Induction of Alimentary Diabetes and Insulin Responses to Glucose in Microtus arvalis Pallas

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ABSTRACT. In Microtus arvalis Pallas, new born voles were fostered to ICR mice and supplied with pellets for mice containing little fiber after weaning. High concentrations of glycosuria accompanied with hyperglycemia were induced in about 50% of voles supplied with pellets for mice. In the glycosuric voles, the fermentation ability and pH of the contents in the forestomach lowered considerably, and large amounts of starch in the diet were considered to be transported to the pyloric stomach without being fermented in the forestomach, and hydrolyzed into glucose to be absorbed in the duodenum. In normal control voles, insulin secretory response to a glucose load was similar to that in mice. In the slight diabetic voles, the plasma insulin levels rose over 200 µU/ml and the insulin secretory response to glucose was remained. But in the serious diabetic voles, the plasma insulin levels lowered under 10 µU/ml and the insulin secretory response was almost absent. According to immunohistochemical studies on pancreatic islet B cells, partial degranulation were observed at the slight diabetic stage. However, at the serious diabetic stage, degranulation and vacuolation of B cells became pronounced. These results clearly indicated that in herbivorous voles, alimentary diabetes were induced by lowering of the fermentation ability in the forestomach and the diabetic voles fell into complete insulin deficiency at the serious stage.—KEY WORDS: alimentary diabetes, fermentation, insulin, Microtus.


Herbivorous voles, Microtus species possess complex stomachs consisted of esophageal sac, fundic stomach and pyloric stomach [8, 13, 14]. There were more then 107 per g aerobic and anaerobic bacteria including cellulolytic bacteria in the contents of the forestomach, i.e., esophageal sac, where bacteri- al fermentation took place to produce volatile fatty acids (VFAs) and other organic acids as in ruminants, and the bacterial flora was changed easily when diet was varied [9, 10, 11].

In our laboratory, spontaneous glycosuria was sometimes found out in about 5% of voles supplied with only pellets for herbivore without feeding grass. Kudo and Oki [12] induced glycosuria accompanied with hyperglycemia in voles by the series of experimental treatment; new born voles derived from hysterectomy of conventional pregnant Microtus were fostered to SPF mice and fed with mice pellets for 7 or 8 weeks after weaning. In such glycosuric voles, the fermentation ability and pH were considerably low in the forestomach. Glucose tolerance lowered with the progress of the diabetes induced by modification of the above method [1].

In the present paper, the mechanism of the alimentary diabetes was studied especially about the fermentation abilities in the digestive tracts and changes of insulin secretory responses to glucose with the progress of the disease. Changes of the pancreatic islets of the diabetic voles were also observed immunohistochemically.

MATERIALS AND METHODS

Animals: Microtus arvalis Pallas, bred in our laboratory, were used for this experiment. 12 normal control voles were supplied with pellets for herbivore (ZC, crude fiber 12.5%:...
Oriental Yeast Co.) and cubed hay after weaning [1, 13]. In the experiment to induce glycosuria, 204 new born voles were fostered to ICR mice and supplied with pellets for mice (CMF, crude fiber 3.5%; Oriental Yeast Co.) after weaning. 10 C57BL/6J mice were also examined as control in analyses of insulin responses to glucose load.

Measurements: Glycosuria was examined with Tes Tape (Eli Lilly Co.) once a week after three weeks old.

Animals were killed by decapitation and blood samples were taken in heparinized tubes. Blood glucose concentrations were measured with glucose oxidase method [7]. Plasma FFA were measured with a commercial kit, NEFA Test Wako (Wako Pure Chem.). Plasma insulin levels were measured with micro ELISA sandwich method, using microplate coated with anti-insulin antibody and β-D-galactosidase labeled anti-insulin antibody. In this method, only 20 μl of plasma was enough to measure the insulin level [2].

