THE ROLE OF PANCREATIC GLUCAGON IN
THE PATHOGENESIS OF ACUTE PANCREATITIS

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(Received 21 April 1972)

SUMMARY

1. A reliable, reproducible and specific method for determination of pancreatic glucagon in plasma by radioimmunoassay is described and plasma glucagon was measured in normal subjects and patients with acute pancreatitis.

2. In patients with acute pancreatitis the normal relationship between glucagon and glucose was impaired and disturbance of alpha-cell function was indicated by relative hyperglucagonaemia in patients with moderately severe disease.

3. In patients with severe pancreatitis glucagon concentrations were low and the possible relationship between hyper- and hypo-glucagonaemia and the pathogenesis of acute pancreatitis is discussed.

4. Insulin values in acute pancreatitis, although increased, were not as high as would be expected for the raised glucose concentrations.

5. It is concluded that both alpha- and beta-cell dysfunction may account for the high incidence of carbohydrate intolerance in acute pancreatitis.

Key words: pancreatic glucagon glucose and insulin, pancreatitis, alpha- and beta-cell dysfunction.

Paloyan, Paloyan & Harper (1967) reported high plasma concentrations of glucagon-like substances in patients with acute pancreatitis. Subsequent work, however, cast doubt on the validity of their results as glucagon was assayed by radioimmunoassay without the use of a kallikrein inhibitor (Trasylol) and with an antiserum which may not have been specific for pancreatic glucagon. The omission of Trasylol may result in spuriously high values of glucagon-like substances caused by damage to the radioiodinated hormone during incubation with antiserum (Eisentraut, Whissen & Unger, 1968). With the development of antisera which are specific to pancreatic glucagon and the use of Trasylol, it is possible to obtain more valid results about the role of pancreatic glucagon in pathological states (Unger, Aguilar-Parada, Muller & Eisentraut, 1970; Heding, 1971).

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It has been shown that glucagon suppresses pancreatic exocrine secretion in man (Zajtchuk, Amato & Paloyan, 1967) and the hormone has been used in the treatment of acute pancreatitis (Knight, Condon & Smith, 1971). Edwards & Taylor (1970) have suggested that energy may be required to retain endogenous glucagon within the alpha cell and it is possible therefore that damage to the alpha cell might be associated with release of glucagon. If this is correct, alpha-cell damage which might occur in acute pancreatitis could result in high-circulating glucagon concentrations. Ultimately severe cell injury might result in a subsequent fall in the concentration of glucagon as was demonstrated in rabbits after administration of Neutral Red (Nevis, Stiller, Woll & Lawrence, 1968). In view of the effect of glucagon on the exocrine function of the pancreas, it is of importance to discover whether circulating concentrations of glucagon are related to the progression of untreated pancreatitis.

METHODS

Immunoassay

Antisera to porcine crystalline glucagon (Sigma) were raised in rabbits by the method of Assan, Rosselin, Drouet, Dolais & Tchobroutsky (1965). Glucagon was iodinated by using $^{125}$I by the method of Hunter & Greenwood (1962) for growth hormone. $^{125}$I-labelled glucagon was separated from free iodine by absorption to microfine precipitated silica (Q.U.S.O., Philadelphia Quartz Co.) and elution with acid–alcohol. Standard or unknown solutions (0.2 ml) of glucagon were incubated with Trasylol (0.1 ml), $^{125}$I-labelled glucagon (0.1 ml) and antiserum (0.1 ml) for 48 h. All dilutions were made with phosphate buffer (0.05 M, pH 7.4) containing human serum albumin (0.2%). Separation of free and bound hormone was performed by alcohol precipitation (Taylor, Howell, Montague & Edwards, 1968). To allow for variation in protein concentration, control tubes which contained normal rabbit serum and excess of antiserum were used as described by Makulu, Vichick, Wright, Sussman & Pao-Lo (1969) for the insulin assay.

The precision ($s$) of the assay was calculated from the formula $s = \sqrt{\Sigma D^2/N(n-1)}$ when $D =$ difference between replicate values and their means, $N =$ number of standard curves and $n =$ number of replicate values. Sensitivity was calculated from the formula, $ts/\sqrt{(N)}$, where $t = 1.96$.

