Plasma Insulin and Glucagon and Their Release from Pancreatic Islet in Hyperosmolar Diabetic Rat

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Abstract. In order to investigate the metabolic abnormalities in hyperosmolar diabetes from the viewpoint of insulin or glucagon, experimental hyperosmolar diabetes was produced by a combination of cortisol injection and water deprivation or only by the latter in streptozotocin-induced moderately hyperglycemic rat. They had a high blood glucose level and high plasma osmotic pressure. Fasting plasma insulin tended to decrease in the dehydrated state whether diabetic or not. Fasting plasma glucagon was increased to 0.047 ± 0.009 nmol/l (P<0.05) in the non-diabetic dehydrated state (normal 0.026 ± 0.004 nmol/l), and a similar high level of plasma glucagon was observed in the dehydrated diabetic rat (0.052 ± 0.020 nmol/l), especially after cortisol treatment. In isolated rat islet, insulin released from the dehydrated diabetic rat at a high concentration of glucose was to some extent lower than that of diabetic rat, and released IRG vice versa. The insulin: glucagon ratio in the presence of high glucose was significantly lower in the dehydrated diabetic rat than in the normal rat (P<0.01). In the diabetic rat this ratio was not significantly different. This finding was also consistent with the results of in vivo experiments. Thus more catabolic hormonal changes were found in in vivo and in vitro studies in the hyperosmolar diabetic rat.

HYPEROSMOLAR nonketotic diabetic coma (HNKC) is generally found in the aged and untreated non-insulin dependent diabetics who have been treated with diuretics, glucocorticoids, mannitol or tube feeding in the case of cerebro-cardiovascular disease or surgical operation [1-3], but its pathogenesis is still not fully clarified and the dehydration is considered to be one of the most important factors contributing to that state. The production of experimental hyperosmolar diabetic rat has already been reported [4, 5]. Till recently no report has been published on the secretion of insulin (IRI) and glucagon (IRG) in these states.

In this study, we developed an experimental hyperosmolar diabetic rat and investigated plasma immunoreactive insulin (IRI) and glucagon (IRG), and the amounts of them in the pancreas. Moreover in vitro studies with pancreatic islets have been done from the view point of the release of IRI and IRG.

Materials and Methods

Experimental hyperosmolar diabetic rat

Male Wistar rats weighing 200 to 300 g were used in all experiments. Rats were made moderately diabetic by the iv administration of 40 mg streptozotocin (STZ) per kg body weight after overnight fasting. These animals were not used until at least four weeks after the onset of diabetes. All the diabetic rats were maintained without exogenous insulin injection. Cortisol-treated rats received 5 mg cortisol i.m. for six days. Production of the hyperosmolar diabetic rat was accomplished by injecting cortisol i.m. into the moderately hyperglycemic diabetic rats followed by water deprivation starting on the fourth day of cortisol injection [4] or by water deprivation only. The
Table 1. Experimental group

The experimental group was divided into eight groups (A to H) according to whether treated with STZ, cortisol or dehydration.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>STZ</th>
<th>cortisol</th>
<th>dehydration</th>
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<tbody>
<tr>
<td>A</td>
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<tr>
<td>B</td>
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<td>G</td>
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<tr>
<td>H</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

The experimental group was divided into eight groups (A-H) according to the treatment with STZ, cortisol or dehydration (Table 1). The experiment was done after fasting overnight on the seventh day, and blood was taken from the tail vein for glucose determination. Rats were anesthetized by the intraperitoneal administration of sodium pentobarbital and blood was taken from the abdominal aorta, and transferred to the test tube containing EDTA and aprotinin (500 U/ml), and centrifuged. Plasma was frozen until hormonal immunoassay or osmotic pressure determinations. A piece of pancreas was obtained from the tail of the pancreas and the tissue extracted with acid alcohol to determine pancreatic IRI and IRG and the extract was assayed as already reported [7]. The urinary ketone body in diabetic rat was checked by ketostix, and all were negative.

