β-Cell Function and Replication in Spontaneously Hypertensive Rats

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We examined β-cell function and replication in spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto rats (WKY). Rats were subjected to 90% pancreatectomy (Px) or sham operation at the age of 8 weeks, and islet function and regeneration were examined 4 weeks after surgery. Plasma glucose levels were higher in SHR than in WKY (509 ± 38 *v* 325 ± 109 mg/dL, $P < .0001$) 1 week after Px and throughout the experimental period. Plasma glucose responses to intravenous injection of glucose (0.5 g/kg body weight) were not different in the sham-operated animals of the two strains, whereas plasma insulin responses were greater in SHR than in WKY. No insulin responses to glucose were observed in either strain of Px rats. The insulin content of the remnant equivalent (6.7 ± 2.1 *v* 4.2 ± 0.4 μg, $P < .05$) and whole pancreas (156.7 ± 10.7 *v* 123.8 ± 23.5 μg, $P < .01$) in sham-operated rats was greater in SHR than in WKY. However, insulin content was lower ($P < .05$) in Px-SHR (1.0 ± 0.2 μg) than in Px-WKY (3.9 ± 1.7 μg). Histological examination showed that fibrotic degeneration of islets was much greater in Px-SHR than in Px-WKY. These data strongly suggest that the β cells of SHR were more vulnerable to reduction of islet mass than those of WKY. Our data also suggest that hyperinsulinemia and/or insulin resistance in SHR has a deleterious effect on β-cell replication.

TYPE II (NON–INSULIN-DEPENDENT) diabetes mellitus is frequently accompanied by hypertension. Several lines of evidence have suggested that insulin resistance may play an important role in the pathogenesis of essential hypertension,[1-3] although this connection is still controversial.[4] Previous studies[5,6] demonstrated that insulin resistance and impaired glucose tolerance are found in spontaneously hypertensive rats (SHR), which are regarded as an experimental model of hypertension in humans. However, the relationship between insulin resistance and the function of the endocrine pancreas in SHR has not been fully investigated.

Reaven and Ho[7] reported that low-dose streptozotocin (STZ) induced diabetes in SHR but not in normotensive Wistar-Kyoto rats (WKY). Sato et al[8] and Iwase et al[9] showed greater pathologic changes of pancreatic islets in neonatally STZ-treated SHR than in similarly treated WKY. These data strongly suggest that β cells in SHR are more sensitive and vulnerable to a β-cytotoxic agent than those in WKY, and that β-cell regeneration in SHR may be hampered after a reduction in islet mass. To examine this hypothesis, we studied β-cell function and regeneration following 90% pancreatectomy in SHR and WKY.

MATERIALS AND METHODS

Animals

SHR and WKY were purchased from Charles River Laboratories (Atsugi, Japan) and delivered to our laboratory at the age of 6 weeks. The animals were housed in air-conditioned quarters at 24°C under artificial lighting (lights on 8 AM to 8 PM). Tap water and chow pellets (Japan Clea, Tokyo, Japan) were given ad libitum. At the age of 8 weeks, animals were divided into sham-operated rats (sham) and 90% partially pancreatectomized (Px) rats. The rats were anesthetized with pentobarbital (50 mg/kg body weight intraperitoneally), and a midline abdominal incision was made. All of the tail portion and most of the head of the pancreas were removed by gentle abrasion with cotton applicators as described by Bonner-Weir et al.[10] The pancreatic remnant is defined as that portion lying between the common bile duct and the first loop of the duodenum. The corresponding portion of the pancreas in sham-operated rats is referred to as the remnant equivalent. Sham-operated rats underwent laparotomy, and their pancreata were ablated from the mesentery and gently rubbed between the fingertips. The animals were divided into four groups as follows: group 1 (n = 10), sham-operated SHR; group 2 (n = 8), sham-operated WKY; group 3 (n = 10), Px-SHR; group 4 (n = 8), Px-WKY.

Sampling and Glucose Tolerance Test

Weekly blood samples were obtained in the fed state by snipping the tail. At the end of 4 weeks after surgery, an intravenous glucose tolerance test (0.5 g/mL/kg body weight) was performed as described previously.[11] Food was removed at 9 AM on days when measurements were taken, and all procedures were initiated 5 hours later as indicated. Rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg body weight), and then glucose (50% solution) was injected into the saphenous vein. Blood samples were obtained immediately before and 5, 10, 15, and 30 minutes after glucose administration.

