Response of Extrapancreatic Glucagon to Glycemic Changes

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Synopsis

Since the secretion of pancreatic glucagon is largely influenced by changes in the blood glucose level, the response of extrapancreatic glucagon to glycemic changes was investigated in man and dogs. Neither insulin-induced hypoglycemia nor arginine infusion caused a rise in plasma glucagon in two patients with total pancreatectomy, but plasma glucagon increased transiently in one of the two patients after oral glucose load. Glucose-induced hyperglycemia did not alter plasma glucagon in the portal vein in a group of 4 depancreatized dogs. When blood glucose fell lower than 50 mg/100 ml after insulin infusion in a group of 7 depancreatized dogs, plasma glucagon in the portal vein did not increase significantly. The administration of 2-deoxyglucose did not cause any changes in plasma glucagon in the portal vein in a group of 5 pancreatectomized dogs. After the ligation of the hepatic artery, blood glucose decreased gradually in a group of 5 pancreatectomized dogs with portocaval shunt, but plasma glucagon in the vena cava was not altered significantly. It is concluded from the present study that regulation of extrapancreatic glucagon differs from that of pancreatic glucagon.

Methods

Since there is no may of method discriminating extrapancreatic glucagon from pancreatic glucagon (Srikant et al., 1977), the response of extrapancreatic glucagon was investigated in totally depancreatized patients and animals.

Clinical study

Two totally depancreatized patients were subjects of this study. Patient K K, a 52-year-old man, had undergone pancreatectomy 6 years previously because of insulinoma at the head of the pancreas, and had been treated with 40 U of insulin. Another patient T F, a 46-year-old man, had undergone total pancreatectomy because of cancer of the stomach 10 years previously and had been treated with 16 U of insulin. A glucose tolerance test was performed after an overnight fast, as described previously (Goto et al., 1960). In an insulin test, monoclonal Actrapid® (Novo Industri A/S, Denmark) was intravenously administered in a dose of 0.1 U/kg and blood specimens were obtained at various
intervals. Furthermore, an arginine infusion test was carried out, as reported elsewhere (Ohneda et al., 1975). Blood sample for blood glucose were obtained from the ear lobe. Blood for hormone assay was drawn from the antecubital vein.

Animal experiment
Mongrel dogs, weighing 15-22 kg, were subjected to the animal experiments. After an overnight fast, the dogs were anesthetized with pentobarbital sodium and the abdomen was opened by a midline incision. The pancreas and a small portion of the duodenum were resected. A polyethylene catheter was inserted into the portal vein through a mesenteric vein, allowing us to obtain blood from the intestine as well as from the stomach in all experimental groups with the exception of the portocaval shunt experiment, in which a plastic catheter was inserted into the vena cava. Immediately after surgery, physiological saline solution was infused into the femoral vein at a constant rate of 2 ml/min. Approximately one hour after the completion of the operation the experiments were commenced. After the base line samples were drawn, various stimuli were given and blood specimens were obtained from the femoral artery and the portal vein or the vena cava at various intervals.

Analytical methods
For hormone assay, 4 ml of blood was obtained with a heparinized syringe and poured into glass tubes containing Trasylol® (1000 U, Bayer Co.). Plasma was separated by centrifugation and kept at \(-20^\circ\text{C}\) until assay. Blood glucose was measured by the glucose oxidase method (Teller, 1956). Plasma insulin was determined by the Morgan-Lazarow method (1962). Plasma glucagon was measured by radioimmunoassay using an antiserum (G 21), specific for "pancreatic" glucagon (Ohneda et al., 1975). In this study the mean values and the standard errors of the mean were calculated.

Results
A. Clinical study.
Glucose tolerance test
In patient K. K., blood glucose increased from the initial level of 100 mg/100 ml and remained higher than 250 mg/100 ml during the test. Plasma glucagon was 140 pg/ml at fasting and rose transiently to a peak of 700 pg/ml at 60 min. 

