Control of Gastric Glucagon Secretion in the Acutely Pancreatectomized Rat

M. MARRE, E. BOBBIONI, M. SUAREZ, G. REACH, M. P. DUBOIS, AND R. ASSAN

SUMMARY

Glucagon immunoreactivity (IRG) was measured in portal plasma from control and pancreatectomized rats and in arterial plasma from eviscerated rats with a functional liver. Portal IRG was 0.41 ± 0.02 ng/ml in control rats and 0.22 ± 0.01 in pancreatectomized rats. After evisceration, values of 0.08 ± 0.01 ng/ml were found (unextracted plasma, antiserum 30 K). Acid-ethanol plasma extracts demonstrated lower values, but a similar stepwise decrease was observed after pancreatectomy, then gastrectomy. Rat gastric extracts contained a low concentration of IRG (approximately 1/1200 the C-terminal IRG concentration of the corresponding pancreas). No IRG-positive cells were detected by immunofluorescence in the gastric mucosa.

In the pancreatectomized rats, portal IRG remained stable for 75 min in the absence of further manipulation. From IRG concentrations and hepatic blood flow estimation in both control and pancreatectomized rats, the contribution of the stomach to portal IRG in the basal state could be estimated as 20% of the total. Gastric IRG release was increased by acute hypoglycemia (peak value 0.75 ± 0.18 ng/ml; N = 10; $P < 0.01$) and by 2-deoxyglucose infusion (0.45 ± 0.15 ng/ml; N = 4; $P < 0.05$). Administration of glucose + insulin induced a decrease in portal IRG (0.13 ± 0.01 ng/ml; N = 4; $P < 0.001$). Vagal stimulation and arginine infusion induced a rise in portal IRG: 0.84 ± 0.27 ng/ml (N = 10; $P < 0.05$) and 0.31 ± 0.03 ng/ml (N = 9; $P < 0.01$), respectively, while portal insulin remained low or undetectable (0–18 μU/ml). A rise in blood glucose accompanied the increase of plasma IRG. A concomitant insulin-induced hypoglycemia (36 ± 5 mg/dl) strongly potentiated the effects of both arginine infusion (1.46 ± 0.47 ng/ml; N = 6; $P < 0.005$) and vagal stimulation (1.39 ± 0.47 ng/ml; N = 4; $P < 0.005$).

Higher IRG values were observed after pancreatectomy in alloxan-diabetic rats: 0.36 ± 0.02 ng/ml (N = 3; $P < 0.001$).

We conclude therefore that: (a) the rat stomach contributes to the release of IRG in blood but to a limited extent, (b) the factors controlling this release appear very similar to those controlling pancreatic A cells; and (c) gastric IRG may be hyperglycemic in the rat.

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The contribution of the stomach to the circulating glucagon immunoreactivity (IRG) can vary, depending on the species and the metabolic state. In the totally depancreatized man, plasma IRG has been found neglible[1,2] or present and not negligible.[3–5] In depancreatized dogs, large amounts of IRG are present in both the plasma[6–10] and the gastric mucosa;[11] this IRG seems to aggravate the metabolic abnormalities observed in these dogs.[12] The control of dog gastric IRG release in vitro has been well documented.[13] A few studies have been devoted to the persistence of IRG in rat plasma after pancreatectomy,[14,15] and even after evisceration under specific conditions.[16–18] It has been suggested that gastric IRG can contribute to some extent to the metabolic disorders present in the pancreatectomized rat.[15] The aim of the present study is to evaluate the contribution of the stomach to plasma IRG in acutely depancreatized rats and to document the factors that control IRG release by the stomach.

MATERIALS AND METHODS

Experimental procedure. Male Wistar rats, 250–300 g, fasted for 14 h, were anesthetized with thiopental (50 mg/kg i.p.). After removal of the jejunum, ileum, and colon, a catheter (Biotrol no. 4) was inserted in the stump of the mesenteric vein so that the tip lay in the portal vein, thus allowing for repeated blood sampling without interruption of the flow (control rats). In rats with a similar portal catheter, the pancreas, duodenum, and spleen were removed. The stomach as well as its vessels (left gastric, right gastroepiploic, and fundic short vessels) remained intact. Thus, the portal blood

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From the Department of Diabetology, Hôtel-Dieu, Paris, France, and U_{36}-INSERM; Institute of Medical Pathology, Parma, Italy; University of Bogota, Colombia; and I.N.R.A., Nouzilly (Tours), France.

