IMMUNOREACTIVE SOMATOSTATIN LEVELS IN PLASMA OF NORMAL AND ALLOXAN DIABETIC DOGS

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1. Introduction

The recent discovery by Luft and associates [1] and by Dubois [2] of somatostatin-containing islet cells, subsequently identified as D-cells [3–5], has opened a new phase in the physiology and pathophysiology of the endocrine pancreas. It is now recognized that diabetes resulting from a deficiency of insulin-secreting cells, such as human juvenile type diabetes and streptozotocin diabetes in rats, is characterized by an apparent increase in somatostatin-containing D-cells per islet [6] and that the content of immunoassayable somatostatin in the islets of such rats is high [7]. However, it has not been determined if these abnormalities in the islets are reflected by hypersomatostatinemia – indeed, it is not known if under normal circumstances somatostatin is released into the circulation from those tissues in which its presence has been demonstrated [8,9]. In this study, we attemped to determine, first, if the levels of immunoreactive somatostatin-like material (IRS) in the venous plasma of organs with a substantial somatostatin content are higher than in peripheral venous plasma and, second, if hypersomatostatinemia is present in dogs with alloxan-induced deficiency of B-cells.

2. Materials and methods

We employed a modification of the radioimmunoassay of Arimura et al. [10], using a double antibody system with R-101 antisomatostatin serum of Arimura and a goat anti-rabbit globulin. To prevent damage of the ^{125}I-labeled tyr^{I}-somatostatin during the incubation [11], dog plasma was collected in tubes containing 1000 Kallikrein inhibitor units (KIU) of Trasylol and 1.2 mg EDTA/ml whole blood, and an additional 200 kIU were present per ml reaction mixture. With this assay, recovery of synthetic somatostatin incubated in canine plasma for 3 h was 88%. The minimum sensitivity of the assay was 50 pg/ml; above this level, differences of 30 pg/ml could be measured with 95% confidence. The coefficient of variation within and between plasma assays was 15% and 31%, respectively. Because dilution slopes of plasma specimens with a high endogenous IRS level were slightly less steep than those obtained with hypersomatostatinemic plasma collected during the intravenous infusion of synthetic somatostatin, endogenous IRS levels were expressed as 'pg equiv./ml' rather than as pg/ml. Insulin and glucagon (IRG) were measured by modification of previously described technics [12,13].

3. Results

To determine the ability of the assay system to detect plasma increments of exogenous somatostatin, we infused synthetic somatostatin into conscious dogs at a rate of 50 ng/min, 100 ng/min, and 250 ng/min for 30 min. The mean IRS of three plasma specimens obtained during each infusion period increased above the preinfusion baseline value of

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96.2 ± 11.2 pg/ml by 61.7 ± 3.3 pg/ml during the 50 ng/min infusion, 85.0 ± 13.2 pg/ml during the 100 ng/min infusion, and 260.0 ± 20.8 pg/ml during the 250 ng/min infusion.

We next compared the plasma IRS levels of venous plasma specimens obtained in five anesthetized dogs from the effluent of organs known to have a high tissue concentration of immunoreactive somatostatin [8,9] with those of the inferior vena cava before and during the infusion of glucose. IRS measurements were at all times significantly higher in pancreaticoduodenal vein plasma than in plasma obtained simultaneously from the inferior vena cava (fig.1). Although mean IRS levels were higher in the short gastric vein than in the inferior vena cava, the differences were not significant. In the gastroepiploic and mesenteric venous plasma, IRS levels were approximately the same as in the inferior vena cava.

Intravenous infusion of glucose at a rate of 1 g/kg per h caused a significant increase in IRS concentration only in the pancreaticoduodenal vein (fig.1).

In six conscious dogs with an indwelling portal vein catheter, portal vein IRS levels were also consistently higher than in crural vein plasma (table 1).

We measured fasting IRS levels in plasma obtained from the crural vein of conscious alloxan diabetic dogs fasted for 20 h and deprived of insulin for 40 h, and once again in these dogs during their usual diet and insulin treatment, which consisted of 8–16 U of NPH insulin twice daily. Following insulin deprivation and prolonged fast, their IRS levels averaged 285 ± 22.8 pg equiv./ml, significantly greater ($p<0.001$) than the mean IRS of 159 ± 9.9 pg equiv./ml in the same dogs when they were receiving insulin. The IRS levels of both treated and untreated animals were significantly greater than the 119 ± 7.6 pg equiv./ml average of a group of 12 conscious normal dogs ($p<0.005$ and $p<0.001$) (table 1).

4. Discussion

These results, among the first reported measurements of immunoreactive somatostatin or somatostatin-like material in plasma [11,14], reveal a gradient of IRS only across the pancreas and the gastric fundus, but the latter gradient was not statistically significant.

