Responses of plasma cyclic AMP, serum immunoreactive insulin, C-peptide immunoreactivity and blood sugar levels to glucagon in patients with liver diseases.

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Abstract

Levels of plasma cyclic AMP, serum immunoreactive insulin (IRI), serum c-peptide immunoreactivity (CPR) and blood sugar (BS) were determined 0, 15, 30, 45 and 60 min after a glucagon injection (0.01 mg per kg body weight) in normal controls, patients with acute hepatitis and liver cirrhosis. Plasma cyclic AMP responses to glucagon in liver disease patients varied widely in peak value, and only in patients with fulminant hepatitis and decompensated liver cirrhosis with poor prognosis was the response suppressed. The peak response of BS was found significantly later in liver cirrhosis patients than in normal controls. IRI and CPR responses to glucagon were lower in acute hepatitis patients than in normal controls and liver cirrhosis patients. IRI levels and their sum were also lower in acute hepatitis patients, although CPR levels were not significantly different. Thus, the ratio of the sum of CPR from 0 to 60 min to that of IRI was significantly higher in acute hepatitis, indicating impaired pancreatic secretion of insulin to glucagon stimulation as well as increased uptake of insulin by the liver in acute hepatitis.

KEYWORDS: liver diseases, glucagon, cyclic AMP, immunoreactive insulin, c-peptide immunoreactivity

*PMID: 3000142 [PubMed - indexed for MEDLINE]
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RESPONSES OF PLASMA CYCLIC AMP, SERUM IMMUNOREACTIVE INSULIN, C-PEPTIDE IMMUNOREACTIVITY AND BLOOD SUGAR LEVELS TO GLUCAGON IN PATIENTS WITH LIVER DISEASES

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Received April 18, 1985

Abstract. Levels of plasma cyclic AMP, serum immunoreactive insulin (IRI), serum c-peptide immunoreactivity (CPR) and blood sugar (BS) were determined 0, 15, 30, 45 and 60 min after a glucagon injection (0.01 mg per kg body weight) in normal controls, patients with acute hepatitis and liver cirrhosis. Plasma cyclic AMP responses to glucagon in liver disease patients varied widely in peak value, and only in patients with fulminant hepatitis and decompensated liver cirrhosis with poor prognosis was the response suppressed. The peak response of BS was found significantly later in liver cirrhosis patients than in normal controls. IRI and CPR responses to glucagon were lower in acute hepatitis patients than in normal controls and liver cirrhosis patients. IRI levels and their sum were also lower in acute hepatitis patients, although CPR levels were not significantly different. Thus, the ratio of the sum of CPR from 0 to 60 min to that of IRI was significantly higher in acute hepatitis, indicating impaired pancreatic secretion of insulin to glucagon stimulation as well as increased uptake of insulin by the liver in acute hepatitis.

Key words: liver diseases, glucagon, cyclic AMP, immunoreactive insulin, c-peptide immunoreactivity.

We have reported that the response of the plasma cyclic AMP level to glucagon is reduced in advanced liver cirrhosis and in fulminant hepatitis while it is rather enhanced in uncomplicated acute hepatitis (1). Several reports have appeared since, indicating the usefulness of the glucagon-cyclic AMP test in the assessment of the maximum functional capacity of injured livers (2-11). Some conflicting results have also been reported (12).

In the present study, we analyzed, by referring to liver function test and liver histology, the cyclic AMP responses to glucagon administration in cases of liver diseases,

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together with the responses of immunoreactive insulin (IRI), c-peptide immunoreactivity (CPR) and blood sugar (BS).

The results indicated a wide variation in peak value of cyclic AMP response not only in the liver disease groups but also in the control group, although markedly suppressed responses were seen in patients with fulminant hepatitis and liver cirrhosis with poor prognosis. Interestingly, the responses of IRI and CPR were significantly reduced in acute hepatitis patients, but not in liver cirrhosis patients, suggesting the possible application of insulin for the treatment of acute hepatic injury.