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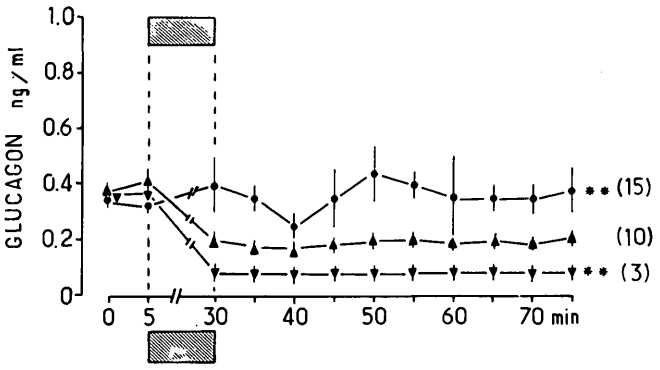


FIGURE 1. Plasma glucagon immunoreactivity in control (●), totally pancreatectomized (▲), and eviscerated rats (▼). Samples were collected from portal vein in (●) and (▲), and from carotid artery in (▼). Number of animals is indicated between parentheses. Results are presented as mean values ±SEM. Asterisks (**) denote a statistically significant difference (P < 0.01) between (▲) and each of the other groups. The hatched area represents the time of surgical procedure.

flow consisted exclusively of gastric venous blood. A catheter was inserted in the pylorus for drainage of gastric juice. The pneumogastric nerves were not touched, unless mentioned. This group of rats was denominated pancreatectomized rats. Other groups of rats were submitted to: subtotal pancreatectomy (leaving approximately 5% of the gland intact); gastrectomy (leaving the pancreas and the duodenum intact); or abdominal evisceration, except for the liver and its arterial blood supply.

Rats were rendered diabetic by treatment with alloxan (30 mg i.v. in 1 ml citrate buffer, pH 4). Two days later, severe diabetes mellitus was demonstrated by massive glucosuria, ketonuria, and weight loss (mean: 10 ± 2% body wt); the portal vein of each was then catheterized and the rats were maintained as such, or pancreatectomized or gastrectomized.

All rats were maintained under a mixture of $O_2 + CO_2$ (95–5%). Careful hemostasis was performed. Blood samples were quantitatively supplemented with fresh blood drawn from fasted normal donor rats. Intraabdominal temperature was monitored with an electrical thermometer (Yellow Spring Scientific Instruments, Yellow Spring, Ohio) and kept normal by the use of a warm pad. Supplementary catheters were inserted in the main right jugular vein for infusion of blood, chemicals, and hormones. All catheters were filled with heparin diluted in physiologic saline solution to maintain patency.

Stimulatory procedure. Arginine, glucose, 2-deoxyglucose, and insulin were administered intravenously with a preliminary bolus followed by a constant rate infusion, using a Braun infusion pump (Melsungen, West Germany), and three-way stopcocks connected with appropriate syringes. The doses were: arginine, 25 μmol bolus, then 5 μmol/min; insulin (Actrapid glucagon-free Novo, Copenhagen, Denmark), 1 mU/min, alone or concomitant with glucose; glucose, 100 mg bolus, then 10 mg/min; and 2-deoxyglucose (Sigma, Saint Louis, Missouri), 100 mg bolus, then 10 mg/min. Vagus nerves were electrically stimulated at the level of the cardia by means of a Hugo Sachs stimulator (Hugsteffen, Breisgau, West Germany) that provided biphasic pulses of 20 V, 1 ms, 20 Hz. In some experiments, the vagus nerves were cut, and then hypoglycemia was induced by intravenous insulin infusion.

Determinations. Portal blood samples (0.5 ml each) were collected with a heparinized syringe. Aprotinin (Iniprol, Choay, Paris, France), 2000 U/ml, was immediately added. Samples were kept at +5°C for less than 20 min and centrifuged at +4°C; plasmas were rapidly frozen and stored at −20°C.

Glucose was assayed by the glucose-oxidase method,[19] using a Beckman analyzer (Beckman Instruments, Fullerton, California). Hepatic blood flow was estimated by a Brome sulfone phtalein (BSP) extraction procedure.[20] BSP was administered intravenously with a bolus of 2.5 mg/100 g body wt followed by a constant rate infusion of 5 mg/100 g body wt for 30 min before the first blood sampling and continuing throughout the experiment. The fluid infusion required for BSP addition did not exceed a total of 1 ml. BSP was measured spectrophotometrically at 578 nm after alkalinization, and the hematocrit by a microcentrifugation method. Hepatic blood flow (EHBF) was estimated according to the formula:

$$EHBF = \frac{R}{0.01 \times (P) - (H)} \times \frac{1}{1 - (\text{hematocrit})}$$

where R is the hepatic removal rate in mg/min, P the peripheral plasma concentration in mg/ml, and H the hepatic venous concentration. Catheterization of the choledocus appeared necessary for correct EHBF estimation.

