Glucagon Immunoreactivities and Amino Acid Profile in Plasma of Duodenopancreatectomized Patients

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ABSTRACT Glucagon immunoreactivity (IRG) was measured in plasma of duodenopancreatectomized subjects with a nonspecific (K-4023) and a specific (30-K) glucagon antiserum. After an overnight fast, plasma IRG (K-4023) was significantly (P < 0.05) higher in the subjects without pancreas, averaging 782±79 (SEM) pgeq/ml, than in the controls (482±80 pgeq/ml). IRG (30-K) of 162±68 pg/ml did not change during an infusion of arginine (450 mg/kg per 40 min). Insulin deprivation during 3 d in one patient did not restore the IRG response during 3 d in one patient did not restore the IRG response to arginine as reported in depancreatized dogs.

Bio-Gel P-30 column chromatography revealed that virtually all IRG (30-K) measured in whole plasma was of different molecular weight than glucagon, and primarily of a mol wt ≥ 40,000. Intravenous arginine did not significantly alter the chromatographic pattern of these plasmas. Thus, as postulated by others, duodeno-pancreatectomized humans have virtually no circulating 3,500-dalton glucagon. Hence, the presence of 3,500-dalton glucagon in plasma is not a condition for the diabetic state. It might, nevertheless, when present in normal or excessive amounts, worsen the metabolic state of diabetic patients.

Among 14 amino acids measured in plasma of these patients, the concentrations of alanine, serine, ornithine, and arginine were significantly (P < 0.05) elevated to approximately twice that of normal: alanine and serine are both substrates for gluconeogenesis, whereas ornithine and arginine are involved in the formation of urea, the second product of hepatic gluconeogenesis. As the concentrations of branched chain amino acids were not grossly altered, it is hypothesized that this amino acid pattern is a consequence of glucagon deficiency rather than secondary to the diabetic state of these patients.

INTRODUCTION

The attractive hypothesis proposed by Unger and Orci (1), that diabetic metabolism is not solely a result of insulin deficiency but also requires absolute or relative excess of glucagon, is based on the observation that plasma levels of glucagon are elevated in most forms of diabetes mellitus. This is true for human diabetes (2–5), as well as in many forms of spontaneous and experimental diabetes in animals (6–10), particularly in pancreatectomized dogs (11–15).

For pancreatectomized humans, interpretation of the available information remains confusing. On the one hand, there is agreement that, in these patients, arginine fails to elicit an increase in plasma glucagon (16–18), even though such an increase is readily seen in insulin-deficient pancreatectomized dogs (11–15, 19). On the other, the question of whether or not plasma of humans without pancreas retains near-normal basal levels of glucagon remains controversial. In a previous study, Muller et al. (16) found basal glucagon values of 35 pg/ml, whereas Barnes et al. (17, 18) reported essentially no detectable glucagon in pancreatectomized patients. This discrepancy may be a result of the antiserum used for glucagon immunoassay: Barnes et al. used an antiserum raised at Hammersmith Hospital, London, whereas we employed antiserum 30-K obtained from Dr. R. H. Unger, Dallas, Tex. This study; by investigating glucagon immunoreactivity (IRG) with antiserum

1 Abbreviations used in this paper: GLI, glucagon-like immunoreactivity; IRG, glucagon immunoreactivity.

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30-K in distinct fractions of plasma obtained from duodenopancreatectomized patients, both in the basal state and after an arginine infusion; brings new facts and, hopefully, some answers. This study also examined the possibility that glucagon-like immunoreactivity (GLI) from the gut might be elevated in the plasma of patients without pancreas, as shown by Matsuyama and Foa in pancreatectomized dogs (13). Finally, this study was intended to define whether pancreatectomy results in a disturbance of the profile of amino acid levels in plasma and, thus, presumably in a disturbance of protein metabolism.