PATIENTS AND METHODS

The glucagon stimulated increase in plasma cyclic AMP (glucagon-cyclic AMP test) was studied in 7 volunteers (4 males and 3 females, 21-41 years old) with normal liver function tests, 15 patients with acute viral hepatitis (icteric cases with a mean serum alanine aminotransferase (GPT) level of 921 ± 655 Karmen units, 5 males and 10 females, 21-48 years old) and 8 patients with liver cirrhosis of posthepatic etiology (a mean K ICG value of 0.067 ± 0.031, 7 males and 1 female, 40-56 years old) without encephalopathy. The diagnoses were made by laboratory test and/or liver histology. Body weights of the patients, expressed as a percent of the ideal body weight, were not significantly different among the three groups (means ± SD for normal controls, acute hepatitis patients and liver cirrhosis patients were 104 ± 13, 113 ± 26 and 96 ± 14, respectively). Fasting blood sugar levels were all less than 120 mg/dl. One patient with fulminant hepatitis, one with decompensated liver cirrhosis having encephalopathy and one with obstructive jaundice from pancreas cancer were studied separately only for the cyclic AMP response. In 3 cases of acute hepatitis and in a case of obstructive jaundice, the glucagon-cyclic AMP test was performed before and after normalization of liver function tests in natural recovery from acute hepatitis or in percutaneous transhepatic bile drainage (PTBD).

The glucagon-cyclic AMP test was performed in the morning after overnight fasting. After collection of venous blood samples for estimation of basal levels of plasma cyclic AMP, serum immunoreactive insulin (IRI), c-peptide immunoreactivity (CPR), plasma glucagon and blood sugar (BS), a bolus intravenous injection of 0.01 mg glucagon (Novo) per kg body weight in 5 ml of isotonic saline was given at 0 min in about 10 seconds and blood samples were drawn at 15, 30, 45 and 60 min. The samples of plasma for assay of immunoreactive glucagon were immediately separated at 4°C from blood collected in tubes containing ethylenediamine tetraacetic acid (EDTA) and aprotinin (2000 units), frozen and assayed with antibody against 30K glucagon at Otsuka Assay Laboratory (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan). The samples of plasma for assay of cyclic AMP were obtained at 4°C from blood collected in tubes containing EDTA, pH 7.4 to give a final concentration of 10 mM, and their cyclic AMP concentrations were determined by radioimmunoassay using Yamasa Cyclic AMP assay kit (Yamasa Syoyu Co., Ltd., Chiba, Japan)(13). Sera obtained from the remaining blood samples were stored at −20°C and used for determinations of IRI with an Insulin Ria kit (Dinabott Radioisotope Laboratory, Tokyo, Japan) with a low detection limit of 5 μu/ml, and for determinations of CPR with a C-peptide kit (Daiichi Radioisotope Laboratories Co., Ltd., Tokyo, Japan).

Statistical analyses were made by a non-parametric, distribution free approach to determine
the significance levels of differences using Mann-Whitney’s U-test, chi-square test and Fisher’s exact probability test (14). We used Mann-Whitney’s U-test unless otherwise indicated. The results were given as the mean ± SD. The sum of measured values from 0 to 60 min was expressed as sigma (Σ), and the difference between the peak and basal values (0 min) was expressed as delta (Δ).

RESULTS

Plasma cyclic AMP concentrations in healthy subjects, acute hepatitis and cirrhotic patients following the glucagon injection are shown in Fig. 1. The peak of plasma cyclic AMP response was found at 15 min in all the subjects, although the peak level varied considerably, particularly in the groups of liver diseases. The mean peak levels of cyclic AMP were slightly higher in the liver disease groups, although the differences were not significant. On the other hand, cyclic AMP levels 30, 45 and 60 min after the glucagon injection in acute hepatitis patients and at 45 min in liver cirrhosis patients were significantly higher than those in healthy controls (p < 0.05), indicating a delayed clearance of cyclic AMP from the plasma in liver diseases. The basal cyclic AMP level in liver cirrhosis patients was higher than that in normal controls or acute hepatitis patients as we reported previously (p ≤ 0.002) (15).

