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Plasma Glucose, Insulin, Pancreatic, and Enteroglucagon Levels in Normal and Depancreatized Dogs[1] (38288)

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Extracts of the upper gastrointestinal tract with hyperglycemic and glycogenolytic properties similar to those of pancreatic glucagon were prepared many years ago (2–4). More recently, similar extracts were found to contain a material capable of binding to antisera raised against pancreatic glucagon (5). This glucagon-like material or enteroglucagon appears to react with the pancreatic glucagon receptors of the B cell (6) and of the hepatocyte (7), a property that may explain the insulinogenic (8) and glycogenolytic actions of the intestinal extracts (2–4, 9). The biologic characteristics of enteroglucagon and the fact that it is secreted in response to the introduction of glucose (9), fat (10), or calcium (11) into the lumen of the small intestine suggest that this hormone may share a role in metabolic regulation with its pancreatic counterpart. In view of the fact that experimental insulin deficiency is associated with levels of pancreatic glucagon that are either high or inappropriate to the existing concentration of glucose (12, 13), we thought it would be interesting to measure plasma enteroglucagon levels in depancreatized dogs. This paper describes the results of our observations.

Materials and Methods. Healthy mongrel dogs weighing 11.5 – 24.4 kg were used. Some of them were depancreatized under pentobarbital[2] anesthesia (35 mg/kg iv), as described previously (14). The dogs received no insulin, except as indicated below, were fed commercial dog food *ad libitum*, but were fasted for about 24 hr before the acute experiments. Blood samples were obtained from the antecubital vein with heparinized syringes before and after pancreatectomy. In 4 dogs, blood samples were collected following a single injection of regular insulin (0.4 U/kg, im) given 3 days after pancreatectomy and after a 24-hr fast. In 3 of these animals, this procedure was repeated after 4 additional days.

The following acute experiment was performed in normal dogs and in depancreatized dogs 4–10 days after surgery: under pentobarbital anesthesia, an intestinal loop was prepared by tying both ends of a 30-cm intestinal segment and by identifying its venous drainage. In some dogs, the cephalic ligature was placed about 30 cm below the ligament of Treitz (jejunal loops); in other dogs the ligature was placed about 30 cm above the ileocecal valve (ileal loops). A soft plastic catheter, to be used for the introduction of glucose, was inserted into the cephalic end of the intestinal loop and this was covered with gauze moistened with 0.9% NaCl solution. Blood samples were obtained from a mesenteric vein draining the loop (but no other portions of the gut) before and for 15 min after the introduction of 50 ml of a 5% glucose solution into the loop itself. This short period of observation was chosen because it had been demonstrated adequate to produce a significant enteroglucagon response (9) while minimizing the possible effect of excessive bleeding (15). The specimens of heparinized blood were transferred immediately into chilled tubes containing lyophilized Trasylol[3], an inhibitor of proteolysis, in amounts sufficient to provide 1000 kallikrein inactivator units per ml of blood. The tubes were kept in ice, centrifuged as soon as possible at 4°, and the plasma was separated and stored at −20° until analyzed. Glucagon was measured in 0.1 ml of

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[2] Nembutal, Abbott Laboratories, North Chicago, Illinois 60064.

[3] FBA Pharmaceuticals, Inc., 425 Park Avenue, New York, NY 10022.

plasma using a double-antibody radioimmunoassay previously described (16), except that the buffer was 0.1 *M* barbital–saline, pH 8.6, containing human serum albumin (0.25%)[4], instead of phosphate buffer containing bovine serum albumin. The labeled hormone was ^{125}I-glucagon[5], and the standards were prepared with crystalline pork glucagon (Lot No. GLF 599A)[6]. Two rabbit anti-glucagon (AGS) were used: AGS 10 which binds both pancreatic and enteroglucagon and, hence, measured "total glucagon" or GLI, and AGS 18 which measures mostly pancreatic glucagon or IRG. The specificity of AGS 18 was determined by means of extracts of dog intestine prepared with the method of Kenny (17) and assayed as described in a recent publication (18). The curves shown in Fig. 1 demonstrate that this serum binds insignificant amounts of enterglucagon when this is present in low concentrations and only 5–8% when present in concentrations of 1000 pg/ml or higher. Enteroglucagon was considered to be the difference between GLI, as measured with AGS 10, and IRG, as measured with AGS 18 after correction for the estimated cross-reactivity of the antiserum. The following formulas were used for this correction:

$$C = A - \alpha D \text{ and } D = B - C,$$

where A = IRG (measured with AGS 18); B = GLI (measured with AGS 10); C = corrected IRG; D = enteroglucagon; $\alpha = 0$, when B was less than 1000 pg/ml; and $\alpha = 0.07$, when B was 1000 pg/ml or more. These corrections were applied only to the assays of peripheral blood plasma, since the conditions in which the mesenteric blood was collected precluded the direct admixture of pancreatic blood. Thus, the pancreas could not have contributed to the observed increment in plasma GLI. In any case, we wish to emphasize that the data thus calculated are only reasonable approximations. The AGS–glucagon complex was precipitated by means of goat anti-rabbit gamma globulin[7]. Plasma glucose was measured enzymatically using the AutoAnalyzer; immunoreactive insulin (IRI) was assayed with the method of Hales and Randle (19).

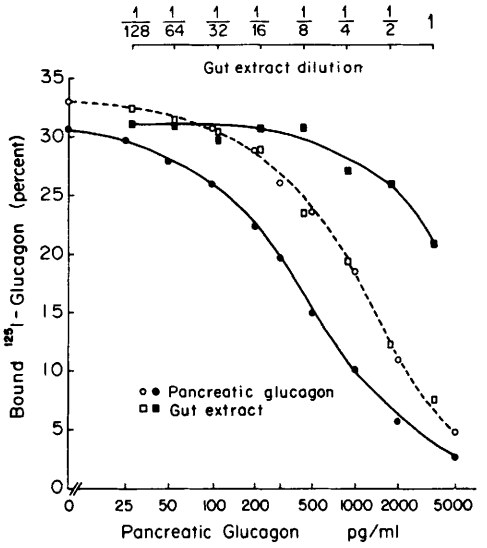

FIG. 1. Standard curves obtained with AGS 18 (solid lines; final dilution 1:22,000) and AGS 10 (dotted line; final dilution 1:63,000).

Results. As expected, pancreatectomy was followed by a progressive decline in the level of plasma IRI and by a progressive increase in plasma glucose. Within 1 or 2 days, the concentration of GLI, IRG, and enteroglucagon increased significantly (Table I). When glucose was injected into the ileum of a normal dog, it caused a greater rise in GLI and a less pronounced hyperglycemia than when it was introduced into the dog's jejunum (Fig. 2). The small rise in IRG noted in these experiments could be accounted for by the cross-reactivity of AGS 18. Thus, the increment in GLI was represented by enteroglucagon. The level of IRI did not change significantly. When a 5% glucose solution was injected into the ileum, the GLI response was significantly greater in the depancreatized than in the normal dogs (Fig. 3) and increased progressively after pancreatectomy, as indicated by the peak value and by the sum of all observed increments (Table II). Again, the IRG values rose no more than could be expected from the cross-reactivity of AGS 18. Thus, also in this case, the GLI increment was represented by the enteroglucagon fraction. The effect of pancreatectomy on plasma IRG and GLI was re-

[4] Hyland, Costa Mesa, California 92626.

[5] Cambridge Nuclear Corporation, Billerica, Massachusetts 01821

[6] Gift of Dr. Mary Root, Lilly Research Laboratories, Indianapolis, Indiana 46206.

[7] Nutritional Biochemicals Corporation, Cleveland, Ohio 44128.

TABLE I. Plasma Levels of IRG, GLI, Enteroglucagon, Glucose, and IRI After Pancreatectomy in (*N*) Dogs, Mean ± SEM.

