Absence of Islet Alpha Cell Function in Pancreatectomized Patients

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Summary. Plasma immunoreactive glucagon, C-peptide and substrates (glucose, lactate, and alanine) were measured in 21 pancreatectomized patients and 28 patients with chronic calcifying pancreatitis during arginine infusion. Results were compared with those obtained in control and in insulin-dependent diabetic subjects, and in pancreatectomized subjects receiving a combined infusion of glucagon and arginine or somatostatin and arginine. Plasma immunoreactive glucagon in the pancreatectomized patients was 230 ± 26 pg/ml (control subjects 100 ± 13 pg/ml, $p < 0.001$), but was unchanged following arginine or somatostatin. Following ethanol extraction of plasma it became undetectable. Similar results were obtained in patients with chronic pancreatitis. In contrast to the insulin-dependent diabetic subjects, no changes in blood glucose, lactate, and alanine concentrations were found during arginine infusion in the pancreatectomized or pancreatitis patients. Addition of glucagon restored the metabolic response to arginine in the pancreatectomized patients. Our results confirm previous smaller studies that in pancreatectomized patients, A cell function is absent or insignificant.

Key words: Glucagon, A cell, pancreatectomy, diabetes, arginine, somatostatin, pancreatitis, glucose, big plasma glucagon.

It is well established that in several animal species glucagon-like material can be released from extra-pancreatic sites [1–6]. In the dog, the stomach produces substantial amounts of glucagon which is immunologically [7] and biologically [8, 9] indistinguishable from pancreatic glucagon. In human subjects, however, the situation is less clear, although several authors have considered that pancreatectomized sub-

jects represent a model of diabetes without glucagon [10–12]. The final solution of this problem is fraught with difficulties: 1) totally pancreatectomized subjects are not often available for metabolic and hormonal studies; 2) in many cases, the stomach and duodenum, as potential sources of extra-pancreatic glucagon [13], have been removed with the pancreas; and 3) cross-reacting substances of gastrointestinal origin [14, 15] and the presence in plasma of an interfering globulin [16] or 'big plasma glucagon' [17] may render the interpretation of radioimmunoassays difficult. In order to clarify this problem, 21 pancreatectomized subjects were submitted to arginine infusion and plasma glucagon immunoreactivity (IRG) was measured with a C-terminal antibody before and after ethanol extraction. The metabolic responses to arginine were assessed by measurement of blood glucose, lactate and alanine levels. The patients were compared with normal subjects, patients with chronic calcifying pancreatitis, and insulin-dependent diabetics free of detectable chronic pancreatitis.

Subjects and Methods

Subjects

The main clinical characteristics of the subjects are shown in Table 1. Of the 21 pancreatectomized subjects, two had undergone operation because of exocrine pancreatic carcinoma, and one because of an intractable juxtaoduodenal insulinoma. All the others had presented either with acute necrotizing pancreatitis or with a chronic pancreatitis. Total pancreatectomy was performed by a standardised technique, totally removing the pancreatic gland, the duodenum, the spleen and the antropyloric two-thirds of the stomach [18]. In the second group the diagnosis of chronic pancreatitis was based on the presence of chronic diarrhoea and steatorrhoea, abdominal pain, and radiological evidence of pancreatic calcification. Most of these patients were chronic abusers of ethanol. Six had been submitted to a partial resection (four-fifths) of the pan-

Table 1. Clinical details of the groups studied

Group	Age (years)	Sex (M : F)	% Ideal body weight	Duration of the disease (years)	Daily insulin dose (U)
Control subjects (<i>n</i> = 31)	36 ± 3	15 : 16	99 ± 1	–	–
Pancreatectomized subjects (<i>n</i> = 21)	39 ± 4	10 : 11	97 ± 1	2 ± 1	35 ± 6
Chronic pancreatitis (<i>n</i> = 28)	52 ± 2	18 : 10	93 ± 3	7 ± 3	30 ± 8
Diabetic subjects (<i>n</i> = 12)	42 ± 2	6 : 6	96 ± 3	7 ± 2	50 ± 4

Table 2. Basal values (08.00 h) for circulating substrates and hormones in the groups studied

