The Serum Glucose Response to Glucagon Suppression with Somatostatin, Insulin or Antiglucagon Serum in Depancreatized Rats

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Summary. Total immunoreactive glucagon (IRG) and immunoreactive glucagon of A cell origin (IRG_a) were measured in the serum of normal, sham-operated and depancreatized rats, after the administration of three glucagon antagonists: insulin (5–200 mU/rat/h), somatostatin (SRIF; 100 $\mu\text{g/kg/h}$) and antiglucagon serum (AGS, enough to bind three times the calculated total amount of circulating IRG). Since no differences were noted between the responses of normal and sham-operated animals, the values were pooled and used as controls. Pancreatectomy caused a significant increase in serum glucose, IRG_a and total IRG and a significant decrease in serum insulin. AGS and SRIF significantly decreased serum glucose in control, but not in depancreatized rats, even though SRIF caused a significant decrease of IRG_a in all animals. SRIF significantly decreased plasma insulin in control rats, but did not modify total IRG secretion in either group. In control rats the minimum effective hypoglycaemic dose of insulin (5 mU/rat/h) may have decreased serum IRG_a , but not total IRG. At higher doses (20 mU/rat/h) insulin stimulated glucagon secretion. In depancreatized animals, higher doses of insulin (200 mU/rat/h) were needed to lower serum glucose. On the other hand, a dose of 100 $\mu\text{U/rat/h}$ was sufficient to lower the serum IRG. We conclude that although hyperglucagonaemia may contribute to the hyperglycaemia of the untreated depancreatized rats, the excessive secretion of glucagon is secondary to insulin insufficiency and that, at least in this animal model, the hypoglycaemic action of insulin is only minimally dependent upon its ability to suppress glucagon secretion.

Key words: Glucagon, somatostatin, insulin, antiglucagon serum, glucagon suppression, serum glucose.

High serum levels of immunoreactive glucagon (IRG) associated with hyperglycaemia have been observed not only in patients with glucagon producing tumours [17, 22] but also in animals and in patients with a variety of debilitating conditions [1, 26] and with diabetes mellitus [4, 13, 21, 30]. In particular, persistent or elevated levels of circulating IRG have been found in depancreatized dogs [19, 20, 33] and in some [23, 32], but not all, [2] depancreatized patients. The purpose of this study was to evaluate the role of extrapancreatic IRG in the pathogenesis of hyperglycaemia in the rat. We used depancreatized animals in whom the only known sources of IRG are the gastrointestinal tract, salivary glands and possibly the kidneys [6, 15, 16, 25]. Hyperglycaemic, ketotic rats, with high serum immunoreactive glucagon levels, were treated with somatostatin (SRIF), antiglucagon serum (AGS) and insulin, three inhibitors of glucagon secretion or glucagon action [3, 5, 10, 35].

Materials and Methods

Definitions

Since most substances with glucagon-like immunoreactivity found in blood and tissues are poorly characterized, we have reserved the name “glucagon” for the chemically defined pancreatic hormone and have called all other materials “immunoreactive glucagon” or IRG, with qualifications. Thus, total IRG is the fraction measured with a “non-specific” antiserum that reacts with glucagon and with extracts of intestinal mucosa and IRG_a is the material secreted by the A cells found in the pancreas and elsewhere and measured with an antiserum “specific” for pancreatic glucagon. Appropriate words, e.g. pancreatic, intestinal, or salivary indicate the probable sources of IRG.