Extraction of Insulin

Following the intravenous glucose tolerance test, pancreata were removed immediately, lyophilized, and weighed. Pancreata from sham-operated rats were divided into the remnant equivalent and the rest. The pancreas was homogenized in 30 mL cold acid-ethanol (0.18 mol/L HCl in 75% vol/vol ethanol) and kept for 24 hours at 4°C. After centrifugation at 3,000 rpm for 20 minutes at 4°C, the supernatant was collected and the precipitate was further extracted in the same manner. Then both supernatants were combined and stored at −70°C until assayed.

Histological Examination

The pancreas was fixed in Formalin and embedded in paraffin for sectioning. Five consecutive 4-μm sections were obtained at intervals of 250 μm throughout the block. These sections were stained for insulin by the strepto-avidin-biotin method using a SAB-PO Kit (Nichirei, Tokyo, Japan).

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Measurements

Systolic blood pressure was measured by the tail-cuff method (automatic blood pressure recorder UR-1000 type; Ueda, Tokyo, Japan) after prewarming to 37°C for 10 minutes.[12] The average of five readings was taken. Plasma glucose level was measured by the glucose oxidase method using a glucose analyzer (Fuji Film, Tokyo, Japan). Immunoreactive insulin level was measured by specific radioimmunoassay[13] using rat insulin (Novo, Bagsvaerd, Denmark) as a standard. The sensitivity of this assay was 0.31 ng/mL, and the interassay coefficient of variation was 10%.

Statistics

Data are presented as the mean ± SD. Data were analyzed by ANOVA in combination with Student's t test. P less than .05 was considered significant.

RESULTS

Blood pressure was greater in SHR than in WKY before the operation (183 ± 11 *v* 135 ± 7 mm Hg, $P < .01$). Blood pressure increased with aging in SHR, and there were no differences between sham-operated SHR and Px-SHR (209 ± 18 *v* 201 ± 17 mm Hg).

There were no differences in body weight between sham-operated SHR and sham-operated WKY throughout the experimental period (Fig 1). However, body weight was significantly decreased 1 week after Px in both strains of rats and then progressively increased in WKY but not in SHR. Body weights of Px-WKY reached preoperative levels 4 weeks after the operation, whereas the body weight of Px-SHR was lower than that of Px-WKY during the experimental period.

Figure 2 shows changes in nonfasting plasma glucose concentration throughout the course of the experiment. Plasma glucose in sham-operated WKY was slightly but not significantly higher than in sham-operated SHR. However,

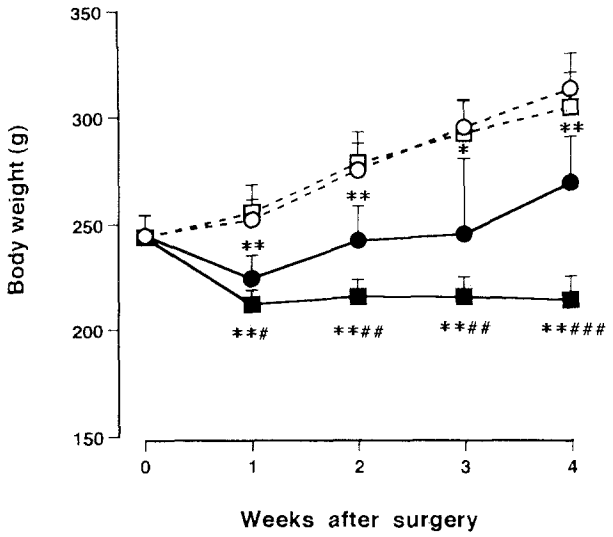

Fig 1. Time course of body weight in sham-operated WKY (○, n = 8), sham-operated SHR (□, n = 10), Px-WKY (●, n = 8), and Px-SHR (■, n = 10). Mean ± SD. *$P < .0005$, **$P < .0001$: *v* corresponding sham-operated rats. #$P < .05$, ##$P < .01$, ###$P < .0001$: *v* Px-WKY.