Islet experiment

In in vitro studies pancreatic islets were isolated by a modification of the method of Lacy and Kostianovsky [8]. For each preparation, the pancreas was inflated via the bile duct with Hanks salt solution. The distended total pancreas was minced and incubated with collagenase (CLS-IV, Worthington Biochemical Corp., NJ, USA) with vigorous shaking. After several washes, the sediment was examined under a dissecting microscope and individual islets were transferred to small beakers containing incubation medium in a randomized fashion. In the experiment on the release of IRI and IRG from isolated pancreatic islets, a batch of 10 islets was used. The islets were first preincubated in 0.5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2 mg/ml bovine plasma albumin (BPA) and 3.3 mmol/l glucose for 30 min at 37°C in a shaking water bath with a gas phase of 95% O₂-5% CO₂. At the end of the 30 min preincubation period, the medium was changed, and the islets were washed once with the incubation medium. The islets were further incubated (95% O₂-5% CO₂) for 90 min at 37°C in 0.5 ml of the test medium containing 500 U aprotinin. At the end of the experiment, the medium was transferred to stock tube and was stored at -20°C.

Assays

IRI in plasma and pancreatic extract was determined by the two-antibody immunoassay of Morgan and Lazarow [9] with rat standard insulin, and IRG by the polyethylene glycol method [10] with 30 K antiglucagon serum specific for pancreatic glucagon. Blood glucose was determined by the glucose oxidase method with an auto-analyser, and plasma osmotic pressure was determined by means of Auto & Stat (Kyoto Dainichi-kagaku Co., Ltd., Kyoto).

Each value was expressed as the mean ± SE. Statistical analysis was performed by Student's t-test and a probability of less than 5% was considered to be significant.

Results

Fasting blood glucose and plasma osmotic pressure

Fasting blood glucose (FBE) in each group is

Assay results were presented in Fig. 1.

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**Fig. 1.** Fasting blood glucose of experimental rats. Fasting blood glucose of experimental rats was determined. Blood was taken from the tail vein. The groups A to H are explained in the Table 1. The values are the mean ± SE, and ***: p<0.01 vs normal control (A).**
Plasma osmotic pressure of experimental rats was determined in groups A to H. Blood was obtained from the aorta according to the method described in Materials and Methods. The values are the mean ± SE, and ***: p<0.01 vs normal control (A) shown in Fig. 1. The FBG values in the non-diabetic control group (A) and in the STZ-rat (E) were 5.8±0.6 mmol/l and 20.8±2.7 mmol/l. In the non-diabetic group, there was no significant difference in FBG among four groups within this range of experiment whether treated with cortisol or by water deprivation. On the other hand, the levels of FBG in the STZ-rat were significantly high in the dehydrated state (F and H) compared with those in the non-dehydrated state (E and G). The FBG levels in groups F and G were 35.1±6.4 mmol/l and 34.9 ± 3.9 mmol/l. Plasma osmotic pressure was determined (Fig. 2). Plasma osmotic pressures for the non-diabetic control group (A) and the non-diabetic and water-deprived one (B) were 305 ± 4 mOs/l and 307 ± 4 mOs/l. Pressure increased significantly to 318 ± 3 mOs/l (p<0.05 vs A) in STZ-rat (E). A particularly high level was observed in diabetes with dehydration (F) reaching 362 ± 10 mOs/l or 359 ± 10 mOs/l in the diabetic rat injected with cortisol or with water deprivation, respectively.

Plasma IRI and IRG in the rat

Fasting plasma IRI and IRG were determined in each group (Fig. 3). Fasting plasma IRI in the non-diabetic rat (A was 0.33±0.12 nmol/l, and 0.21±0.07 nmol/l in the non-diabetic and dehydrated rat (B). In the STZ-rat, plasma IRI in the E group was 0.09±0.03 nmol/l (p<0.01), and it decreased to 0.08±0.02 nmol/l in the G group, but not significantly.

On the other hand, plasma IRG increased to 0.047±0.009 nmol/l in the non-diabetic dehydrated state (p<0.05) compared with normal fasting IRG (A: 0.026±0.004 nmol/l), and a similar high level of plasma IRG (0.052±0.020 nmol/l) was observed at in the dehydration state in STZ-rat (F) especially after cortisol treatment (H: 0.190±0.055 nmol/l).

