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Variations in Insulin Sensitivity in Spontaneously Hypertensive Rats From Different Sources

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We investigated the possibility of variations in the genetic transmission of insulin sensitivity in the offspring of spontaneously hypertensive rats (SHRs) and Wistar Kyoto rats (WKYs) obtained from different sources (Charles River, Tokyo, Japan [NCrj]; and Funabashi Farm, Chiba, Japan [Izm]) with the insulin suppression test (IST) using a somatostatin analog, glucose, and insulin. The steady-state blood glucose (SSBG) in the IST and the glucose infusion required (GIR) in the euglycemic-hyperinsulinemic clamp differ significantly between obese and lean Zucker rats, indicating that both methods are useful for identifying insulin resistance. The fasting blood glucose and SSBG of the IST were significantly higher in SHR/Izm than in WKY/Izm. We did not observe a significant difference between SHR/NCrj and WKY/NCrj. These results indicate that the genetic transmission of hypertension and impaired insulin sensitivity may be variable and that insulin resistance does not play an important role in the pathogenesis of hypertension in the SHR.

STUDIES HAVE SHOWN that insulin resistance is related to the development of hypertension,[1,2] although the underlying mechanism has not been clarified. Insulin resistance leads to hyperinsulinemia, resulting in sodium retention, sympathetic hyperactivity, and alterations in lipid metabolism that may contribute to the development of hypertension and to an increased risk of cardiovascular disease.[3]

The results of studies examining insulin sensitivity in the spontaneously hypertensive rat (SHR), a model for investigating essential hypertension, have not been consistent. In some studies, SHRs have not exhibited insulin resistance,[4-6] whereas other studies have found impaired insulin sensitivity in SHRs.[7-10] Discrepancies in the study results may be related to differences in the methods used to assess insulin sensitivity, the age of animals studied, and the experimental conditions,[11,12] but the possibility that insulin sensitivity differs among SHRs cannot be ruled out.

We evaluated insulin sensitivity in SHRs and Wistar Kyoto rats (WKYs) obtained from different sources to determine whether there are variations in the genetic transmission of hypertension and insulin resistance.

MATERIALS AND METHODS

Animals

As a preliminary experiment, we investigated the validity of the insulin suppression test (IST) versus the euglycemic insulin clamp for assessment of insulin sensitivity in 8-week-old male obese (fa/fa) Zucker rats (Charles River, Kingston, NY), an insulin-resistant and hyperinsulinemic animal model.[13] The control group consisted of lean (fa/?) Zucker rats.

We obtained 8-week-old male SHRs and WKYs from Charles River Japan (Tokyo, Japan: SHR/NCrj and WKY/NCrj) and from Funabashi Farm (Chiba, Japan: SHR/Izm and WKY/Izm). The rats were from different litters. They were maintained in individual cages with a 12-hour light/dark cycle with access to water and normal rat chow ad libitum for 1 week before the experiments.

Mean Arterial Pressure and Heart Rate

Polyethylene catheters (PE-50; Intramedic, New York, NY) were inserted in a unilateral left carotid artery and the left jugular vein under light ether anesthesia. The catheters were subcutaneously exteriorized through the skin incision between the scapulae, and were filled with an isotonic (154 mmol/L) saline solution containing heparin (500 U/mL) and sealed with steel nails. The animals were allowed to rest for 1 day. Experiments were performed on minimally restrained and awake animals. Animals fasted for 12 hours before the beginning of the experiment.

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Table 1. Body Weight, Fasting Blood Sugar, SSBG, and SSPI in Lean and Obese Zucker Rats

Group	No. of Rats	BW (g)	FBS (mg/dL)	SSBG (mg/dL)	SSPI (μU/mL)
Lean	6	235.8 ± 9.3	110.9 ± 6.1	92.4 ± 14.9	134.1 ± 110
Obese	6	299.2 ± 20.6	138.1 ± 26.0	304.5 ± 63.7*	201.7 ± 72.3

Abbreviations: BW, body weight; FBS, fasting blood glucose.
*$P < .001$ *v* lean.

After at least 30 minutes of rest, the direct mean arterial blood pressure (MAP) and the pressure wave–triggered heart rate (HR) were measured via the carotid catheter with a polygraph (RM-6100; Nihon Koden, Tokyo, Japan).