Insulin test
Blood glucose and plasma glucagon in the insulin test are shown in Figure 1. The intravenous administration of insulin elicited a decrease in blood glucose with a nadir of 19 mg/100 ml in patient KK and of 34 mg/100 ml in patient TF. The plasma glucagon was 150 and 45 pg/ml at fasting, respectively, and did not show any significant changes during the insulin test.

Arginine test
For comparison, an intravenous arginine test was carried out in the two patients and the results are shown in Figure 2. The blood glucose levels were 240 and 97 mg/100 ml at fasting in patient KK and patient TF, respectively, and did not change after the arginine infusion. The plasma glucagon
B. Animal experiment

Glucose-induced hyperglycemia

In a group of 4 depancreatized dogs, glucose was administered into the femoral vein as a priming dose of 1 g followed by a constant infusion at the rate of 400 mg/min for 20 min. The change in blood glucose, plasma insulin and plasma glucagon are shown in Figure 3. The mean blood glucose rose from the base line of 121 ± 2 to a peak of 241 ± 21 mg/100 ml 20 min after glucose infusion and thereafter declined. Plasma insulin did not change throughout the experiment. Plasma glucagon in the portal vein was 58 ± 23 pg/ml at the base line and revealed no significant change during hyperglycemia.

Insulin-induced hypoglycemia

Since hypoglycemia is known to be a potent glucagon secretion stimulant (Ohneda et al., 1969), insulin was administered as a priming dose of 0.6 U/kg followed by a constant infusion of 0.02U/kg/min for 2 hours in a group of 7 depancreatized dogs. The changes in blood glucose and plasma glucagon are shown in Figure 4. Blood glucose fell gradually from the base line of 127 ± 11 mg/100 ml and reached a nadir of 47 ± 7 mg/100 ml 2 hours after the insulin injection. Plasma glucagon in the portal vein was 69 ± 15 pg/ml at the base line and it was 89 ± 26 pg/ml at 120 min, indicating no significant changes.

2-Deoxyglucose

It has been reported that insulin reduces elevated plasma glucagon in totally depan-
creatized dogs (Matsuyama and Foa, 1974; Vranic et al., 1974). Therefore, in order to obtain an intracellular glucose deficiency, 2-deoxyglucose (Wako Pure Chemical Industries, Osaka, Japan) was injected intravenously in a dose of 300 mg/kg in a group of 5 dogs. The changes in blood glucose, plasma insulin and plasma glucagon are shown in Figure 5. Blood glucose increased abruptly from the base line of 114 ± 25 mg/100 ml to a peak of 185 ± 26 mg/100 ml 5 min after 2-deoxyglucose administration and remained elevated for 90 min. Plasma insulin was near zero and did not fluctuate during the experiment at all. Plasma glucagon was 80 ± 27 pg/ml at the initial level and did not reveal any significant change during the experiment.

**Portocaval shunt with ligation of hepatic artery**

In order to obtain another type of hypoglycemia, a portocaval shunt was performed in a group of 5 dogs. The portocaval anastomosis was carried out above the renal vein and the portal vein was ligated just under the liver after the pancreas was removed. The results are shown in Figure 6. The ligation of the hepatic artery resulted in a gradual decrease in blood glucose from the base line of 150 ± 18 to 99 ± 20 mg/100 ml at 120 min. Plasma insulin was less than 10 μU/ml throughout the experiment. Plasma glucagon in the vena cava was 75 ± 25 pg/ml and did not alter significantly throughout the experiment.

**Discussion**

Previous studies performed by our laboratory demonstrated that the secretion of a pancreatic glucagon was controlled in connection with the blood glucose levels in con-
Fig. 6. Changes in blood glucose (BG) in the fomoral artery (FA) and plasma insulin (IRI) and glucagon (IRG) in the vena cava (VC) after ligation of the hepatic artery in a group of 5 pancreatectomized dogs with portocaval shunt.

