Extrapancreatic Glucagon and Glucagonlike Immunoreactivity in Depancreatized Dogs

A QUANTITATIVE ASSESSMENT OF SECRETION RATES AND ANATOMICAL DELINEATION OF SOURCES

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ABSTRACT The anatomical sites and the rates of extrapancreatic secretion of glucagon and of glucagonlike immunoreactivity (GLI) were assessed in dogs 2 h after pancreatectomy by catheterization of the gastroplenic and mesenteric veins.

Glucagon release from the gastroplenic area approximated one-fourth that of a normal pancreas and rose from 0.25 to 1.0 ng/kg per min during arginine stimulation. Intestinal glucagon secretion was small and did not respond to arginine, suggesting that the stomach is the only important extrapancreatic source of glucagon.

Glucagon concentrations attained by gastroplenic secretion were in close proportion to those obtained during the administration of exogenous glucagon, indicating similar clearance rates of extrapancreatic and pancreatic glucagon, approximating 10 ml/kg per min.

GLI secretion (0.3 ng eq/kg per min) was limited to the intestinal area and was transiently stimulated by arginine and exogenous glucagon. Base-line GLI clearance approximated 1 ml/kg per min. No insulin secretion could be detected. Gastrointestinal glucose uptake rose from 0.56 to 2.2 mg/kg per min after glucagon administration suggesting that as much as 10% of total glucose production can be taken up by the gastrointestinal tract.

In two dogs both the stomach and pancreas were removed. Intestinal glucagon release remained small and did not increase during arginine administration. By contrast, GLI release was stimulated by both arginine and exogenous glucagon.

INTRODUCTION

The evidence that immunoreactive glucagon originates not only from the pancreas but also from the gastrointestinal tract (1) explained the well-known observations that depancreatized dogs do not exhibit symptoms of glucagon deficiency. Indeed, in depancreatized insulin-deficient dogs, plasma immunoreactive glucagon does not decrease, but increases markedly (1–3).

The possibility that the increased plasma concentrations of glucagon in patients with diabetes mellitus (4) could be in part of gastrointestinal origin, became, therefore, apparent. In man, A-like cells have been described in the duodenum (5) which usually is removed when pancreatectomy is performed.

Given the difficulty of easy access to the gastrointestinal tract in humans, the release of extrapancreatic glucagon has been studied mainly in animals. In the dog (6), the gastric mucosa contains most of the material reacting with the glucagon antiserum 30-K (Dr. R. H. Unger, Dallas, Tex.), designated as "pancreatic-glucagon specific antiserum" and reported to react with the C-terminal end of the glucagon molecule (7). Small concentrations of glucagon, however, have also been found throughout the gastrointestinal tract. Several biochemical characteristics of such material originating from porcine duodenum were investigated (8), and virtually no differences were found when compared with glucagon of pancreatic origin. The same findings were
also reported using gastric extracts from dogs (6, 9, 10).

Histologic examination of gastric mucosa has revealed cells that are indistinguishable from the glucagon-producing pancreatic A cells (8, 11, 12).

Several authors (3, 9, 13–15) demonstrated that in the depancreatized dog arginine could stimulate the release of gastric glucagon provided that the animal was insulin deficient. This was achieved by stopping insulin treatment of depancreatized dogs after days or weeks of treatment. Secretion rates of extrapancreatic glucagon have not yet been studied.

In the present study the secretion rates of immunoreactive glucagon were measured separately from the stomach and intestines in anesthetized dogs 2 h after pancreatectomy. Similarly, the secretion of glucagon-like immunoreactivity (GLI) of gastrin and intestinal areas was assessed. This is the material cross-reacting in assays using “nonspecific” antisera against glucagon, reported to react principally with the N-terminal end of the glucagon molecule (16). In addition, gastrectomy was performed to verify the importance of the stomach and, in its absence, that of the intestines. Finally, in view of the claims postulating the existence of extrapancreatic insulin (17), the secretion of extrapancreatic insulin was assessed.