Normal, depancreatized and sham-operated male Sprague-Dawley rats (300–350 grams) were used. One-stage pancreatectomy and sham surgery, consisting of laparotomy and manipulation of pancreas and intestines, were performed under pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Illinois) anaesthesia (30 mg/kg, IP). Immediately after surgery, the animals received 0.5 ml of an antibiotic (Distrycillin A.S., E.R. Squibb and Sons, Princeton, New Jersey) and 10 ml of NaCl (0.154 mol/l) solution subcutaneously. Thereafter, the rats were kept in individual metabolic cages with water and food *ad libitum*. Urinary glucose and ketones were estimated with Labstix (Ames Company, Elkhart, Indiana). Glycosuria (2–3+) and ketonuria (1–2+) appeared on the first postoperative day and became progressively more severe thereafter. On the second postoperative day, the animals began to eat and to lose weight. Four to 5 days after surgery, after a 24 hour fast, the rats appeared well groomed and in fair health although they had suffered an average weight loss of about 15%. At this time, they were anaesthetized again with sodium pentobarbital and both external jugular veins were cannulated. One cannula was used for the removal of blood, the other for the introduction of experimental solutions. A rabbit antiglucagon serum (AGS 28), which had a binding capacity of 20 ng of pancreatic glucagon per ml and did not distinguish between the latter and glucagon-like materials extracted from intestinal mucosa [9], was injected intravenously in a single dose sufficient to bind at least 3 times the total amount of circulating IRG. This amount, up to 3.0 ml per rat, was calculated from the serum levels of total IRG measured with a similar antiserum (AGS 10), assuming a blood volume equal to 10% of body weight. In two depancreatized rats we injected 6.0 ml of AGS. At the end of the experiment the animal's serum could still bind glucagon *in vitro*, indicating that the injected dose of AGS was in excess of the amount needed. Control rats received an equal volume of normal rabbit serum. SRIF (cyclic somatostatin, 100 µg/kg/h, Ayerst Laboratories, New York, New York), insulin (5–200 mU/rat/h) and NaCl (0.154 mol/l) solution were infused over a 2 h period with the aid of a pump (Harvard Apparatus Company, Millis, Massachusetts). One ml of blood was collected before the beginning of the infusion; other samples were 0.25 ml, except the last (120 min) sample which was 2.0 ml. The blood was placed immediately into chilled test tubes and spun in a refrigerated centrifuge. The serum was transferred to a tube containing aprotinin (Trasylol, FBA Pharmaceuticals Inc., New York, New York), an inhibitor of proteolysis (1000

Kallikrein inactivator units per ml) and stored at –20°C. Glucose was measured enzymatically (GOD Perid method, Boehringer Mannheim Corp., New York, New York) and glucagon by means of a double-antibody radioimmunoassay previously described [28], using ¹²⁵I-labelled porcine glucagon (New England Nuclear, Boston, Massachusetts) and porcine standards (prepared from lot #258-D30-138-4, gift of Dr. Mary Root, Lilly Research Laboratories, Indianapolis, Indiana). Two antiglucagon sera were used: AGS 10, which reacts with pancreatic glucagon and with intestinal glucagon-like materials and measures total immunoreactive glucagon and AGS 18, which reacts minimally with extracts of intestinal mucosa and measures IRG secreted by the A cells of the pancreas and of other organs (IRG_a). The characteristics of these antisera have been described previously [9]. Insulin was measured with the method of Hales and Randle [12] using porcine insulin standards and ¹²⁵I-labelled porcine insulin. The latter was prepared from lot #PJ 5682 (gift of Dr. Mary Root, Lilly Research Laboratories, Indianapolis, Indiana) according to Greenwood and Hunter [11]. We have determined previously [18] that our anti-insulin serum binds less rat insulin than porcine insulin, though at values lower than 20 µU/ml the difference becomes insignificant. In any case all IRG and IRI values should be considered porcine hormone equivalents. The results are expressed as means ± SEM. Their statistical significance was calculated using Student's *t* test.

Results

No significant differences were noted between the results obtained in normal and in sham-operated rats; therefore, the two groups of values were combined and used as control data. Removal of the pancreas resulted in a significant increase in the serum levels of glucose, total IRG and IRG_a and in a significant decrease in serum IRI, which, however, did not disappear completely (Table 1).

The injection of AGS, but not normal rabbit serum, into normal rats caused a significant decrease in serum glucose that reached a nadir in about 30 min, persisted for about 1 h, thereafter returning to the preinfusion and, in some cases, higher values. Neither serum significantly modified the serum glucose concentration of depancreatized rats (Fig. 1). This was true also in the two depancreatized rats which received twice as much AGS (6 ml) as the others (data not recorded).