IST

After measurements of MAP and HR were obtained, insulin sensitivity was assessed by the IST and a euglycemic-hyperinsulinemic clamp in separate groups of rats. The IST was performed as follows. After administration of a bolus injection of 50 mg/kg octreotide (Sandostatin; Sandoz, Osaka, Japan), the following substances were infused via the jugular catheter for 150 minutes using an infusion pump (EP 60; Eicon, Tokyo, Japan): 16 $mg \cdot kg^{-1} \cdot min^{-1}$ glucose, 5 $mU \cdot kg^{-1} \cdot min^{-1}$ human insulin (Novolin R; Novo Nordisk, Chiba, Japan), and 0.077 $\mu g \cdot kg^{-1} \cdot min^{-1}$ octreotide. Blood samples were obtained via the carotid catheter for measurement of blood glucose and plasma insulin concentrations. In a pilot study, there was no significant difference in blood glucose levels at 150 and 180 minutes in SHR/NCrj, WKY/NCri, and obese Zucker rats. Therefore, we defined steady-state blood glucose (SSBG) as the blood glucose level at 150 minutes (data not shown).

Euglycemic-Hyperinsulinemic Clamp

In addition to the catheters implanted in the left carotid artery and left jugular vein, an additional catheter was implanted in the jugular vein under light ether anesthesia. The clamp was performed under conscious conditions 1 day after catheter implantation. One jugular catheter was used for infusion of human insulin (Novolin R) and the other for infusion of a 20% glucose solution. Insulin was infused according to the following protocol. After a bolus injection of 3.6 mU/kg, human insulin was infused at 3 $mU \cdot kg^{-1} \cdot min^{-1}$ for 75 minutes (low-dose), and then after a 9.6-mU/kg bolus injection, at 8 $mU \cdot kg^{-1} \cdot min^{-1}$ (high-dose). During the infusions, blood samples were obtained from the carotid catheter and the blood glucose level was measured every 5 minutes. The glucose infusion rate was adjusted to maintain the fasting glucose level, and the glucose infusion required (GIR) during the high-dose stage was calculated. Blood samples were obtained at the end of the high-dose stage for measurement of the serum insulin concentration.

Data Analysis

Blood glucose levels were measured using an electrode system (Antsense; Ames, Osaka, Japan). Serum insulin concentrations were determined using a monoclonal radioimmunoassay technique.

The data were analyzed using Student's *t* test. A *P* value less than .05 was considered statistically significant. Results are expressed as the mean ± SD.

RESULTS

The SSBG of the IST was significantly higher in obese versus lean Zucker rats (Table 1). Blood glucose and serum insulin concentrations were similar in lean and obese Zucker rats during the glucose clamp study (Table 2). The GIR was significantly decreased in obese Zucker rats.

There were no significant differences in body weight and HR among the groups of SHR and WKY from different sources (Table 3). The MAP was similar in SHR/NCrj and SHR/Izm and was significantly higher in SHR groups than in the control WKY groups. The fasting glucose level was significantly higher in SHR/Izm than in WKY/Izm.

The steady-state plasma insulin (SSPI) of the IST did not differ significantly among groups (Table 4). The SSBG was significantly higher in SHR/Izm compared with WKY/Izm.

DISCUSSION

The conventional IST originally proposed by Mondon and Reaven[7] uses propranolol and epinephrine to suppress intrinsic insulin secretion. These agents may not be useful for assessing insulin sensitivity in the SHR model, because changes in skeletal muscle blood flow influence insulin sensitivity and the sensitivity of peripheral arterioles to these agents may differ in hypertensive models.

Somatostatin suppresses insulin secretion but does not influence hemodynamics and glucose metabolism.[14-16] We used a somatostatin analog instead of propranolol and epinephrine to avoid alterations in hemodynamics.

The SSBG of the IST and the GIR of the euglycemic-hyperinsulinemic clamp differ significantly between lean and obese Zucker rats, indicating that both methods are useful for identifying insulin resistance. The IST is less complicated than the euglycemic-hyperinsulinemic clamp because only one infusion solution is necessary, the infusion rate is determined according to body weight and is kept constant throughout the study, and only one blood sample for measurement of glucose and insulin levels is required. In contrast, the glucose infusion rate has to be adjusted frequently based on the initial blood glucose level to determine the GIR with the euglycemic-hyperinsulinemic clamp method.[17,18] The high standard deviation values observed in our sample of lean Zucker rats on both

Table 2. Results of the Euglycemic-Hyperinsulinemic Insulin Clamp in Lean and Obese Zucker Rats

Group	No. of Rats	BW (g)	FBS (mg/dL)	Blood Glucose (mg/dL)	Plasma Insulin (μU/mL)	GIR ($mg \cdot kg^{-1} \cdot min^{-1}$)
Lean	5	234.0 ± 15.6	130.0 ± 14.7	123.0 ± 16.8	153.2 ± 86.1	27.1 ± 27.5
Obese	5	294.0 ± 22.4	127.0 ± 23.0	120.0 ± 21.5	156.9 ± 99.1	3.6 ± 4.0*

Abbreviation: FBS, fasting blood sugar.
*$P < .001$ *v* lean Zucker rats.