METHODS

Adult mongrel dogs weighing between 25 and 39 kg of either sex were used. The animals were fed commercial dog food ad lib. and were fasted 18–24 h before the experiment. To reduce bronchial secretion the animals were premedicated with 2 mg of atropine. Anesthesia was induced with barbiturate rates and succinylcholine and maintained by continuous slow infusion of these drugs as well as by inhalation of nitrous oxide. Ventilation was controlled by a positive pressure respirator after endotracheal intubation, and was adjusted so as to maintain blood pH, arterial $P_{aO_2}$, $P_{aCO_2}$, and base excess (measured with a Corning Eel blood-gas analyzer, Corning Glass Works, Science Products Div., Corning, N.Y.) within physiological range; in addition, sodium bicarbonate was infused when needed. Body temperature was maintained at 38±0.5°C with a thermostated heating blanket. Arterial pressure and heart rate were continuously monitored on a Beckman polygraph (Beckman Instruments, Inc., Spincro Div., Palo Alto, Calif.). After midline incision on the abdomen, the pancreas was removed completely, leaving in place the entire intestine and the spleen.

After pancreatectomy, catheters were inserted into the gastrosplenic vein and into the common mesenteric vein draining all the blood between jejunum and rectum (Fig. 1). Each of these catheters was shunted into one of the femoral veins allowing for the measurement of blood flow (Table 1) and for sampling. In addition, one of the femoral arteries was catheterized for blood sampling. The decreasing blood flow in the gastrosplenic vein reflects the contraction of the spleen and illustrates the importance of measuring blood flow when hormone secretion is discussed. In two dogs, the stomach was removed as well as the pancreas; the spleen was left in place.

In these dogs, only one venous catheter (mesenteric vein) was inserted.

After the operative procedures, the anesthetized dogs were allowed to recover for approximately 2 h before the experiments were started. After a control period of 20 min arginine was infused for 30 min into the femoral vein at a rate of 12 mg/kg per min. Thereafter the dogs rested for 50 min. Then exogenous glucagon was infused at 4 mg/kg per min for another 30 min. Blood was simultaneously withdrawn from the two venous catheters and from the artery. Blood flow was measured individually in the two venous catheters at each sampling as described earlier (18). 6 ml of blood were sampled and transferred into chilled tubes containing 0.1 ml of Trasylol (3,000 KIU, a gift from Drs. Ruf and Aman, Bayer AG, Zurich, Switzerland). 10 mg EDTA (Na$_2$) and 100 IU of lithium heparin. The tubes were centrifuged and the plasma frozen until assayed, not later than 2 wk after the experiments. The hematocrit was measured at every second sampling to calculate plasma flow. It fell from 59±8 at the beginning to 39±6% at the end of the experiment. Autopsies were carried out in all cases to verify completeness of pancreatectomy.

**Analyses.** Plasma glucose was measured by the glucose oxidase method (19) (glucose oxidase was donated by Prof. F. H. Schmidt, Boehringer Mannheim, Germany). Insulin, glucagon, and GLI were determined by immunoassay using charcoal separation (20, 21). The following antisera were used: insulin antiserum (a gift from Dr. P. Wright,
TABLE I

Blood Flows in the Gastrosplenic and Mesenteric Veins during infusions of Arginine (n = 6) and Glucagon (n = 4) in Dogs 2 h after Pancreatectomy

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
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<tr>
<td>Gastrospenic vein</td>
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<td></td>
<td></td>
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<tr>
<td>Mean</td>
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<td>129</td>
<td>176</td>
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<tr>
<td>SEM</td>
<td>52</td>
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<td>35</td>
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<tr>
<td>Mesenteric vein</td>
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<tr>
<td>Mean</td>
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<td>189</td>
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<td>188</td>
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<td>170</td>
</tr>
<tr>
<td>SEM</td>
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<td>18</td>
<td>17</td>
<td>16</td>
<td>9</td>
<td>14</td>
<td>13</td>
<td>22</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

Indianapolis, Ind.); glucagon antiserum (30-K donated by Dr. R. H. Unger, Dallas, Tex.), and the nonspecific antiserum K-4023 (obtained from Novo Industri, Copenhagen, Denmark). The minimal detectable concentrations of the assays were: insulin 3 µU/ml, glucagon 30-K 40 pg/ml, GLI K-4023 40 pg/ml.

For ease of understanding and to avoid another abbreviation, the term "glucagon" is used throughout the text for what is measured in plasma by antiserum 30-K.

The values of GLI were calculated by subtracting the value of glucagon (30-K) from that obtained with the nonspecific antiserum (K-4023), as read against a glucagon standard curve. The values are expressed as equivalent of glucagon. Fig. 2a demonstrates that the addition of glucagon did not alter significantly the GLI values measured in a plasma sample, and Fig. 2b shows that added glucagon was quantitatively recovered in the measurements using antiserum K-4023. This is in contrast with the findings of Srikant et al. (22) where in canine pancreatic extracts the measurements of GLI by the antiserum 78-J were influenced by the presence of glucagon. K-4023 yields linear dilution curves with purified gut GLI. 3 Dilutions 1:2 yielded 58±11% (SEM) of the original values.

