuyama *et al.*, 1978; Muller *et al.*, 1978), or by the transplantation of hypophysis (Yoshida, 1977). Gut GLI is inhibited by insulin injection, but is

promoted by the withdrawal of insulin therapy (Matsuyama and Foa, 1974; Vranic *et al.*, 1974) or by the administration of arginine and exogenous glucagon (Muller *et al.*, 1978).

Since it is still obscure whether the secretion of gut GI and gut GLI is influenced by the parasympathetic nervous system, the effect of acetylcholine and atropine on gut GI and gut GLI was investigated in pancreatectomized dogs.

As shown in Fig. 1 and 2, a large administration dose of acetylcholine (10 μ M/kg/min) was found to produce a stimulatory effect on gut GI but not on gut GLI, and atropine to inhibit the effect of acetylcholine. Such a stimulatory effect of acetylcholine on the secretion of gut GI has not been shown either in vitro or in vivo previously.

Concerning pancreatic glucagon, Iversen (1972), and Kaneto and Kosaka (1974a) reported that acetylcholine stimulated the release of pancreatic glucagon, and the infusion of atropine completely abolished the stimulatory effect of acetylcholine on pancreatic glucagon. Bloom *et al.* (1974a) and Kaneto *et al.* (1974b) reported that vagal stimulation increased plasma glucagon level. Bloom *et al.* (1974b) showed that the infusion of atropine produced a significant fall in fasting plasma pancreatic glucagon, and that after arginine infusion, atropine reduced the normal rise of glucagon. But in our present study as shown in Fig. 3, gut GI and gut GLI were not influenced by one shot administration of atropine.

On the other hand, Lindsey *et al.* (1974) and Wilmore *et al.* (1974) reported that plasma glucagon rose in the shock state, such as trauma, bleeding and burn. Although systolic blood pressure was also measured in this experiment, its average pressure slightly decreased during the infusion of acetylcholine. So, this showed that these dogs were not in the shock state, and such a minute change of blood pressure

observed in this experiment would not affect glucagon level.

Some workers (Botha *et al.*, 1977; Matsuyama *et al.*, 1978) reported that the kinetics of the extrapancreatic GI to various stimulants and suppressants was different from that of pancreatic glucagon. However, from our present study, it is concluded that the parasympathetic nervous system might play an important role in the control mechanism of the secretion of gut GI as well as in that of pancreatic glucagon.

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