Response of Extrapancreatic Glucagon
to Glycemic Changes under Chronic
Insulin Deficiency in Dogs

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Since the secretion of pancreatic glucagon is largely influenced by the changes in the blood glucose level, the response of extrapancreatic glucagon was investigated in totally pancreatectomized dogs under chronic insulin deficiency. Insulin-induced hypoglycemia did not alter plasma glucagon in the portal vein in a group of 5 pancreatectomized dogs, but the decrease of the blood glucose was small, by 70 mg/100 ml, in spite of a large amount of insulin. The administration of 2-deoxyglucose did not cause any changes in plasma glucagon in the portal vein in a group of 6 pancreatectomized dogs. Glucose-induced hyperglycemia, both transient and continuous, did not cause any changes in plasma glucagon in the portal vein, although blood glucose was significantly elevated. It is concluded that regulation of extrapancreatic glucagon differs from that of pancreatic glucagon.

Since the establishment of radioimmunoassay for glucagon, a number of studies have been done concerning the regulation of the secretion of pancreatic glucagon. There have been, however, a few reports on the secretion of extrapancreatic glucagon, whose presence was demonstrated in 1974. The secretion of pancreatic glucagon is affected by changes in blood glucose (Ohneda et al. 1969), and glycemic changes are the most important among the various factors influencing the secretion of pancreatic glucagon. Therefore, we have studied the effect of glycemic changes upon the secretion of extrapancreatic glucagon in animals. Since there is no method to discriminate extrapancreatic glucagon from pancreatic glucagon (Srikant et al. 1977), the response of extrapancreatic glucagon has been investigated in totally pancreatectomized animals.

We have already reported the response of extrapancreatic glucagon to glycemic changes immediately after pancreatectomy in dogs. However, different responses of extrapancreatic glucagon may possibly be evoked several days after pancreatectomy, because the circulating extrapancreatic glucagon increases about

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days, especially under insulin deficiency. Therefore, the present study was intended to determine the effect of glycemic changes upon the secretion of extrapancreatic glucagon in animals which were fed under insulin deficiency for a week.

**Materials and Methods**

Healthy mongrel dogs, weighing 15 to 23 kg, were the subjects of this study. After an overnight fast, the dogs were anesthetized with pentobarbital sodium and the abdomen was opened by a midline incision. The pancreas was removed carefully from the adjacent tissues. After the operation, the dogs were fed under insulin deficiency. To avoid ketoacidosis and extreme hyperglycemia, the dogs were administered with a small dose of insulin, if necessary, but the insulin administration was withdrawn 2 days before the experiment. About a week after pancreatectomy, the dogs were anesthetized with pentobarbital sodium after an overnight fast, and the abdomen was opened by a midline incision again. In order to collect blood from the intestine as well as the stomach, a polyethylene catheter was inserted into the portal vein up to the hepatic hilus through a branch of the mesenteric vein. Plastic needles were inserted into the femoral artery and the femoral vein for the purpose of blood sampling and drug infusion, respectively. Immediately after surgery, physiological saline was infused into the femoral vein at a constant rate of 2 ml per min. Approximately 1 hr after the completion of the operation, the experiments were commenced.

In a group of 5 pancreatectomized dogs, insulin (Actrapid,® Novo Institute, Denmark) was administered into the femoral vein as a priming dose of 0.4 U/kg followed by a constant infusion of 0.04 U/kg for 120 min. In this experiment, the level of blood glucose was monitored, and a bolus injection of insulin was added when the lowering of blood glucose was inadequate.

In order to obtain an intracellular glucopeny, 2-deoxyglucose (Waco Pure Chemical Industries, Osaka) was injected intravenously in a dose of 300 mg/kg in a group of 6 pancreatectomized dogs.

Furthermore, in order to examine the response of extrapancreatic glucagon to hyperglycemia, 20% glucose solution in a dose of 12 g was administered into the femoral vein in a group of 5 pancreatectomized dogs, successively following the experiment with the insulin induced hypoglycemia. In another group of 6 pancreatectomized dogs, glucose was administered into the femoral vein in a priming dose of 1 g, and constant infusion of 400 mg/min successively after the experiment with 2-deoxyglucose.

After the base line samples were drawn, various stimuli were given and blood specimens were obtained from the femoral artery and the portal vein at various intervals.