The basal glucagon levels were elevated in both liver disease groups (Table 1). The responses of plasma cyclic AMP in some cases of acute hepatitis examined after the recovery from acute hepatic injury were reduced as compared with the initial responses (Fig. 2).
TABLE 1. BASAL GLUCAGON LEVELS IN PLASMA OF NORMAL SUBJECTS AND LIVER DISEASE PATIENTS

<table>
<thead>
<tr>
<th></th>
<th>No. of subjects</th>
<th>Glucagon (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>126.3 ± 48.1</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>13</td>
<td>363.5 ± 212.2</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>8</td>
<td>348.4 ± 120.3</td>
</tr>
</tbody>
</table>

* p<0.05, *** p≤0.002

Fig. 2. Responses of plasma cyclic AMP level to glucagon injection in 3 patients with acute hepatitis before and after recovery. Closed symbols (○, ●, and ▲ for different cases) represent the values in the acute stage, and the corresponding open symbols represent the values for the respective cases after recovery.

In a case of fulminant hepatitis and a case of liver cirrhosis with encephalopathy, both of them having died of hepatic failure shortly after, the cyclic AMP responses were much smaller and the maximum increases were less than 100 nmoles/dl (Fig. 3).

The glucagon-cyclic AMP test was performed twice, before and after PTBD, on a case of obstructive jaundice with pancreas cancer (Fig. 4). The peak values of cyclic AMP before and after the drainage were not different; however, the retarded clearance of cyclic AMP was markedly improved following the bile drainage.
Fig. 3. Responses of plasma cyclic AMP level to glucagon injection in a case of fulminant hepatitis (▲) and a case of decompensated liver cirrhosis with hepatic encephalopathy (●).

Fig. 4. Responses of plasma cyclic AMP level to glucagon injection in a case of obstructive jaundice before PTBD (●) and after PTBD (○).

BS, IRI and CPR levels before and after glucagon injection in healthy subjects, acute hepatitis and liver cirrhosis patients are shown in Figs. 5, 6, 7. The
peak of the BS level in normal controls in the glucagon test was found mostly at 15 min, and in acute hepatitis and liver cirrhosis patients at 30 min. The difference between normal subjects and cirrhotic patients was significant by Fisher’s exact probability test (p<0.03). The increments of BS at 15 min in the liver disease patients were thus smaller than in the healthy controls, the difference between normal controls and liver cirrhosis patients being significant (p<0.05). No other values of BS given in Table 2 and Fig. 5 were significantly different among the groups studied.

The peak concentrations of IRI and CPR were found at 15 min in most cases independent of the presence of liver disease. Thus, the peak responses of IRI and CPR were found before those of BS (p<0.005, chi-square test) in liver disease patients in contrast with the normal group, in which the peaks of BS, IRI and CPR were found mostly at 15 min (Figs. 5, 6 and 7). In acute hepatitis patients, the IRI response

| Table 2. The sum of measured values from 0 to 60 min (Σ) and the difference between the peak and basal values (Δ) of plasma cyclic AMP (cAMP), IRI, CPR and BS |

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Σ cAMP (nmoles/dl)</th>
<th>Δ cAMP (nmoles/dl)</th>
<th>Σ BS (mg/dl)</th>
<th>Δ BS (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1346.7 ± 369.1</td>
<td>890.3 ± 242.6</td>
<td>467 ± 66</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>2169.0 ± 1171.7</td>
<td>1223.6 ± 720.3</td>
<td>457 ± 87</td>
<td>22 ± 13</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>1970.9 ± 1130.0</td>
<td>1143.1 ± 731.5</td>
<td>488 ± 68</td>
<td>26 ± 17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Σ IRI (µu/ml)</th>
<th>Δ IRI (µu/ml)</th>
<th>Σ CPR (ng/ml)</th>
<th>Δ CPR (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>91.2 ± 23.1</td>
<td>35.6 ± 14.1</td>
<td>11.1 ± 2.2</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>15</td>
<td>10.7 ± 7.8</td>
<td>10.8 ± 4.8</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>8</td>
<td>31.4 ± 18.6</td>
<td>13.3 ± 3.0</td>
<td>1.5 ± 0.9</td>
</tr>
</tbody>
</table>

# No. of subjects was 6. ** p≤0.01, *** p≤0.002
IRI: Immunoreactive insulin, CPR: C peptide immunoreactivity, BS: Blood sugar

Fig. 5. Responses of blood sugar level to glucagon injection in normal controls, acute hepatitis and liver cirrhosis patients. Column with horizontal bar indicates mean ± SD.

