The Effect of Hypophysectomy and Hypophysis-Transplantation on The Secretion of Gut Glucagon Immunoreactivity and Gut Glucagon-Like Immunoreactivity in Depancreatized Dogs

TOSHIHIDE YOSHIDA AND MOTOHARU KONDO
First Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kamikyo-ku, Kyoto 602, Japan

Synopsis

The role of hypophysis in the regulation mechanism of 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 depancreatized dogs.

Plasma glucose, gut GI and gut GLI levels were found to increase in totally depancreatized dogs. The former two showed a significant decrease after hypophysectomy, and were reversed by the hypophysis-transplantation, while gut GLI was not affected either by hypophysectomy or hypophysis-transplantation. Intramuscular injections of human growth hormone (HGH) or adrenocorticotropic hormone-Z (ACTH-Z) to depancreatized-hypophysectomized dogs had no effect on plasma glucose level or gut GI.

It is concluded that hypophysis may promote the secretion of gut GI after pancreatectomy, but not of gut GLI. Gut GI seems to regulate plasma glucose level after pancreatectomy. However, the precise regulation mechanism of gut GI by the hypophysial hormone after pancreatectomy is not clarified yet.

It has been demonstrated that 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, increased markedly in depancreatized dogs untreated with insulin (Matsuyama and Foa, 1974; Vranic et al., 1974; Mashiter et al., 1975). However, the regulation mechanism of the secretion of extrapancreatic glucagon has not been clearly explained, except that gut GI is inhibited by the injection of insulin (Matsuyama and Foa, 1974; Vranic et al., 1974), and by the infusion of somatostatin (Sakurai et al., 1975). In contrast, the secretion of 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) and by vagal stimulation (Marre et al., 1979). Gut GLI is also inhibited by insulin injection and promoted by the withdrawal of insulin therapy (Matsuyama and Foa, 1974; Vranic et al., 1974) and by the administration of arginine and exogenous glucagon (Muller et al., 1978). The present investigation was designed to find a part of the regulation mechanism of the secretion of gut GI and gut GLI in connection with hypophysis,
and also to examine the relation between gut GI and plasma glucose level after pancreatectomy.

**Materials of Methods**

Thirty mongrel dogs weighing 9.0-14.3 kg, fasted for 24 hr, received total pancreatectomy under ketamine hydrochloride (Ketalar®, Sankyo Co., Japan) and thiopental sodium (Ravonal®, Tanabe Pharmaceutical Co., Japan) anesthesia, and had polyethylene catheters inserted into the femoral veins for blood sampling. Ten dogs were fed on dog food without using insulin therapy for eight days as a control group. The rest were also fed without insulin, and on the fourth day they received hypophysectomy through the temporal approach. On the sixth day, four of them were transplanted with hypophysis obtained from other normal dogs into the hypoderm of the head. The other sixteen dogs were divided into three groups, and were given intramuscular injection with 4 IU of human growth hormone (HGH) (Crescormon®, KABI Co., Sweden), 1.0 mg of adrenocorticotropic hormone-Z (ACTH-Z) (Cortrosyn-Z®, Organon Co., Holland), or saline for three consecutive days, respectively. But, all dogs could not eat dog food as much as usual because of vomiting.

Blood samples were obtained from a polyethylene catheter into the femoral vein every morning in their fasting state, and transferred immediately into chilled tubes containing 1.2 mg of EDTA (ethylenediamine tetraacetate) and 1000 KIU of aprotinin (Trasylol®, Bayer, Germany) per ml of blood. The sample was centrifuged immediately and the plasma was stored at -20°C until examined. Glucagon was measured by radioimmunoassay using the talc method (Sakurai et al., 1973). Two kinds of anti-glucacon 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) to assay total glucagon-like immunoreactivity (total GLI). Gut GLI was calculated by subtracting GI from total GLI. Insulin was measured by radioimmunoassay using the double antibody method (Hales and Randle, 1963), and glucose by the glucose-oxidase method.

In our experiment, the completeness of the operation was confirmed at autopsy by microscopic and macroscopic examination of the tissues in the sella turcica and the stalk region. The clinical confirmation was made by demonstrating the decrease of plasma ACTH (using ACTH radioimmunoassay kit, CEA, France), and thyroid stimulating hormone (using thyroid stimulating hormone radioimmunoassay kit, CEA, France) levels after hypophysectomy, and by the increase after hypophysis-transplantation. All data were shown as mean±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 pancreatectomy on plasma glucose, GI and gut GLI in dogs.