For hormone assay, 4 ml of blood was obtained with a heparinized syringe and poured into glass tubes containing 1000 U of Trasylol® (Bayer Co.). Plasma was separated by centrifugation and kept at −20°C until assay.

Blood glucose was measured by the glucose oxidase method (Teller 1956). Plasma insulin (IRI) was determined by the Morgan-Lazarow method (1962). Plasma glucagon (IRG) was measured by radioimmunoassay using an antiserum (G21), specific for C-terminus of glucagon (Ohneda et al. 1975).

In this study, mean ± s.e. was calculated. The statistical analysis was performed by the Student’s t-test.

**Results**

*Effect of insulin-induced hypoglycemia upon circulating extrapancreatic glucagon*

Since hypoglycemia is known to elicit glucagon secretion (Ohneda et al.
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1969), insulin was administered to a group of 5 pancreatectomized dogs in a priming dose of 0.4 U/kg and constant infusion of 0.04 U/kg/min for 120 min followed by a bolus dose of insulin, if necessary. The changes in blood glucose and plasma IRG are shown in Fig. 1. The mean blood glucose fell gradually from the base line of 275±30 mg/100 ml and reached a nadir of 205±44 mg/100 ml 120 min after the insulin injection (p <0.02). Plasma IRG in the portal vein was 346±59 pg/ml at the base line and it was 276±71 pg/ml at 120 min, indicating no significant changes at all. Plasma IRI at the base line was near zero and it rose and remained at the level more than 800 µU/ml during insulin infusion.

Effect of 2-deoxyglucose upon circulating extrapancreatic glucagon

It was reported that elevated plasma glucagon in totally pancreatectomized dogs was reduced by a small amount of insulin (Matsuyama and Foà 1974 ; Vranic et al. 1974). Therefore, in order to obtain intracellular glucopenia by another stimulus than insulin, 2-deoxyglucose was administered to a group of 6 pancreatectomized dogs. The changes in blood glucose, plasma IRI, and IRG following 2-deoxyglucose administration are shown in Fig. 2. Blood glucose slightly increased from the base line of 315±25 mg/100 ml to a level of 329±19 mg/100 ml at 5 min and reached a peak of 342±11 mg/100 ml at 60 min. However, these changes were not significant. Plasma IRI was near zero and did not fluctuate throughout the experiment. Plasma IRG was 294±103 pg/ml at the initial level and did not elicit any significant changes during the experiment.
Effect of glucose-induced hyperglycemia upon circulating extrapancreatic glucagon

It is well known that the secretion of pancreatic glucagon is inhibited by hyperglycemia. To see the effect of glucose-induced hyperglycemia upon the release of extrapancreatic glucagon, glucose was administered under different experimental conditions.

**Transient hyperglycemia.** The changes in blood glucose and plasma IRG are shown in Fig. 3-A. The mean blood glucose rose from the base line of 205±44 mg/100 ml to a peak of 374±38 mg/100 ml ($p<0.01$) 10 min after glucose administration (12 g, one shot) in a group of 5 pancreatectomized dogs. Thereafter, blood glucose declined but remained still elevated at 60 min ($p<0.05$). Plasma IRG in the portal vein was 276±71 pg/ml at the base line and did not elicit any significant changes for 60 min.

**Continuous hyperglycemia.** In order to maintain hyperglycemia for longer time, glucose was given to a group of 6 pancreatectomized dogs as a bolus injection (1 g) followed by a constant infusion (400 mg/min) for 20 min. The changes in blood glucose, plasma IRI and plasma IRG are shown in Fig. 3-B. The mean blood glucose rose abruptly from the base line of 305±14 mg/100 ml to a peak of
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DISCUSSION

As far as the release of pancreatic glucagon is concerned, insulin-induced hypoglycemia is widely accepted as one of the most important stimuli. However, in the present studies, blood glucose decreased by only 70 mg/100 ml in spite of a large amount of insulin, and plasma IRG did not rise significantly. These results suggest that resistance to insulin develops in pancreatectomized dogs which were fed under insulin deficiency. A failure of the response of extrapancreatic glucagon observed in this study might be due to a small and gradual decrease of blood glucose and an inhibition of extrapancreatic glucagon by insulin itself. Hypoglycemia-induced glucagon-release is considered as a result of intracellular glucopenia in the A cell of the islet of Langerhans, as this concept was supported by glucagon increase with 2-deoxyglucose (Müller et al. 1971). In contrast, however, the administration of 2-deoxyglucose did not induce any changes in extrapancreatic glucagon.

