Hyperinsulinemic Yellow KK Mice and Norepinephrine Turnover

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Abstract

To clarify whether hyperinsulinemia accelerates sympathetic nervous system (SNS) activity, norepinephrine (NE) turnover, a reliable indicator of SNS activity, was measured in the interscapular brown adipose tissue (IBAT) and heart of hyperinsulinemic yellow KK and normoinsulinemic C57BL control mice at 12 weeks of age. The yellow KK mice were more obese and had higher levels of plasma glucose (about 2.3 times) and of plasma insulin (about 24 times) than did the control mice. In IBAT, the rate of NE turnover following blockade of NE synthesis with α-methyl-p-tyrosine (α-MPT) was significantly slower in yellow KK mice than in C57BL mice, although in heart, no significant difference between both groups was observed in NE turnover. These results suggest that hyperinsulinemia does not always increase NE turnover, and furthermore that the reduced NE turnover in IBAT of yellow KK mice may be one of the important factors in the development of obesity of this animal, as it is recognized that brown adipose tissue is a main effector of diet-induced thermogenesis and its defect or absence would predispose to obesity.

Previously we reported (Yoshida et al., 1985b) that sympathetic nervous system (SNS) activity is decreased in insulin-deficient streptozotocin (STZ) diabetic rats, and that this effect is partially but significantly prevented by replacement insulin treatment. In contrast, Yoshida and Bray (1984) reported that SNS activity is increased in ventromedial hypothalamus (VMH)-lesioned obese rats with hyperinsulinemia at the dynamic phase, and Rowe et al. (1981) reported that insulin increases SNS activity in the absence of changes in plasma glucose, using the glucose clamp technique in man. Therefore, in the present study, to clarify whether hyperinsulinemia accelerates SNS activity, norepinephrine (NE) turnover, a reliable indicator of SNS activity (Spector et al., 1965; Young and Landsberg, 1977a, b; Avakian and Horvath, 1981; Yoshida et al., 1983), was measured in the interscapular brown adipose tissues (IBAT) and hearts of hyperinsulinemic yellow KK and normoinsulinemic C57BL control mice at 12 weeks of age. The yellow KK mice were more obese and had higher levels of plasma glucose (about 2.3 times) and of plasma insulin (about 24 times) than did the control mice. In IBAT, the rate of NE turnover following blockade of NE synthesis with α-methyl-p-tyrosine (α-MPT) was significantly slower in yellow KK mice than in C57BL mice, although in heart, no significant difference between both groups was observed in NE turnover. These results suggest that hyperinsulinemia does not always increase NE turnover, and furthermore that the reduced NE turnover in IBAT of yellow KK mice may be one of the important factors in the development of obesity of this animal, as it is recognized that brown adipose tissue is a main effector of diet-induced thermogenesis and its defect or absence would predispose to obesity.

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Materials and Methods

The 18 male yellow KK mice from our mice stock, through the courtesy of Takeda Chemical Industries, Ltd. (Japan), and 18 male C57BL mice maintained at our university were used in this experiment. The mice were weaned at 4 weeks and subsequently fed a commercial powdered chow (Charles River Japan Inc.) and tap water ad libitum. The animals were housed in cages containing six mice each under conditions of controlled temperature (22±2°C) with artificial light from 0700 h to 1900 h each day. The mice were examined 12 weeks after birth. They were weighed and their nasoanal length measured. The Lee index was then calculated individually. Food intake was assessed for five days before the experiment by weighing the food administered and subtracting the amount remaining at the end of a 24 hour period in each group. Blood samples were obtained from the orbital vein plexus of the fed mice with capillary glass. Blood glucose was measured by the glucose-oxidase method. Plasma insulin was determined by radioimmunoassay using the double-antibody method (Hales and Randle, 1963). Two days after measurement of these parameters, the study of NE turnover began between 0900 and 1000 hours, by determining the concentration of NE in IBAT and heart at 0, 3, 6 hours following intraperitoneal injection of the methyl ester of α-methyl-p-tyrosine (80 mg/kg, Sigma Chemical Co., St. Louis, MO, USA). This drug blocks tyrosine hydroxylase and prevents synthesis of NE (Spector et al., 1965; Avakian and Horvath, 1981). The IBAT and heart were rapidly removed and dissected from connective tissue. Specimens were then frozen on dry ice and stored at −70°C for later determination of NE. At the time of the assay (usually within 2 weeks), the frozen tissues were weighed and homogenized in ice-cold 0.1 N perchloric acid containing 0.1 mM reduced glutathione in a Brinkman polytron and centrifuged at 0°C. Aliquots of the supernatant were analyzed radioenzymatically for NE using a minor modification (Yoshida et al., 1983) of the method of Peuler and Johnson (1977). The sensitivity of the assay is 1~2 pg for NE. It is based on the use of an isolated catechol-O-methyl-transferase to transfer a radioactive methyl group from adenosyl-L-methionine, S-(methyl-3H) to an endogenous catecholamine receptor to form a radioactive O-methyl catecholamine derivative.

