NOTE

Nature of Plasma Immunoreactive Glucagon in Man

KENJI SHIMA, TATSUO MATSUYAMA, SEIICHIRO TARUI
AND MITSUO NISHIKAWA

Central Laboratory for Clinical Investigation and the Second Department of
Internal Medicine, Osaka University Medical School, Osaka

Synopsis

The effect of differing crossreactivities of antiglucagon sera with pancreatic and
gut glucagon on the content of immunoreactive glucagon (IRG) in plasma has been
investigated.

Two types of antisera employed in the study gave identical dilution curves for
pancreatic glucagon, but different ones for gut glucagon. The mean value of IRG of
seven plasma samples determined using antiserum less reactive to gut glucagon was
0.57 ± 0.19 μg/ml, but 0.77 ± 0.22 μg/ml when measured with another antiserum.
Similar findings were observed in IRG determination in the plasma samples obtained
from a pancreatectomized patient containing gut glucagon alone. The recovery of gut
glucagon added to plasma assayed with one antiserum differed from that using another,
but those of pancreatic glucagon were the same without reference to antiserum employed.

These facts suggest a possible explanation for the widely divergent results reported
for circulating glucagon levels.

Since an immunoassay technique was first
applied for measurement of glucagon, many
studies on circulating glucagon levels under
various conditions have been conducted. Their results, however, are not necessarily
unanimous, as suggested by the fact that more
than twenty fold differences in plasma im-
umnoreactive glucagon (IRG) values in fast-
ing healthy subjects have been reported.
(Schalch, 1966; Aguilar-Parada et al., 1969)

Several factors are considered to be re-
sponsible for the wide range of reported
plasma IRG concentrations in the basal
condition. One possible factor may be con-
ected with the problem of the concomitant
presence in plasma of an IRG of extrapan-
creatic as well as pancreatic origin, as sug-
gested in the studies of Samols et al. (1966)
and Valverde et al. (1967, 1970 a and b).

Plasma IRG levels of normal subjects re-
ported by Aguilar-Parada et al. (1969), using
an antiglucagon serum which reacted poorly
with gut glucagon, were notably lower than
those determined with antisera which might
crossreact to a moderate degree with gut

These facts show that an unequal
affinity of antiglucagon sera employed by
various investigators for endogenous gut and
pancreatic glucagon might be one of the
causes for the striking differences in normal
plasma IRG levels.

The present study was undertaken to throw
light on this problem by using two different
antiglucagon sera which reacted with gut

Materials and Methods

Antiserum to glucagon was prepared in rabbits
using a modification of the method of Assan et al.
(1965) Two antisera, #150 and #159, employed for

Received for publication September 27, 1972.
the experiment were useful in a final dilution of 1:3,000. Crystalline pancreatic glucagon was obtained from Lilly Research Laboratory, Indianapolis, Indiana. Anti rabbit γ-globulin goat serum was purchased from Dainabot ‘RI Research Laboratory, Tokyo. Crude acid alcohol extract of human jejunum was prepared by the method of Kenny (1955). Plasma samples were obtained from normal volunteers and a pancreatectomized patient, after an overnight fast or a 100 g glucose load, by collecting blood in heparinized syringes and immediately separating the plasma. The samples were stored frozen with Trasylol (4,000 KIU/ml of plasma) until the assay. The buffer utilized for a dilution of plasma and crude gut extract as well as for the assay was 0.04M phosphate buffer, pH 7.4 containing 1% bovine serum albumin and 0.01% merthiolate.

The concentration of glucagon was determined by the double antibody radioimmunoassay described previously (Shima and Foa, 1968).

Results

Figure 1 shows the crossreactivities of antiglucagon sera, #150 and #159, with pancreatic glucagon and a crude gut extract. Both antisera gave similar dilution curves for pancreatic glucagon, showing the same degree of affinity for pancreatic glucagon. However, percentages of bound radioactivity were markedly different between these two antisera at any given amounts of the gut extract, higher with #150 than with #159.

The mean contents of IRG in 250 μg of the gut extract assayed with #150 and #159 were 2.15 ± 0.24 and 3.72 ± 0.25 μg, respectively; the significantly higher value was given by the assay with #159. Similar findings were observed for the assay of 125 and 62.5 μg of the gut extracts (Table 1).