Fig. 6. Responses of serum immunoreactive insulin (IRI) level to glucagon injection in normal controls, acute hepatitis and liver cirrhosis patients. Column with horizontal bar indicates mean ± SD.

Fig. 7. Responses of serum c-peptide immunoreactivity (CPR) level to glucagon injection in normal controls, acute hepatitis and liver cirrhosis patients. Column with horizontal bar indicates mean ± SD.
Table 3. Ratios of Measured Parameters Related to IRI Secretion

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Σ CPR/Σ IRI</th>
<th>Δ CPR/Δ IRI</th>
<th>Σ IRI/Σ BS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.132 #</td>
<td>0.063</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>± 0.026</td>
<td>± 0.027</td>
<td>± 0.057</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>0.259 **</td>
<td>0.290</td>
<td>0.092 **</td>
</tr>
<tr>
<td></td>
<td>± 0.103</td>
<td>± 0.228</td>
<td>± 0.025</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>0.131 **</td>
<td>0.047</td>
<td>0.243 **</td>
</tr>
<tr>
<td></td>
<td>± 0.043</td>
<td>± 0.029</td>
<td>± 0.126</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Δ IRI/Δ BS</th>
<th>Σ IRI/Σ cAMP</th>
<th>Δ IRI/Δ cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.529</td>
<td>0.067</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>± 0.803</td>
<td>± 0.038</td>
<td>± 0.029</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>0.639 **</td>
<td>0.026 **</td>
<td>0.014 **</td>
</tr>
<tr>
<td></td>
<td>± 0.665</td>
<td>± 0.019</td>
<td>± 0.014</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>1.356 **</td>
<td>0.071 **</td>
<td>0.035 **</td>
</tr>
<tr>
<td></td>
<td>± 0.767</td>
<td>± 0.037</td>
<td>± 0.022</td>
</tr>
</tbody>
</table>

# No. of subjects was 6. * p<0.05, ** p<0.01, *** p<0.002
IRI, CPR, BS: See Table 2.

to glucagon was significantly lower at 15 (p<0.05) and 30 min (p<0.002) than in normal controls, and at 0 (p<0.05), 15 (p<0.002), 30 (p<0.002) and 45 min (p<0.002) than in liver cirrhosis patients. Both Σ IRI and Δ IRI were significantly lower in the acute hepatitis group than in the other groups (Table 2). The CPR response was also reduced in acute hepatitis patients, although CPR levels varied widely and were not significantly different from the other groups. Thus, the Δ CPR in the acute hepatitis group was smaller than in controls, and the Σ CPR and the serum levels of CPR were not different among the three groups (Table 2 and Fig. 7). When the glucagon-stimulated insulin secretion in acute hepatitis was analyzed by taking the ratios of measured parameters (Table 3), the Σ CPR/Σ IRI and the Δ CPR/Δ IRI ratios were high, although the latter was not statistically significant. The results indicated the impaired secretion of insulin as well as the increased uptake of insulin in acute hepatitis. Σ IRI was diminished in acute hepatitis independent of BS (see Σ IRI/Σ BS in Table 3). It may also be seen from the ratios given in Table 3 that in acute hepatitis the response of IRI to glucagon was impaired as compared with that of cyclic AMP to glucagon (see Σ IRI/Σ cyclic AMP and Δ IRI/Δ cyclic AMP in Table 3).

When the correlations among the above parameters were analyzed independently of the disease groups, significant positive correlations were found in Σ IRI v.s. Δ BS (r = 0.478, p<0.02), Σ CPR v.s. Σ BS (r = 0.429, p<0.05), but not in Σ cyclic AMP or Δ cyclic AMP v.s. Σ BS or Δ BS. Thus, the mechanisms of BS release from the liver and of the secretion of CPR to glucagon stimulation appear to be different
as far as the plasma cyclic AMP level is concerned.