All data are presented as the mean±SEM. Statistical analyses were performed using the analysis of variance and of covariance (Zar, 1974). In studies of NE turnover, the data were plotted semilogarithmically. The slope of the decline in endogenous NE was calculated by the method of least squares. The statistical significance of each computed regression line was assessed by analysis of variance. Fractional turnover rates were compared by analysis of covariance. The NE turnover rate (ng/organ/h) was calculated as the product of the fractional turnover rate (k) times the endogenous NE content at the zero time point. 95% confidence intervals were determined for the NE turnover rates as described (Taubin et al., 1972).

Results

Table 1 shows body weight, nasoanal length, Lee index, blood glucose, plasma insulin, food intake and the core temperature of yellow KK and C57BL control mice 12 weeks after birth. The body weight of yellow KK mice was significantly heavier than that of controls (p<0.001); their nasoanal length was also significantly greater (p<0.001). The yellow KK mice also had higher levels of plasma glucose than did the controls (p<0.001), their plasma insulin was about 24 times higher (p<0.001), they ate more (p<0.001) and their core temperature was markedly lower than in the controls (p<0.001).

Norepinephrine (NE) turnover data from interscapular brown adipose tissues (IBAT) and hearts are summarized in Table 2 and Figure 1. The IBAT weight of yellow KK mice was greater than that of the controls (p<0.001). Endogenous NE content was markedly lower in IBAT of yellow KK mice.
Table 1. Morphometric Data, Plasma Glucose, Plasma Insulin, Food Intake and Core temperature of Yellow KK and C57BL Mice at 12 Weeks of Age

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Body Weight (g)</th>
<th>Nasoanal Length (cm)</th>
<th>Lee Index$^#$</th>
<th>Plasma Glucose (mg/dl)</th>
<th>Plasma Insulin ($\mu$U/ml)</th>
<th>Food Intake (g/day)</th>
<th>Core Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow KK</td>
<td>34.9±0.6*</td>
<td>9.7±0.1*</td>
<td>336.0±2.0*</td>
<td>320.7±17.0*</td>
<td>547.3±27.5*</td>
<td>6.3±0.2*</td>
<td>36.2±0.1*</td>
</tr>
<tr>
<td>C57BL</td>
<td>23.6±0.5</td>
<td>9.1±0.1</td>
<td>315.0±2.5</td>
<td>140.3±3.4</td>
<td>23.0±2.3</td>
<td>3.0±0.1</td>
<td>37.5±0.2</td>
</tr>
</tbody>
</table>

* p<0.001 vs C57BL
$^\#$ Lee index = $\frac{\sqrt{3} \times \text{Body weight (g)}}{\text{Nasoanal length (cm)}} \times 1000$

Table 2. NE Content and NE Turnover in Interscapular Brown Adipose Tissues and Hearts of Yellow KK and C57BL Mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Organ Weight (g)</th>
<th>Endogenous NE (ng/organ)</th>
<th>$k$ (%/h)</th>
<th>NE Turnover Rate (ng/organ/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow KK</td>
<td>0.158±0.006***</td>
<td>66.1±2.6***</td>
<td>2.1±1.6*</td>
<td>1.4 (2.5–0.3)</td>
</tr>
<tr>
<td>C57BL</td>
<td>0.098±0.004</td>
<td>107.3±3.9</td>
<td>10.6±0.9</td>
<td>11.4 (12.8–10.0)</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow KK</td>
<td>0.121±0.005*</td>
<td>115.8±4.1**</td>
<td>4.2±1.1</td>
<td>4.9 (6.4–3.5)</td>
</tr>
<tr>
<td>C57BL</td>
<td>0.104±0.003</td>
<td>89.4±5.1</td>
<td>4.6±0.9</td>
<td>4.1 (5.2–3.1)</td>
</tr>
</tbody>
</table>

The fractional NE turnover rate ($k$) is expressed as mean±SEM. The NE turnover rate is expressed as mean with 95% confidence limits. Six mice were used at each time point to obtain turnover data. Endogenous NE is value at 0 time point.

Statistical significance: *p<0.01; **p<0.005; ***p<0.001 vs C57BL

Discussion

The purpose of the present study was to determine whether hyperinsulinemia is associated with an increase in sympathetic nervous system (SNS) activity. Our data showed that yellow KK mice were much more hyperphagic, hyperglycemic, hyperinsulinemic and obese than the C57BL controls. In particular, plasma insulin levels of yellow KK mice were about 24 times higher. These results were consistent with previous reports (Iwatsuka et al., 1970; Fujita et al., 1983). However, the NE turnover in IBAT of hyperinsulinemic yellow KK mice was reduced significantly compared to that of controls, although the heart NE turnover was similar in both groups. We found previously (Yoshida et al., 1987a, b) that the NE turnover rate obtained by NE biosynthesis inhibition with $\alpha$-methyl-p-tyrosine was consistent with that obtained by $[^3$H]–NE administration. In the present study, we used the NE biosynthesis inhibi-
Fig. 1. NE turnover in IBAT and hearts of yellow KK (○) and C57BL (●) mice. NE turnover was measured in IBATs and hearts from yellow KK and C57BL mice fed ad libitum for 12 weeks after birth. All data are plotted as the mean ± SEM for endogenous NE in tissues from six animals in each group at 0, 3 and 6 hours after injection of α-methyl-p-tyrosine (80 mg/kg). In IBAT, the slope for yellow KK mice differed significantly from that for C57BL mice. As for the heart, group slopes did not differ significantly.

