Response of Extrapancreatic Glucagon to Arginine in Dogs

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Since an elevation of plasma immunoreactive glucagon, measured with antisera specific for pancreatic glucagon, was demonstrated (Vranic et al. 1974; Matsuyama and Foa 1974), the presence of extrapancreatic forms of glucagon has been established. Morphological studies using the electron microscope or using specific immunofluorescein stains have also identified that the mucosal cells in the gastrointestinal tract of dogs appear to contain a glucagon or true glucagon-like substance (Sasaki et al. 1975; Larsson et al. 1975; Baetens et al. 1976). Although the distribution of extrapancreatic glucagon in the gastrointestinal tract varies in different species (Moody 1972), a large amount of extrapancreatic glucagon has been demonstrated in the gastric fundus of dogs (Sasaki et al. 1975; Larsson et al. 1975; Shima 1976; Muñoz-Barragan et al. 1976). According to the recent
studies, gastric glucagon cannot be distinguished from pancreatic glucagon in view of its immunochemical character, physical property or biological action (Sasaki et al. 1975). An elevation of fasting plasma glucagon and/or hyperresponsiveness of pancreatic glucagon to arginine have been demonstrated in diabetes mellitus or other diseases (Unger 1974; Seino et al. 1977). However, it is difficult to identify how much the extrapancreatic glucagon participates in the hyperresponsiveness of plasma immunoreactive glucagon to various stimuli in diabetes mellitus. This is particularly so as no discriminating assay methods are available to distinguish extrapancreatic glucagon from pancreatic glucagon with certainty.

Therefore, in order to clarify the regulation of secretion of extrapancreatic glucagon, plasma glucagon response to arginine was investigated in normal and alloxan diabetic dogs, whose pancreas was acutely removed. Furthermore, glucagon response to arginine was also studied in the dogs, in which both the pancreas and the stomach were removed.

**Materials and Methods**

In this experiment 16 mongrel dogs weighing 13 to 24 kg were subjected to examination and were divided into three experimental groups.

In order to investigate the response of extrapancreatic glucagon to arginine in the normal state, five normal dogs were anesthetized with Nembutal® after an overnight fast and the abdomen was opened by a middle incision. A polyethylene catheter was inserted into the portal vein through a mesenteric vein. This allowed blood sampling from the region of the intestine as well as from the stomach. Plastic needles were inserted into the femoral artery and vein. After the base line samples were obtained from the portal vein and the femoral artery, 60 ml of a 10% arginine solution were infused through the femoral vein for 10 min. Blood samples were collected at various intervals for 60 min. The pancreas was quickly excised and, approximately one hour later, the blood samples were drawn and the arginine infusion test was repeated. Throughout the experiment saline solution was continuously infused through the femoral vein at a constant rate of 2 ml per min.

In order to determine the response of extrapancreatic glucagon in diabetic dogs, alloxan was injected intravenously at a dose of 60 mg/kg of body weight in a group of five dogs a week prior to the experiment. After an overnight fast, an arginine infusion test was performed before and after the acute total pancreatectomy in these alloxan diabetic dogs, as mentioned above in the normal dogs.

To maintain an increased level of plasma extrapancreatic glucagon, total pancreatectomy was carried out in another group of five dogs and a small amount of insulin (4 to 8 U/day) was administered for a week. After an overnight fast, they were laparotomized again under Nembutal® anesthesia. A polyethylene catheter was implanted into the portal vein through a mesenteric vein. Plastic needles were inserted into the femoral artery and vein. After base line samples were obtained, the arginine infusion test mentioned above was carried out for 60 min. Thereafter the stomach, from the cardia to the pylorus, was quickly resected. Thirty min after gastrectomy, the arginine infusion test was repeated. In one dog, pancreatectomized one week earlier, an arginine infusion test was performed before and after the sham gastrectomy.

Blood samples were obtained from the femoral artery and blood glucose was measured by the glucose oxidase method (Teller 1956). Blood samples for hormone assay were drawn from the portal vein and the femoral artery with heparinized syringes and were poured into the glass tubes containing 1000 U of Trasylol® (Bayer Co.). The samples were chilled in ice and plasma was separated by centrifugation immediately after the completion of the experiment. The plasma was kept at -20°C until analyzed. Plasma insulin was
determined by the Morgan-Lazarow method (1963). Plasma immunoreactive glucagon was determined with an antiserum (G 21) specific for pancreatic glucagon (Ohneda et al. 1975), and here was designated as plasma glucagon. In addition, plasma immunoreactive glucagon was measured using an antiserum (G 25), which cross-reacts with both pancreatic glucagon and gut glucagon-like immunoreactive substance (GLI) and was designated as plasma total immunoreactive glucagon (IRG).