Group	Blood glucose (mmol/l)	Blood lactate (mmol/l)	Blood alanine (mmol/l)	Plasma IRG Unextracted plasma (pg/ml)	Plasma IRG Extracted plasma (pg/ml)	Plasma C-peptide (ng/ml)
Control subjects (<i>n</i> = 31)	4.4 ± 0.1	0.80 ± 0.01	0.47 ± 0.03	100 ± 13	45 ± 5	2.80 ± 0.20
Pancreatectomized subjects (<i>n</i> = 21)	8.7 ± 1.0 ^c	1.86 ± 0.13 ^c	0.60 ± 0.05 ^a	230 ± 26 ^c	28 ± 5 ^a	0.22 ± 0.05 ^c
Chronic pancreatitis (<i>n</i> = 28)	8.7 ± 0.8 ^c	1.35 ± 0.08 ^c	0.78 ± 0.09 ^b	242 ± 13 ^c	48 ± 10	0.72 ± 0.10 ^c
Diabetic subjects (<i>n</i> = 12)	8.7 ± 1.6 ^c	0.82 ± 0.05	0.58 ± 0.14	191 ± 26 ^c	135 ± 29 ^c	0.99 ± 0.10 ^c

Results are presented as mean values ± SEM

Significance of differences from control values is denoted by: ^a*p* < 0.05, ^b*p* < 0.01, ^c*p* < 0.001

creas, but gastrectomy had been performed in none of them. Liver function was assessed by measurement of the coagulation factors, of serum alkaline phosphatase concentration and bromsulphthalein excretion. The third group consisted of insulin-dependent diabetic patients, free of detectable chronic calcifying pancreatitis. Insulin was administered as either two daily injections of Rapitard insulin or three daily injections of Actrapid insulin, the last dose being given at 20.00 h, 12 h before the tests (unless otherwise stated). Pancreatectomized patients and subjects with chronic pancreatitis were placed on a carbohydrate-rich (300 g/day) and low fat (20 g/day) isocaloric diet. None of them received biguanides or drugs other than insulin. Pancreatic enzyme supplements (Eurobiol) were administered routinely to all pancreatectomized and pancreatitis patients. Tests were performed at 08.00 h after an overnight fast, the morning insulin injection being omitted. Chronic pancreatitis patients with chronic ethanol ingestion had been abstinent for at least 12 h. Informed consent was obtained from all subjects and the investigation was approved by the INSERM Ethical Committee.

Protocol

Arginine infusion test: Arginine HCl (25 g), dissolved in 400 ml sterile water, was infused at constant flow rate for 30 min into an antecubital vein, using an Infusomat pump (Braun, Melsungen, FGR). Blood samples were obtained from a contralateral ante-cubital vein, two to four basal samples being collected during the 30 min before infusion, then at 15, 30, 45, 60 and 90 min after the start of infusion. Blood samples (5 ml each) were drawn with a heparinized syringe and distributed into chilled tubes, containing 2000 U aprotinin (Trasylol) and 1.2 mg EDTA/ml blood for hormone assays or 1 ml of 10% perchloric acid/ml blood for substrate determinations. Samples were centrifuged at +4 °C, then the plasma was rapidly frozen and kept at –20 °C until assay. A total of 31 control subjects, 21 pancreatectomized patients, 28 with chronic pancreatitis and 12 diabetic subjects free of detectable pancreatitis were tested.

Somatostatin + Arginine infusion: Cyclic somatostatin (Clin Midy, Paris, France) was dissolved in 0.154 mol/l saline solution immedi-

ately before use. It was then injected IV as a bolus of 1 mg followed by an infusion of 1 mg over 30 min, at a constant flow rate, mixed with arginine; four control subjects and eight pancreatectomized subjects underwent this test.

Glucagon + Arginine infusion: Crystalline glucagon (Novo, Copenhagen) was dissolved in 0.154 mol/l saline solution immediately before use. A bolus IV dose of 8 µg was given, followed by 0.57 µg/min for 30 min, mixed with arginine. These doses have been shown to produce glucagon concentrations in plasma comparable to those measured in uncontrolled diabetic patients [19]. Four pancreatectomized patients received this infusion.

Other tests: Arginine (25 g) was administered orally, after an overnight fast, to six pancreatectomized patients; glucose (50 g) to four pancreatectomized patients. Interruption of insulin therapy for 36 h was performed in one pancreatectomized patient, after which a IV arginine infusion was performed.

Assays

Glucagon immunoreactivity: IRG was determined in plasma samples using the 30 K antiserum (Dr. R. H. Unger, Dallas, Texas) [20] the standard curves being constructed in 0.2 mol/l glycine buffer containing 5 g/l serum albumin and 10 g/l sheep serum. The lowest amount of glucagon standard which gave a significant variation of the bound/free ratio from the zero standard was 7 pg/ml, corresponding in whole plasma to a detection limit of 21 pg/ml. Inter-assay variation was less than 5%. Aliquots of plasma were also submitted to ethanol fractionation [21]: 0.2 ml plasma were mixed with 0.36 ml ethanol 96% (v/v) at 5 °C, then centrifuged at 4 °C; the supernatant was lyophilized and resuspended in 0.2 ml glycine buffer (0.2 mol/l, pH 8.6) containing 2.5 g/l bovine serum albumin. Recovery was 75%–85%: the values determined after the extraction procedure were corrected accordingly.