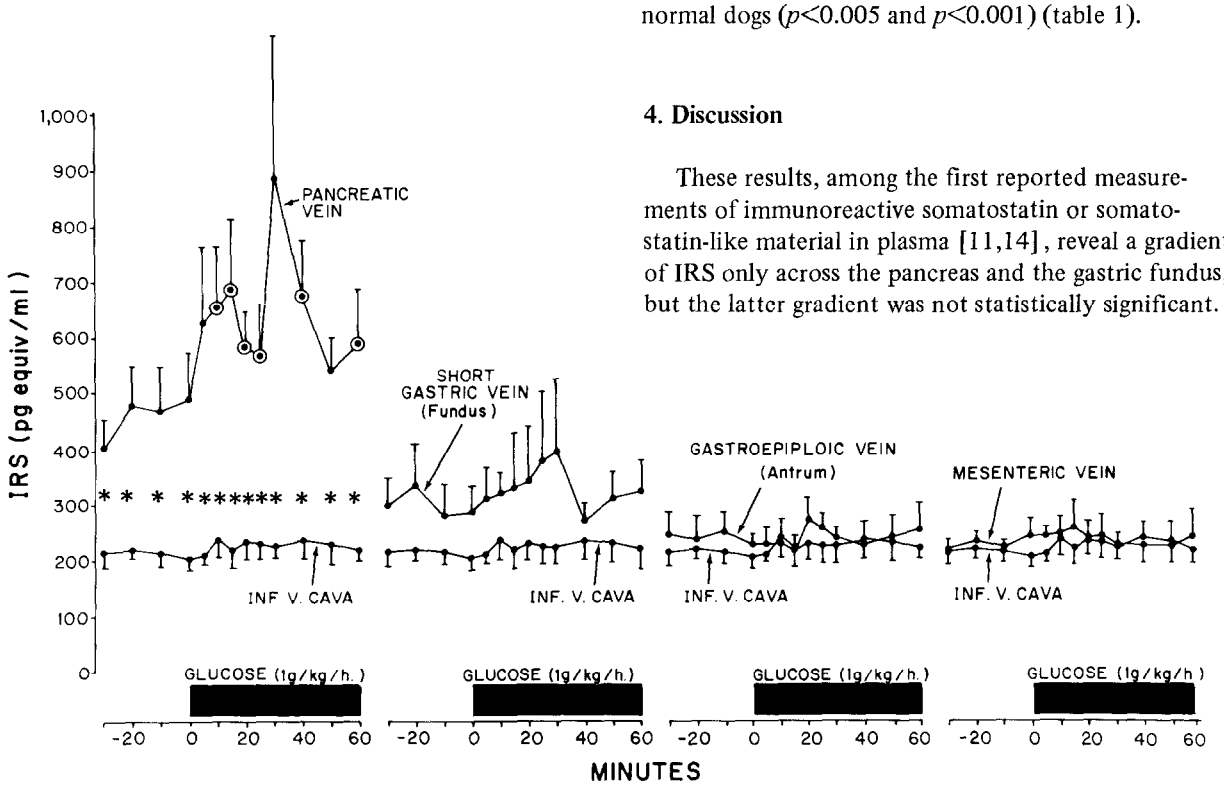

Fig.1. Immunoreactive somatostatin (IRS) concentration in the pancreatic, short gastric, gastroepiploic, and mesenteric vein plasma and in inferior vena caval plasma before and during intravenous glucose infusion in five anesthetized dogs. (○) Represents significance of at least $p<0.05$ above the mean of the baseline values. Asteriks indicate a statistical significance of at least $p<0.05$ above the mean inferior vena caval level.

Table 1
Plasma somatostatin (IRS), glucose, glucagon (IRG), and insulin (IRI) levels in conscious normal and alloxan diabetic dogs

	Normal dogs		Alloxan diabetic dogs (peripheral vein)	
	Peripheral vein (N = 12)	Portal vein (N = 6)	Insulin deprived (N = 8)	Insulin treated (N = 7)
Glucose (mg/dl)	76.3 ± 1.9	80.9 ± 3.0	320.0 ± 14.7	250.0 ± 14.7
IRI (μU/ml)	11.4 ± 1.1	11.5 ± 0.8	8.4 ± 0.7	11.8 ± 1.2
IRG (pg/ml)	83.5 ± 7.2	248.6 ± 28.2[a]	180.6 ± 23.1[b]	149.0 ± 13.9[a]
IRS (pg equiv./ml)	119.7 ± 7.6	275.1 ± 12.9[a]	284.6 ± 22.8[a,d]	159.2 ± 9.9[c]

[a] $p<0.001$ versus normal dogs, peripheral vein level
[b] $p<0.01$ versus normal dogs, peripheral vein level
[c] $p<0.005$ versus normal dogs, peripheral vein level
[d] $p<0.001$ versus insulin treated alloxan diabetic dogs
Values are means ± SEM

This would suggest that the pancreas is a major contributor to the circulating IRS. The demonstration of increased pancreaticoduodenal vein IRS levels during glucose infusion confirms in vitro studies showing a stimulatory effect of glucose upon pancreatic IRS release [15–17].

The study also demonstrates significantly increased fasting plasma IRS levels in insulin-deprived and, to a less degree, insulin-treated alloxan diabetic dogs. The latter finding would fit with earlier demonstrations of increased tissue IRS levels and IRS-containing D-cells in other forms of diabetes in which B-cells are deficient [6,7].

In view of the possible endocrine roles of pancreatic somatostatin [18,19], these findings may be of physiologic and pathophysiologic significance.

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