		IRG (pg/ml) (*A*)	GLI (pg/ml) (*B*)	Corrected IRG* (pg/ml) (*C*)	"Enteroglucagon"* (pg/ml) (*D*)	Glucose (mg/100 ml)	IRI (μU/ml)
Control	(12)	74 ± 15	390 ± 60	74 ± 15	320 ± 60	87 ± 6	32 ± 4
1 Hour	(12)	44 ± 8	340 ± 50	44 ± 8	300 ± 40	142 ± 10[c]	21 ± 2[a]
2 Hours	(12)	48 ± 9	290 ± 40	48 ± 9	240 ± 40	142 ± 12[c]	20 ± 2[a]
3 Hours	(11)	45 ± 10	290 ± 50	45 ± 10	250 ± 50	160 ± 15[c]	18 ± 3[a]
4 Hours	(12)	51 ± 10	300 ± 50	51 ± 10	250 ± 50	208 ± 16[c]	17 ± 3[b]
1 Day	(12)	87 ± 13	550 ± 50[a]	87 ± 13	460 ± 50	293 ± 21[c]	15 ± 3[b]
2 Days	(11)	235 ± 42[b]	900 ± 80[c]	214 ± 41[b]	680 ± 70[c]	329 ± 26[c]	15 ± 2[b]
3 Days	(10)	337 ± 60[c]	1070 ± 150[c]	300 ± 52[c]	770 ± 130[b]	336 ± 24[c]	14 ± 3[b]
4 Days	(5)	284 ± 70[a]	970 ± 190[a]	247 ± 57[b]	730 ± 150[a]	318 ± 33[c]	12 ± 5[b]
5 Days	(1)	620	910	620	290	410	11
6 Days	(3)	460 ± 120[a]	1300 ± 340[a]	420 ± 110[b]	880 ± 340	345 ± 36[c]	7 ± 4[c]
7 Days	(4)	370 ± 150	1300 ± 440	330 ± 120[a]	970 ± 330	342 ± 31[c]	11 ± 5[b]
8 Days	(2)	580 ± 330	1770 ± 1040	510 ± 260	1260 ± 790	383 ± 80[b]	13 ± 2[b]
9 Days	(2)	1060 ± 490	2710 ± 1190	930 ± 430	1780 ± 760	458 ± 153[a]	10 ± 4[b]

[a] $P < 0.05$.; [b] $P < 0.01$.; [c] $P < 0.001$.

* These values were calculated from the original experimental data, not from the mean values.

versed, in part, by insulin given in a dose sufficient to cause a significant elevation of the plasma IRI level but only a moderate decrease in plasma glucose (Fig. 4). Two hours after the injection of insulin, the calculated enteroglucagon level also decreased, from 630 ± 100 to 350 ± 70 pg/ml ($P < 0.05$).

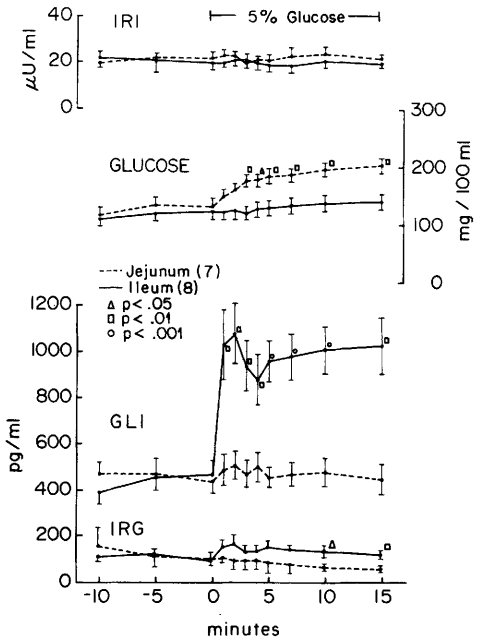

FIG. 2. Plasma IRG, GLI, glucose, and IRI in the regional mesenteric blood following the injection of 50 ml of a 5% glucose solution into a loop of small intestine of normal dogs (mean ± SEM). *P* values compare the responses to jejunal and ileal loops. Number of experiments in parentheses.