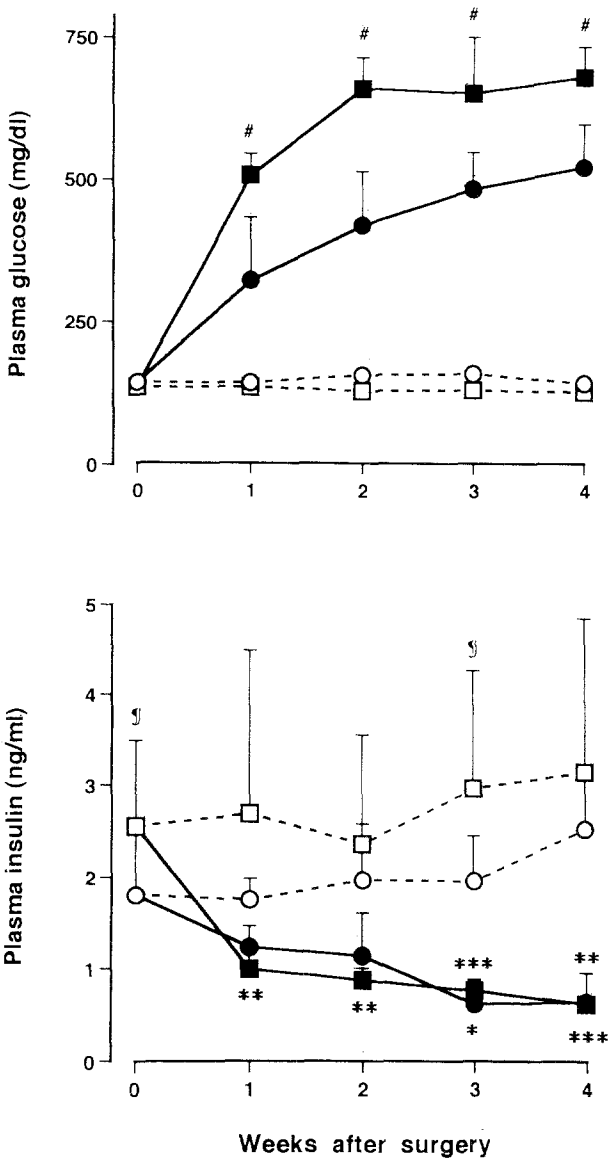

Fig 2. Time course of glucose (A) and insulin (B) levels following sham operation and Px. (○) Sham-operated WKY; (□) sham-operated SHR; (●) Px-WKY; (■) Px-SHR. Mean ± SD. #$P < .0001$ *v* Px-WKY. ¶$P < .05$ *v* sham-operated WKY. *$P < .01$, **$P < .005$, ***$P < .0001$: *v* corresponding sham-operated rats.

after Px plasma glucose levels were significantly increased in both strains of rats as compared with corresponding sham-operated animals. Plasma glucose levels were greater in Px-SHR than in Px-WKY 1 week after Px (509 ± 38 *v* 325 ± 109 mg/dL, $P < .0001$) and during the experimental period.

Nonfasting plasma insulin concentrations were slightly but significantly higher in SHR than in WKY before the operation (2.6 ± 0.9 *v* 1.7 ± 0.7 ng/mL, $P < .05$). After Px, plasma insulin concentration was decreased, but insulin secretory activity was to some extent preserved in the residual pancreas.

After a 5-hour fast, there were no differences between plasma glucose concentrations in sham-operated WKY and