METHODS

Eight patients, six males and two females (mean age 57±4 yr [SEM], ranging from 37 to 71 yr) were admitted to either the Hôpital Cantonal, Geneva, Switzerland, the Medizinische Universitätsklinik E, Düsseldorf, West Germany, or the Chirurgische Universitätsklinik, Klinikum Mannheim, West Germany. A total pancreatectomy with splenectomy and partial resection of the stomach (procedure according to Whipple et al. [20]) was performed because of cancer of the exocrine pancreas (five patients) or because of intractable pain associated with chronic pancreatitis (three patients), between 4 mo and 3 yr before study (median interval: 8 mo). The diabetes, a consequence of the operation, was treated in all cases by one daily injection of insulin. During the months preceding investigation, three patients had gained weight, two had remained constant, and three had lost weight; the average being a gain of 0.643±1.001 kg/yr (normalized range: 1.3–4 kg/yr). Diarrhea was rare; none of the patients was treated by irradiation or cytostatic drugs. At the time of study, none of the pancreatectomized patients exhibited an elevated serum bilirubin level, although alkaline phosphatase was slightly elevated in four. The control group consisted of six patients admitted to the hospital for diseases unrelated to metabolic or gastrointestinal disorders (mean age 51±3 yr, four males, two females). After detailed orientation about the experimental procedures and the possible risks involved, informed consent was obtained. The studies were performed in the morning, after a 14- to 17-h fast, and 24 h after the last insulin injection. Arginine hydrochloride was infused into a forearm vein as a 10% solution at a rate of 450 mg/kg per 40 min. At all time points indicated, 6 ml of blood was drawn from a vein of the contralateral forearm and transferred to chilled centrifuge tubes containing 10 mg EDTA Na2, 0.1 ml (3,000 kallikrein inhibitor units) Trasylol (a gift from Doctors Ruf and Aman, Bayer AG, Zurich, Switzerland), and 100 IU lithium heparin, which resulted in optimal anticoagulation. The tubes were then centrifuged, and the plasma was separated and frozen for further examination.

Analyses. Glucose was measured with glucose oxidase (21); lactate (22), acetocetate (23), and β-hydroxybutyrate (24) were also measured enzymatically. IRG was assayed in plasma according to Falona and Unger (25) using, as already stated, specific glucagon antiserum 30-K donated by Dr. Unger. Nonspecific antiserum K-4023 was obtained from Novo Research Institute, Copenhagen, Denmark. Authentic glucagon was used as standard in both instances, and the values obtained with antiserum K-4023 were expressed accordingly as “glucagon equivalents” (pg/g). Partially purified intestinal extracts with GLI yield linear dilution curves with pancreatic glucagon, as reported by Heding et al. (26). The binding of radio-labeled glucagon by the patients plasma above that of buffer was 6.2±0.7% (range: 4.5–8.6%). Plasma amino acid levels were measured after deproteinization with sulfosalicylic acid using a Durrum amino acid analyser, model D-500 (Durrum Instrument Corp., Sunnyvale, Calif.) with a ninhydrin detection system.

Column chromatography. Plasma components were separated according to molecular size with Bio-Gel P-30 (Bio-Rad Laboratories, Richmond, Calif.), using a slightly modified procedure from that described by Valverde et al. (27) and Kuku et al. (28). The columns measured 80 × 1 cm, the elution buffer was 0.2 M glycine, pH 8.8, containing 0.25% human serum albumin and 500 kallikrein inhibitor units Trasylol/ml. For calibration of the columns, 125I-glucagon, 125I-insulin, and 125I-proinsulin were used. The void volume was derived from the presence of protein in each individual column fraction, as measured by ultraviolet light absorption at 280 nm. 2 ml of plasma were applied to the columns and eluted by gravity. Eluate volumes of 1 ml were collected, and samples of each were assayed in duplicate using a sensitive modification of IRG assay: 0.2-ml sample in a total volume of 0.6 ml containing 8 pg 125I-glucagon and antiserum 30-K (1977 batch) at a dilution of 1:40,000. The sensitivity obtained was 20 pg/ml. For recovery calculations, values of 20 pg/fraction were included, whereas values <20 pg/fraction were discarded. Statistical analyses were done according to Snedecor and Cochran (29).

RESULTS

Plasma concentrations of IRG, glucose, ketone bodies, and lactate, before and after infusion of arginine. Base-line IRG in the eight duodenopancreatectomized patients averaged 162±68 pg/ml (range: 20–500 pg/ml, a value not significantly different from normal) and 152±61 pg/ml after 30 min of the arginine infusion (Fig. 1). By contrast, plasma IRG in all six control subjects rose about threefold in response to arginine infusion. Individual values are given. The black area shows plasma IRG in a normal, control group. X = mean.
arginine from a mean base line of 121±24 to 327±32 pg/ml after 30 min.