Antiserum specificity

Antisera were incubated with porcine crystalline glucagon (Sigma and M.R.C. standard), insulin, pancreozymin and secretin (Boots), and extracts of human pancreas and of various parts of human gastrointestinal tract obtained at operation, immediately frozen, homogenized and extracted with acid–alcohol and ether–alcohol (Kenny, 1955).

Extraction of plasma samples

In view of the non-specific variations in binding caused by molarity (Day, 1972) and globulin (Assan, Rosselin, Tchobroutsky & Freychet, 1969), all samples were extracted by a modification of the spherocil-absorption method used for oxytocin (Boyd & Chard, 1971). Plasma was mixed with spherocil (50 mg/ml), centrifuged and the resultant precipitate washed with 3 ml of water and 3 ml of HCl (1 m) and then eluted with 80% acetone. The acetone eluate was evaporated to dryness. The final residue was redissolved in phosphate buffer. The overall yield
of added $^{125}$I-labelled glucagon was 75·0% (±9%) and of unlabelled glucagon was 86·0% (±12%). The dilution characteristics of the immunological material obtained were compared with those of crystalline glucagon and the molecular weight characteristics assessed by gel filtration (Sephadex G-50). Plasma samples were obtained from eight normal subjects while they were fasting and during the oral glucose tolerance test (50 g), after a 3-day high carbohydrate diet.

Samples were taken from a further fifteen normal subjects while they were fasting and twenty-six patients with acute pancreatitis, as soon as the diagnosis was established. The diagnosis of acute pancreatitis was made at operation (nine cases), on the typical clinical picture and raised plasma amylase values (seventeen cases). Six cases were classified as having haemorrhagic pancreatitis. The normal subjects and patients with pancreatitis were matched for age, sex and weight. All plasma samples were measured for concentrations of glucagon, glucose (glucose oxidase method) and insulin (Hales & Randle, 1963), amylase and calcium.

RESULTS

The sensitivity, precision and reproducibility of the glucagon immunoassay are indicated in Table 1. The lowest concentration of glucagon detectable was 0·13 ng/ml in the incubation tubes and 0·07 for plasma samples which have been concentrated twice. The dilution curves of human pancreatic extract and standard glucagon solutions were identical. No glucagon-like activity could be detected when the antiserum was incubated with insulin, pancreozymin or secretin, or extracts of human stomach, duodenum, small intestine or colon. These results are compared with glucagon-like activity found in pancreatic extract and pancreatic adenomas (Table 2). The dilution curves of an extract of 50 ml of plasma and standard glucagon solutions were immunologically identical. The elution pattern from Sephadex G-50 of the immunological glucagon-like activity in such extracts was identical to that of glucagon added to glucagon-free plasma (outdated blood-bank plasma). No significant immunological activity was detected in the molecular weight range 5000–16 000. The inverse relationship between glucagon and glucose concentrations in normal subjects during the oral glucose tolerance test is illustrated in

**Table 1. Glucagon radioimmunoassay: details of six successive standard curves**

<table>
<thead>
<tr>
<th>Glucagon concentration (ng/ml)</th>
<th>% of $^{125}$I bound to glucagon (Mean±SEM)</th>
<th>Precision of individual standard curves (%)</th>
<th>Sensitivity of individual curves ($P&lt;0.05$) (%)</th>
<th>Equivalent glucagon concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49·19±1·15</td>
<td>1·89</td>
<td>1·51</td>
<td>0·10</td>
</tr>
<tr>
<td>0·25</td>
<td>45·11±1·38</td>
<td>2·57</td>
<td>2·06</td>
<td>0·13</td>
</tr>
<tr>
<td>0·50</td>
<td>41·13±1·64</td>
<td>2·50</td>
<td>2·00</td>
<td>0·15</td>
</tr>
<tr>
<td>1·0</td>
<td>38·46±1·84</td>
<td>2·73</td>
<td>2·18</td>
<td>0·36</td>
</tr>
<tr>
<td>2·0</td>
<td>33·38±1·67</td>
<td>2·70</td>
<td>2·16</td>
<td>0·37</td>
</tr>
<tr>
<td>4·0</td>
<td>24·21±1·07</td>
<td>1·51</td>
<td>1·2</td>
<td>0·95</td>
</tr>
<tr>
<td>8·0</td>
<td>19·86±1·33</td>
<td>2·50</td>
<td>2·0</td>
<td>1·9</td>
</tr>
</tbody>
</table>
Table 2. Glucagon-like material in various tissues

