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THE INSUFFICIENCY OF INSULIN SECRETION IN SPONTANEOUSLY HYPERTENSIVE RATS

Chihiro Fujimoto M. D.

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Summary: The influence of the sympathetic nervous system on insulin secretion was investigated in both spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). In an oral glucose tolerance test (2g/Kg body weight), the SHR had a significantly lower serum insulin level and a significantly higher level of pancreatic insulin content than the WKY, suggesting that an inhibitory mechanism of insulin secretion existed in the SHR. The noradrenaline (NA) contents in both pancreatic tissue and plasma of SHR were significantly higher than the NA contents of the WKY. In conclusion, these results indicate that SHR are in a state of insufficient insulin secretion which is largely caused by dominant sympathetic nervous activity in their pancreatic tissues.

Key Words: insulin secretion, spontaneously hypertensive rats, sympathetic nervous system, catecholamine

Hypertension is more frequent in diabetic patients than in the nondiabetic population [1-4]. Similarly, the prevalence of diabetes mellitus is higher in hypertensives than in normotensives [4, 5]. Spontaneously hypertensive rats (SHR), which are known to be useful animal models for the study of essential hypertension in humans, were bred from normotensive Wistar-Kyoto rats (WKY) through selective sib-breeding and grown up accompanying by sympathetic hyperfunction [6]. SHR have greater sympathetic activity in the peripheral tissues, such as the heart and the blood vessels [7], higher levels of the catecholamine in the plasma or urine [8], and stronger activities in the splanchnic or renal nerves [9] than WKY.

Regarding insulin secretion in SHR, there has been no agreement on the serum insulin levels of SHR. The majority of reports indicate hypoinsulinemia as compared with normal insulin levels in either WKY [10-12] or normotensive local Wistar rats [13], and the minority [14, 15, 42] demonstrate hyperinsulinemia in SHR as compared with normal insulin levels in the control rats. However, the mechanisms of this hypoinsulinemic phenomenon have not been fully understood.

The pancreatic islets are richly supplied with the autonomic nerves. The fibers follow the arterioles, penetrate the islets and terminate close to the endocrine cells. This morphological relationship makes it likely that the nerves are involved in the physiological regulation of islet function. Basal insulin release, like glucose-induced insulin release, is decreased by sympathetic nerve stimulation in several species [16-19]. Sympathetic neural activity has a direct effect on the endocrine pancreas through release of noradrenaline.

In the present study, the influence of the adrenergic nerves on insulin secretion in both SHR and WKY was investigated biochemically.

Materials and Methods

A total of 59 male SHR and 49 age-matched male WKY, obtained from Charles River Japan Inc. (Ihatagi, Japan), were used in this study. Six-week-old SHR were applied to prehypertensive stage study and twenty-week-old SHR were applied to hypertensive stage study. The rats were housed to mate in colony cages under conditions of controlled temperature (24 ± 2 °C), humidity (55 ± 2%) and artificial light from 8 a.m. to 6 p.m. each day at the Laboratory Animal Center for Biomedical Research, Nagasaki University. They were fed with chow and tap water ad libitum. The systolic blood pressure was measured with an indirect tail-cuff method (autonomic blood pressure recorder UR-1000 type, Ueda Manufactory, Japan).

Oral glucose tolerance tests (OGTT)

OGTT was carried out after 12-hour overnight fasting. Glucose solution (2 g/Kg body weight) was administered through a metal gastric tube attached to a syringe. Plasma glucose, serum immunoreactive insulin, serum immunoreactive glucagon, pancreatic immunoreactive insulin content and pancreatic immunoreactive glucagon content were measured during fasting and at 30 and 120 minute intervals after glucose administration. Blood was collected by cardiac puncture under ether anesthesia. The whole pancreas was excised, being free from the adipose tissue and lymph nodes. The tissues were immediately weighed, cut by scissors in amount of need, and frozen at −80 °C. Plasma glucose: Plasma glucose was determined by the glucose-
dehydrogenase method [21]. Serum immunoreactive insulin (serum IRI): Serum IRI was determined by a double-antibody radioimmunoassay with rat insulin standard (Incstar Corporation, U. S. A.), using a commercial insulin assay kit (Incstar Corporation, U. S. A.). Serum immunoreactive glucagon (serum IRG): Serum IRG was measured by a double-antibody radioimmunoassay, using a commercial glucagon assay Kit (Daiichi Radioisotope LABS., LTD., Japan). Pancreatic immunoreactive insulin content (pancreatic IRI) and pancreatic immunoreactive glucagon content (pancreatic IRG): Insulin and glucagon were extracted by the acid-ethanol method [22]. The weighed tissue was put in 5 ml of cold acid ethanol (78.2 ml ethanol, 1.5 ml Conc. HCl, 20.3 ml distilled water) and homogenized by a polytron homogenizer (Model PTA-7, Kineumatica, Switzerland). After overnight preservation in a cold room, the extracts were centrifuged at 2,000-3,000 rpm for 20-30 mins. in cold air. The supernatants were removed to another tube and the pH adjusted to 7.4-7.8, using IN-NH₄OH solution. Each extract was diluted with a Borate buffer and kept at −20 °C until the insulin and glucagon were assayed in the same way as in the measurement of sera IRI and IRG.