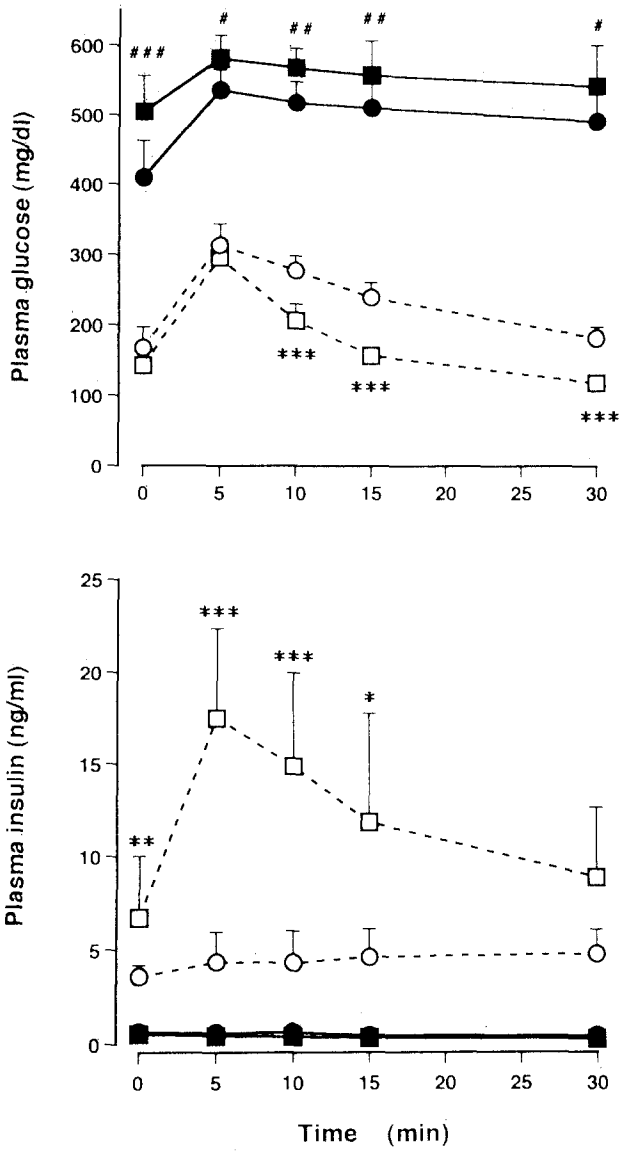

Fig 3. Plasma glucose (A) and insulin (B) responses to intravenous injection of glucose (0.5 g/kg body weight) in SHR and WKY with sham operation or Px. (○) Sham-operated WKY; (□) sham-operated SHR; (●) Px-WKY; (■) Px-SHR. Mean ± SD. #P < .05, ##P < .001, ###P < .0001: v Px-WKY. *P < .005, **P < .001, ***P < .0001: v sham-operated WKY.

SHR (Fig 3). Intravenous injection of glucose resulted in significant increases in plasma glucose concentration at 10, 15, and 30 minutes in sham-operated WKY as compared with sham-operated SHR. Plasma glucose concentrations in the fasting state and after an intravenous glucose load were higher in Px-SHR than in Px-WKY. Plasma insulin responses in sham-operated animals showed differences between WKY and SHR. Although 5-hour fasting insulin levels were not statistically significantly different, plasma insulin concentrations in SHR were significantly higher than in WKY at 5, 10, and 15 minutes after an intravenous glucose load. There was no insulin response to an intravenous glucose load in Px-WKY and Px-SHR.

There were no significant differences in dry weight of the whole pancreas in sham-operated animals (Table 1). Dry weight of the pancreas was greater in Px-WKY than in Px-SHR (62.4 ± 12.6 v 40.0 ± 10.0 mg, P < .01). As compared with the corresponding remnant equivalent, the regeneration ratio was estimated to be 64% (62.4/38.0 × 100) in Px-WKY and 33% (40.0/30.0 × 100) in Px-SHR. The insulin content of the remnant equivalent and whole pancreas in SHR was slightly but significantly higher than in WKY. In addition, the insulin content per dry weight of the pancreas in sham-operated SHR was also greater than that of sham-operated WKY. Four weeks after Px, there was no increase in insulin content in WKY (3.9 ± 1.7 μg), whereas it was markedly decreased in SHR (1.0 ± 0.2 μg). Thus, Px resulted in a greater decrease (P < .0001) in pancreatic insulin stores in Px-SHR (14.9%, 1.0/6.7 × 100) as compared with corresponding Px-WKY (92.9%, 3.9/4.2 × 100).

There were no structural changes in the islets of the pancreas in sham-operated rats (Fig 4). In Px rats, by contrast, the β cells were scattered throughout the islet and were no longer arranged in trabecular strands as observed in normal islets. In both Px-SHR and Px-WKY, islets lost their normal structure and had a reduced size, although Px-SHR showed more serious atrophic changes.