Plasma insulin: glucagon ratio (I/G ratio) in experimental animals

The molar ratio of fasting plasma IRI and IRG (I/G ratio) was calculated in each experimental group (Table 2). It was decreased in the dehydrated group of both normal and diabetic rats. Especially in the dehydrated diabetic rat with cortisol administration, the value was very low compared with the normal A group (p<0.01) or diabetic E group (p<0.02).
Table 2. Plasma insulin: glucagon ratio in experimental animals

Molar ratio of fasting plasma IRI and IRG was calculated in experimental animals. Each value is the mean ± SE, and n is the number of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
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<tr>
<td></td>
<td>18.10</td>
<td>7.96</td>
<td>26.80</td>
<td>13.20</td>
<td>4.31</td>
<td>1.88</td>
<td>2.00</td>
<td>0.78*</td>
<td></td>
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<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
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<td>8.01</td>
<td>3.79</td>
<td>17.30</td>
<td>3.79</td>
<td>1.29</td>
<td>0.67</td>
<td>0.32</td>
<td>0.47</td>
<td></td>
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</tbody>
</table>

*: p<0.01 vs A  **: p<0.02 vs E

Release of IRI from pancreatic islet

The release of IRI from pancreatic islet was studied in the normal and diabetic rat with or without dehydration (Fig. 5a). In these islet experiments, FBG values for the diabetic rat and dehydrated diabetic rat before starting dehydration were 18.0 ± 5.4 mmol/l (n: 12) and 17.4 mmol/l (n: 12). The osmotic pressure and FBG of the former and the latter in the experiment were 322.2 ± 3.3 mOs/L or 413.3 ± 18.8 mOs/L and 15.7 ± 2.1 mmol/l or 26.2 ± 2.3 mmol/l, respectively. In diabetic rat, the secretion of IRI decreased significantly at a low or high concentration of glucose compared with that in the normal islet. At a high concentration of glucose (16.7 nmol/l), IRI secretion in the normal islet was 2.81 ± 0.55 pmol/islet/90 min (fourteen rats) and in the diabetic or dehydrated diabetic islet was 0.85 ± 0.19 (twelve rats) or 0.50 ± 0.11 pmol/islet/90 min (twelve rats), respectively. Between the dehydrated and non-dehydrated diabetic rat, there was tendency for the insulin to be low in the former in the presence of high glucose, but not significant.

Release of IRG from pancreatic islet

The release of IRG from pancreatic islet was studied in the normal and diabetic rat with or without dehydration (Fig. 5b). In the normal rat, the values for the release of IRG at the basal or high concentration of glucose, or arginine were 0.091 ± 0.028, 0.085 ± 0.015 or 0.098 ± 0.020 pmol/islet/90 min, respectively. In the dehydrated diabetic rat, a higher value for the release of IRG was found at high concentration of glucose compared with normal controls (0.141 ± 0.058 vs 0.085 ± 0.015 pmol/islet/90 min) but it was not statistically significant. IRG release from the normal, diabetic and dehydrated diabetic rat was 0.085 ± 0.015, 0.066 ± 0.001 and 0.141 ± 0.058 pmol/islet/90 min.

Comparison of insulin: glucagon ratio (I/G ratio)

The molar ratio of released IRI to IRG (I/G ratio) was compared among the normal, diabetic and diabetic rats with dehydration. As shown in Table 3, the I/G ratio was very low in the diabetic or dehydrated diabetic rat compared with that of normal rat. In comparison with the normal rat, the value in the dehydrated diabetic rat at a high
Fig. 5. Release of IRI and IRG from pancreatic islet. Ten pancreatic islets from normal, diabetic and diabetic rat with
dehydration were incubated for 90 min at 37°C and IRI and IRG in the medium were determined (eight experiments). The number of each kind of rat was 14, 12 or 12, respectively. Each value is the mean ± SE, and
in parentheses is shown the number of flasks. a) Release of IRI from pancreatic islet, b) Release of IRG from
pancreatic islet.