Other methods: C-peptide was determined [23] using an antiserum directed against human synthetic C-peptide (BYK. Mallinckrodt, Dietzenbach, FGR); the lowest amount of C-peptide standard giv-

ing a significant variation of the bound/free ratio from zero standard was 0.2 ng/ml (whole plasma). The inter-assay variation was less than 4%.

Glucose was measured by a glucose-oxidase method [24] adapted for the Beckman analyzer (glucose analyzer Mark 3G A₂, Beckman Instruments, Fullerton, CA). Alanine and lactate were determined by enzymatic methods [25, 26].

Calculations

Results are presented as mean $\pm$ SEM. Statistical analysis was performed employing Student's *t* test for unpaired samples [27].

Results

Basal Values (Table 2)

After an overnight fast, blood glucose was significantly higher in all diabetic groups than in control subjects. Blood lactate and alanine were significantly higher in both the pancreatectomized patients and the subjects with chronic pancreatitis than in the two other groups. This difference was particularly marked for lactate in the pancreatectomized group.

Plasma C-peptide values were lower in all diabetic groups than in control subjects. In all pancreatectomized patients they were undetectable. Plasma IRG levels in unextracted plasma samples were significantly higher in all diabetic groups than in the control subjects. However, following extraction by ethanol, IRG levels were 28 ± 5 pg/ml in the pancreatectomized patients, almost at the limit of sensitivity and significantly lower than in control subjects ($p < 0.001$). In the patients with chronic pancreatitis, extracted plasma IRG levels were not significantly different from controls: 45 ± 5 versus 48 ± 10 pg/ml. Liver function tests were within normal limits in all subjects.

Arginine Infusion Test

In control subjects (Fig. 1) arginine infusion induced the expected significant rise in C-peptide and IRG concentrations. Blood lactate decreased significantly, while no significant changes occurred in glucose and alanine.

In the insulin-dependent patients arginine induced a rise in glucagon concentration, more pronounced than in controls, while the C-peptide level remained lower than normal and did not increase significantly. Blood glucose rose, while lactate and alanine concentrations declined, these changes being significant and related in time to the rise of plasma IRG.

In the pancreatectomized patients (Fig. 2) plasma IRG (unextracted samples) did not change signifi-

Fig. 1. Effect of arginine infusion on plasma hormone and blood substrate concentrations in control subjects ($n = 15$) and insulin-dependent diabetics ($n = 17$). $\blacksquare$ = arginine infusion. Results are presented as mean values $\pm$ SEM. * statistical significance of differences with basal value in the same group ($p < 0.01$). Basal values were calculated as the mean of results in two to six samples collected for the 30 min pre-infusion period in each patient. Plasma glucagon (IRG) values are given for samples not submitted to extraction with ethanol

cantly during the arginine infusion. C-peptide levels remained undetectable. No significant changes in blood glucose, lactate and alanine concentrations were found. The subjects with chronic pancreatitis displayed a pattern similar to that of pancreatectomized patients. However, a slight but significant rise of IRG level occurred at 30 and 90 min ($p < 0.05$); C-peptide values, although measurable, did not rise. Blood glucose, lactate and alanine concentrations did not change significantly.

The pattern of plasma IRG was markedly changed by ethanol extraction of samples (Fig. 3). In control subjects, IRG values in extracted samples rose acutely during the stimulation period and were not different from the values obtained before extraction over this period. In contrast, extractable IRG in the pancreatectomized patients remained below the

Fig. 2. Effect of arginine infusion on plasma hormone and blood substrate concentrations in pancreatectomized patients ($n = 21$) and patients with a chronic pancreatitis ($n = 28$). Same presentation as in Fig. 1

Fig. 3. Effect of arginine infusion on glucagon (IRG) concentrations measured in plasma samples before (●) and after (▲) extraction with ethanol in 31 control subjects, 21 pancreatectomized patients and 28 patients with chronic pancreatitis. Same presentation as in Fig. 1

limit of sensitivity of the assay and did not rise during the stimulatory period. In the patients with pancreatitis, values were higher than in the pancreatectomized patients but did not change significantly (Fig. 3).