Calculations. Net balance of hormones was calculated by the formula

\[ \text{balance} = (C_r - C_a) \times \text{blood flow} \times \frac{100 - Hct}{100} \times \frac{1}{BW} \]

where \( C_r \) equals venous concentration (picogram per milliliter or microunits per milliliter), \( C_a \) equals arterial concentration (picograms per milliliter or microunits per milliliter), blood flow is in milliliters per minute, Hct equals hematocrit (percent), BW equals body weight (kilogram). Positive values for the balance indicated release from the tissues, negative values uptake. The balance from the "gastrosplenic area" was calculated from the concentration difference between arterial blood and that sampled in the gastrosplenic vein; similarly, the "intestinal area" refers to the blood obtained from the mesenteric vein. Statistical analyses were done according to Snedecor and Cochran (23). Means and SEM are indicated in the text, figures, and tables. For ease of reading, Tables I and II give the values at intervals of 10 min only; Fig. 3–7 show the calculated values at every sampling for secretion rates.

RESULTS

Pancreatectomy, secretion of insulin, and concentration of glucose. To study the amount of glucagon

\[ ^3 \text{Heding, L. G. Personal communication.} \]

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TABLE II

Plasma Concentrations of Glucagon, GLI*, Glucose, and Insulin during Infusions of Arginine (n = 6) and Glucagon (n = 4) in Depancreatized Dogs

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Arginine</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>0</td>
<td>73</td>
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<td>10</td>
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<td>50</td>
<td>152*</td>
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<tr>
<td>60</td>
<td>204*</td>
<td>36</td>
</tr>
<tr>
<td>70</td>
<td>201</td>
<td>37</td>
</tr>
<tr>
<td>80</td>
<td>133</td>
<td>33</td>
</tr>
</tbody>
</table>

* GLI is a calculated value, (i.e. the value measured with antiserum K-4023 minus that measured with antiserum 30-K). The experiments were started 2 hours after pancreatectomy. Arginine (12 mg/kg per min) was infused between min 20 and 50, glucagon (4 mg/kg per min) between min 100 and 130. Means±SEM are given.

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released from extrapancreatic sites, it is essential that the pancreatic tissue, the veins of which might anastomose with the ones draining the gastrointestinal tissues, be completely removed. The measurements of insulin balance across gastroplenic and intestinal areas provided a useful functional test for completeness of pancreatectomy. 2 h after pancreatectomy the arterial plasma concentrations of insulin averaged 7±2 μU/ml with a range between 3 and 15 μU/ml. During the infusions of arginine or glucagon, the concentrations of insulin remained unchanged in the artery, the gastroplenic and the mesenteric veins (Table II). Calculation of exchange rates showed that at no time was insulin secreted from these sites (Fig. 3). The plasma concentrations of glucagon are given in Table II. By contrast to the findings of Ross et al. (15), the glycemia was not affected by the infusion of arginine, whereas the administration of glucagon was accompanied by a rise in the arterial levels of glucose from 120±34 to 190±32 mg/dl, presumably because
reaching vessels (Table II). After completion of the infusion, the glucagon concentration gradually fell in all three vessels.

**Secretion of glucagon in pancreatectomized gastrectomized dogs.** Because gastric glucagon secretion seems to be important only after the pancreas has been removed (1), the question was raised whether glucagon secretion from the distal gastrointestinal tract might be increased in the absence of both the pancreas and the stomach. To test this the stomach was removed in two additional dogs concurrently with pancreatectomy. Fig. 5 demonstrates that a small amount of glucagon (0.05 and 0.17 ng/kg per min) was secreted from the intestinal area, confirming the findings that in the dog immunoreactive glucagon is present throughout the gastrointestinal tract (6). The infusion of arginine was, however, without effect (Fig. 5).

**Glucagon disappearance from the circulation.** To

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**Figure 3** Insulin balance across the gastrosplenic (upper panel) and the intestinal areas (lower panel) in depancreatized dogs during infusions of arginine \(n = 6\) and of glucagon \(n = 4\). Positive values represent secretion, negative numbers uptake of hormone.