DISCUSSION

The original idea of the glucagon-cyclic AMP test was to assess hepatic function by specifically stimulating the adenyl cyclase system with glucagon. Since our initial report on the glucagon-cyclic AMP test in patients with acute hepatitis and liver cirrhosis (1), a number of similar works have been carried out in different laboratories (6-11). General conclusions are in agreement with ours that the glucagon-cyclic AMP test differentiates patients with severe hepatic injury from those with mild or no injury. Only fulminant hepatitis and decompensated liver cirrhosis with poor prognosis gave low responses of cyclic AMP. Identical results have been reported by Desgrez et al. (4), Francavilla et al. (5), Matsuda et al. (7) and Miyakoshi et al. (8). Lower responses of cyclic AMP in liver cirrhosis patients (compensated or decompensated) as compared with those in normal controls have been reported by some groups of workers (9-11). The difference between the opposing results can not be explained by the difference in modality of the glucagon-cyclic AMP test (one shot i.v., dripping infusion or i.m.). The discrepancy appears to be accountable only by the difference in the cases studied. In view of the large variation observed in normal controls in the present study, it is rather difficult to expect a clear-cut difference between two groups differing slightly in disease activity.

The variation observed in the healthy subjects may result from the difference in dietary condition. Since the extent of cyclic AMP response to glucagon depends mainly on the hepatic thyroid status (16), fasting would give a low T3 status (17) and reduce the cyclic AMP response. In hepatic injury the T4 to T3 conversion is also affected (18), and this may cause altered responses to glucagon. Refractoriness to exogenous glucagon is known to be present in cases with sustained elevation of endogenous glucagon (19). Thus, the elevated glucagon levels in hepatitis and cirrhosis patients could constitute another factor causing variation in the cyclic AMP response, although no negative correlation was found between the fasting glucagon level and the subsequent cyclic AMP response to the exogenous glucagon in the present study.

When considering the physiological importance of the cyclic AMP response, it is rather unreasonable to expect markedly suppressed cyclic AMP responses in mild hepatic injuries, particularly when a near maximum-dose glucagon stimulation is given as in the present study. Conversely, a small-dose glucagon stimulation might differentiate between small changes in the so-called reserved hepatic function as were reported by Maekubo et al. (6). The hyper-response of cyclic AMP to glucagon found in acute hepatitis patients in this and other studies (4, 6, 7) is similar to that observed in cholestasis patients in that a delayed clearance of cyclic AMP was observed in patients with either pathological condition of the liver.
Thus, the cholestasis appears to be important as a common underlying factor to enhance the cyclic AMP response as revealed by Francavilla et al. (20). However, no significant correlation was found between the cyclic AMP response and the serum levels of alkaline phosphatase and bilirubin. Discrepant results have also been reported (11). It is also to be noted that the response of BS to glucagon was reduced in liver cirrhosis patients in spite of unaltered cyclic AMP responses. This could be due to the reduced glycogen content in the cirrhotic liver.

The reduced insulin response to glucagon in acute hepatitis demonstrated in the present work is interesting in that the glucose-stimulated insulin secretion in liver diseases is generally reported to be enhanced (21-26). The reduced insulin secretion is not secondary to the smaller BS elevation since the insulin peak preceded the BS rise in liver injury groups. In acute hepatitis, not only Δ IRI but also Δ CPR following glucagon injection was low, indicating that the secretion of insulin from the pancreas is suppressed. In addition, although the interpretation of the change in the peripheral c-peptide to insulin molar ratio requires several assumptions, such as a fixed metabolic clearance ratio of insulin and c-peptide, the high Σ CPR/Σ IRI ratio in acute hepatitis might indicate the increased uptake or clearance of insulin by the injured liver (27). Also, the increased uptake of insulin would contribute to the lower response of IRI in acute hepatitis. These reciprocal changes in insulin response to different stimuli, namely glucose and glucagon, may be explained by assuming that the mechanisms of insulin secretion by glucose and glucagon are different, and that only the glucagon-dependent system is impaired in acute liver injury. Since the mean basal BS level in acute hepatitis was not significantly different from others, it is not the basal BS level that potentiates the secretion of insulin by glucagon. In stimulation of insulin secretion by glucose, the insulin resistance in liver injury (28, 29) results in higher blood glucose levels, which in turn stimulate the pancreatic β-cells and cause the hyper-response of insulin to occur. Accordingly, the use of glucagon as a stimulant of β-cells is more reasonable for the assessment of pancreatic dysfunction in acute hepatitis.

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Plasma Cyclic AMP in Glucagon Test


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