DISCUSSION

Previous studies have suggested that pancreatic β cells of SHR are much more sensitive to β-cytotoxic agents such as STZ than those of WKY.[7-9] However, those studies did not quantitatively assess the regeneration ratio of β cells, because it is impossible to estimate how many β cells were killed by STZ. Therefore, 90% pancreatectomy was used in the present study to determine the function of the residual pancreas. Compared with our previous observation[14] and another report,[15] marked hyperglycemia occurred in SHR

Table 1. Dry Weight, Insulin Content, and Insulin Content per Dry Weight in SHR and WKY 4 Weeks After Surgery

Group	No. of Rats	Dry Weight (mg)	Content (μg)	Content/ Dry Weight (μg/mg)
WKY				
Sham-operated				
Remnant equivalent	5	38.0 ± 10.9	4.2 ± 0.4	0.15 ± 0.06
Whole pancreas	5	486.0 ± 106.4	123.8 ± 23.5	0.28 ± 0.05
Px	5	62.4 ± 12.6‡	3.9 ± 1.7	0.08 ± 0.05
SHR				
Sham-operated				
Remnant equivalent	6	30.0 ± 12.6	6.7 ± 2.1*	0.25 ± 0.07*
Whole pancreas	6	405.0 ± 38.5	156.7 ± 10.7*	0.39 ± 0.05†
Px	5	40.0 ± 10.0†	1.0 ± 0.2*§	0.03 ± 0.01§

NOTE. Values are the mean ± SD.

*P < .05, †P < .01: v corresponding WKY.

‡P < .005, §P < .0001: v corresponding remnant equivalent of sham-operated rats.