Following the infusion of SRIF we noted a sig-

Table 1. Serum glucose, insulin, A cell glucagon (IRG_a) and total glucagon (total IRG) in normal and depancreatized rats. Ave. $\pm$ SEM. Number of rats in parentheses

	Glucose mg/100 ml	Insulin μ u/ml (porcine equivalents)	Glucagon pg/ml (porcine equivalents)	
			IRG _a	Total IRG
Normal	92 $\pm$ 3 (11)	41 $\pm$ 6 (10)	62 $\pm$ 9 (15)	446 $\pm$ 66 (11)
Depancreatized	453 $\pm$ 52 ^b (19)	13 $\pm$ 2 ^a (18)	152 $\pm$ 40 ^a (17)	1079 $\pm$ 136 ^a (15)

^a $p < 0.01$; ^b $p < 0.001$ **Table 2.** Serum IRG_a, total IRG and IRI before and 120 minutes following somatostatin infusion (100 μ g/kg/hr) in normal and depancreatized rats. Ave. $\pm$ SEM. Number of rats in parentheses

	IRG _a		Total IRG		IRI	
	0	120	0	120	0	120
Normal	71 $\pm$ 11 (11)	33 $\pm$ 7 (5)	334 $\pm$ 24 (7)	328 $\pm$ 78 (7)	36 $\pm$ 5 (11)	20 $\pm$ 3 ^a (5)
Depancreatized	152 $\pm$ 40 (17)	18 $\pm$ 4 ^a (6)	1239 $\pm$ 108 (6)	980 $\pm$ 123 (6)	—	—

^a = $p < 0.05$ vs 0 time **Fig. 1.** Effect of antilucagon serum (AGS) and of normal rabbit serum (NRS) on serum glucose in normal (a) and depancreatized (b) rats. Ave. initial serum glucose (mg/100 ml) in normal rats: AGS-treated 95; NRS-treated 104; in depancreatized rats: AGS-treated, 448; NRS-treated 460. * = $p < 0.05$ **Fig. 2.** Effect of somatostatin infusion (100 μ g/kg/h) on serum glucose in normal and depancreatized (DX) rats. Ave. initial serum glucose values (mg/100 ml): normal rats, 106; depancreatized rats 381. * = $p < 0.05$; ** = $p < 0.01$

nificant decrease in the level of serum IRI in normal rats and in the level of serum IRG_a in normal and depancreatized rats. This reduction in IRG_a accounted for the effect of SRIF on total IRG, but it was not statistically significant, due to the large standard error (Table 2). Serum glucose decreased significantly in normal, but not in depancreatized rats (Fig. 2).

The effect of insulin on the serum glucose con-

centration of normal rats varied with the dose (Fig. 3). The smallest dose (5 mU/rat/h) caused a slight, non-significant decrease in total IRG and IRG_a in normal animals. However, when insulin was infused in larger amounts, the resulting hypoglycaemia was accompanied by a significant stimulation of IRG_a and consequently of total IRG (Table 3). Insulin caused a significant decrease in IRG_a and total IRG of depancreatized rats only in doses of 100 mU/

Fig. 3. Effect of insulin infusion on serum glucose in normal rats. Ave. initial serum glucose, 104 mg/100 ml. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Fig. 4. Effect of insulin infusion on serum glucose in depancreatized rats. Ave. initial serum glucose, 518 mg/100 ml. ** = $p < 0.01$; *** = $p < 0.001$

Table 3. Serum IRG_a and total IRG in normal and depancreatized rats 120 minutes following insulin infusion. Ave $\pm$ SEM. Number of rats in parentheses

	mU/rat/h	IRG _a	Total IRG
Normal	0	75 $\pm$ 24 (4)	446 $\pm$ 66 (11)
	5	46 $\pm$ 19 (4)	343 $\pm$ 58 (3)
	20	447 $\pm$ 72 (4) ^b	712 $\pm$ 143 (5) ^a
Depancreatized	0	186 $\pm$ 55 (7)	1007 $\pm$ 133 (15)
	80	166 $\pm$ 30 (4)	893 $\pm$ 228 (6)
	100	49 $\pm$ 16 (9) ^a	476 $\pm$ 91 (13) ^b
	200	61 $\pm$ 16 (7) ^a	540 $\pm$ 80 (6) ^b

^a $p < 0.05$ vs 0 control; ^b $p < 0.01$ vs 0 control

rat/h or more (Table 3). On the other hand, a significant lowering of serum glucose required a dose of at least 200 mU/rat/h (Fig. 4).