<table>
<thead>
<tr>
<th>Tissue extracted</th>
<th>Specimen</th>
<th>Weight extracted (g)</th>
<th>Final dilution (g/ml)</th>
<th>Concentration of glucagon-like material (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>Post mortem</td>
<td>21</td>
<td>0.0015</td>
<td>4333</td>
</tr>
<tr>
<td></td>
<td>Post mortem</td>
<td>23</td>
<td>0.0015</td>
<td>5430</td>
</tr>
<tr>
<td>Insulinoma</td>
<td>Operation</td>
<td>0.22</td>
<td>0.02</td>
<td>530</td>
</tr>
<tr>
<td></td>
<td>Operation</td>
<td>0.32</td>
<td>0.03</td>
<td>310</td>
</tr>
<tr>
<td>Stomach</td>
<td>Fundus</td>
<td>44</td>
<td>1.0</td>
<td>&lt;0.13</td>
</tr>
<tr>
<td>Antrum</td>
<td>Operation</td>
<td>12</td>
<td>1.0</td>
<td>&lt;0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
<td>&lt;1.3</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Operation</td>
<td>1</td>
<td>0.1</td>
<td>&lt;1.3</td>
</tr>
<tr>
<td>Colon</td>
<td>Operation</td>
<td>5.2</td>
<td>0.5</td>
<td>&lt;0.26</td>
</tr>
</tbody>
</table>

Fig. 1. The changes in glucose, glucagon and insulin concentration after administration of 50 g of glucose to eight normal subjects. ■, Concentration of glucose (mg/100 ml); ●, concentration of insulin (µunits/ml); ○, concentration of glucagon (ng/ml). The bars represent the SEM.
Glucagon and acute pancreatitis

The concentration of glucagon (mean ± SEM) 15 min after glucose administration fell significantly from 0.49 (± 0.08) ng/ml to 0.27 (± 0.03) ng/ml. Associated glucose concentrations were 71 (± 2) mg/100 ml and 101 (± 6) mg/100 ml respectively.

The mean plasma glucagon concentration in fifteen fasting normal subjects was 0.48 (± 0.08) ng/ml and the corresponding plasma glucose concentration was 81 (± 3) mg/100 ml. In the patients with pancreatitis (n = 26) the mean glucagon concentration was 0.45 (± 0.07) ng/ml and corresponded to a plasma glucose concentration of 163 (± 6) mg/100 ml. Although the absolute glucagon concentration was similar in the two groups, the glucose values in the pancreatic subjects were considerably increased (162 mg/100 ml). There was no inverse correlation between glucose and glucagon concentrations in those patients with pancreatitis (r = -0.25).

Six of the twenty-six patients were classified as having severe haemorrhagic pancreatitis and all had low glucagon concentrations (mean = 0.28 ± 0.09 ng/ml).

In patients sampled on more than one occasion glucagon concentrations were unchanged in six, showed a significant rise in three (0.19, 1.26 and 0.43 ng) and a fall in one (−0.5 ng).

The mean concentration of insulin in normal fasting subjects was 12 (± 1) μunits/ml. After administration of 50 g of glucose this rose to 50 (± 10) μunits/ml (n = 8).

In the patients with pancreatitis the mean insulin concentration was 30 (± 7) μunits/ml. The correlation of insulin and glucose concentrations gave a coefficient of +0.44 (P < 0.05). There appeared to be some relationship between glucose and insulin concentrations, but the insulin response to increased glucose concentration in the patients with pancreatitis appears somewhat less than the response of normal subjects to a lower glucose concentration.

There was no correlation between the concentration of glucagon and those of calcium and amylase.

DISCUSSION

The role of the alpha cell in acute pancreatitis has not been adequately assessed because of the non-specificity of assay procedures which have been used. We believe that the assay and extraction procedures we have described provide valid measurements of plasma pancreatic glucagon.

The normal plasma glucagon values in fasting subjects (0.48 ± 0.08 ng/ml) are slightly higher than those (0.1–0.3 ng/ml) reported by Unger et al. (1970), and Heding (1971), who also used pancreatic specific antiserum, but are within the range described by Langslow & Hales (1970) who used a bioassay.