The digestive tracts were removed immediately after the animals were killed. PH of the contents in the digestive tracts were measured with Toyo Test Papers (Toyo Roshi Co.). After the pH measurement, fermentation products in the digestive tracts were analyzed with gas chromatography. Starch amounts of the contents in the fundic and pyloric stomachs were detected with the application method using the iodo-starch reaction [1].

Analyses of insulin responses to glucose: Insulin responses to glucose load were analyzed in 10 normal voles and 10 C57BL/6J mice as control, and 14 diabetic voles which were classified to slight (7) and serious (7) stages according to the length of glycosuria appearance and the average blood glucose concentrations.

1 g/kg glucose was administered intraperitoneally to each animal anaesthetized with 40 mg/kg sodium pentobarbital after fasting for 18 h. Blood samples were taken from the orbital venous plexus in heparinized microcapillary tubes at 0, 2, 5, 15, 30, 45 min after the glucose administrations. These samples were centrifuged for 6 min at 8,000 rpm and 20 μl of separated plasma were used for insulin assay.

Immunohistochemical observations of the pancreatic islets: 4 normal control voles, 5 slight diabetic and 4 serious diabetic voles were used for immunohistochemical observations of the pancreatic islets. These diabetic voles were typical cases experimentally induced with the above method. Voles were killed by decapitation, and as quickly as possible the pancreas were removed to be fixed in Bouin’s fluid and embedded in paraffin. The pancreas were used for successive sections about 5 μm.

Immunohistochemical changes of pancreatic islets were observed by enzyme immunoassay (EIA) using peroxidase labeled antibody from guinea-pig [17, 18]. The first antibody was anti-insulin serum from guinea-pig and second antibody was peroxidase labeled anti-guinea-pig IgG serum from goat (Japan Immunoresearch Lab.).

RESULTS

Induction of glycosuria: In normal control voles supplied with pellets for herbivore and cubed hay, glycosuria was not detected. However, high concentrations of glycosuria (++: 1/4%, +++, 1/2%; Tes Tape) were induced in 54.9% of voles which were fostered to ICR mice and supplied with pellets for mice containing little fiber after weaning (Table 1). Glycosuria appeared three or four weeks after the weaning and continued for over five weeks. There was no sex difference in the appearance ratio of glycosuria. Glycosuric voles were generally 20 or 30% heavier than normal control voles. In such glycosuric voles, concentrations of blood glucose and plasma FFA were three or four fold of those in normal control (Table 2).
Table 1  Incidence of glycosuria in *Microtus arvalis* Pallas fostered to ICR mice

<table>
<thead>
<tr>
<th></th>
<th>No of voles examined</th>
<th>No of glycosuria</th>
<th>Ratio of glycosuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>105</td>
<td>60</td>
<td>57.1%</td>
</tr>
<tr>
<td>Female</td>
<td>99</td>
<td>52</td>
<td>52.5%</td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
<td>112</td>
<td>54.9%</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

a) Supplied with pellets for mice for over ten weeks after the fosterage to ICR mice and the weaning.

Table 2  Comparison of the concentrations of blood glucose, plasma FFA, fermentation products in the digestive tracts and starch in the contents of fundic and pyloric stomachs between normal and diabetic voles