Glucagon and insulin were measured by the radioimmunologic method,[21] with slight technical adaptations.[22,23] Glucagon was assayed with the 30 K antiserum (R. H. Unger) without prior ethanol extraction of plasma samples, unless mentioned. A few plasma samples were extracted with acid-ethanol[24] then assayed by using the 30 K antiserum (which binds the C-terminal part of the molecule) and a second antiserum that is turned toward the N-terminal part of the molecule.[25]

The stomach and the pancreas were dissected from normal and diabetic rats, rapidly washed with chilled physiologic saline solution, sonicated (Ultrasonic desintegrator MK2-MGF, Crowley, England), and then submitted to acid-ethanol extraction;[26] the extracts were serially diluted in gly-

TABLE 1
Estimated hepatic blood flow (EHBF), hematocrit, and estimated pancreatic hormone release in the control and pancreatectomized rats

Groups	N	EHBF (ml/min/100 g body wt)	Hematocrit (%)	IRG release (ng/min/100 g body wt)	Insulin release (μU/min/100 g body wt)
Control rats	10	7.62 ± 0.61*	45 ± 2	1.85 ± 0.01*	630 ± 80*
Pancreatectomized rats	4	3.90 ± 0.74	51 ± 1	0.42 ± 0.01	26 ± 2

Results are presented as mean values ± SEM. N means the number of animals in each group.* Statistically significant difference (P < 0.01).

TABLE 2
Glucagon immunoreactivity (ng/ml) measured in plasma and in acid-ethanol plasma extracts using a C-terminal antiserum (30 K) and a N-terminal antiserum (PVP8)

Group	N	Unextracted plasma (30 K)	Extracted (30 K)	Extracted (PVP8)
Control rats	10	0.41 ± 0.02	0.31 ± 0.07	0.27 ± 0.07
Pancreatectomized rats	9	0.22 ± 0.01	0.11 ± 0.04	0.13 ± 0.03
Eviscerated rats	10	0.08 ± 0.01	0.02 ± 0.00	0.08 ± 0.02

Samples were collected from the portal vein in control and pancreatectomized rats, and from the carotid artery in the eviscerated animals. N means the number of animals in each group. Same presentation as in Table 1.

cine buffer plus human serum albumin 0.25% before assaying.

Results are presented as mean values ±SEM. Statistical significance of differences was calculated by the Student's *t* test for unpaired groups.[27]

Immunofluorescence studies. The stomachs from normal and diabetic rats were similarly dissected, washed, and fixed in Bouin-Hollande solution. They were then studied for glucagon and somatostatin immunofluorescence.[28]

RESULTS

HORMONE AND GLUCOSE LEVELS IN PORTAL PLASMA BEFORE AND AFTER PANCREATECTOMY

In control rats, portal plasma insulin was 150 ± 20 μU/ml, blood glucose was 84 ± 5 mg/100 ml, and glucagon (IRG) was 0.41 ± 0.02 ng/ml (N = 10). These levels remained stable for 75 min in the absence of further manipulation. After total pancreatectomy, insulin fell to 14 ± 2 μU/ml and remained low even in the presence of various stimuli. IRG decreased to 0.22 ± 0.01 ng/ml and remained stable for 75 min, in the absence of further manipulation (Figure 1). Blood glucose rose progressively averaging 144 ± 8 mg/dl for the 40 min after pancreatectomy and 159 ± 9 mg/dl for the subsequent 40 min. Estimated hepatic blood flow (EHBF) was 7.62 ± 0.61 ml/min/100 g body wt before pancreatectomy and 3.90 ± 0.74 ml/min/100 g body wt after removal of the pancreas. This resulted in a significant decrease of the estimated IRG release (Table 1). Evisceration was followed by a further decrease in circulating IRG: 0.08 ± 0.01 ng/ml; insulin, 10 ± 2 μU/ml; and blood glucose, 61 ± 6 mg/dl at 100 min after evisceration. Plasma samples submitted to acid-ethanol extraction demonstrated a similar stepwise decrease after the removal of the pancreas, then of the stomach. The assays of C-terminal and N-terminal IRG gave comparable values except for the plasma extracts from eviscerated animals (Table 2).