The antiserum which was used showed no cross-reaction with insulin, pancreozymin or secretin and did not detect the contaminating gastrointestinal glucagon-like material which has been reported by Assan et al. (1969) to be present in Boots’ preparations of pancreozymin and secretin. Further evidence of specificity is provided by the failure of extracts of human stomach, duodenum, ileum and colon to suppress the binding of 125I-labelled glucagon with antiserum. Valverde, Rigopoulou, Marco, Faloona & Unger (1970) have demonstrated that at least part of the gastrointestinal glucagon-like material has a higher molecular weight than crystalline pancreatic glucagon in spite of immunological similarity. No such material was detected by immunoassay with our antiserum. Assays of glucagon-like material which measure both pancreatic and gastrointestinal ‘glucagon’ have shown a rise in the concentration of glucagon-like material after oral glucose administration (Lawrence, 1966; Samols, Tyler, Megyesi & Marks, 1966); with pancreatic-specific antiserum a fall has been demonstrated.
following oral glucose administration (Heding, 1971) and our results in the normal subjects confirm this inverse relationship between glucagon and glucose concentrations. Since other non-specific factors such as globulin concentration (Assan et al., 1969) and salt and urea concentration (Day, 1972) may interfere with the assay, an extraction was used which excluded these factors and allowed concentration of the samples without loss of immunological activity.

In the group of patients with pancreatitis the normal inverse relationship between glucagon and glucose appeared to be impaired, indicating alpha-cell dysfunction. The concentrations of glucagon in pancreatitis, although not significantly different from those of normal fasting subjects, were high when related to their glucose values. In contrast all patients with haemorrhagic pancreatitis had low glucagon concentrations. These findings agree with the hypothesis that mild or moderate damage to the alpha cell is associated with a relative hyperglucagonaemia but once the disease has progressed severe damage to the alpha cell results in depletion of glucagon. On the other hand, the low concentrations in severe pancreatitis could be attributed to destruction of glucagon by proteolytic enzymes, but there is no evidence at present to support this alternative. It is known that acute oedematous pancreatitis progresses to haemorrhagic pancreatitis in some patients and this has also been demonstrated experimentally in animals (Pfeffer, Stasior & Hinton, 1957). We have shown that non-severe pancreatitis is associated with a relative hyperglucagonaemia. In view of these findings and the known inhibitory effect of glucagon on pancreatic exocrine secretion, we suggest that a fall in plasma glucagon concentration may contribute to progression of pancreatitis by allowing secretion of pancreatic juice to continue unchecked in the inflamed gland.

The variation in glucagon concentrations in four of the subjects with moderate pancreatitis in whom glucagon concentration was measured on more than one occasion would appear to indicate that the alpha cell is still capable of changing its rate of secretion. This, in addition to the hyperglucagonaemia in the group of patients with pancreatitis as a whole, is similar to the findings of Aguilar-Parada, Eisentraut & Unger (1969), who reported hyperglucagonaemia in patients with diabetes mellitus. They suggested that this was due to alpha-cell dysfunction on the basis of hyperglucagonaemia that was not suppressed by high glucose concentrations. Their subjects were nevertheless capable of increasing their plasma glucagon concentrations in response to arginine infusion. In some instances of experimental diabetes in animals hyperglucagonaemia occurred and this was reversed by insulin administration (Muller, Faloona & Unger, 1971). In our patients with pancreatitis insulin concentrations were higher than in normal fasting subjects. The insulin concentrations showed some correlation with those of glucose ($r = 0.44$). However, the increase in insulin concentration observed in acute pancreatitis was lower than would normally be expected if the elevated glucose concentrations were considered. Insulinolysis seems unlikely since Pi-Sunyer, Byrne & Freinkel (1968) were unable to demonstrate evidence of this in experimental acute pancreatitis. The other alternative is impairment of insulin secretion. The combination of alpha- and beta-cell dysfunction may explain the increased incidence of carbohydrate intolerance during acute pancreatitis (Williams, 1960).

**ACKNOWLEDGMENTS**

We should like to express our thanks to Professor J. Anderson for his help and support in this work, Miss J. Summerhayes for her technical assistance, Miss D. Tillman for secretarial assistance and the Lister Institute for the supply of human albumin.
References