<table>
<thead>
<tr>
<th></th>
<th>Normal voles&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diabetic voles&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>70.8±10.9&lt;sup&gt;4&lt;/sup&gt;</td>
<td>215.8±82.9</td>
</tr>
<tr>
<td>Plasma FFA (meq/l)</td>
<td>0.58±0.21</td>
<td>2.11±1.26</td>
</tr>
<tr>
<td>Forestomach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.7±0.1</td>
<td>2.8±1.2</td>
</tr>
<tr>
<td>Total VFA (m moles/dl)</td>
<td>4.98±0.93</td>
<td>2.02±1.01</td>
</tr>
<tr>
<td>Lactic acid (m moles/dl)</td>
<td>1.54±1.03</td>
<td>1.33±1.12</td>
</tr>
<tr>
<td>Fundic and pyloric stomachs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>1.8±0.3</td>
<td>2.0±0.6</td>
</tr>
<tr>
<td>Total VFA (m moles/dl)</td>
<td>0.54±0.23</td>
<td>0.44±0.28</td>
</tr>
<tr>
<td>Starch in the content (mg/g of the content w.t.)</td>
<td>0.59±0.39</td>
<td>10.20±7.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cecum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.9±0.1</td>
<td>7.0±0.3</td>
</tr>
<tr>
<td>Total VFA (m moles/dl)</td>
<td>11.22±1.90</td>
<td>9.24±2.84</td>
</tr>
</tbody>
</table>

<sup>a</sup> n=12  
<sup>b</sup> n=30.  
<sup>c</sup> Mean±S.D.  
<sup>d</sup> n=12.

Table 3. Relation between severity of the diabetes and plasma insulin levels

<table>
<thead>
<tr>
<th>Severity of diabetes</th>
<th>Blood glucose (mg/dl)</th>
<th>Plasma insulin (μU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slight (n=6)</td>
<td>130.2±46.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>237.0±102.5</td>
</tr>
<tr>
<td>Middle (n=6)</td>
<td>127.8±23.3</td>
<td>23.5±11.5</td>
</tr>
<tr>
<td>Serious (n=6)</td>
<td>300.6±77.9</td>
<td>9.1±7.6</td>
</tr>
<tr>
<td>Normal (n=30)</td>
<td>70.7±11.8</td>
<td>21.5±6.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> means±S.D

In the forestomachs of the diabetic voles, total VFA dropped below half of normal values and pH also lowered considerably (P<0.001). Meanwhile, lactic acid did not decrease significantly in the forestomach. In the fundic and pyloric stomachs, there were
no significant differences between normal and diabetic voles in the aspects of pH and total VFA (Table 2). In the cecum, total VFA of the diabetic voles decreased about 20% lower than that of the normal control, and lactic acid was not detected in either normal or diabetic voles. However, relative amounts of starch of the contents in the fundic and pyloric stomachs in diabetic voles were 15- to 20-fold of those in normal control voles (Table 2).

Changes of insulin responses with the progress of the diabetes: Diabetic stages were classified into three stages, slight, middle and serious, according to the length of glycosuria and the average blood glucose concentrations. In the slight diabetic voles, one or two weeks after appearance of glycosuria, the blood glucose concentrations ranged between 100 and 150 mg/dl. In the middle diabetic voles, high concentrations of glycosuria appeared discontinuously for five or seven weeks, and the blood glucose concentrations were 120 to 150 mg/dl. In the serious diabetic voles, high concentrations of glycosuria continued for over six weeks, and the blood glucose concentrations were average 300 mg/dl.

In the slight diabetic voles, the plasma insulin levels increased to over 200 μU/ml, about ten fold of normal control. In the middle diabetic voles, the plasma insulin levels were almost normal, although blood glucose concentrations were considerably high. In the serious diabetic voles, plasma insulin levels decreased to below 10 μU/ml.

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**Table 4. Effects of glucose administration on plasma insulin levels in the diabetic cases in Microtus arvalis Pallas**

<table>
<thead>
<tr>
<th>Severity of diabetes</th>
<th>Plasma insulin levels (μU/ml) after glucose b) i.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Slight (n=7)</td>
<td>76.7±21.2c)</td>
</tr>
<tr>
<td>Serious (n=7)</td>
<td>4.9±3.6</td>
</tr>
<tr>
<td>Normal (n=10)</td>
<td>9.1±1.1</td>
</tr>
</tbody>
</table>

a) Fasted for 18 h before glucose administrations.
b) Glucose was administered with 1 g/kg intraperitoneally.
c) Mean±S.D.
Fig. 2. Pancreatic islets of a normal control vole stained with EIA.
(a) Normal islet reacted with normal Guinea-pig serum.
(b) Normal islet reacted with Guinea-pig anti-insulin serum.
The horizontal bars represent 20 μm.