STIMULATION AND SUPPRESSION OF GASTRIC IRG RELEASE

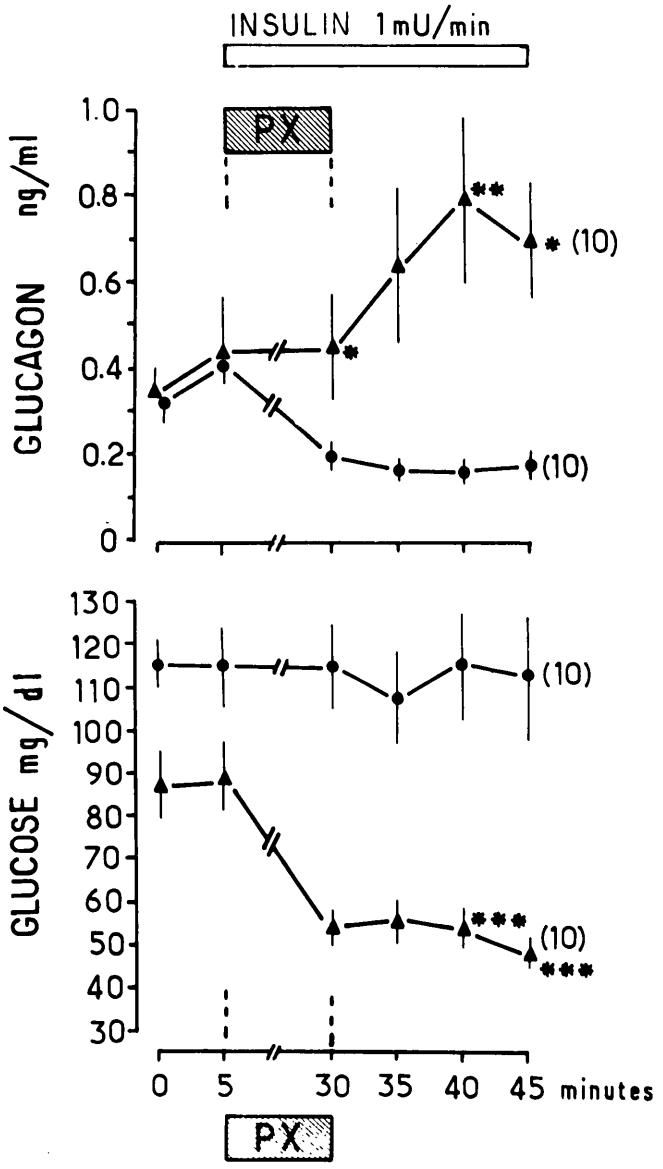
Acute variations in blood glucose level and glucose availability. Acute insulin-induced hypoglycemia provoked, in control rats, a prompt rise in portal IRG, reaching 1.50 ± 0.20 ng/ml at 20 min (N = 10; P < 0.001 vs. basal value; concomitant blood glucose 30 ± 5 mg/dl). In the pancreatectomized rats, acute hypoglycemia also provoked an IRG rise to 0.75 ± 0.18 mg/ml at 20 min (N = 10; P < 0.01 vs. basal; concomitant blood glucose 47 ± 5 mg/dl) (Figure 2). A significant negative correlation between the corresponding IRG and blood glucose values (r = 0.71; P < 0.01) appeared.

Infusion of glucose plus insulin, resulting in high blood glucose levels (350–400 mg/dl), partially suppressed portal IRG (0.13 ± 0.01 mg/ml; N = 4; P < 0.001). The abrupt termination of insulin infusion was followed by a slight IRG rebound to 0.31 ± 0.06 ng/ml in spite of high blood glucose levels (350 ± 10 mg/dl).

Infusion of 2-deoxyglucose induced an increase in IRG in pancreatectomized rats (0.30 ± 0.06 ng/ml; N = 4; P < 0.05 vs. controls) while blood glucose rose from 110 ± 2 to 196 ± 5 mg/dl.

Electrical stimulation of vagus nerves. In control rats,

FIGURE 2. Blood glucose and IRG level in portal plasma, after intravenous infusion of saline (●) or insulin (▲) in pancreatectomized rats. The hatched area represents the duration of total pancreatectomy. The white rectangle represents the infusion of insulin or, in control rats, of saline physiologic solution. Number of animal figures in parentheses. Results are presented as mean values ±SEM. Statistically significant differences between (▲) and (●) are: *, P < 0.05; **, P < 0.01; ***, P < 0.001.