Somatostatin + Arginine, and Glucagon + Arginine Infusions

As shown in Figure 4, addition of somatostatin to the arginine infusion did not modify the pattern observed in the pancreatectomized patients. No change was found in plasma IRG, blood glucose, lactate, alanine and plasma C-peptide concentrations. In control subjects, the same combined infusion induced the expected suppression of IRG and C-peptide levels followed by a rebound at the end of infusion. In contrast, when exogenous glucagon was added to the arginine infusion in pancreatectomized subjects, a significant increase in blood glucose occurred, accompanied by significant decreases in blood lactate and alanine concentrations.

Other Tests

Interruption of insulin therapy: In one pancreatectomized patient, insulin therapy was interrupted for 36 h, and this patient was submitted to a complete fast for 24 h. At end of this period, IRG concentration in plasma (unextracted samples) was 318 pg/ml (mean of two samples; NS versus overnight fasting state). Arginine infusion test at that time, when blood glucose was 23 mmol/l, did not induce any rise in IRG.

Ingestion of arginine (Table 3): Oral ingestion of 25 g arginine HCl did not significantly modify the plasma IRG concentration in six pancreatectomized patients.

Oral glucose tolerance test (Table 3): Ingestion of 50 g glucose induced, in a group of four pancreatectomized subjects, a slight but significant increase in IRG concentration ($p < 0.05$), as measured by the 30 K antiserum in plasma samples not submitted to extraction by ethanol.

Discussion

Our results show that in pancreatectomized patients plasma IRG was detectable before ethanol extraction but disappeared or remained close to the limit of assay sensitivity following this extraction. Furthermore, plasma IRG in unextracted samples did not rise during arginine infusion in these patients and remained barely detectable in extracted samples. Somatostatin infusion did not influence this pattern in eight patients. The modifications of blood glucose, lactate, alanine which follow arginine infusion in insulin-dependent diabetics were not observed in the pancreatectomized group, suggesting the absence of release of biologically active glucagon. In the patients with

chronic pancreatitis, whose stomachs were still present, the hormonal and metabolic pattern was very similar to that observed in pancreatectomized (and partially gastrectomized) patients. Infusion of exogenous glucagon in pancreatectomized subjects reproduced the changes in glucose, lactate and alanine which are observed when endogenous glucagon is released under influence of IV arginine, in the absence of any

concomitant insulin release. These points are consistent with the concept of an exclusive pancreatic source of glucagon in man.

At least 17 papers have been published on the debated point of extra-pancreatic glucagon in man, based on approximately 52 pancreatectomized subjects and 65 patients with chronic pancreatitis and/or partial resection of the pancreas (Table 4). The majority of published results suggest that extra-pancreatic sources of immunoreactive and biologically active glucagon are insignificant in man: plasma glucagon in the basal state was either undetectable, or significantly lower than in normal and in diabetic subjects [9, 11, 12, 15, 29–36]. Furthermore a deficient or absent plasma IRG response occurred following IV infusion of amino acids [9, 11, 15, 28, 30, 33, 34, 36], interruption of insulin therapy [9] or insulin-induced hypoglycaemia [29, 30]. This latter test is however less meaningful than the others, since diabetic A cells are not as sensitive to hypoglycaemia as normal A cells [37]. The lack of suppression of plasma IRG by somatostatin [34] is another line of evidence for the non-hormonal nature of 'big plasma glucagon'. In most of these papers a C-terminal antiserum was used, usually the 30 K antiserum, and various procedures were employed to avoid non-specific plasma interference. These include extraction procedures [9, 11, 12, 15, 31, 33, 38] and/or construction of standard curves with 'deglucagonized' plasma [9, 11]. It must be emphasized, however, that proving the absence of a factor is much more difficult and problematic than confirmation of its existence. The particular metabolic features observed in these patients may be more significant. These include high blood alanine levels in the basal state [38], the delayed rise of blood glucose and ketone bodies following cessation of insulin therapy [9, 12] and the absence of a rise in blood glucose during arginine infusion [6, 11, 15, 34]. The present study is consistent with these previous results and documents further the presence of high lactate levels in the basal state and the persistence of blood alanine during arginine infusion. These metabolic features may repre-

Fig. 4. Effect of arginine infusion on plasma hormone and blood substrate concentrations in pancreatectomized patients submitted concomitantly to an IV infusion of somatostatin (left panel, $n = 8$) and an IV infusion of glucagon (right panel, $n = 4$). Same presentation as in Fig. 1

Table 3. Plasma IRG values (pg/ml; unextracted plasma samples) measured in pancreatectomized patients submitted to an oral arginine load and oral glucose tolerance test