In this study, the mean values and the standard errors of the mean were calculated. Statistical analyses were performed by the paired t-test between the base line and experimental values.

**RESULTS**

*Pancreatectomized normal dogs*

Fig. 1 shows the changes of blood glucose, plasma insulin, glucagon and total IRG in response to arginine infusion prior to and after total pancreatectomy. Following arginine infusion carried out prior to pancreatectomy, blood glucose in the femoral artery increased from the base line of 83±4.0 to 92±5.0 mg/100 ml at 5 min (p<0.05), and returned to the initial level in 50 min. Plasma insulin in the portal vein rose after arginine infusion from the initial level of 21±4.8 to a peak of 89±32.1 μU/ml at 5 min, then decreased gradually. Plasma glucagon in the portal vein increased following arginine infusion from the base line of 225±20 to a peak of 497±100 pg/ml at 5 min (p<0.05). Plasma total IRG in the portal vein

Fig. 1. Changes in blood glucose, plasma insulin, total IRG and glucagon in response to arginine infusion performed (A) prior to and (B) after pancreatectomy in a group of 5 normal dogs. Double circles represent significant changes from the base line value. Mean±s.e.m.
rose slightly after arginine administration from the base line level of 1.24±0.20 to 1.85±0.13 ng/ml at 10 min (p<0.025).

The changes in blood glucose, plasma insulin, glucagon and total IRG were generally minimal in response to arginine infusion performed after total pancreatectomy (Fig. 1B). Blood glucose did not change through 60 min. Plasma insulin decreased to undetectable level after pancreatectomy and did not change significantly following arginine infusion. Plasma glucagon in the portal vein decreased to 142±34 pg/ml after pancreatectomy and showed a slight increase in response to arginine infusion (55 pg/ml). However, plasma glucagon tended to increase gradually through 60 min after arginine infusion. In contrast to plasma glucagon, plasma total IRG decreased only slightly after total pancreatectomy. Furthermore, plasma total IRG increased slightly from the basal level of 0.74±0.09 to 1.12±0.23 ng/ml 10 min after arginine infusion, indicating a response of gut GLI to arginine.

**Pancreatectomized alloxan diabetic dogs**

Fig. 2 shows the changes in blood glucose, plasma insulin, glucagon and total IRG in response to arginine infusion carried out prior to and after total pancreatectomy in animals previously rendered diabetic with alloxan. Blood glucose in the femoral artery appeared to rise from the base line of 227±17 to a peak of 244±19 mg/100 ml at 20 min and returned to the initial level at 60 min, although the change was not significant because of wide deviation. Plasma insulin in the
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The portal vein was 4.2±1.7 μU/ml at the initial level and did not change at all during and after arginine infusion. Plasma glucagon in the portal vein rose from the initial level of 213±27 to 471±229 pg/ml 30 min after arginine infusion. Plasma total IRG in the portal vein increased from the base line of 1.03±0.41 ng/ml transiently after arginine infusion. However, these changes in plasma glucagon and total IRG were not significant because of deviation.

After total pancreatectomy, blood glucose in the femoral artery was 243±21 mg/100 ml but it did not show any rise in response to arginine infusion. Plasma insulin in the portal vein decreased to non-detectable levels after total pancreatectomy and did not change during the arginine infusion. In contrast, plasma glucagon in the portal vein was still 292±73 pg/ml after pancreatectomy and rose to a peak of 485±219 pg/ml 20 min after arginine. Plasma total IRG in the portal vein was 1.38±0.51 ng/ml and increased after pancreatectomy, reaching a peak of 2.60±1.05 ng/ml 5 min after arginine infusion. These changes in plasma glucagon and total IRG were not significant because of large deviation.

Gastrectomized dogs one-week post-pancreatectomy

The changes in blood glucose, plasma insulin, glucagon and total IRG are shown in Fig. 3. Prior to gastrectomy blood glucose in the femoral artery did not show any rise in response to arginine infusion. Plasma glucagon in the portal vein was still 292±73 pg/ml after pancreatectomy and rose to a peak of 485±219 pg/ml 20 min after arginine. Plasma total IRG in the portal vein was 1.38±0.51 ng/ml and increased after pancreatectomy, reaching a peak of 2.60±1.05 ng/ml 5 min after arginine infusion. These changes in plasma glucagon and total IRG were not significant because of large deviation.