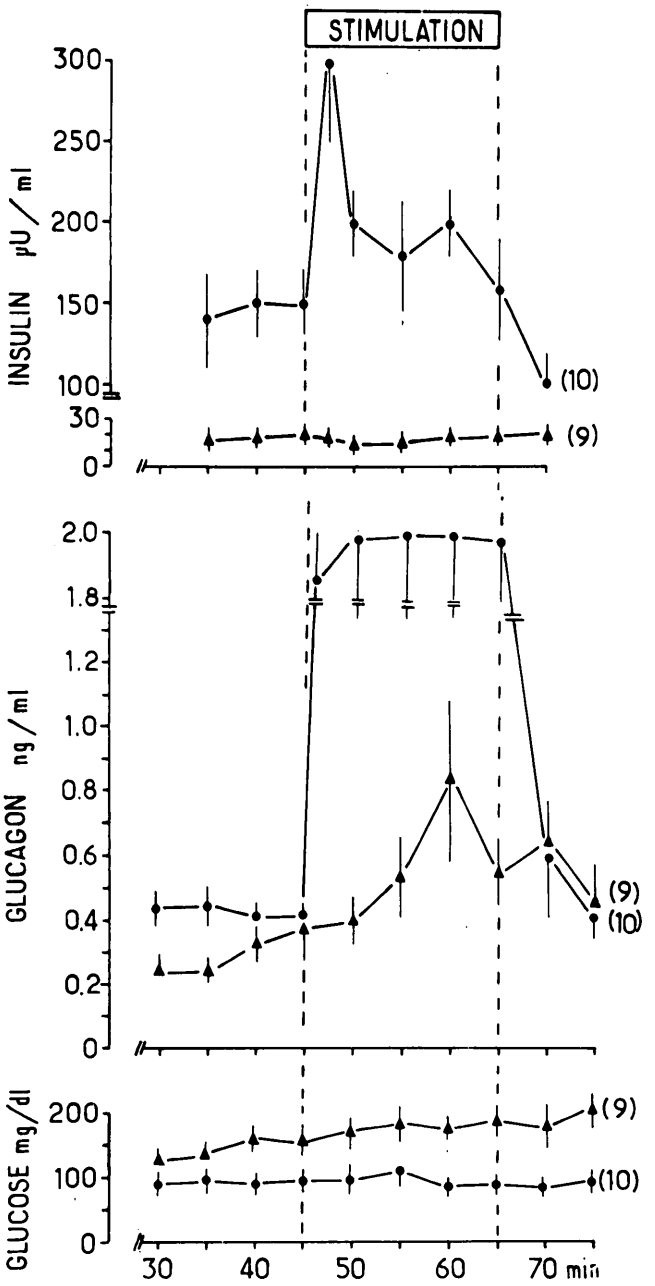


FIGURE 3. Plasma insulin, glucagon, and glucose levels in control (●) and pancreatectomized (▲) rats during electrical stimulation of both vagus nerves. Samples were obtained from portal vein. Results are presented as mean values ±SEM. Number of rats is in parentheses.

vagal stimulation induced a release of both insulin (300 ± 50 mU/ml at 5 min) and IRG (2.02 ± 0.02 ng/ml; N = 10; P < 0.001). Blood glucose did not vary significantly. In the pancreatectomized rats (Figure 3), insulin remained below 18 μU/ml while IRG rose to 0.84 ± 0.27 mg/ml (N = 9; P < 0.05). Glucose rose progressively to 208 ± 12 mg/dl at the end of stimulation.

The complete section of both vagus nerves at the cardiac level reduced substantially the effect of acute hypoglycemia on portal IRG (0.31 ± 0.02 ng/ml).

Arginine infusion. Arginine induced a prompt biphasic insulin and glucagon rise in control rats, while blood glucose did not vary significantly (Figure 4). In the pancreatectomized rats, insulin remained low while glucagon rose progressively to 0.45 ± 0.15 ng/ml (N = 9; P < 0.01 vs. basal). Blood glucose rose to 195 ± 20 mg/dl.

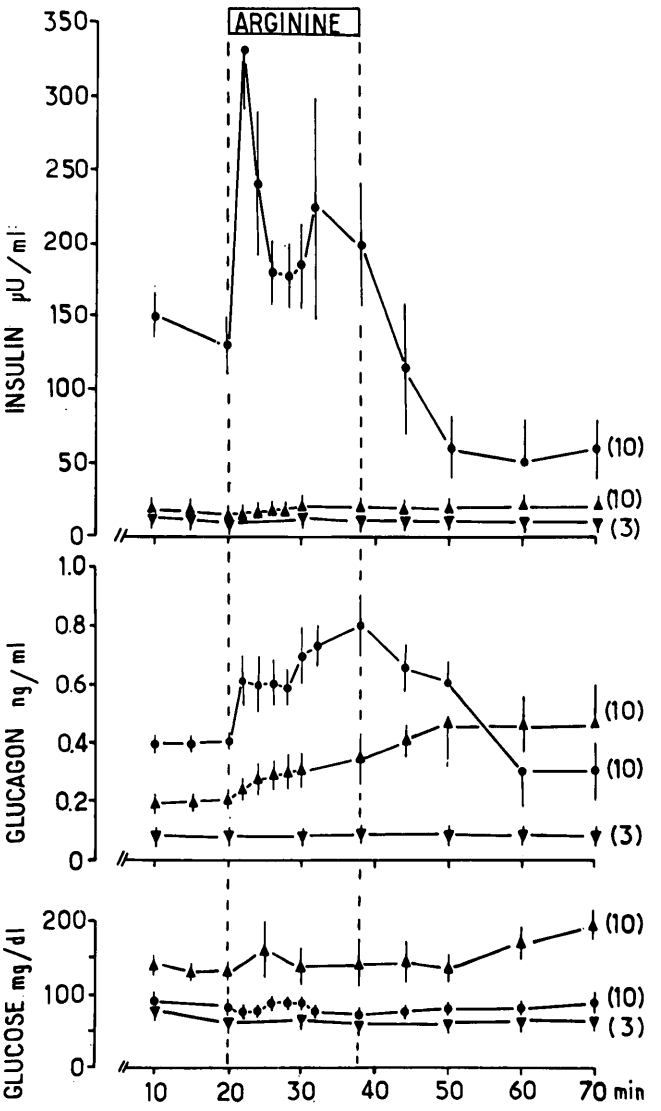
In eviscerated rats, arginine infusion did not induce a change in either arterial plasma insulin (10 ± 3 μU/ml) or IRG (0.08 ± 0.01 ng/ml) concentrations.