Table 3. Baseline Characteristics of SHR and WKY Groups

Strain	No. of Rats	BW (g)	FBS (mg/dL)	MAP (mm Hg)	HR (bpm)
SHR/NCrj	6	198.0 ± 10.0	133.1 ± 14.2	161.0 ± 22.0*	367.0 ± 33.6
WKY/NCrj	6	208.8 ± 2.7	121.4 ± 5.9	110.0 ± 2.4	321.0 ± 31.8
SHR/Izm	10	213.0 ± 0.9	170.8 ± 8.8†	162.2 ± 9.7†	348.0 ± 29.4
WKY/Izm	10	224.0 ± 8.5	133.7 ± 7.9	119.0 ± 13.3	370.5 ± 24.3

*$P < .05$ *v* WKY/NCrj.
†$P < .05$ *v* WKY/Izm.

tests suggest that it may be part of the genetic heterogeneity of these rats.

Despite the fact that lean and obese Zucker rats received the same amount of glucose and insulin, obese rats had a higher SSBG, suggesting an impaired insulin metabolism and confirming previous data.[13]

Although insulin resistance is common in patients with essential hypertension, the results of studies examining insulin sensitivity in the SHR are not consistent.

Mondon et al[7,8] and Hulman et al[9] observed insulin resistance in anesthetized and restricted SHRs. However, their results may have been influenced by the experimental conditions, because anesthetics and/or restriction activate the sympathetic nervous system[12,19] and thus may alter insulin sensitivity. Hulman et al also detected insulin resistance in conscious, minimally restrained SHRs using the euglycemic-hyperinsulinemic clamp method.[10]

Frontoni et al[6] observed an increased insulin-glucose uptake and muscle glycogenic rate in SHRs compared with WKYs when a physiological dose of insulin was infused, but not when high-dose insulin was infused, in a euglycemic clamp study. Buchanan et al[4] also failed to detect insulin resistance in conscious and unstressed SHRs using the euglycemic clamp method. Tsutsu et al[5] reported that the glucose disappearance rate was increased in conscious 22-week-old SHRs compared with age-matched normotensive WKYs during an oral glucose tolerance test. The discrepancies in study results may be related to differences in the methods used to assess insulin sensitivity, the experimental conditions, and the age of the animals studied.

Table 4. SSBG and SSPI for IST in SHR and WKY Groups

Strain	No. of Rats	SSBG (mg/dL)	SSPI (μU/mL)
SHR/NCrj	6	122.3 ± 26.9	78.8 ± 10.3
WKY/NCrj	6	138.5 ± 26.7	62.5 ± 15.4
SHR/Izm	10	157.8 ± 17.7*	88.2 ± 19.9
WKY/Izm	10	121.8 ± 29.7	63.7 ± 11.4

*$P < .05$ *v* WKY/Izm.

The SHR is descended from WKYs, but studies using DNA-fingerprinting techniques have shown that the genotypes of these strains differ.[20,21] When SHRs were obtained from Kyoto University, they were not genetically purified because the genotype could not be identified at that time. Therefore, their offspring may not be genetically uniform. For example, Calhoun et al[22] reported that SHRs from Taconic Farm are salt-sensitive (SHR-S), whereas SHRs from Charles River are salt-resistant (SHR-R). We previously found that chronic administration of insulin increased the blood pressure response to hyperinsulinemia also in a variable manner in SHRs from different sources.[23]

Maternal diabetes induced by streptozotocin or environmental conditions has been found to influence blood pressure.[24] Plasma glucose levels at fasting and after glucose loading have been found to be significantly lower in the offspring of diabetic versus control SHR dams.[25]

These findings suggest that different genotypes and/or environmental factors may explain the variation in insulin sensitivity in SHRs from different sources.

In the present study, insulin sensitivity did not differ between SHR/NCrj and WKY/NCrj, but was significantly impaired in SHR/Izm compared with WKY/Izm. There was no significant difference in SSBG between WKY/NCrj and WKY/Izm, suggesting that the SHR/Izm is an insulin-resistant hypertensive model.

Blood pressure was similar in SHR/NCrj and SHR/Izm, suggesting that there may be differences in the way hypertension and impaired insulin sensitivity are transmitted.

In conclusion, insulin sensitivity in the SHR varied among offspring, and this variation may be linked to differences in the genetic transmission of hypertension and insulin resistance. Differences in insulin sensitivity did not appear to contribute to hypertension in the SHR.