Fig. 3. Changes in blood glucose, plasma insulin, total IRG and glucagon in response to arginine infusion performed (A) prior to and (B) after gastrectomy in a group of 5 dogs, whose pancreas was removed a week previously. Plasma glucagon and total IRG were determined using blood samples obtained from the portal vein (PV) (•--•) and the femoral artery (FA) (○--○). Double circles represent significant changes in comparison with the base line value. Mean±S.E.M.
not show any changes following arginine infusion but increased gradually throughout the experiment. Plasma insulin in the portal vein was minimal and did not change following arginine infusion at all. Plasma glucagon in the portal vein increased from the initial level of 268±70 to a peak of 544±100 pg/ml at 5 min (p<0.01) and returned to the fasting level at 30 min. Plasma total IRG in the portal vein rose from the base line of 0.78±0.085 ng/ml after arginine infusion and reached a peak of 1.35±0.117 ng/ml (p<0.05). The changes in plasma glucagon and total IRG in the femoral artery were similar to those in the portal vein.

To the contrary, response of plasma glucagon and total IRG to arginine were minimal after total gastrectomy (Fig. 3 B). Blood glucose increased gradually throughout the experiment rather than responding to arginine infusion. Plasma glucagon in the portal vein was 148±16 pg/ml at the base line and did not change after arginine infusion. There were no changes of plasma total IRG in the portal vein in response to arginine infusion. The plasma levels of both glucagon and total IRG in the femoral artery did not change significantly after arginine infusion.

To determine the effect of manipulation of the gastrointestinal tract at gastrectomy, a totally pancreatectomized dog underwent arginine infusion prior to and after a sham gastrectomy. The changes of blood glucose, glucagon and total IRG are presented in Table 1. Changes in blood glucose, plasma insulin, glucagon and total IRG after the sham operation were identical with those performed before the sham operation.

| Table 1. Changes in blood glucose, plasma insulin, glucagon and total IRG following arginine infusion prior to and after sham operation for gastrectomy in a dog depancreatized previously |
|-----------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Measurements* Sources† Min after start of arginine infusion |  |  |  |  |  |  |  |
| A. Prior to sham operation |  |  |  |  |  |  |  |
| Glucose FA | 285 | 291 | 279 | 293 | 294 | 289 | 288 | 311 | 306 |
| Insulin PV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Glucagon PV | 240 | 180 | 325 | 375 | 300 | 375 | 300 | 375 | 250 |
| Glucagon FA | 175 | 170 | 220 | 280 | 300 | 290 | 210 | 160 | 206 |
| Total IRG PV | 0.84 | 0.95 | 1.06 | 0.75 | 0.92 | 1.66 | 1.30 | 1.12 | 1.07 |
| Total IRG FA | 0.58 | 0.71 | 0.73 | 0.89 | 0.83 | 0.85 | 0.82 | 0.65 | 0.73 |
| B. After sham operation |  |  |  |  |  |  |  |  |
| Glucose FA | 287 | 298 | 287 | 292 | 299 | 294 | 281 | 287 | 293 |
| Insulin PV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Glucagon PV | 260 | 150 | 290 | 350 | 300 | 225 | 410 | 260 | 260 |
| Glucagon FA | 170 | 175 | 225 | 275 | 338 | 325 | 175 | 166 | 225 |
| Total IRG PV | 1.25 | 0.98 | 1.18 | 1.26 | 1.26 | 1.15 | 1.04 | 1.46 | 1.03 |
| Total IRG FA | 0.70 | 0.68 | 0.85 | 0.92 | 0.88 | 0.97 | 0.69 | 0.68 | 0.81 |

* Glucose: mg/100 ml, Insulin: μU/ml, Glucagon: pg/ml, Total IRG: ng/ml.
† FA; femoral artery, PV; portal vein.
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DISCUSSION

The base line value of plasma glucagon in the portal vein after total removal of the pancreas remained at a half of the pre-pancreatectomy level in the normal dogs. In addition, the plasma glucagon levels in the portal vein tended to increase approximately 2 hr after total pancreatectomy. Furthermore, the mean level of 270 pg/ml of plasma glucagon was observed in the portal vein of one-week post-pancreatectomized animals. These increased levels of plasma glucagon after pancreatectomy cannot be attributed to persistence of pancreatic glucagon or cross-reacting materials in circulation. These facts indicate the presence of extrapancreatic glucagon and are compatible with the results reported previously (Vranic et al. 1974; Matsuyama et al. 1974; Mashiter et al. 1975). Furthermore, the present study clearly demonstrates that the base line level of plasma glucagon of extrapancreatic origin increased markedly in one-week post-pancreatectomized animals compared with those of one-hour post-pancreatectomized animals in the normal as well as alloxan diabetic state.