Table I characterizes the diabetic state of the subjects studied: plasma glucose, ketone bodies (sum of acetoacetate and β-hydroxybutyrate), as well as lactate were elevated in the pancreatectomized patients. Furthermore, and as previously reported by Barnes et al. (18), the administration of arginine failed to affect the concentration of any of these metabolites.

Because Cherrington et al. (19) have shown in pancreatectomized dogs that arginine does stimulate gastric IRG secretion, but only coincident with insulin deficiency, insulin treatment was withheld for 3 d in one patient (patient 2) suffering from hypoglycemic symptoms occurring every other night. Fluid and electrolyte losses were carefully measured and replaced. The patient felt well, was ambulatory around the ward, and was able to take her meals. Plasma glucose was 290 mg/dl 24 h after the last insulin injection, rose to 390 mg/dl after 48 h, and remained at approximately this level up to 72 h. As shown in Table II, there was moderate ketosis by 72 h, whereas the concentration of branched chain amino acids rose quite markedly, an indication of insulin deficiency (30, 31). IRG, however, remained constant at about 570 pg/ml and, again, failed to rise in response to arginine infusion. In human subjects, therefore, it would seem that all alpha cells that might be stimulated by arginine, even after 72 h of insulin deficiency, are eliminated by duodenopancreatectomy.

IRG in plasma fractions obtained by Bio-Gel P-30 column chromatography before and after arginine infusion. As shown in Fig. 1 and Table II, two pancreatectomized patients exhibited substantial plasma concentrations of IRG, even though these IRG levels could be increased no further by arginine infusion. Accordingly, the possibility of IRG linked to plasma components of molecular size different from glucagon was considered. Plasma samples from duodenopancreatectomized patients were, therefore, analyzed with Bio-Gel P-30 column chromatography, as previously proposed by others (27, 28).

Fig. 2A shows the elution pattern obtained for plasma from the five pancreatectomized patients with IRG levels >70 pg/ml. Clearly, no IRG eluted with the 125I-glucagon, as observed in normal subjects (Fig. 2B). The bulk of IRG appeared in the void volume, which implies a molecular weight equal to, or in excess of, 40,000. Relatively less IRG was detected with molecules smaller than 125I-glucagon in most instances.

To rule out conversion of the large molecular moiety to glucagon by the arginine infusions, column chromatography was performed on plasma obtained after infusions in the same patients. As apparent from Fig. 2, no significant conversion of one IRG molecular species into another was induced by arginine.

In patient 2, column chromatography of plasma was carried out 24 h (Fig. 3A) and 3 d (Fig. 3B) after insulin withdrawal. After 3 d, a small IRG peak coeluted with 125I-glucagon, although this was not seen after 24 h or even after 3 d during arginine infusion.

IRG recoveries during column chromatography are shown in Table III. Although the mean recoveries of plasma samples obtained before arginine were 111 ±33%, and 133±33% for those obtained after arginine infusion, both recoveries do not differ significantly from the IRG recovery of 98±6% obtained for plasma (range: 68–128%) from normal subjects and from a patient with glucagonoma. There was, however, very wide individual variation in the recoveries; reaching as much as 217% (Table III). A statistically significant relationship did exist between the fraction of IRG eluted with the void volume and total IRG recovery (r = 0.886, P < 0.05). This is possibly secondary to the fact that the dilution of IRG eluted with the void volume was not linear (Table IV), as it always was for dilution for the glucagon standard. It is not surprising, therefore, that void-volume IRG yielded false, often spuriously high, values.

Plasma concentration of GLI in duodenopancreatectomized patients. Matsuyama and Foa (13) have

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**Table I**

**Blood Energy Substrates in Duodenopancreatectomized Subjects**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Before arginine</th>
<th>At 30 min of arginine infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>274±38</td>
<td>254±30</td>
</tr>
<tr>
<td>Ketone bodies, mM</td>
<td>0.67±0.20</td>
<td>0.82±0.25</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>1.06±0.09</td>
<td>1.04±0.09</td>
</tr>
</tbody>
</table>

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**Table II**

**Energy Substrates and IRG in Blood of a Duodenopancreatectomized Patient after Insulin Withdrawal**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Insulin withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>290</td>
</tr>
<tr>
<td>Ketone bodies, mM</td>
<td>0.6</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.92</td>
</tr>
<tr>
<td>Leucine, μM</td>
<td>201</td>
</tr>
<tr>
<td>Isoleucine, μM</td>
<td>132</td>
</tr>
<tr>
<td>Valine, μM</td>
<td>415</td>
</tr>
<tr>
<td>IRG, pg/ml</td>
<td>590</td>
</tr>
</tbody>
</table>