Potentiation, by hypoglycemia, of vagal and arginine effects on IRG level. When the pancreatectomized rats were rendered hypoglycemic by a slow intravenous insulin infusion, the IRG increments during vagal stimulation were markedly higher than in nonhypoglycemic rats (1.39 ± 0.47 ng/ml; N = 4; P < 0.005 vs. basal). The same occurred during arginine infusion: IRG rose to 1.47 ± 0.34 ng/ml (N = 6; P < 0.005 vs. basal; concomitant blood glucose value 38 ± 5 mg/dl). These results are summarized in Figure 5.

INFLUENCE OF SUBTOTAL PANCREATECTOMY AND OF GASTRECTOMY ON PORTAL IRG

Total pancreatectomy is a stressful surgical procedure. In order to make valid comparisons with similarly stressed rats in the presence of functional pancreatic A cells, sub-

FIGURE 4. Plasma insulin, glucagon, and glucose, in control (●), pancreatectomized (▲), and eviscerated rat (▼) submitted to intravenous arginine infusion. Blood samples were obtained from portal vein in (●) and (▲), and the carotid artery in (▼). Same presentation as in the preceding figures.



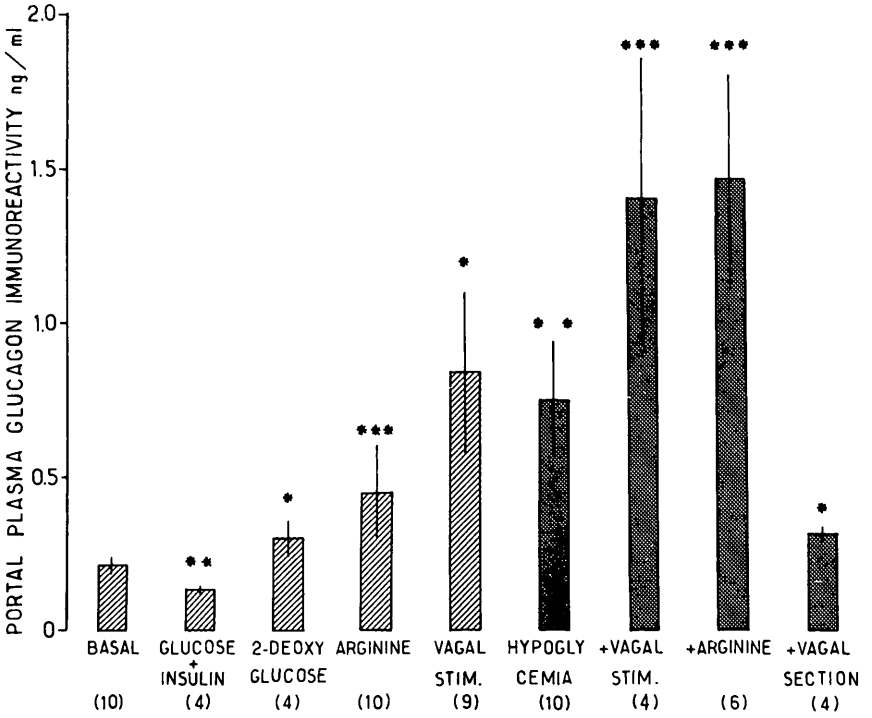


FIGURE 5. Portal plasma IRG in depancreatized rats in the absence of stimulation (basal) and during various stimulatory procedures. The values are the mean of peak values measured in individual experiments ±SEM. Asterisks denote statistically significant differences with the "basal" state: *, $P < 0.05$; **, $P < 0.01$, and ***, $P < 0.005$. Darker columns indicate the presence of a concomitant hypoglycemia. Number of animals is indicated in parentheses.

total pancreatectomies were performed. In subtotally depancreatized rats, portal IRG was 0.63 ± 0.10 ng/ml (N = 4; $P < 0.01$ vs. control rats and $P < 0.01$ vs. totally pancreatectomized rats). This portal IRG level was increased by vagal stimulation (1.46 ± 0.18 ng/ml) and by arginine infusion (0.91 ± 0.02 ng/ml). Portal insulin was 5 ± 3 μU/ml at the end of the surgical procedure and 96 ± 21 μU/ml during arginine infusion. Plasma glucose was 204 ± 8 mg/dl.

In the gastrectomized rats with a pancreas present, portal IRG was 0.93 ± 0.17 ng/ml and insulin 164 ± 36 U/ml at the end of surgery, rising to 3.07 ± 0.80 ng/ml and 404 ± 37 μU/ml, respectively, during arginine infusion. These results are summarized in Tables 3 and 4.

INFLUENCE OF ACUTE ALLOXAN DIABETES ON PORTAL IRG

Alloxan-diabetic rats displayed high glucose (650 ± 50 mg/dl) and glucagon (1.83 ± 0.21 ng/ml) concentrations and a low insulin concentration (6 ± 2 μU/ml). Arginine infusion induced a supplementary IRG rise (4.71 ± 1.05 ng/ml; N = 10) while insulin remained significantly lower than in control rats (42 ± 22 μU/ml).