Fig. 3. Pancreatic islet of diabetic voles reacted with Guinea-pig anti-insulin serum.
(a) The moderately enlarged islet reveals partial degranulation of B cells (slight stage). Peripheral portions of B cells stain apparently.
(b) Complete degranulation and vacuolation of the B cells in a moderately enlarged islet are observed (serious stage).
The horizontal bars represent 20 μm.
and some of them had no plasma insulin (Table 3). Insulin responses to glucose in normal voles and mice were shown in Fig. 1. The plasma insulin levels decreased to about 10 μU/ml in both the voles and mice after fasting for 18 h. Glucose administrations stimulated the secretion of insulin in the voles, and plasma insulin levels increased from the basal levels, 10 μU/ml, to a mean peak level of 18.6 μU/ml 2 min after the administration, then it decreased immediately and remained between 12 and 13 μU/ml. Similar insulin secretory response was observed in the mice administered with glucose.

The insulin secretory responses to glucose load were analyzed at various stages of the diabetes in voles (Table 4). In the slight stages, the plasma insulin levels were considerably high and glucose administration stimulated insulin secretion. At 5 min, plasma insulin levels reached the peak, 50% higher than the basal level. In the serious stages, the plasma insulin levels in fed were below 10 μU/ml and some of them had no plasma insulin. The glucose administration did not stimulate the insulin secretion and plasma insulin levels were kept very low.

Histochemical observations on the pancreatic islets: Insulin levels in the pancreatic islets were histochemically compared using EIA between normal and diabetic voles (Fig. 2 and 3).

In the islet of normal voles, stained with EIA using control serum (Normal guinea-pig serum) as the first serum, B cells were not stained (Fig. 2-a). In the case using anti-insulin serum as the first serum, insulin in the islet B cells reacted with anti-insulin serum and staining reaction was observed broadly around the cells in the islets, but the reaction was not observed at the peripheral region of the islet (Fig. 2-b).

Fig. 3-a shows the typical figure of the islet of slight diabetic voles, one or two weeks after onset of the diabetes. The blood glucose concentrations of these voles were 218.8±49.7 mg/dl (n=5). The islet revealed moderately enlargement and partial degranulation of the B cells. The peripheral portion of B cells were stained apparently by the reaction with anti-insulin serum.

Fig. 3-b shows the typical figure of the islet of serious diabetic voles over ten weeks after onset of the diabetes. The blood glucose concentrations of these voles were 367.5±43.1 mg/dl (n=4), over five times as high as normal control. In these voles, completely degranulation and vacuolation of the B cells in a moderately enlarged islet were observed. In these degenerated cells, pyknosis and disappearance of nuclei became pronounced.

DISCUSSION

In herbivorous Microtus species, the bacterial flora in the forestomach changed easily when the diet varied [9, 11]. In voles supplied with high carbohydrate ration, i.e., potato alone, large amount of lactic acid, 5- or 6-fold of normal control, was produced and pH dropped considerably in the forestomach [11].

As previously reported [1], normal control voles supplied with high fiber containing rations did not show glycosuria, but, high concentrations of glycosuria were induced in voles supplied with mouse pellets containing high carbohydrate and low fiber. Glycosuria accompanied with hyperglycemia was also induced in about 50% of voles supplied with acidified water (pH 1.7) [1]. In the present experiment, diabetic voles showed considerable decrease of pH (1.8-4.0) in the forestomach. In the diabetic voles, the contents in the forestomachs became muddy owing to a lack of fiber in the diet, and it was supposed that gastric juice flowed backward to the forestomach easily and pH decreased considerably in the forestomach. In such low pH (under 3.0), most bacteria were not considered to be alive. In the consequence, the lowering of fermentation ability in the fore-
stomach was supposed to be one of the most important factors to induce the alimentary diabetes in the voles.