Discussion

The untreated depancreatized animal is a convenient experimental model for the study of extrapancreatic glucagon since, when given no insulin, it rapidly develops not only hyperglycaemia and ketosis, but also high serum levels of IRG_a and total IRG. In our depancreatized rats (we could not use dogs for obvious reasons of AGS economy) circulating glucagon rose significantly, even though measurable amounts of endogenous IRI were still present. Four explanations may be offered for these findings: decreased rates of hormone degradation or excretion; release of previously stored hormones; hormone secretion by extrapancreatic tissues; and failure to remove the entire pancreas. We know of no evidence suggesting that pancreatectomy alters the normal processes of insulin or glucagon disposal, or the release of tissue bound hormones. On the other hand, there is evidence that the pancreas is not the sole source of insulin and of glucagon since IRI, IRG_a and total IRG have been found in the salivary glands [6, 14, 15, 25], in the kidneys [6, 16], and in the gastrointestinal tract [8, 27, 29, 31].

Finally, even though we may have failed to remove small portions of the pancreas in some rats, all of them developed marked hyperglycaemia, glycosuria and ketosis and, in any case, subtotal pancreatectomy and the resulting hyperglycaemia could explain the presence of residual IRI, but not the rise in pancreatic IRG. In any case, IRI and IRG do not disappear entirely from the blood even after total evisceration [6, 24], a procedure which makes total pancreatectomy a virtual certainty. We suggest that, by removing the pancreas, we may have removed the inhibitory influence of insulin and pancreatic glucagon on the release of IRG from any residual pancreatic A cells or from extrapancreatic sources [7].

The binding of IRG by AGS resulted in a significant lowering of the blood glucose level in fasted normal rats. This effect lasted 30 to 60 min, or about as long as the hyperglycaemic effect of a glucagon injection [34], suggesting that a biologically significant portion of the circulating IRG had indeed been bound and subsequently released by the antibody. On the other hand AGS had no effect in depancreatized rats whose serum glucose continued to increase during the 2 h period of observation. The reason for this different response is not

readily apparent: perhaps, in the normal rat, a decrease in IRG_a allows insulin to exert its full effect, while in the depancreatized, insulin-deficient animal, this is not possible. A crucial role of insulin and a relatively minor role of glucagon are suggested also by the observation that the suppression of IRG_a by SRIF failed to decrease the level of serum glucose in depancreatized rats, although it had a hypoglycaemic effect in normal animals.

Progressively larger doses of insulin caused a progressively larger decrease in the serum glucose of normal rats, but only the lowest doses caused a slight non-significant decrease in serum IRG_a and total IRG. Larger doses caused greater hypoglycaemia associated with an increased level of both IRG fractions. Thus, although insulin may have inhibited IRG secretion in normal rats, this effect was masked by the stimulatory effect of hypoglycaemia. On the other hand, insulin significantly decreased the serum levels of IRG_a and total IRG in depancreatized rats, even when the dose was insufficient to cause a significant decrease in their serum glucose. Thus, the suppression of IRG_a by SRIF or of total IRG (including IRG_a) by insulin was not necessarily associated with a reduction in serum glucose, indicating that glucagon did not play an essential role in the pathogenesis of hyperglycaemia in depancreatized rats. On the other hand, while in the normal animals insulin-induced hypoglycaemia was associated with a rise in IRG_a , in the depancreatized ones it was associated with a decrease in IRG_a . These findings suggest that in the insulin-deprived rat insulin is capable of suppressing glucagon secretion, at least as long as the serum glucose does not reach hypoglycaemic levels. We believe that our data are consistent with the conclusions that, in the rat, insulin is required for the proper function of the pancreatic and extrapancreatic glucagon-secreting cells; that although hyperglucagonaemia may contribute to the hyperglycaemia of the depancreatized animal, the excessive secretion of glucagon is secondary to insulin insufficiency and that, at least in this species, the hypoglycaemic action of insulin does not depend primarily upon its ability to suppress glucagon secretion.

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