S. R. No. 1

EXTRAPANCREATIC GLUCAGON

125

mg/dl

180

140

100

60

20

0

10 0 30 60 90 120 min

BG (FA)

IRI (VC)

IRG (VC)

Ligature of hepatic artery

N = 5

M±SEM

Hyperglycemia suppresses glucagon secretion, while hypoglycemia enhances it. Thereby a reversed correlation (r = -0.603) was observed between the changes in the glucagon levels in the pancreatic vein and the blood glucose levels. In contrast, hyperglycemia induced by intravenous glucose administration did not alter the levels of extrapancreatic glucagon in totally depancreatized dogs. Therefore, the present study does not support the theory of the presence of a glucoreceptor in the glucagon-secreting cells of the gastrointestinal tract, this being clearly demonstrated in the A cell of the pancreatic islets (Matschinsky et al., 1975, Grodsky et al., 1975.)

It has been widely recognized that hypoglycemia causes a significant rise of the secretion of pancreatic glucagon in man as well as experimental animals (Unger et al., 1962; Ohneda et al., 1969; Ohneda et al., 1972; Gerich et al., 1973). On the contrary, insulin-induced hypoglycemia less than 50 mg/100 ml did not induce any change in extrapancreatic glucagon in depancreatized dogs. However, these results do not necessarily exclude the possibility that hypoglycemia enhances the secretion of extrapancreatic glucagon, for the following reasons: (1) insulin itself decreases the secretion of pancreatic glucagon, demonstrating a negative feedback system (Samols et al., 1972) and (2) elevated extrapancreatic glucagon is reduced by insulin administration (Vranic et al., 1974; Matsuyama and Foa, 1974). In addition, hypoglycemia did not cause any increase in extrapancreatic glucagon in two depancreatized patients. In these cases also, the failure of extrapancreatic glucagon to respond to hypoglycemia might be attributed to the presence of insulin in the circulation. However, the fact that hypoglycemia does not induce any rise in extrapancreatic glucagon secretion, indicates the difference between the physiological role of extrapancreatic glucagon and pancreatic glucagon.

Since it has been reported that intracellular glucose deficiency induced by 2-deoxyglucose enhances the glucagon secretion in the normal dogs (Müller et al., 1971), 2-deoxyglucose was administered intravenously in depancreatized dogs, resulting in no significant change in extrapancreatic glucagon. In order to obtain another type of hypoglycemia, hepatic exclusion was carried out by ligation of the hepatic artery in pancreatectomized dogs with portocaval shunt. Although the mean blood glucose decreased by 50 mg/100 ml at 120 min, plasma extrapancreatic glucagon did not increase at all. These results also suggest a difference between the regulation of the secretion of extrapancreatic glucagon and that of pancreatic glucagon.

In the present study, the oral glucose load
elicited a rise in plasma glucagon in a patient who had undergone pancreatectomy. This increase in plasma glucagon cannot be attributed to its cross-reactivity with gut glucagon-like immunoreactive substance, because the antiserum (G21) used in the study is specific for glucagon and cross-reacts with gut glucagon-like immunoreactive substance less than 3% (Ohneda et al., 1975). As demonstrated by Blazquez et al. (1976), intragastric instillation of arginine induced a prompt increase in extrapancreatic glucagon in the gastric vein of the depancreatized dogs. Therefore, these results, along with our exprimental results, suggest promotion of the secretion of extrapancreatic glucagon by intraluminal instillation of the nutrients.

Although it is well known that extrapancreatic glucagon extracted from the gastrointestinal tract elicits a biological action identical with pancreatic glucagon (Srikant et al., 1977), the present study indicates that glycemic changes seem unlikely to affect the secretion of extrapancreatic glucagon. Therefore, an important role of extrapancreatic glucagon in glucose homeostasis could not be established from the present study. The biological significance of extrapancreatic glucagon still remains to be clarified.

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References