Discussion

Using the technique in measuring the NE turnover, which has been shown to be a reliable indicator of SNS activity (Young and Landsberg, 1977a, b; Yoshida et al., 1983). Therefore, our results suggest that the SNS activity in IBAT of yellow KK mice is reduced, but not in the heart. Although the cause of the discrepancy between the NE turnover in IBAT and heart is unknown, it is suggested that IBAT may be regulated more strongly by SNS than in the heart (Young et al., 1982; Yoshida et al., 1985a). The decreased core temperature in yellow KK mice can be easily explained by the reduced SNS activity in IBAT of this animal, since brown adipose tissue (BAT) is known to be a main effector of cold-induced non-shivering thermogenesis (Foster and Frydman, 1979). Yellow KK mice showed reduced NE turnover in IBAT, while wet weight of IBAT was remarkably increased. Generally in obese animals, BAT cells fill with lipid when lipid stores in the body are large (Himms-Hagen, 1983). Knehans and Romsov (1983) report increased wet weight and reduced IBAT NE turnover in genetically obese (ob/ob) mice, results similar to our own in yellow KK mice. Previously we reported (Yoshida et al., 1985) that insulin-deficient experimental diabetes have a reduced NE turnover, and that this effect is prevented by replacement insulin therapy. Rowe et al. (1981) and Christensen et al. (1984) suggested that insulin stimulated the
sympathetic nervous system by acting on the central nervous system. Oomura et al. (1981) reported that the activity of the hypothalamus could be influenced by blood-borne insulin and that the activity of glucose-receptor neurons in the ventromedial hypothalamus (VMH), which was not only a center of satiety, but also a center of the SNS (Inoue and Bray, 1979; Bray and York, 1979), was facilitated by simultaneous application of insulin and glucose. Furthermore, insulin has been thought to play an important role in coordinating SNS activity with dietary intake (Yoshida et al., 1985; Young et al., 1983; Seydoux et al., 1983; Rothwell and Stock, 1981). However, our present results do not appear to be in agreement with these reports, because the yellow KK mice had reduced NE turnover despite the marked hyperinsulinemia. A possible explanation for this discrepancy might be decreased sensitivity to insulin at the particular site (probably hypothalamus) of the central nervous system as well as in peripheral tissue in yellow KK mice, since insulin resistance is a characteristic feature of this animal (Iwatsuka et al., 1970). The report (Cunningham et al., 1983) that the development of insulin resistance may interfere with dietary thermogenesis during cafeteria feeding in rats, which was related to the stimulation of SNS activity, supports this hypothesis.

On the other hand, since in hypothalamic obesity insulin resistance is absent or mild, although it can be found in obesity of prolonged duration (Bray and York, 1979), hyperinsulinemia might contribute to increased NE turnover in VMH-lesioned rats at the dynamic phase, as reported by Yoshida and Bray (1984).

In animals with severe diabetes such as STZ diabetic rats, it is possible that decreased NE turnover may be a direct result of weight loss and negative caloric balance (Yoshida et al., 1985b). Also, our recent report (Yoshida et al., 1987b) shows that STZ diabetic rats exhibited a decrease in motor nerve conduction velocity and SNS activity. Therefore, it is possible, too, that peripheral sympathetic nerve dysfunction as a consequence of diabetic neuropathy, may cause decreased NE turnover in yellow KK mice. This seems unlikely, however, since the decrease in NE turnover is observed only in IBAT, and not in the heart. If peripheral nerve disorders were causative factors, a diffused change in SNS activity would have been observed (Giachetti, 1978; Yoshida et al., 1985b). Therefore, decreased NE turnover in the IBAT of yellow KK mice presumably represents a primary alteration in central sympathetic outflow, although no studies in the current report specifically addressed this question.

It is well established that when hyperphagia is induced in experimental animals by other techniques, there is often a marked increase in NE turnover (Young and Landsberg, 1977a; Young et al., 1982). However, the yellow KK mice in the present study had a reduced NE turnover in IBAT despite hyperphagia. On the other hand, it is now recognized that brown adipose tissue (BAT), which is mainly controlled by the SNS (Jansky, 1973), is a main effector of diet-induced thermogenesis as well as cold-induced non-shivering thermogenesis and that a defect in or an absence of BAT would predispose to obesity (Rothwell and Stock, 1979), although the relevance of brown adipose tissue to thermogenesis per se, let alone the pathogenesis of obesity, remains unknown in humans. It is therefore suggested also that reduced SNS activity in IBAT may be one of the important factors in the development of obesity in yellow KK mice.

In conclusion, our present data indicate that hyperinsulinemia does not always increase NE turnover and that the reduced NE turnover in IBAT of yellow KK mice may be one of the important factors in the development of obesity in this animal.
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