The intravenous administration of arginine has been presumed as one of the most powerful stimuli to the A cells of the pancreatic islets (Ohneda et al. 1972). In contrast, extrapancreatic glucagon did not respond to arginine, when the pancreas was intact, since plasma glucagon in the portal vein did not increase remarkably in one-hour post-pancreatectomized animals, as shown in Fig. 1 B. Furthermore, plasma glucagon of extrapancreatic origin responded to arginine infusion in one-week post-pancreatectomized dogs or alloxan diabetic animals, indicating an increase in its responsiveness to arginine in a state of insulin deprivation. However, it was suggested that in totally pancreatectomized patients diabetic state is much more important in the responsiveness of extrapancreatic glucagon to arginine infusion than insulin deficiency itself (Barnes and Bloom 1976; Villanueva et al. 1976). In fact, the response to arginine of extrapancreatic glucagon was demonstrated more prominently in one-week post-pancreatectomized dogs than alloxan diabetic dogs, in the former the mean level of fasting blood glucose being higher than that of the latter.

On the other hand, clinical observations have demonstrated the elevated response of the A cells in the pancreas to amino acids in diabetes mellitus (Unger et al. 1970; Gossain et al. 1974; Ohneda et al. 1975). However, no information is available as to how much extrapancreatic glucagon contributes to hyperglucagonemia observed in diabetes mellitus. The fact that extrapancreatic glucagon responded to arginine infusion in alloxan diabetic dogs, suggests the possible participation of extrapancreatic glucagon in hyperglucagonemia in diabetes mellitus. Distribution of extrapancreatic glucagon, determined by antisera specific for pancreatic glucagon, varies in different species (Larsson et al. 1975; Dobbs et al. 1975; Shima 1976; Villanueva et al. 1976; Holst 1977). In dogs the stomach is major source of extrapancreatic glucagon (Sasaki et al. 1975; Larsson et al. 1975; Shima 1976; Munoz-Barragan 1976). Therefore, to investigate whether gastric glucagon has an important role in the secretion of extrapancreatic glucagon, arginine was
administered intravenously prior to and after gastrectomy in one-week post-pancreatectomized animals. Plasma glucagon in the portal vein was reduced markedly after total gastrectomy and glucagon response was abolished. In addition, plasma glucagon did increase in response to arginine in a sham-operated dog, indicating that a failure to increase plasma glucagon after gastrectomy may not be due to manipulation of the upper gastrointestinal tract. Therefore, the results clearly demonstrate that an increase of extrapancreatic glucagon in response to arginine derives mostly from the stomach in such circumstances, as reported previously (Blazquez et al. 1976). However, the fact that the glucagon level of approximately 100 pg/ml was still observed after gastrectomy, indicates other glucagon-secreting organs than the pancreas and the stomach. Immunoreactive glucagon was detected in the extract from the gastrointestinal tract (Holst 1977). In addition, it was reported that plasma glucagon was measurable in totally eviscerated rats (Penhos et al. 1975) and recently immunoreactive glucagon was demonstrated in the salivary glands of various species (Lawrence et al. 1976). Therefore, those organs might be responsible for a small amount of glucagon in the circulation even after the removal of the pancreas and the stomach.

Somatostatin or insulin suppresses the secretion of extrapancreatic glucagon as well as pancreatic glucagon (Mortimer et al. 1974; Valverde et al. 1975). Hyperglycemia or insulin-induced hypoglycemia did not affect the secretion of extrapancreatic glucagon (Blazquez et al. 1976; Ohneda, unpublished observation). In addition, extrapancreatic glucagon responds to arginine only when it is administered to insulin-deprived animals, as shown in the present study and by others (Mashiter et al. 1975; Blazquez et al. 1976), indicating a difference in the secretion of extrapancreatic glucagon from pancreatic glucagon. In this context, the response of gut glucagon-like immunoreactivity (GLI) was also exaggerated when the pancreas was removed both in normal and alloxan diabetic dogs. Therefore, these results suggest that pancreatectomy or insulin deficiency elicits a peculiar circumstance for extrapancreatic glucagon or GLI in the gastrointestinal tract. However, the regulation of extrapancreatic glucagon and its physiological significance still remain to be solved.

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References

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