Total pancreatectomy in diabetic rats was followed by a decrease in IRG (0.36 ± 0.02 ng/ml). This value remained significantly higher than in nondiabetic pancreatectomized rats ($P < 0.001$).

Gastrectomy in diabetic rats was followed by a large increase in portal IRG (3.92 ± 0.09 ng/ml). Intravenous arginine infusion induced an even larger IRG rise 6.42 ± 0.52 ng/ml (Table 3).

IRG CONTENT OF THE STOMACH AND PANCREAS

IRG concentration in the stomach was 21 ± 0.4 ng/g wet tissue, as calculated from the 0.1 dilution of extracts (N-terminal assay system; N = 4; mean weight of stomach, 1.53 g). The serial dilution of extracts did not follow the standard curves. The C-terminal assay gave lower values (4.00 ± 0.03 ng/g). IRG concentration in pancreas of the same animals was 4.95 ± 0.87 μg/g wet tissue for the N-terminal IRG and 4.49 ± 0.87 μg/g wet tissue for the C-terminal IRG. Serially diluted extracts followed the standard curve in both assay systems.

No glucagon-positive cells could be detected in any part of the four stomachs studied, while somatostatin positive cells were detected in all of them. Glucagon positive cells

TABLE 3
Plasma glucagon immunoreactivity (IRG, ng/ml) in the various groups studied

Group of rats	Number of experiments	Basal state	Peak value during arginine infusion
Control rats	10	0.41 ± 0.02	1.65 ± 0.25
Depancreatized rats	10	0.22 ± 0.01	0.45 ± 0.15
Subtotally depancreatized rats	4	0.63 ± 0.10	0.91 ± 0.20
Gastrectomized rats	6	0.93 ± 0.17	3.07 ± 0.80
Diabetic rats (nonoperated)	10	1.83 ± 0.21	4.71 ± 1.05
Diabetic rats, depancreatized	3	0.36 ± 0.02	0.36 ± 0.02
Diabetic rats, gastrectomized	6	3.92 ± 0.09	6.42 ± 0.52
Eviscerated rats	3	0.08 ± 0.01	0.08 ± 0.01

Results are presented as mean values ± SEM.

TABLE 4
Plasma insulin (μU/ml) in basal state and during arginine infusion

Group of rats	Number of experiments	Basal state (μU/ml)	Peak value during arginine infusion
Control rats	10	150 ± 20	330 ± 50
Totally depancreatized rats	10	14 ± 2	14 ± 2
Subtotally depancreatized rats	4	5 ± 3	96 ± 21
Gastrectomized rats	6	164 ± 36	404 ± 37
Diabetic (nonoperated) rats	10	6 ± 2	42 ± 22
Eviscerated rats	3	10 ± 2	10 ± 2

Results are presented as mean values ± SEM.

were present in the pancreas, using the same antiserum.

DISCUSSION

The present results show that glucagon immunoreactivity was still present in the plasma of acutely depancreatized rats. Pancreatectomy seems to have been relatively complete: plasma IRI was extremely low, less than one-tenth of the concentration measured in portal plasma prior to pancreatectomy, and even lower than the concentration measured, in peripheral plasma, by others after the pancreatectomy.[15] This IRI concentration, furthermore, was not stimulable. The fact that IRI, albeit very low, was still detectable one full hour after pancreatectomy can be explained by the transient return to plasma of insulin molecules previously combined with membrane receptors, particularly on hepatocytes, or the return of IRI from bile to plasma after ligation of the choledocus, rather than by an extrapancreatic source of IRI.[16] It may also mean that a few islets remained adherent to the portal vein. Most of the extrapancreatic IRG appeared to be released by the stomach since a sharp decrease in IRG followed gastrectomy. The factors controlling gastric IRG release appeared very similar to those that control pancreatic A cells, e.g., acute hypoglycemia, glucopenia, arginine, and vagal stimulation. The hyperglycemic potency of the gastric IRG can only be suggested in the present study, on the basis of a slight supplementary glucose rise induced by both arginine infusion and vagal stimulation in the pancreatectomized rats. The glucose rise was moderate; this may be due to the 12-h fast that preceded all experiments and may have depleted the hepatic glycogen stores. A trend toward hypoglycemia was observed after gastrectomy plus pancreatectomy; this may be related to the suppression of gastric and pancreatic A cells or merely to the extreme reduction in hepatic blood flow after evisceration. But some groups presented evidence that dog gastric glucagon does have biologic activity[6,9,31] and it is reasonable to assume that rat gastric glucagon also has biologic activity.