REFERENCES

1. Swislocki ALM, Hoffman BB, Reaven GM: Insulin resistance, glucose intolerance and hyperinsulinemia in patients with hypertension. Am J Hypertens 2:419-423, 1989

2. Vaaler S: Carbohydrate metabolism, insulin resistance, and metabolic cardiovascular syndrome. J Cardivasc Pharmacol 20:S11-S14, 1992 (suppl)

3. Reaven GM, Lithell H, Landsberg L: Hypertension and associated metabolic abnormalities—The role of insulin resistance and the sympathoadrenal system. N Engl J Med 8:374-381, 1996

4. Buchanan TA, Sipos GF, Madrilejo N, et al: Hypertension without peripheral insulin resistance in spontaneously hypertensive rats. Am J Physiol 262:E14-E19, 1992

5. Tsutsu N, Takata Y, Nunoi K, et al: Glucose tolerance and insulin secretion in conscious and unrestrained normotensive and spontaneously hypertensive rats. Metabolism 38:63-66, 1989

6. Frontoni S, Ohaman L, Haywood JR, et al: In vivo insulin action in genetic models of hypertension. Am J Physiol 262:E191-E196, 1992

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

8. Mondon CE, Reaven GM, Azhar S, et al: Abnormal insulin metabolism by specific organs from rats with spontaneous hypertension. Am J Physiol 257:E491-E498, 1989

9. Hulman S, Falkner B, Chen YQ: Insulin resistance in the spontaneously hypertensive rat. Metabolism 40:359-361, 1991

10. Hulman S, Falkner B, Freyvogel N: Insulin resistance in the conscious spontaneously hypertensive rat: Euglycemic hyperinsulinemic clamp study. Metabolism 42:14-18, 1993

11. Aynsley-Green A, Biebuyck JF, Alberti KGMM: Anaesthesia and insulin secretion: The effects of diethyl ether, halothane, pentobarbitone sodium and ketamine hydrochloride on intravenous glucose tolerance and insulin secretion in the rat. Diabetologia 9:274-281, 1973

12. MacCarty R, Kopin IJ: Alterations in plasma catecholamines and behavior during acute stress in spontaneously hypertensive and Wistar-Kyoto normotensive rats. Life Sci 22:997-1006, 1978

13. Rohner-Jeanrenaud F, Proietto J, Ionescu E, et al: Mechanism of abnormal oral glucose tolerance of genetically obese fa/fa rats. Diabetes 35:1350-1355, 1986

14. Nagulesparan M, Savage PJ, Unger RH, et al: A simplified method using somatostatin to assess in vivo insulin resistance over a range of obesity. Diabetes 28:980-983, 1979

15. Harano T, Ohgaku S, Hidata H, et al: Glucose, insulin and somatostatin infusion for the determination of insulin sensitivity. J Clin Endocrinol Metab 45:1124-1127, 1977

16. Alberti KGMM, Christensen NJ, Christensen SE, et al: Inhibition of insulin secretion by somatostatin. Lancet 2:1299-1301, 1973

17. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979

18. Smith G, Rossetti L, Ferrannini E, et al: In vivo glucose metabolism in the awake rat: Tracer and insulin clamp studies. Metabolism 36:1167-1174, 1987

19. Shah JH, Wongsurawat N, Aran PP, et al: A method for studying acute insulin secretion and glucose tolerance in unanesthetized and unrestrained rats. Diabetes 261:1-6, 1977

20. Lezin E, Simonet L, Pravenec M, et al: Hypertensive strains and normotensive control strains. How closely are they related? Hypertension 19:419-424, 1992

21. Johnson ML, Ely DL, Turner ME: Genetic divergence between the Wistar-Kyoto rat and the spontaneously hypertensive rat. Hypertension 19:425-427, 1992

22. Calhoun DA, Zhu S, Wyss JM, et al: Diurnal blood pressure variation and dietary salt in spontaneously hypertensive rats. Hypertension 24:1-7, 1994

23. Tomiyama H, Kushiro T, Abeta H, et al: Augmentation of salt sensitive hypertension by insulin. Proc Annu Meet Am Heart Assoc Council High Blood Pressure Res 4, Baltimore, MD, 1990 (abstr)

24. Iwase M, Wada M, Wakisaka M, et al: Effects of maternal diabetes on blood pressure and glucose tolerance in offspring of spontaneously hypertensive rats: Relation to birth weight. Clin Sci 89:255-260, 1995

25. Iwase M, Wada M, Shinohara N, et al: Effect of maternal diabetes on longevity in offspring of spontaneously hypertensive rats. Gerontology 41:181-186, 1995