The contents of IRG in seven plasma specimens were determined using antisera #150 and #159. As shown in Table 2, the value measured with #159 was higher than the corresponding one with #150 without exception. Figure 2 shows IRG values measured in plasma samples from a pancreatectomized

<table>
<thead>
<tr>
<th>gut ext. added (μg)</th>
<th>mean IRG ± S.D. (μg)</th>
<th>AGS 150</th>
<th>AGS 159</th>
<th>AGS 159 × 100 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>2.15 ± 0.24</td>
<td>3.72 ± 0.25</td>
<td>173.5 ± 13.9</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>1.74 ± 0.18</td>
<td>2.64 ± 0.17</td>
<td>153.0 ± 10.4</td>
<td></td>
</tr>
<tr>
<td>62.5</td>
<td>1.02 ± 0.08</td>
<td>1.58 ± 0.18</td>
<td>155.5 ± 22.1</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± S.D. of seven determinations.
Table 2. Difference in plasma IRG values measured with different anti-glucagon sera (AGS)

<table>
<thead>
<tr>
<th>Plasma</th>
<th>IRG found (mug)</th>
<th>AGS 159/AGS 150 × 100 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGS 150</td>
<td>AGS 159</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.53</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>0.53</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>0.66</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0.37</td>
</tr>
<tr>
<td>6</td>
<td>0.66</td>
<td>1.07</td>
</tr>
<tr>
<td>7</td>
<td>0.9</td>
<td>0.93</td>
</tr>
</tbody>
</table>

mean 0.57 0.77 142.8
± S.D. 0.19 0.22 28.6

patient before and after oral glucose using both antisera. Here again, similar findings to those in Table 2, were observed. The dilution curve for IRG in the plasma sample obtained from the pancreatectomized patient was shallower than that for pancreatic glucagon, but superimposed on the curve for IRG in the gut extract shown in Figure 3, indicating that IRG contained in the samples from the patient was derived exclusively from gut glucagon.

The recoveries of pancreatic glucagon added to plasma from the pancreatectomized patient were found to be satisfactory, ranging from 90 to 100%, irrespective of the antisera employed (Table 3). On the other hand, there were difference between the recoveries of gut glucagon added to plasma, when measured with #150 and #159, and the values measured with #159 were higher than those with #150 (Table 4).

Discussion

The difficulties in the specific measurement of pancreatic glucagon in plasma by radioim-
munoassay have handicapped research on circulating glucagon levels, resulting in widely divergent findings of plasma glucagon levels by different authors, (Unger et al., 1963; Samols et al., 1965; Schalch, 1966; Lawrence, 1966; Shima and Foà, 1968; Vance et al., 1968; Aguilar-Parade et al., 1969), and have contributed to a confusion of understanding of the physiological role of this material.

The presence of gut glucagon in plasma is now an established fact (Buchanan et al., 1967; Unger et al., 1968). Since pancreatic and gut glucagon have shown appreciable amounts of immunoreactivity by the glucagon immunoassay using ordinary glucagon antiserum (Heding, 1971), the total immunoreactivity on this assay procedure is composed of both immunoreactive pancreatic glucagon and immunoreactive gut glucagon. Therefore, when pancreatic glucagon was adopted as a standard for the assay of gut glucagon and the results were described in the amount of pancreatic glucagon equivalency, variable results would be inevitably found, depending on the difference in crossreactivity of the antiserum with pancreatic glucagon and with gut glucagon.

Two antisera, #150 and #159, employed in the present study gave identical dilution curves for pancreatic glucagon, but different ones for gut glucagon, indicating the varying degrees of their affinity for gut glucagon (Fig. 1). If the contents of IRG in a certain weight of the gut extract are measured with these antisera using pancreatic glucagon as a standard, the values obtained with #150 must be different from those with #159. The results in Table 1 show that this is the case. Furthermore, the fact that the content of IRG in the same plasma sample measured with #159 was higher than that with #150 when expressed in terms of pancreatic glucagon, might be attributed to the concomitant presence in plasma of gut glucagon of which the reading was higher when assayed with #159. The assumption was also confirmed by the results of the recoveries of pancreatic and gut glucagon added to the plasma samples.

Although the present study has revealed one of the factors which may invalidate the radioimmunological determination of glucagon in plasma, more work is needed on this assay in order to clarify other problems such as reactivity of the pancreatic glucagon-specific antiserum with degraded fragments of glucagon (Heding, 1971).

Acknowledgments

The authors wish to recognize the kindness of Dr. Piero P. Foà in supplying the anti-glucagon sera. The technical assistance of Miss Mikiko Kobayashi and Mrs. Mari Goto is gratefully acknowledged.

References