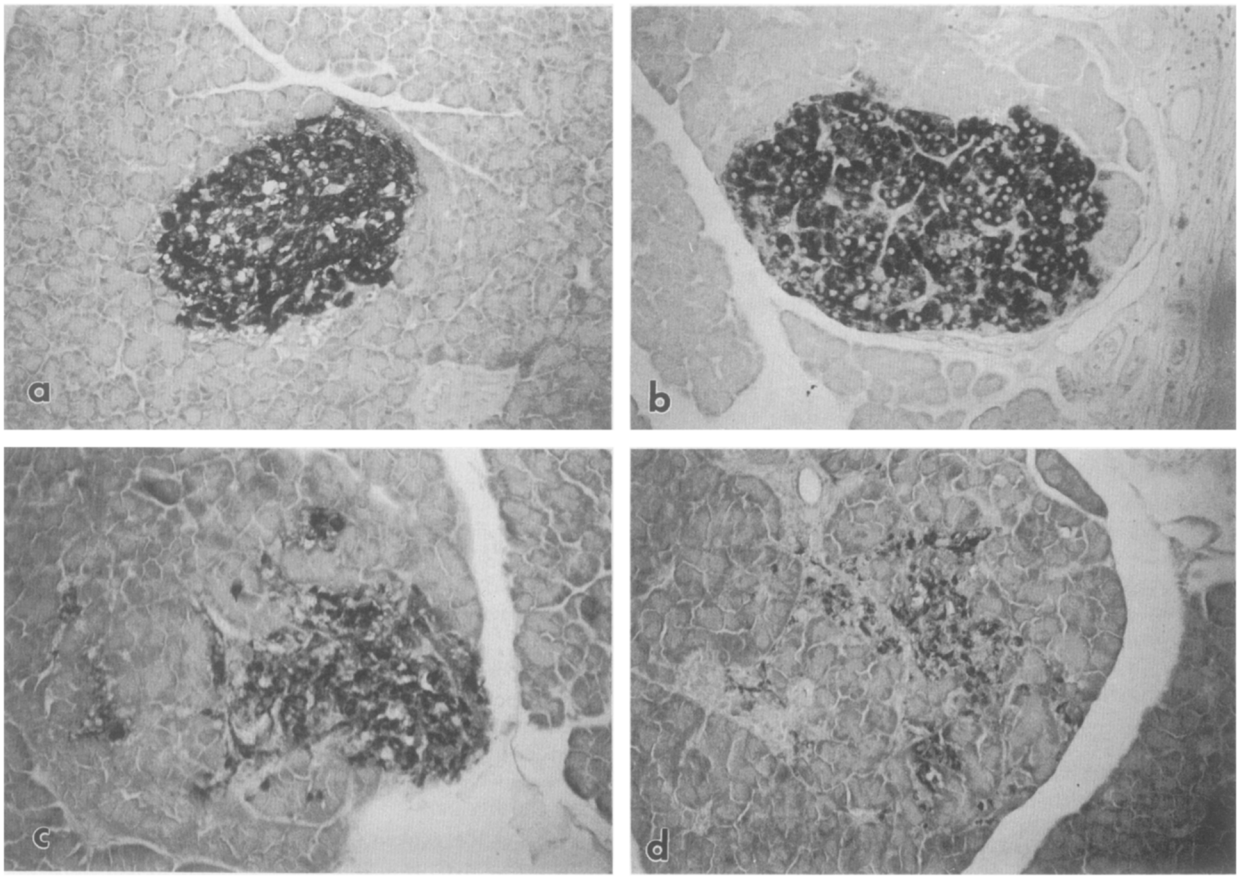

Fig 4. Histological view of pancreatic islets from SHR and WKY with sham operation or Px (original magnification × 100). (a) Sham-WKY, (b) sham-SHR, (c) Px-WKY, and (d) Px-SHR.

and WKY following 90% pancreatectomy. After male Wistar rats[14] or Sprague-Dawley rats[15] underwent 90% pancreatectomy, plasma glucose concentration gradually increased during the following 2 to 3 weeks. Peak concentrations of plasma glucose were approximately 200 to 250 mg/dL. Plasma glucose concentrations then progressively declined due to regeneration of the residual pancreas. However, in the present study, plasma glucose concentrations in both strains of rats were significantly higher than those reported in previous studies.[14,15]

Although the effect of pentobarbital on glucose metabolism in the rat is well known,[16,17] our preliminary study indicated a negligible effect of pentobarbital on plasma glucose concentration following intraperitoneal injection of saline in both WKY and SHR (data not shown). Plasma glucose concentrations after intravenous injection of glucose were not markedly elevated in sham-operated rats, whereas those of Px animals were extremely high as compared with the previous study.[10] Thus, these results suggest that not only SHR but also WKY are vulnerable following reduction of the islet mass, although plasma glucose levels after Px and during the intravenous glucose tolerance test were significantly higher in SHR than in WKY. Another feature of Px-SHR and Px-WKY was a marked reduction in body weight. We recently obtained similar findings in depancreatized 15-month-old Wistar rats (Tanigawa K, et al, unpublished observation, June 1993). In young male Wistar rats, the reduction in body weight 1 week after Px was probably due to surgical stress, but then the animals gained weight and there were no differences in body weight between Px and sham-operated animals by 4 to 5 weeks following the operation. Bonner-Weir et al[10] did not find any difference in body weight between sham-operated and Px rats during 7 weeks after the operation. In the present study, prominent hyperglycemia and diminished insulin secretory response were accompanied by failure to gain body weight, especially in SHR. Taken together, the findings indicate that Px-SHR represents an animal model similar to that observed for type I (insulin-dependent) diabetes.

SHR exhibited exaggerated insulin secretion in response to an intravenous glucose load. The exaggerated insulin secretion may have a basis in the larger area of the islet cells[8] and higher insulin content (Table 1). These data are in good agreement with previous reports.[18,19] In addition, we recently found that glucose-stimulated insulin secretion from the isolated perfused pancreas was far more exaggerated in SHR than in WKY (Tanigawa K, et al, submitted). Chen et al[20] reported that pancreatic islets of SHR had a lower set-point for glucose-induced insulin secretion as compared with those of WKY, indicating higher β-cell sensitivity to glucose in SHR than in WKY. They explained

this greater sensitivity in SHR by enhanced activity of glucokinase and greater expression of glucose transporter (Glut 2) in pancreatic islets as compared with those in WKY. However, whether the changes observed in SHR correspond to a genetic predisposition or an increase in damage to the islets due to hypertension remains unclear.

We did not find any evidence to explain the relationship between exaggerated insulin secretion and vulnerability to reduction of islet mass in SHR in the present study. Whether insulin resistance plays an important role in hypertension in SHR is still a matter of controversy,[5,21,22] but hyperinsulinemia is undoubtedly present in this animal model. Hyperinsulinemia may reflect insulin resistance in euglycemic SHR, and long-term compensation for hyperinsulinemia in SHR may have a deleterious effect on islet function. Thus, the findings obtained by Chen et al[20] may reflect abnormal function of islets in SHR. A comparison between normal Wistar rats and WKY is required.