The administration of arginine resulted in a prompt glucagon rise in the gastrosplenic vein reaching \(777 \pm 225\) SEM pg/ml after 5 min. Fig. 4 shows that this rise corresponded to a net glucagon secretion of \(1.05 \pm 0.42\) ng/kg per min at 5 min which remained near a plateau between 1.0 and 1.3 ng/kg per min throughout the arginine infusion period. Comitantly, the levels in the artery and the mesenteric vein increased from 85 \(\pm 15\) and 95 \(\pm 17\) pg/ml to 201 \(\pm 37\) and 195 \(\pm 29\) pg/ml, respectively, at the end of the arginine infusion (Table II). After completion of the infusion, the glucagon concentration gradually fell in all three vessels.

**Figure 4** Glucagon balance across the gastrosplenic and intestinal areas in depancreatized dogs during infusions of arginine \(n = 6\) and of glucagon \(n = 4\). Positive values represent secretion, negative numbers uptake of hormone.
test whether glucagon secreted from the stomach had a different clearance rate than pancreatic glucagon, exogenous glucagon was infused intravenously during 30 min in four dogs at a rate of 4 ng/kg per min. The levels reached at steady state were used for comparison. The fact that arginine stimulation resulted in a secretory pattern reflecting a constant infusion made such a comparison possible. Table III summarizes these results and compares them with data obtained in an earlier study (24). Assuming that the clearance of either exogenous or endogenous glucagon does not depend on hormone concentration in the range studied as it was shown in humans (25, 26), the two must have a similar plasma half-life.

During the infusion of glucagon, arterial levels varied between 500 and 700 pg/ml (Table II). During this time, a significant net glucagon uptake was observed in both gastroplenic and intestinal areas (Fig. 4). After cessation of the glucagon infusion, a small (statistically insignificant) increase of glucagon release from the gastroplenic area was observed. Similar findings, both uptake and "release," were observed across the peripheral tissues, a phenomenon called "storage by inundation" by Cannon in 1929 (27).

Secretion of GLI. The presence of GLI has been reported in extracts of pancreas (22, 28) and stomach (29). To determine whether it is a secretory product of the endocrine cells present in the canine stomach or A-like cells present in the more distal intestine (8), GLI secretion from the gastroplenic and intestinal areas was assessed. Fig. 6 demonstrates that at no time during the experiments was any GLI release observed from the gastroplenic area, thus ruling out GLI secretion from the stomach. By contrast, GLI release from the intestinal area was substantial: arginine stimulated its secretion in all dogs from an average base line of 0.30±0.12 ng eq/kg per min during the control period.

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**TABLE III**

**Effect of Exogenous and Endogenous Glucagon on Venous Glucagon Concentrations in Normal (24) and Depancreatized Dogs**

<table>
<thead>
<tr>
<th>Dogs</th>
<th>Glucagon source</th>
<th>Plasma glucagon rise above basal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng/kg/min</td>
</tr>
<tr>
<td>Normal</td>
<td>Infusion 3.5 (n=4)</td>
<td>345±44</td>
</tr>
<tr>
<td>Depancreatized</td>
<td>Infusion 4.0 (n=4)</td>
<td>435±119</td>
</tr>
<tr>
<td></td>
<td>Secretion 1.0 (n=6)</td>
<td>100±29</td>
</tr>
</tbody>
</table>

In the normal animals, blood was sampled in the superior vena cava, in the depancreatized dogs in the mesenteric vein. The average glucagon rises, as measured at 30 min of the infusion, are indicated (mean±SEM).
to a peak of \(0.80 \pm 0.24\) ng eq/kg per min \((P < 0.05)\) at 5 min. The elevated secretion rate lasted only 15 min, after which it fell toward base line despite the continuing arginine administration (Fig. 6).

The infusion of glucagon was also accompanied by an increased GLI secretion. At 10 min after the beginning of the infusion, GLI levels were above mean base line in all four dogs. In addition in both dogs in which the pancreas and the stomach were removed, GLI secretion increased markedly during the infusion of glucagon (Fig. 7).

**DISCUSSION**

The present study delineates by functional tests the anatomical region of secretion of extrapancreatic glucagon and of GLI. It measures quantitatively the rates of such secretion and by comparison with an infusion of pancreatic glucagon indicates that in vivo the clearance rates of pancreatic and gastric glucagon are identical. The gastric A cells offer an interesting model for studying the regulation of A-cell secretion in tissues where contiguous B cells are absent. It has been shown previously that, indeed, regulation of gastric A-cell secretion differs from that of the pancreatic A cell (9, 13), suggesting that the anatomical relationships of these two endocrine cells play some role in regulating their function (30).