Discussion. The increase in plasma IRG noted after pancreatectomy is an apparent paradox and was unexpected, especially since at the time of our experiment, the persistent IRG response to arginine in depancreatized dogs (20) had not been reported. We cannot explain this finding with satisfaction: as stated above, the cross-reactivity of AGS 18 could explain presence of some residual IRG-like material, but could account only for a small portion of its increase. Perhaps some of this "IRG" represented immunoreacting fragments of the enteroglucagon molecule or it might have been true IRG secreted by pancreatic tissue inadvertently left behind during surgery. This, however, seems improbable because upon careful post mortem examination we failed to find any pancreatic tissue, because the dogs developed diabetic ketosis and primarily because plasma IRG increased while plasma IRI was decreasing. Other possibilities are that glucagon receptors in the target tissues may function as reversible depots and release the hormone under stress and that a substance immunologically similar to pancreatic glucagon may be produced by other tissues. The small intestine does not appear to be a likely source of

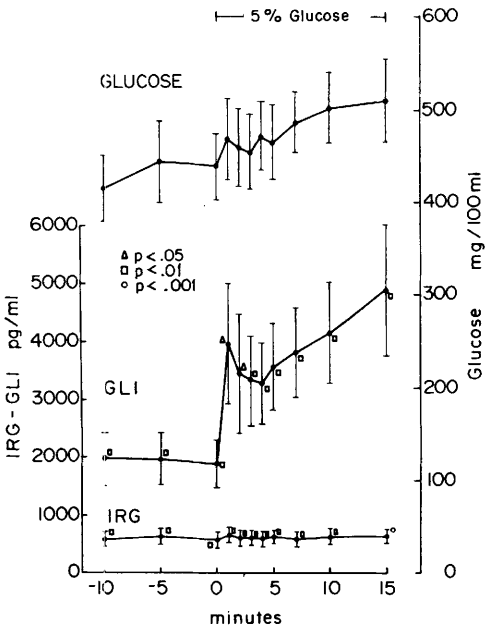

FIG. 3. Plasma IRG, GLI, and glucose in the regional mesenteric blood following the injection of 50 ml of a 5% glucose injection into a loop of ileum in 8 depancreatized dogs (mean ± SEM). *P* values compare the responses of depancreatized and normal dogs.

this hypothetical extra pancreatic IRG, since after the introduction of glucose into the ileum of normal and depancreatized dogs, the increase in plasma IRG was not greater than that which would have been expected from the cross-reactivity of AGS 18 (Figs. 2 and 3).

The abnormally high concentration of GLI and of enteroglucagon, their partial correction by insulin, and the exaggerated response to glucose stimulation noted in the depancreatized animals suggest that insulin may help regulate the secretion of enteroglucagon, as it is known to do for pancreatic glucagon (12, 13). Indeed, Buchanan *et al.* (21) state that they have observed a high GLI response to intrajejunal glucose in dogs 2 hr after pancreatectomy. The failure of plasma IRI to rise in response to the introduction of glucose into the ileum of normal dogs, in spite of the marked increase in plasma GLI, may indicate that the conditions of our experiments (short intestinal loop and removal of most of its venous blood) have prevented enteroglucagon from reaching the pancreas in amounts sufficient to stimulate the B cells. Thus, our data do not help resolve the disagreement between the evidence for (8) and against (22) the insulinogenic action of enteroglucagon: the question of a possible negative feedback between the secretion of insulin and enteroglucagon remains unanswered. The marked GLI, or in this case enteroglucagon, response to the introduction of glucose directly into the ileum may explain the hypersecretion of GLI noted in gastrectomized subjects after the ingestion of

TABLE II. GLI Response to the Injection of 50 ml of a 5% Glucose Solution into a Loop of Small Intestine of 8 Depancreatized and (*N*) Normal Dogs (pg/ml).