In the control group which received pancreatectomy alone, plasma glucose level showed a significant increase from 107±7 mg/dl before the operation to 306±24 mg/dl one day after the operation (p<0.001), and up to 756±124 mg/dl on the eighth day. Insulin level of 14±2 μU/ml before the operation showed a decrease after pancreatectomy (p<0.01). GI rose from the initial value of 70±6 pg/ml to 251±32 pg/ml on the second day after the operation (p<0.001), and showed a gradual increase thereafter to a maximum of 680±167 pg/ml on the eighth day. Gut GLI increased from 175±7 pg/ml before the operation to 452±74 pg/ml (p<0.01) on the second day after the operation, to 648±50 pg/ml (p<0.001) on the third day, and it maintained about 650 pg/ml until the eighth day. (Fig. 1)

2) Effect of hypophysectomy and hypophysis-transplantation on plasma glucose, GI and gut GLI in depancreatized dogs.

In the group of dogs which were hypophysectomized after total pancreatectomy, plasma glucose level decreased significantly from the value before hypophysectomy of 362±22 mg/dl to 278±24 mg/dl one day after hypophysectomy (p<0.05), and to 255±21 mg/dl on two days after (p<0.02). GI showed a significant decrease from 438±36 pg/ml to 305±46 pg/ml one day after the operation (p<0.05), and to 262±52 pg/ml two days after (p<0.02). However, gut GLI showed no significant change after hypophysectomy.

By the hypophysis-transplantation in hypophysectomized dogs following total
Fig. 1. Effect of pancreatectomy on plasma glucose level, insulin, GI and gut GLI in dogs.

Fig. 2. Effect of hypophysectomy and hypophysis-transplantation on plasma glucose level, GI and gut GLI in depancreatized dogs.

3) Effect of HGH or ACTH-Z on plasma glucose level and GI after hypophysectomy following pancreatectomy in dogs.

In the groups to which 4 IU of HGH or 1.0 mg of ACTH-Z were intramuscularly administered daily instead of hypophysis-transplantation, both plasma glucose level and gut GI failed to show significant variations, compared with the control group given an intramuscular injection of saline alone. (Fig. 3)

Discussion

The present control experiment showed that plasma glucose level, GI and gut GLI increased significantly in totally depancreatized dogs. This was in agreement with the observations of Matsuyama and Foa (1974), Vranic et al. (1974) and Mashiter et al. (1975).
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Fig. 3. Effect of the intramuscular injection of HGH or ACTH-Z on plasma glucose level and GI after hypophysectomy following pancreatectomy in dogs.

The present findings showed that the elevated plasma glucose level and gut GI due to pancreatectomy fell down significantly in almost parallel with each other after hypophysectomy. These results were in agreement with the report of Nakabayashi et al. (1978). In this experiment, however, high gut GLI level was not affected. Furthermore, both plasma glucose level and gut GI decreased by hypophysectomy, increased again in almost parallel after hypophysis-transplantation, while gut GLI was not affected. These results suggest that the increased secretion of gut GI after pancreatectomy was accelerated by the hypophysis; that is, the hypophysis might play some roles in the regulation mechanism of the secretion of A cells of gastric fundus that have been considered to secrete gut GI. These findings may lead us to resolve the famous phenomenon of Houssay's dogs that symptoms of experimental diabetes induced by pancreatectomy are improved by the removal of hypophysis (Houssay and Biasotti, 1931). In our study, sham operations of hypophysectomy and hypophysis-transplantation were not performed, so some effects of craniotomy cannot be ruled out.

The intramuscular injections of HGH or ACTH instead of hypophysis-transplantation failed to demonstrate significant changes in plasma glucose or GI as compared with the saline control group. Although GH is known to have a strong species specificity, these results might suggest that GH or ACTH could not be deemed as the hormone to regulate gut GI.

Regarding the relationship between pancreatic glucagon and GH, Farmer et al., (1971) reported that GH did not stimulate glucagon secretion. In contrast, Pek et al. (1971), Goldfine et al. (1972) and Tai and Pek (1976) reported that GH caused the secretion of glucagon. Therefore, further investigations should be done on the relationship between gut GI and GH or ACTH.

On the other hand, our present experiment showed that gut GI regulated plasma glucose level after pancreatectomy as already reported (Sakurai et al., 1975; Tanaka et al., 1979). In contrast, Felig et al. (1976) reported that there was no relation between plasma glucose level and gut GI.

In the present study, it is concluded that hypophysis may promote the secretion of gut GI, but not of gut GLI after pancreatectomy, and that gut GI may regulate plasma glucose level after pancreatectomy. However, the precise regulation mechanism of gut GI by the hypophysial hormone after pancreatectomy is not clarified yet.
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