	Time (min)									
	−15	0	15	30	45	60	75	90	120	
Oral arginine load (25 g) (<i>n</i> = 6)	190 ± 42	187 ± 41	199 ± 45	207 ± 41	207 ± 45	307 ± 82	223 ± 45	214 ± 26	229 ± 42	
Oral glucose tolerance test (50 g) (<i>n</i> = 4)	140 ± 34	149 ± 30	185 ± 42	260 ^a ± 38	216 ± 42	236 ± 39	213 ± 28	180 ± 50	165 ± 27	

Results are presented as mean values $\pm$ SEM

^a $p < 0.05$ versus basal value in corresponding group

Table 4. Published studies of plasma IRG in pancreatectomized patients and patients with pancreatitis. Numbers in parentheses correspond to bibliography of present paper

Reference	No. of patients	Antiserum used	Extraction procedure	Tests used	IRG changes	Metabolic changes
<i>Pancreatectomized patients</i>						
Müller et al. [11, 15]	5	C-terminal and N-terminal	Charcoal	Arginine	Lower IRG reduced rise	No glucose rise
Villanueva et al. [33]	1	C-terminal	Biogel + charcoal	Arginine	No rise	
Barnes et al. [9, 12]	5	C-terminal	Affinity chromatography	Arginine; Interruption of insulin treatment	Basal IRG = zero No rise	No glucose rise (arginine) Delayed glucose and ketone rise
Gerich et al. [34]	1			Arginine; somatostatin	Basal = zero no change	No glucose change
Donowitz [32, 35]	10	C-terminal		Alanine	Low basal	No glucose rise
Nelson et al. [36]	6	C-terminal		Arginine	Low basal No rise	No amino-acid changes
Boden et al. [38]	4		Sephadex	Somatostatin	Basal = zero No change	
Botha et al. [42]	1	C-terminal	Sepharose; Ethanol	Arginine; hypoglycaemia; oral glucose tolerance test; somatostatin	Normal basal level No IRG rise IRG decrease	
Palmer et al. [41, 43]	5	C-terminal	Acetone	Arginine; Oral glucose tolerance test; somatostatin; hypoglycaemia; insulin interruption		Variable changes
Vranic et al. [40]	1	C-terminal			Normal basal IRG	
Mirouze et al. [44]	6	C-terminal		Oral glucose tolerance test + somatostatin		
<i>Pancreatitis subjects</i>						
Aguilar-Parada et al. [28]	1			Arginine	No IRG rise	
Persson et al. [29]	1			Hypoglycaemia	No rise	
Assan et al. [30]	16	N-terminal		Arginine; hypoglycaemia	Lower IRG Reduced rise	Reduced glucose rise Deeper hypoglycaemia
Kalk et al. [31]	6	C-terminal and N-terminal	Ethanol	Arginine; oral glucose tolerance test	Low basal no change	
Kalk et al. [31]	8	N-terminal	Ethanol	Oral glucose tolerance test	Normal IRG level	Glucose rise

sent a pattern diametrically opposite to that described in the glucagonoma syndrome [39].

Six other studies, however, have documented the presence of IRG or glucagon-like immunoreactivity in plasma from pancreatectomized patients (a total of 13 subjects) and from some groups of patients with

chronic pancreatitis [31, 40, 44]; the 30 K antiserum was used in most of these reports and plasma samples were submitted to various extraction procedures in three studies [31, 42, 43]. However plasma IRG was not increased by arginine [42, 43], adrenaline [42] or cessation of insulin therapy [43]; it was paradoxically

increased by oral glucose or meals [43]. It was also not wholly or partially suppressed by somatostatin [43, 44]. All these features suggest that plasma IRG in these patients is very different from that measured in other situations.

The significance of 'big plasma glucagon' remains obscure. Some extra pancreatic sources of IRG, particularly the salivary glands, have been documented in man [45, 46]; most attempts at conversion of 'big plasma glucagon' to 3,500 dalton IRG using proteolytic digestion seem to have yielded fragments of 2,000 daltons rather than 'true' glucagon [33]. Some peptides reacting with the N-terminal antiglucagon antisera are detectable in plasma from pancreatectomized patients and their concentrations may rise after ingestion of glucose [14] but their biological properties remain difficult to interpret. Thus the properties of A cells appear very different in man and also in the macacus Rhesus [47] from that in dogs [2, 3, 5, 6] and several other animal species [4 (review)].

In pancreatectomized patients, the absence of significant glucagon release may contribute to the well-known tendency of these patients to be prone to insulin-induced hypoglycaemia [47].

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