The blood was obtained after an overnight fast. Glucose and lactate were determined in whole blood, the other parameters were measured in plasma. Ketone bodies is the sum of β-hydroxybutyrate and acetoacetate.
DISCUSSION

The pancreatectomized human subject has become essential in the discussion of whether glucagon is an
important factor in the genesis of the diabetic state (1), or whether it changes its plasma concentration in a number of metabolic situations (32, 33) without, however, exerting effective influence. To fully assess the importance of a hormone, it is necessary to observe the results of its total absence. The data presented here suggest that, for glucagon, this condition is fulfilled in patients in whom the pancreas, the duodenum, and the gastric antrum have been removed. They show that in the plasma of these patients 3,500-dalton glucagon, measured with the widely used antiserum 30-K, is virtually absent.

Because Cherrington et al. (19) reported that glucagon stimulation in pancreactectomized dogs was only detected when insulin deficiency was also present, gastrointestinal alpha-cell secretion in duodenopancreatectomized man might have been missed because patients were studied after relatively short periods of insulin deprivation. In patient 2, however, plasma glucagon still failed to respond to arginine after 3 d of insulin withdrawal. The negative proof of the total absence of glucagon is, of course, always open to new experiments. Indeed, one single eluate did possibly show a very small amount of glucagon (Fig. 3B). Nevertheless, and together with the results of Barnes and Bloom (17) and Villanueva et al. (34), our observations suggest that duodenopancreatectomized humans, independent of their metabolic state, exhibit no or almost no circulating 3,500-dalton glucagon.

This study adds another piece of evidence to the work of Weir et al. (35), Valverde et al. (27), and Rubenstein et al. (28, 36), showing that antiserum 30-K does not measure exclusively 3,500-dalton-size glucagon. What, then, are these plasma fractions bearing glucagon immunoreactivity? Are they precursors or degradation products; are they GLI or incidentally crossreacting substances? Because Valverde et al. (27) were able to

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**TABLE III**

Recovery of IRG Applied for Column Chromatography of Plasma from Duodenopancreatectomized Patients

<table>
<thead>
<tr>
<th>Base line</th>
<th>Arginine</th>
<th>IRG recovered in</th>
<th>IRG recovered in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>IRG applied</td>
<td>vv*</td>
<td>vv-G</td>
</tr>
<tr>
<td>1</td>
<td>IRG pg/fraction</td>
<td>600</td>
<td>807</td>
</tr>
<tr>
<td></td>
<td>% of applied</td>
<td>134</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>IRG pg/fraction</td>
<td>1,100</td>
<td>1,834</td>
</tr>
<tr>
<td></td>
<td>% of applied</td>
<td>167</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>IRG pg/fraction</td>
<td>170</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>% of applied</td>
<td>26</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>IRG pg/fraction</td>
<td>184</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>% of applied</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>IRG pg/peak</td>
<td>230</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>% of applied</td>
<td>38</td>
<td>16</td>
</tr>
</tbody>
</table>

Mean±SEM (% of applied) 81±29 21±9 0 13±6 111±28 105±17 20±7 0 23±6 133±33

The IRG eluted was divided into four according to the peaks observed (Fig. 2).

*vv, void volume.
†vv-G, between void volume and five fractions before the 125I-glucagon marker.
‡G±5 fr, glucagon marker plus or minus five fractions.
§G, smaller than five fractions beyond the glucagon marker.

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**TABLE IV**

IRG Measured in the Peak of Void Volume after Column Chromatography: Effect of Dilution before the Glucagon Assay

<table>
<thead>
<tr>
<th>Column chromatography</th>
<th>IRG</th>
<th>Undiluted</th>
<th>1:2</th>
<th>1:4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/ml</td>
<td>pg/ml</td>
<td>pg/ml</td>
<td></td>
</tr>
<tr>
<td>Chromatography 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction 17</td>
<td>320</td>
<td>140</td>
<td>270</td>
<td></td>
</tr>
<tr>
<td>Fraction 19</td>
<td>106</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Chromatography 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction 17</td>
<td>130</td>
<td>65</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Fraction 19</td>
<td>160</td>
<td>50</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