The control SHR and control WKY rats were defined as rats without stress such as fasting, and were used for evaluating the levels of plasma glucose, serum IRI and IRG, pancreatic IRI and IRG contents.

Catecholamine (CA) contents

The concentration of noradrenaline (NA), adrenaline (A), and dopamine (DA) were measured by high-performance liquid chromatographic electrochemical detection (HPLC-ECD) in the pancreas and plasma of both control SHR and control WKY. The weighed tissue were homogenized in 0.4 N perchloric acid containing 5.3 mM sodium pyrosulfite and 1.4 mM EDTA by an ultrasonic probe homogenizer (SONIFIR Model 185, Bronson, U. S. A.). The homogenates were centrifuged at 15,000 rpm at 4°C for 10 min. Isolation of CA from the supernatant was done by absorption in alumina in the presence of 0.5 M Tris-HCl buffer, pH 8.6. Then CA was eluted from alumina with 0.1 N perchloric acid and assayed using the HPLC system [23].

Statistical analysis

All values were expressed as mean ± SD. Statistical analysis was performed by Student’s t test. A p level of < 0.05 was considered to be of statistical significance.

Results

Blood pressure, body weight and pancreatic wet weight.

Systolic blood pressure was significantly higher in the SHR (6 w (n = 6):138 ± 9 mmHg, p < 0.05, 20 w (n = 6):192 ± 27 mmHg, p < 0.01) than in the WKY (6 w (n = 6):126 ± 9 mmHg, 20 w (n = 6):128 ± 19 mmHg). Body weight tended to be heavier in the SHR (6w (n = 6):135 ± 8 g, n. s, 20 w (n = 6):345 ± 17 g, p < 0.01) than in the WKY (6w (n = 6):132 ± 8 g, 20 w (n = 6):313 ± 17 g). There were no differences in pancreatic wet weight between the SHR (6w (n = 6):0.58 ± 0.04 g, n. s, 20 w (n = 6):1.17 ± 0.12 g, n. s) and the WKY (6w (n = 6):0.59 ± 0.03 g, 20 w (n = 5):1.17 ± 0.21 g).

OGTT.

In 6-week-old rats, the plasma glucose levels in the SHR were significantly higher than in the WKY at the three points (during fasting, and at 30 and 120 min. intervals after glucose administration) (Fig. 1A). In 20-week-old rats, the SHR had a tendency toward a high level of plasma glucose (Fig. 1B).
glucose though there was no significant difference from the WKY (Fig. 2A). Serum IRI in the SHR was significantly lower than in the WKY at the three points of measurement in the 6- and 20-week-old rats (Fig. 1B, 2B). The levels of pancreatic IRI in the 6-week-old SHR were significantly higher than those in the age-matched WKY at the three points of measurement. In the 20-week-old SHR, levels were also higher than in the age-matched WKY, but not significantly (Fig. 1C, 2C). Thus the SHR were found to have hypoinsulinemia and a high level of pancreatic IRI content. Furthermore, the control group of SHR had hypoinsulinemia and greater value of pancreatic IRI content than the control group of WKY (Table 1). Assayed at 120 mins., the levels of serum IRG in 6-week-old SHR were significantly higher than those found in age-matched WKY (Fig. 1D, 2D) or in the control rats in 20 weeks of age (Table 1). Assayed during fasting and at 30-min. interval after the termination of fasting, the levels of pancreatic IRG

Fig. 2. Plasma glucose (A), serum IRI (B), pancreatic IRI (C), serum IRG (D) and pancreatic IRG (E) responses to OGTT (2g/Kg body weight) in SHR (0':n = 7, 30':n = 7, 120':n = 7) and WKY (0':n = 5, 30':n = 5, 120':n = 5) at 20 weeks of age, respectively. Results are mean ± SD.
Fig. 2C

![Graph showing insulin secretion in WKY and SHR](image)

Fig. 2D

![Graph showing serum IRG](image)

Fig. 2E

![Graph showing pancreatic IRG](image)

Fig. 2. (continued)

Table 1. Plasma glucose, serum-, pancreatic IRI & IRG in the control SHR & control WKY