2 h after pancreatectomy, glucagon secretion from the gastrosplenic area could be stimulated by arginine in all dogs studied. In contrast to Lefebvre and Luyckx’s (31) findings of a short-lived secretory peak in an isolated stomach preparation, we observed a sustained secretion throughout the 30 min of arginine administration. The difference between the two studies could arise from the fact that in Lefebvre’s study the perfusate was whole blood containing normal insulin concentrations, and this hormone decreases responsiveness of gastric A cells (9, 13).

According to Muñoz-Barragan et al. (14), gastric glucagon secretion becomes quantitatively important only after removal of the pancreas. It was speculated, therefore, that gastrointestinal sources other than the stomach might, in a hierarchic manner, take over the secretion in the absence of both pancreas and stomach. The results presented here (Fig. 5) demonstrate that 2 h after pancreatectomy-gastrectomy, such a mechanism is not operative and shows (Fig. 4, Table II) that in the dog the stomach is the sole important source of extrapancreatic glucagon.

It was one of the aims of this study to assess quantitatively the capacity of this source when compared to the endocrine pancreas. At rest, the gastrosplenic area secreted approximately 0.25 ng/kg per min (Fig. 4) which is approximately one-fourth of a basal secretion from the nonstimulated pancreas (18, 32). Upon stimulation with arginine the secretion rate from the stomach quadrupled to 1 ng/kg per min raising the arterial glucagon levels by approximately 100 pg/ml. A similar fold increase in plasma glucagon levels has been reported in humans during arginine administration (33). In the two dogs in which the pancreas and the stomach were removed, intestinal glucagon secretion amounted to 0.05 and 0.17 ng/kg per min, respectively (Fig. 5). This, in turn, is approximately one-third of gastric secretion in the nonstimulated state of <10% of normal pancreatic secretion.

As an in vivo test for similarity between gastric and pancreatic glucagon, their respective clearance rates were assessed by comparing the levels achieved in venous blood during either endogenous secretion at plateau or constant rate infusion of exogenous glucagon (Table II, Fig. 4). Table III illustrates that endogenous (gastric) glucagon and exogenous (pancreatic) glucagon have a similar plasma half-life, suggesting a high degree of identity between the two (6, 10). According to these data, an infusion or secretion of 1 ng/kg per min elevates plasma levels by 100 pg/ml. The calculated clearance rate for glucagon (when delivered into the systemic circulation) would, therefore, amount to 10 ml/kg per min. This is approximately double that calculated for the base-line period (Table II, Fig. 4). If one considers, however, that the antisemum 30-K measures, besides glucagon, other molecular moieties in plasma of humans (34) and of dogs (35), and this in approximately similar amounts, true glucagon clearance lies probably closer to 10 ml/kg per min.

The catheterization procedure used in this study has proved a useful tool in measuring in vivo the rates of net uptake or release of metabolites or hormones to and from different parts of the gastrointestinal tract. It ruled out the existence of a significant extrapancreatic source of insulin in the gastrointestinal tract of the dog. Such a source has been claimed to exist in the
pig (17) because insulin immunoreactivity was detected in mucosa extracts from porcine stomach.

It was of interest to observe that substantial amounts of glucose were taken up by the intestinal tract in these insulin-deprived animals. Ross et al. (15) calculate a glucose disappearance in depancreatized insulin-deprived dogs of 5.3 mg/kg per min. In our dogs, gastrointestinal glucose uptake amounted to 0.56 mg/kg per min or approximately 10% of Ross’ number. This indicates that the gut can take up a substantial fraction of the overall glucose produced, a fact to be considered when splanchnic balances are discussed.

Finally, this technique allowed us to determine both the source and secretion rates of GLI. As it has been claimed that GLI is stored in gastric mucosa (29), we investigated whether such material was secreted from that site as well. Fig. 5 illustrates that we could not detect any GLI release from the stomach. The sole source of GLI was the intestines. We also observed that arginine and glucagon stimulated GLI secretion (Figs. 6 and 7), and this has not been reported previously. In view of the possibility that GLI might be a precursor of glucagon (36), it was of interest to observe that at base line GLI clearance amounted to approximately 1 ml/kg, which is one-tenth of that calculated for glucagon. As far as our model is representative of normal physiology, this difference in clearance renders unlikely the hypothesis that blood-borne GLI is the only precursor for glucagon.

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