Most of the present results are consistent with other studies concerning gastric IRG.[6–13,15] However, slight differences can be noted. Gastric IRG seems to be less abundant and physiologically less important in the rat than in the dog. In this latter species, a particular abundance of IRG in the gastric mucosa and in plasma after pancreatectomy has been noted.[33] The high content of glucagon in the dog gastric mucosa (which equals that of the pancreas) may be specific to that species:[11] only small amounts of IRG were found in the stomach of primates[35] and rats[36] and few A cells were identified in rat gastric mucosa.[37] Higher IRG levels were measured in plasma of pancreatectomized rats studied several days after the pancreatectomy.[15] This delay may have been long enough to allow for a progressive hypertrophy of gastric A cells. Furthermore, the completion of pancreatectomy may have been less drastic than in our study since the duodenum was still present and nonnegligible insulin concentrations were measured in peripheral plasma (13 ± 2 μU/ml).[15]

The plasma from eviscerated rats contained low amounts of IRG, a result that is at variance with other studies.[16–18] A minor part can be attributed to the globulin interference,[38] as suggested by the comparison of unextracted with extracted plasmas. Acid-ethanol extraction discards mainly the big plasma glucagon (BPG) immunoreactivity associated with globulins.[24] The physiology of BPG is largely unknown, but it has been proposed that it may represent glucagon fragments associated with larger protein (globulin) carriers (Conlon, unpublished observations). The persistence of N-terminal IRG in plasma from eviscerated rats may be due to the presence of glucagon fragments,[24] or to some IRG from another source, e.g., the salivary gland,[18] while C-terminal IRG concentration varied in plasma as expected from A cell secretion. The N-terminal IRG is considered as less specific since its concentration rises in plasma after ingestion of glucose.[24] N-terminal IRG is present in enteric extracts where no "true glucagon" has been detected.[24,25] It may represent hormone(s) distinct from glucagon, although immunochemically related to it. However, it must be stressed that the degradation of glucagon 3500 generates N-terminal immunoreaction fragments.[24] High insulin and IRG values have been measured in plasma from eviscerated rats.[16,17] These rats were kept living for more than a few hours and the choledocus was connected with the rectum, two conditions differing from those used in the present work. A sufficient interval of time may also be necessary for the progressive increase of IRG from extrapancreatic and extradigestive sources.

The factors controlling rat gastric A cells appeared very similar, but not fully identical, to those depicted in other works.[9,13,39,40] Arginine was an efficient stimulator, but no biphasic pattern of release was observed. The gastric IRG release was very moderate in nonhypoglycemic rats. This is at variance with what happened in the isolated perfused dog stomach.[13] In contrast, hypoglycemia alone or in conjunction with arginine or with vagal stimulation appeared to be a major stimulatory factor. Hyperglycemia alone, in the absence of exogenous insulin, did not suppress the gastric IRG release. Furthermore glucopenia, as induced

by 2-deoxyglucose, stimulated gastric A cells. These features are consistent with a predominant role of glucose metabolism, rather than extracellular glucose levels, in controlling A cell function. The B cell may be the glucose sensor of A cells.[5,40] The exquisite sensitivity of gastric A cells to insulin has been well demonstrated by the use of both in vitro preparation[13] and different insulin infusion rates in the pancreatectomized dogs.[7]

A major difference with previous results[13] concerns the effects of vagal stimulation, while no gastric IRG release followed the stimulation of peripheral vagal fibers in the dog stomach perfused in vitro. This induced a frank gastric IRG release in rats; furthermore, vagal section abolished most of the stimulatory effects of hypoglycemia, suggesting that a part of this stimulation was neurally mediated. The effect of acute hypoglycemia after complete section of both vagus nerves, although statistically above the baseline, differed significantly from the IRG rise after hypoglycemia in rats with intact vagi (Figure 5). This would indicate that gastric A cells have a limited intrinsic glucose sensing capability when they are apart from the B cells. Finally, in the rat, no fundamental difference was found between the factors controlling pancreatic A cells and gastric A cells.

The numerical importance of gastric A cells seems relatively limited, at least in acutely depancreatized rats and in rats rendered acutely diabetic by alloxan. The IRG content of the stomach was low. No IRG-positive cells were identified in the stomach by an immunofluorescence technique that stained A cells in the pancreatic islets. An immunochemical difference between gastric IRG and pancreatic glucagon may explain this negative result, as well as the discrepancy in gastric IRG content when measured with two different antisera. It seems clear, in any case, that the stomach is not a major IRG secreting organ in the rat, after acute pancreatectomy, and alloxan diabetes. In these short term conditions, in the rat, the situation may be similar to that observed in the cat,[39] the pig,[42] and the well controlled pancreatectomized man,[1,2,41] but not to that documented in the dog.[6–8,43]

A remarkable increase in basal and stimulated levels of glucagon was observed after gastrectomy, when the pancreas was left intact. This was attributed at a first glance to the superimposition of a serious surgical stress, stimulating A cells. The eventual removal of factors elaborated by the stomach with a suppressive effect on pancreatic A cells, i.e., somatostatin, cannot be excluded; a trend toward an increase in insulin was also detectable in these rats, in basal state, and this became significantly higher after stimulation by arginine (Table 4). The same pattern was observed in some of the alloxan-diabetic rats operated in the same way (data not shown). This insulin rise is hardly consistent with a stress situation and could be better explained by the removal of somatostatin as another suppressive factor.

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