In the diabetic voles, considerable large amount of diet was transported to the pyloric stomach without being fermented in the forestomach. Starch in such diet appeared to be hydrolyzed into glucose and absorbed in the duodenum. It was supposed that the continuous absorption of such glucose caused the hyperglycemia in voles.

It has been well known that glucose administration stimulated insulin secretion in many species [4, 5, 6, 19]. Voles were also stimulated insulin secretion by the glucose administration, showing the similar pattern to insulin response in mice.

On the other hand, diabetes mellitus in dogs was classified into three types on the basal plasma insulin concentration and the insulin response to a glucose load [15]. In our previous report, alimentary diabetes in voles were classified into three stages according to the basal blood glucose concentrations and the length of glycosuria appearance. Glucose tolerance lowered considerably with the progress of the diabetes in voles [1]. The lowering of glucose tolerance was supposed to be caused by the shortage of insulin secretion.

In the present paper, insulin response to glucose lowered considerably with the progress of the alimentary diabetes in voles. In the slight diabetic voles, insulin secretory function in the islet B cells was considered to become active to maintain blood glucose concentrations to normal levels. In the serious diabetic voles, after such hyperfunctional condition continued, the pancreatic islet was exhausted, and the insulin response to glucose became almost absent.

Immunohistochemical observations of the islets revealed that B cells occupied broadly around the center of the islet in normal voles. In mouse or rat, it has been reported that A cells occupied the peripheral region and B cells occupied around the center of the islet [3, 16].

In the slight diabetic voles, the peripheral portions of B cells were stained apparently by the EIA of insulin. It is considered that B cells became hyperfunctional and actively secreted insulin. In the serious diabetic voles which continued high concentrations of glycosuria for over ten weeks, B cells were exhausted, and the complete degranulation and vacuolation in the cells were observed. In this stage, insulin was not secreted. The histochemical feature in such pancreatic islets seemed to be parallel with the analytic results of plasma insulin levels at various stages of the diabetes in voles.

In conclusion, it was suggested that the lowering of the fermentation ability and pH of the contents in the forestomach caused the alimentary diabetes in the herbivorous Microtus species, and the insulin secretory response to glucose was lowered with the progress of the disease, and then the voles fell into complete insulin deficiency accompanied with severe hyperglycemia at the serious stage.

REFERENCES

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要約

草食性ハタネズミにおける食齢性糖尿病の誘発とグルコースに対するインスリン応答：新井敏郎・佐々木稔・大木与志雄・米田嘉彦郎（日本獣医薬産学・生理化学教室、東京医科大学動物実験センター）——食齢性ハタネズミの新生仔（204匹）をマウス用内服剤のマウス用ベレットのみで飼育すると、約50%の個体に高血糖を伴なう食齢性糖尿病が誘発された。糖尿病例では、前胃pHおよび発酵能が著しく低下し、かなりの量の食齢が未発酵のまま十二指腸へ送られ、炭水化物が分解され、グルコースの形で吸収され、白血球が誘発されるものを推察された。正常ハタネズミでは、グルコース摂与2分後に血漿インスリノン値がピークに達し、マウスのそれと類似したパターンを示したが、糖尿病初期のハタネズミでは、インスリン値は、絶食後も正常値（21.5 μU/ml）の2～5倍（50～100 μU/ml）を示し、グルコース摂与により、インスリン分泌がさらに促進された。末期には、インスリン値は著しく低下し、グルコースに対するインスリン応答は、ほとんど消失した。いっぽう、糖尿病初期では、脾島B細胞においてインスリンの盛んな生合成と分泌を示す組織像がみられたが、末期には、細胞の空胞化や核消失などが顕著で、インスリン分泌の著しい低下を示す所見が認められた。