Fig. 3. A: Effect of transient hyperglycemia upon the blood glucose (BG) in the femoral artery and plasma levels of glucagon (IRG) in the portal vein in a group of 5 pancreatectomized dogs under chronic insulin deficiency. B: Effect of continuous hyperglycemia upon the blood glucose (BG) in the femoral artery and the plasma levels of insulin (IRI) and glucagon (IRG) in the portal vein in a group of 6 pancreatectomized dogs under chronic insulin deficiency. Mean±s.e.
Furthermore, it is recognized that hyperglycemia suppresses the release of pancreatic glucagon. In the present study, however, extrapancreatic glucagon did not change following glucose administration, either one shot or continuous infusion.

According to the previous reports (Matsuyama and Foà 1974; Vranic et al. 1974), the circulating level of extrapancreatic glucagon rises during the course after pancreatectomy. In this situation, insulin administration reduces the plasma glucagon level (Matsuyama and Foà 1974; Vranic et al. 1974). These results suggest that increased plasma glucagon after pancreatectomy is mostly due to insulin deficiency. Previous study from our laboratory demonstrated a failure in response of extrapancreatic glucagon to glycemic changes immediately after pancreatectomy in dogs (Ohneda et al. 1980). In that experiment, however, the circulating level of plasma glucagon was still low and there was a possibility that the changes in plasma glucagon could not be detected. Therefore, in the present study the response of extrapancreatic glucagon to glycemic changes was investigated in animals which were fed under insulin deficiency for several days following pancreatectomy. However, the results obtained in the present study were in agreement with those in the previous experiments, in which the effect of glycemic changes was investigated immediately after pancreatectomy, indicating that the release of extrapancreatic glucagon is not affected by glycemic changes. The previous study as well as the present experiments performed several days after pancreatectomy indicates that the release of extrapancreatic glucagon differs entirely from that of pancreatic glucagon, as far as the response to glycemic changes is concerned.

Different distribution of extrapancreatic glucagon was reported in various animal species (Sasaki et al. 1975; Matsuyama et al. 1977). In dogs, the stomach is known as a main origin of extrapancreatic glucagon (Blazquez et al. 1976; Ohneda et al. 1979). According to the histological studies (Sasaki et al. 1975; Orci and Perrelet 1981), glucagon-secreting cells in the gastrointestinal tract are scattered diffusely in the epithelium, unlike A cells in the pancreatic islet, which are closely packed with B and D cells and receive innervation. Therefore, the secretion of extrapancreatic glucagon may be under the minimum influence of B cells (insulin) or D cells (somatostatin). Recent report that hypoglycemia-induced glucagon response was not observed in diabetic patients with autonomic neuropathy (Hilsted et al. 1982) supports a role of the nervous system in glucagon secretion.

Previous studies in our laboratory concerning the regulation of the secretion of extrapancreatic glucagon show that intravenous administration of arginine and diltiazem, a calcium antagonist, promotes the secretion. Furthermore, among the gastrointestinal hormones, gastrin, CCK-octapeptide and caerulein stimulate the secretion of extrapancreatic glucagon, especially during the administration of arginine. In the intraluminal administration of nutrients to pancreatectomized
dogs, only arginine stimulates the secretion of extrapancreatic glucagon. These results suggest that glycemic changes seem unlikely to play a significant role in the regulation of extrapancreatic glucagon secretion.

Sundby and his co-workers (1976) extracted glucagon immunoreactive material from the porcine gut and named glicentin. Later, the amino acid sequence of glicentin was proved. According to their report, glicentin contains the entire sequence of glucagon (Jacobsen et al. 1977; Thim and Moody 1981), and is considered as a precursor of glucagon. It is presumed that pancreatic and extrapancreatic glucagons are produced by the enzymatic cleavage of the identical glicentin-related peptide (Moody et al. 1978; Ravazzola et al. 1979). Extrapancreatic glucagon extracted from the gastrointestinal tract elicits the biological actions identical with pancreatic glucagon (Sasaki et al. 1975; Holst 1977; Srikant et al. 1977). Nevertheless, the response of extrapancreatic glucagon to glycemic changes differs from that of pancreatic glucagon. Therefore, pathophysiological role of extrapancreatic glucagon remains still to be investigated.

References


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