<table>
<thead>
<tr>
<th></th>
<th>Plasma glucose (mg/dl)</th>
<th>serum IRI (ng/dl)</th>
<th>pancreatic IRI (ug/g tissue)</th>
<th>serum IRG (ng/ml)</th>
<th>pancreatic IRG (ug/g tissue)</th>
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<tbody>
<tr>
<td>6 Week-Old Rats</td>
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<tr>
<td>WKY (n = 6)</td>
<td>154.0 ± 16.8</td>
<td>1.92 ± 0.63</td>
<td>11.16 ± 1.09</td>
<td>0.16 ± 0.03</td>
<td>1.19 ± 0.29</td>
</tr>
<tr>
<td>SHR (n = 6)</td>
<td>162.0 ± 34.0</td>
<td>0.90 ± 0.45*</td>
<td>13.75 ± 1.13**</td>
<td>0.16 ± 0.05</td>
<td>1.71 ± 0.31*</td>
</tr>
<tr>
<td>20 Week-Old Rats</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>WKY (n = 5)</td>
<td>142.0 ± 8.3</td>
<td>3.58 ± 0.05</td>
<td>10.09 ± 10.06</td>
<td>0.16 ± 0.04</td>
<td>0.40 ± 0.17</td>
</tr>
<tr>
<td>SHR (n = 7)</td>
<td>148.0 ± 9.8</td>
<td>1.36 ± 0.31***</td>
<td>15.68 ± 4.16</td>
<td>0.27 ± 0.04**</td>
<td>0.53 ± 0.17</td>
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</table>

(Mean ± SD) * p < 0.05, ** p < 0.01, *** p < 0.001 vs. WKY

Table 2. Comparison of catecholamine contents in the pancreas of the control SHR & control WKY

<table>
<thead>
<tr>
<th></th>
<th>Noradrenaline (ng/g tissue)</th>
<th>Adrenaline (ng/g tissue)</th>
<th>Dopamine (ng/g tissue)</th>
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<tbody>
<tr>
<td>6 Week-Old Rats</td>
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<tr>
<td>WKY (n = 6)</td>
<td>510.2 ± 48.6</td>
<td>9.3 ± 3.6</td>
<td>24.8 ± 5.2</td>
</tr>
<tr>
<td>SHR (n = 6)</td>
<td>823.8 ± 182.6**</td>
<td>8.8 ± 1.9</td>
<td>42.8 ± 6.9***</td>
</tr>
<tr>
<td>20 Week-Old Rats</td>
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<td></td>
</tr>
<tr>
<td>WKY (n = 5)</td>
<td>870.1 ± 64.6</td>
<td>11.4 ± 5.1</td>
<td>30.7 ± 8.9</td>
</tr>
<tr>
<td>SHR (n = 5)</td>
<td>1044.4 ± 108.6**</td>
<td>11.7 ± 2.3</td>
<td>38.5 ± 5.6</td>
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</table>

(Mean ± SD) ** p < 0.01, *** p < 0.001 vs. WKY

CA contents.

The CA contents in the pancreatic tissue are shown in Table 2. The NA content was significantly higher in 6- and 20-week-old SHR than in age-matched WKY. The DA content was significantly higher only in 6-week-old SHR. There was no significant difference in A content between the SHR and the WKY. The plasma CA contents are shown in Table 3. The NA content was significantly higher in 6-week-old SHR were significantly higher than the levels found in age-matched WKY (Fig. 1E, 2E) or than those found in the control rats at 6 weeks of aged (Table 1). Among all the rats, the levels of pancreatic IRG were lower at the 20-week stage of growth than at 6-week stage (Fig. 1E, 2E and Table 1).

20-week-old SHR than in age-matched WKY.
These results indicate that SHR are in a state of insufficient facilitation in the pancreas. Insulin is inhibited via an $\alpha_2$-mechanism [29]. The fact that SHR showed a mild glucose intolerance genetically with lower cholesterol levels and smaller body weights was first reported in 1978 [24, 25]. In SHR, increased hormone-induced lipolysis in associated with glucose intolerance and transitory serum insulin enhancement [13]. Sato et al. [12] and Iwase et al. [11] reported that SHR had hypoinsulinemia. Furthermore Iwase et al. showed that serum IRI was significantly lower, whereas pancreatic IRI content tended to be higher in SHR than in WKY. Tsutsu et al. demonstrated that conscious and unrestrained SHR showed slightly but significantly more enhanced glucose tolerance than did WKY. This enhancement was associated with reduced insulin secretion under basal conditions as well as during the I. V. GTT. A possible explanation for the inhibition of the specific function of the insulin-producing tissue in the SHR may be related to the well-known competition between the effects of insulin and catecholamines, which seem to play a significant role in the pathogenesis of the spontaneous hypertension [13, 15, 24] and intracellular and membrane defects in cation transport in SHR [13, 15].