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REFERENCES

1. Ferranini E, Buzzigoli G, Bonadonna R, et al: Insulin resistance in essential hypertension. N Engl J Med 317:350-357, 1987

2. DeFronzo RA: Insulin resistance: The metabolic link between non–insulin-dependent diabetes mellitus, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. Curr Opin Cardiol 5:586-593, 1990

3. Reaven GM: Insulin resistance and compensatory hyperinsulinemia: Role in hypertension, dyslipidemia, and coronary heart disease. Am Heart J 121:1283-1289, 1991

4. McCarty MF: Insulin resistance—not hyperinsulinemia—is pathogenic in essential hypertension. Med Hypotheses 42:226-236, 1994

5. Mondon CE, Reaven GM: Evidence of abnormalities of insulin metabolism in rats with spontaneous hypertension. Metabolism 37:303-305, 1988

6. Reaven GM, Chang H, Hoffman BB, et al: Resistance to insulin-stimulated glucose uptake in adipocyte isolated from spontaneously hypertensive rats. Diabetes 38:1155-1160, 1989

7. Reaven GM, Ho H: Low-dose streptozotocin-induced diabetes in the spontaneously hypertensive rat. Metabolism 40:335-337, 1991

8. Sato T, Nara Y, Note S, et al: New establishment of hypertensive diabetic animal models. Neonatally streptozotocin-treated spontaneously hypertensive rats. Metabolism 36:731-737, 1987

9. Iwase M, Nunori K, Kikuchi M, et al: Morphometrical and biochemical differences of endocrine pancreata between spontaneously hypertensive and normotensive rats with or without neonatal streptozotocin-induced diabetes. Lab Invest 60:102-106, 1989

10. Bonner-Weir S, Tren DF, Weir GC: Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. J Clin Invest 71:1544-1553, 1983

11. Portha B, Picon L, Rosselin G: Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes. Diabetologia 17:371-377, 1979

12. Ikeda K, Nara Y, Yamori Y: Indirect systolic and mean blood pressure determination by a new tail cuff method in spontaneously hypertensive rats. Lab Anim 25:26-29, 1991

13. Desbuquios B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound hormones in radioimmunoassay. J Clin Endocrinol Metab 33:732-738, 1977

14. Inoue Y, Tanigawa K, Nakamura S, et al: Nicotinamide, a poly(ADP-ribose)synthetase inhibitor, ameliorates B-cell function in partially depancreatized rats. Diabetes Res Clin Pract 22:19-27, 1993

15. Brockenbrough JS, Weir GC, Bonner-Weir S: Discordance of exocrine and endocrine growth after 90% pancreatectomy in rats. Diabetes 37:232-236, 1988

16. Lang CH, Bagby GJ, Hargrove DM, et al: Alterations in glucose kinetics induced by pentobarbital anesthesia. Am J Physiol 253:E657-E663, 1987

17. Clark PW, Jenkins AB, Kraegen EW: Pentobarbital reduces basal liver glucose output and its insulin suppression in rats. Am J Physiol 258:E701-E707, 1990

18. Gaboury GL, Karanja N, Holcomb SR, et al: Patterns of insulin secretion and responsiveness in Wistar-Kyoto and spontaneously hypertensive rats. Am J Hypertens 4:661-666, 1991

19. Buchanan TA, Youn JH, Campese VM, et al: Enhanced glucose tolerance in spontaneously hypertensive rats: Pancreatic B-cell hyperfunction with normal insulin sensitivity. Diabetes 41:872-878, 1992

20. Chen C, Hosokawa H, Bumbalo L, et al: Mechanism of compensatory hyperinsulinemia in normoglycemic insulin-resistant spontaneously hypertensive rats. J Clin Invest 94:399-404, 1994

21. Finch D, Davis G, Bower J, et al: Effect of insulin in renal sodium handling in hypertensive rats. Hypertension 15:514-518, 1990

22. Buchanan TA, Sipos GF, Madrilejo N, et al: Hypertension without peripheral insulin resistance in spontaneously hypertensive rats. Am J Physiol 22:E14-E19, 1992