	Basal Value	Maximal Response	Total Response (15 min)	Days after pancreatectomy
Depancreatized dogs—ileum	790	2790	14660	4
	1050	2310	11470	6
	1560	2640	10310	6
	900	3120	23170	8
	1080	3480	25580	8
	3620	8470	47250	9
	2710	8360	54060	10
	3850	9460	50460	10
Mean ± SEM	1950 ± 450	5080 ± 1090[a]	29620 ± 6450	
Normal dogs—ileum (8), mean ± SEM	460 ± 60[b]	1140 ± 130[b,c]	7610 ± 1240[b]	
Normal dogs—jejunum (7), mean ± SEM	460 ± 40	550 ± 70[d]	1890 ± 250[e]	

[a] $P < 0.05$ vs basal value.
[b] $P < 0.01$ vs corresponding values in depancreatized dogs.
[c] $P < 0.001$ vs basal value.
[d] $P < 0.01$ vs ileum.
[e] $P < 0.001$ vs ileum.

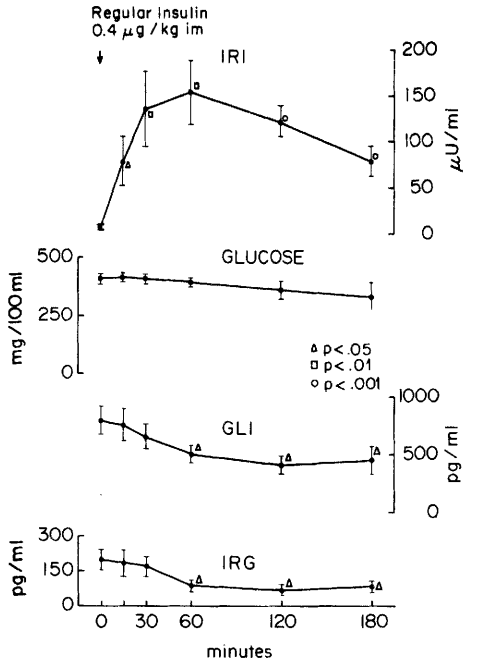

FIG. 4. Plasma IRG, GLI, glucose, and IRI in the peripheral blood of 4 depancreatized dogs following a single injection of regular insulin (7 experiments, mean ± SEM).

glucose (23–25). On the other hand, the relatively small jejunal response may have been the result of either insulin or secretin-induced inhibition. We favor the second possibility because the level of plasma IRI did not change significantly during the experiment and because secretin, which is found in much greater amounts in the jejunum than in the ileum (26), can inhibit the glucose-induced secretion of enteroglucagon (27), at least in man. The fact that regional hyperglycemia was smaller after the injection of glucose into the jejunum than after its injection into the ileum, even though the corresponding GLI response was greater, lends support to the notion that the release of enteroglucagon depends more upon its contact with the intestinal mucosa than upon its absorption (28). Our results do not contradict the finding that rat "jejunum" secretes GLI *in vitro* (29), since the preparation used in those experiments reached 65 cm from the pylorus, a distance that in the rat probably includes most of the ileum.

Summary and Conclusion. Pancreatic glucagon (IRG), total glucagon-like immunoreactive material (GLI), enteroglucagon (calculated from GLI and IRG values), glucose, and insulin (IRI) were measured in the systemic plasma of normal and depancreatized dog. IRG, GLI, and enteroglucagon began to increase 1 or 2 days after pancreatectomy, respectively, reaching values that were several times greater than those observed before the operation. Glucose, already elevated 1 hr after pancreatectomy, continued to increase thereafter; IRI showed a progressive decline. In other experiments, 50 ml of a 5% glucose solution were injected into 30-cm loops prepared from the upper portion of the jejunum or the lower portion of the ileum and blood samples were obtained from the regional mesenteric veins. Plasma enteroglucagon rose more and plasma glucose less when glucose was introduced into the ileum than when it was introduced into the jejunum. The enteroglucagon responses to glucose were much greater in depancreatized than in normal dogs. In the depancreatized animals, a single injection of regular insulin (0.4 U/kg; im), sufficient to produce a significant elevation of the plasma IRI level but only a moderate decrease in plasma glucose, caused a prompt and significant decrease in the systemic plasma IRG, GLI, and enteroglucagon levels. We conclude that in the dogs, the ileum is a major source of enteroglucagon and that removal of the pancreas increases the secretion of this hormone, a phenomenon reversed in part by insulin therapy.

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