Table 3. Insulin: glucagon ratio in islet experiment
The molar ratio of IRI and IRG released into the
medium was calculated in the normal, diabetic and
diabetic rats with dehydration. Each value is the
mean ± SE, and in parenthesis is shown the number
of experimental flasks.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Glucose 3.3 mmol/l</th>
<th>Glucose 16.7 mmol/l</th>
<th>Arginine 20 mmol/l</th>
<th>Glucose 3.3 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16.2±5.6 (15)</td>
<td>93.3±23.0 (23)</td>
<td>14.9±3.8 (22)</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>6.1±2.9 (15)</td>
<td>53.3±14.4 (21)</td>
<td>16.7±4.3 (24)</td>
<td></td>
</tr>
</tbody>
</table>
| Diabetic with
dehydration            | 9.2±1.7 (15)       | 25.2±5.5* (21)    | 21.9±6.7 (21)      |

*: p<0.01 vs normal (glucose 16.7 mmol/l)

concentration of glucose was lower than that in the
diabetic compared with normal rat (former p <
0.01, the latter p>0.05).

Discussion
In hyperosmolar nonketotic diabetic coma of
human subjects, most of the patients exhibit
hypernatremia and hyperchloremia together with
hyperosmolarity, a high plasma glucose level and
some ketonemia. In this study, similar biochemical
findings were obtained in the diabetic rat with
simple water deprivation or together with cortisol
injection. FBG increased greatly in the dehydrated
diabetic rat. From the viewpoint of FBG there was
no difference in inducing the hyperosmolar diabetic
state whether dehydration was accompanied by
cortisol injection or not in this series of experi-
ments, but notable differences were a high plasma
IRG level and a very low insulin value: the
glucagon ratio found in STZ rat deprived of water
together with the injection of cortisol. Although
Marco et al. [11] failed to alter plasma IRG by
means of iv injection of prednisolone, a high level
of plasma IRG has been reported in chronically
glucocorticoid-treated rat [12–14]. Therefore the
administration of cortisol to dehydrated diabetic rat would be an important contributory factor in inducing a hyperosmolar diabetic state.

In the insulin deficient state, plasma IRG is reported to increase greatly and even the administration of an amount of insulin insufficient to decrease of blood sugar decreased IRG rapidly [15, 16]. A significant increase in fasting plasma IRG was observed in the dehydrated diabetic rat, probably most of it from the pancreas and some from the intestines. This was suggested by the higher amount of IRG in the pancreas in the dehydrated state.

Plasma IRI had a tendency to increase in cortisol-treated non-diabetic rat and to decrease in water-deprived rat, and in STZ rat there was no remarkable change in plasma IRI due to its β cell destruction. In spite of this, in dehydrated diabetic rat there was a tendency for plasma IRI to decrease, probably due to a more striking metabolic derangement or to a decrease in food intake associated with dehydration.

In the in vivo experiment, the fasting plasma IRI: IRG ratio was generally decreased in the dehydrated state, especially in the dehydrated diabetic rat. Similar findings were found in in vitro pancreatic islet experiments. In islet experiments, the IRI released from dehydrated diabetic islet at a high concentration of glucose was rather low compared with that from diabetic islet, whereas the opposite was true of released IRG. Therefore the insulin: glucagon ratio at a high concentration of glucose in dehydrated diabetic rat islet compared with that in normal islet was significantly lower than that of diabetic islet compared with the normal one. The insulin: glucagon ratio varies inversely with the need for endogenous glucose production and the inability of diabetics to increase their I/G ratio parallels the catabolic state [17]. Thus in the hyperosmolar diabetic state due to dehydration, the tendency to secrete less IRI and more IRG would increase plasma blood sugar and aggravate the metabolic state of diabetes. It is possible that the higher FBG value observed in the dehydrated diabetic rat in the islet experiment, irrespective of the similar starting levels of FBG, might be responsible for the lower I/G ratio. But the same tendency in the I/G ratio was observed when a comparison was made of a similar range of FBG in both groups (results not recorded).

The reason why glucagon secretion had a tendency to increase in dehydrated and hyperosmolar diabetes is unknown. Hypersomolarity itself was reported to decrease the secretion of IRG in the perfused dog pancreas [18], but in vivo and in vitro studies showed lower insulin response in the above states probably due to the loss of appetite or to dehydration. A larger amount of IRG was found in the pancreas as shown in Fig. 4. Therefore, these factors are considered to contribute to the higher secretion of IRG in such diabetes.

Thus in the hyperosmolar diabetic state, the more catabolic hormonal changes from the viewpoint of the hormonal value would raise the blood sugar level and aggravate the metabolic state and might worsen the consciousness of the patients.

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References