Chromatography 1 refers to the blood sample from patient 2 before arginine infusion. Chromatography 2 refers to the blood sample of patient 2 after arginine infusion.
convert void-volume IRG into IRG close to 3,500 daltons by tryptic digestion in vitro, the question has been raised whether one part of this fraction, in our patients in vivo, could represent a precursor which, when stimulated by arginine, would be converted into glucagon, leaving the total IRG unchanged. Fig. 2 demonstrates that this was not the case: arginine failed to significantly alter the chromatographic pattern of these plasmas. One might argue, of course, that antiserum 30-K lacks specificity and should not be used. Although our results, as well as those just mentioned, do demonstrate that a 30-K measurement in a given plasma sample needs to be qualified, this conclusion should not be fully accepted as long as it has not been proven that seemingly nonglucagon IRG fractions in plasma really are unrelated to glucagon. Thus, Srikant et al. (37) showed that a large molecular fraction obtained from extracts of canine gastric fundus of approximately 65,000 daltons in size, was active in liberating glucose from an isolated, perfused rat liver. Unfortunately, there was not sufficient plasma available from our patients to perform bioassays with different fractions.

The differences seen with the two antisera (Fig. 4) are of interest. Just as Matsuyama and Foa (13) have found in depancreatized dogs, IRG measured with a nonspecific antiserum (K-4023) was significantly elevated in our patients without pancreas. A relatively small variability was seen in pancreatectomized patients when their total IRG was appreciated with K-4023 antiserum.

The argument that diabetes following pancreatectomy is clinically milder than juvenile onset diabetes is not new (38). Although reduced food intake or impaired absorption might be the cause of this “mildness”, neither weight loss nor diarrhea were important in our patients. The relatively benign metabolic decompensation of patient 2 after 3 d of insulin withdrawal supports the view that this form of diabetes might, in fact, be less prone to ketosis than juvenile onset-type diabetes. Similarly, Barnes et al. (39) showed a much slower rise in blood glucose and ketone bodies in pancreatectomized patients after insulin deprivation than in juvenile-type diabetics, which reflects the slowed onset of ketoacidosis in the juvenile-type diabetics of Gerich et al. (40) during glucagon suppression by somatostatin. The administration of glucagon accelerated the development of ketoacidosis back to where it was before somatostatin administration (40). Thus, the hypothesis of Unger and Orci (1) should be corrected in the sense that the presence of chemically defined glucagon is not a requirement for diabetic metabolism, even though it most likely does aggravate the metabolic state of insulin-deficient diabetes. Whether small glucagon elevations such as those described in diabetics under “ordinary” diabetic treatment (2–5) worsen the metabolic control appreciably or not, remains to be established (41, 42).

An additional metabolic alteration reported in this
study is the significant elevation in the plasma of duodenopancreatectomized patients of the concentration of several amino acids (Fig. 4). The levels of alanine, the “key” glucogenic amino acid (43), and serine, another substrate for gluconeogenesis, were twice those found in the plasma of normal postabsorptive humans. In addition, the plasma concentrations of arginine and ornithine were significantly greater in these patients. These two amino acids are involved in the production of urea, the second metabolic end product of hepatic gluconeogenesis. It is unlikely that the diminished nutritional state commonly observed in cancer patients was the cause of this amino acid pattern; first, because our patients did not lose weight appreciably and, second, because nutritional deficiency is accompanied by a lowering rather than an elevation of most amino acids (44). By contrast to the data presented here, in the presence of glucagon excess plasma amino acids are characteristically reduced: for example, in patients with glucagonoma the levels of most amino acids are substantially diminished (45); likewise, the infusion of exogenous glucagon is accompanied by a reduction in the levels of many amino acids (46). It is suggested, therefore, that this alteration of the pattern of plasma amino acids is a consequence of glucagon deficiency. On the other hand, absence of significant elevation of branched chain amino acids, leucine, isoleucine, and valine, suggests that insulin deficiency in our patients was relatively modest (30, 31). Only after 3 d of insulin withdrawal (patient 2) did the branched chain amino acids substantially increase (Table II).

In conclusion, duodenopancreatectomized humans have virtually no circulating 3,500-dalton glucagon. They are, nonetheless, diabetic. The marked elevation of some amino acids involved in gluconeogenesis suggests a role for glucagon in the intermediary metabolism of amino acids.

ACKNOWLEDGMENTS
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