The results of this present study were summarized as follows: 1) SHR have hypoinsulinemia and a high level of pancreatic IRI content. 2) The NA contents in both pancreas and plasma were significantly higher in SHR than in WKY. Because the pancreatic islets have markedly higher NA content in the pancreas than age-matched WKY, the pancreatic IRI content of the insulin-producing tissue in the SHR may be related to the well-known competition between the effects of insulin and catecholamines, which seem to play a significant role in the pathogenesis of the spontaneous hypertension [13, 15, 24] and intracellular and membrane defects in cation transport in SHR [13, 15].

The results of this present study were summarized as follows: 1) SHR have hypoinsulinemia and a high level of pancreatic IRI content. 2) The NA contents in both pancreas and plasma were significantly higher in SHR than in WKY. Because the pancreatic islets have markedly higher NA content than exocrine pancreas [26], the NA content in the pancreas would reflect the NA content in the pancreatic islets. Furthermore, SHR have morphometrically more hyperinnervation of adrenergic fibers in the pancreatic islets and around the intrapancreatic arteries than WKY (according to the author's data to be published).

In the pancreas of both the rat [27] and the dog [28], the $\beta_1$-adrenoceptors responsible for enhancing insulin secretion have been found to be of the $\beta_1$ type. Two kinds of postsynaptic $\alpha$-receptors have been defined on the basis of agonist-antagonist binding studies. Several groups have found that insulin is inhibited via an $\alpha$-mechanism [29]. These results indicate that SHR are in a state of insufficient insulin secretion which is largely caused by sympathetic facilitation in the pancreas.

Discussion

The fact that SHR showed a mild glucose intolerance genetically with lower cholesterol levels and smaller body weights was first reported in 1978 [24, 25]. In SHR, increased hormone-induced lipolysis in associated with glucose intolerance and transitory serum insulin enhancement [13]. Sato et al. [12] and Iwase et al. [11] reported that SHR had hypoinsulinemia. Furthermore Iwase et al. showed that serum IRI was significantly lower, whereas pancreatic IRI content tended to be higher in SHR than in WKY. Tsutsu et al. demonstrated that conscious and unrestrained SHR showed slightly but significantly more enhanced glucose tolerance than did WKY. This enhancement was associated with reduced insulin secretion under basal conditions as well as during the I. V. GTT. A possible explanation for the inhibition of the specific function of the insulin-producing tissue in the SHR may be related to the well-known competition between the effects of insulin and catecholamines, which seem to play a significant role in the pathogenesis of the spontaneous hypertension [13, 15, 24] and intracellular and membrane defects in cation transport in SHR [13, 15].

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Twenty-two-week-old SHR have enlarged islets and an increased number of $\beta$ cells compared to age-matched WKY [30]. This enlargement was not encountered in 6-week-old SHR. The enlarged islets with increased $\beta$ cell population in older SHR are probably due to compensatory hyperplasia of $\beta$ cell against hypoinsulinemia. However, Postnov et al. [15] showed that the $\beta$ cell component of the pancreatic islet tissue is considerably smaller in the young SHR at the prehypertensive and early hypertensive stages than in the normotensive Wistar rats. The decrease in mass of the insulin-producing tissue does not seem to belong to the primary manifestations of the genetic pathology but, rather, is secondary in character, it may be caused by the lower insulin requirement of the tissue, primarily, of the cell membranes. Sato et al. showed that the enlarged islets in the SHR occurred without increased $\beta$ cell population [12]. In contrast, Iwase et al. demonstrated that pancreatic islets were morphometrically similar in SHR and WKY [11].

Six-week-old SHR have higher levels of IRG in the serum and pancreas than age-matched WKY. Glucagon secretion is known to be stimulated by electrical activation of the sympathetic nerves in the dog, calf, pig, cat, and rat [31-40]. Furthermore, glucagon secretion from a perfused dog pancreas has been shown to be stimulated by both $\alpha$- and $\beta$-adrenoceptor agonists [20]. For example, adrenaline or noradrenaline in the presence of propranolol ($\beta$-blocker) increases glucagon secretion; this response is inhibited by phenoxybenzamine [41]; isoprenaline, a $\beta$-adrenoceptor agonist, also stimulates glucagon secretion [28]. The finding that selective $\beta_1$-adrenoceptor agonism stimulates glucagon secretion and that selective $\beta_1$-adrenoceptor antagonist fails to inhibit isoprenaline-induced glucagon secretion makes it likely that a $\beta_1$-receptor subtype mediates this effect [28].

In conclusion, SHR are in a state of insufficient insulin secretion which is largely caused by sympathetic facilitation in the pancreas. The greater sympathetic activities in the pancreas can be taken into consideration as the cause of glucose intolerance in essential hypertension. It seems that SHR can provide an animal model of insufficient insulin secretion which, through future research, can help to clarify pathogenesis in humans.

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C. Fujimoto: Insufficiency of insulin secretion in SHR

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