Insulin Secretion and Biosynthesis by the Perfused Pancreas of Spontaneously Hypertensive Rats

Keiichiro Tanigawa, Yasushi Inoue, and Katsuhiro Tamura

Insulin secretory activity was compared in the spontaneously hypertensive rat (SHR) versus the normotensive Wistar-Kyoto (WKY) rat and Wistar rat. When the isolated pancreas was perfused with 16.7 mmol/L glucose, insulin release was significantly greater in the SHR versus the other groups. On the other hand, there was no difference in arginine (19 mmol/L)-induced insulin secretion among the three groups. To determine insulin biosynthesis during glucose stimulation, the pancreas was perfused with 16.7 mmol/L glucose for 180 minutes. Insulin secretion was greater in SHR versus WKY and Wistar rats, but the net increase in insulin content was not different between the three groups. These results strongly suggest that in vivo hyperinsulinemia in the SHR is associated with increased in vitro insulin secretion in response to glucose. The mechanisms by which enhanced glucose-induced insulin secretion is linked to hypertension in the SHR remain unclear.

SEVERAL LINES OF EVIDENCE suggest an association between insulin resistance and hypertension.[1-3] The spontaneously hypertensive rat (SHR) has been used as an experimental model of hypertension in humans. Previous studies[4-6] reported insulin resistance and augmented insulin secretion in the SHR, although this matter is still controversial.[7,8] The relationship between insulin resistance and the function of the endocrine pancreas in the SHR has not been fully investigated. In the present study, we examined insulin secretory activity of the endocrine pancreas in the SHR compared with the normotensive strain-matched control, the Wistar-Kyoto (WKY) rat. Conventional Wistar rats were used as controls for the SHR and WKY rats.

MATERIALS AND METHODS

Male SHR and WKY rats were purchased from Charles River Laboratories (Atsugi, Japan), and male Wistar rats were purchased from Shizuoka Laboratory Center (Hamamatsu, Japan). The animals were housed in air-conditioned quarters at 24°C under artificial light (lights on 8 AM to 8 PM). Tap water and chow pellets were provided ad libitum, and the animals were used for experiments starting at 10 weeks of age.

The nonfasted rats were anesthetized with pentobarbital (50 mg/kg body weight intraperitoneally). Isolation and perfusion of the pancreas was performed using the technique described by Goto et al.[9] Briefly, the pancreas plus duodenum block was placed in an incubator and perfused through the aorta via an inserted cannula. All perfusions were performed with Krebs-Ringer bicarbonate (KRB) buffer containing 0.25% bovine serum albumin and 4.6% dextran (molecular weight 70,000). The medium was gassed with 95% O_2/5% CO_2 and maintained at pH 7.4 and 37°C. The flow rate was kept constant at 2.2 mL/min. After an equilibration period of 20 minutes with KRB buffer containing 4.4 mmol/L glucose, the glucose concentration was increased to 16.7 mmol/L via a side pump without changing the flow rate. After the perfusion with 16.7 mmol/L glucose for 20 minutes, the glucose level was decreased to the initial concentration of 4.4 mmol/L and perfusion with 19 mmol/L arginine was performed for 20 minutes. Effluent from the portal vein was collected every minute in chilled tubes, immediately frozen, and stored at −70°C until assayed. Total insulin secretion during the stimulation period was calculated by multiplying the insulin concentration in the perfusate (nanograms per milliliter) by the flow rate (2.2 mL/min).

In the second series of experiments, 16.7 mmol/L glucose was perfused for 180 minutes after an equilibration period, and the perfusate was collected every minute for the first 10 minutes, then every 20 minutes for 170 minutes, and finally every 5 minutes for 10 minutes. Perfusion with 16.7 mmol/L glucose was followed by a final 5 minutes of perfusion with 4.4 mmol/L glucose. Using the method reported by Curry,[10] insulin biosynthesis was calculated as IB = IR + ICe − ICb, where IB is insulin biosynthesis during glucose stimulation and IR is the amount of insulin secreted by the pancreas throughout the entire stimulatory period. ICe and ICb are the insulin content of the pancreas at termination of the perfusion and the insulin content of pancreases that were not perfused, respectively.

Following the perfusion, the pancreas was immediately removed, minced, 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 1,800 × *g* for 20 minutes at 4°C, the supernatant was collected and the precipitate further extracted in the same manner. Then, both supernatants were combined and stored at −70°C until assayed.

Systolic blood pressure was measured by the tail-cuff method using an automatic blood pressure recorder (UR-1000; Ueda, Tokyo, Japan) after prewarming at 37°C for 10 minutes.[11] The average of 10 readings was used. Plasma glucose levels were measured by the glucose oxidase method using a glucose analyzer (Fuji Film, Tokyo, Japan). The immunoreactive insulin level was measured by specific radioimmunoassay[12] using rat insulin (Novo, Bagsvaerd, Denmark) as a standard. The assay sensitivity was 0.31 ng/mL and the interassay coefficient of variation was 10%.

The data are presented as the mean ± SE. Data were analyzed by one-way ANOVA followed by Fisher's multiple-comparison test. A P level less than .05 was considered significant.

RESULTS

Table 1 shows the characteristics of the experimental animals. Body weight was lower in SHR versus the other rats. Blood pressure was significantly higher in SHR versus WKY rats. Nonfasting plasma glucose was lower in SHR compared with WKY rats but higher than in Wistar rats, while plasma insulin levels were greater in SHR versus the other rats.

When the isolated pancreas was perfused with 16.7 mmol/L glucose, insulin secretion was markedly greater in SHR versus WKY (Fig 1). Statistical significance was obtained 4 minutes after glucose stimulation began (7.8 ± 0.8 *v* 2.3 ± 0.2 ng/mL, $P < .05$). Not only first-phase but also second-phase insulin

From the Department of Clinical Nutrition, Suzuka University of Medical Science, Mie; and Department of Surgery, Shimane Medical University, Izumo, Japan.
Submitted March 16, 1995; accepted July 21, 1998.
Address reprint requests to Keiichiro Tanigawa, MD, Department of Clinical Nutrition, Suzuka University of Medical Science, Kishioka-cho 1001-1, Suzuka, Mie 510-02, Japan.

0026-0495/99/4801-0002$03.00/0

Table 1. Body Weight, Systolic Blood Pressure, Fed Plasma Glucose, and Insulin in the Experimental Animals

Rat Group	No.	Body Weight (g)	Blood Pressure (mm Hg)	Plasma Glucose (mg/dL)	Plasma Insulin (ng/mL)
WKY	10	293 ± 3	163 ± 4	177 ± 6	1.7 ± 0.3
SHR	10	255 ± 3†	220 ± 7†	158 ± 3*	2.6 ± 0.3*
Wistar	8	312 ± 3	ND	138 ± 4	1.6 ± 0.3

NOTE. Values are the mean ± SE.
Abbreviation: ND, not determined.
*$P < .05$, †$P < .005$ *v* WKY and Wistar rats.

secretion was profoundly higher in SHR versus WKY rats. The total amount of insulin released during glucose stimulation was sixfold greater in SHR compared with WKY (262.8 ± 19.3 *v* 46.4 ± 3.8 ng/20 min, $P < .001$).

To determine whether islet function in the SHR is augmented or islet function in the WKY rat is impaired, the isolated pancreas of Wistar rats was also perfused. Glucose-induced insulin secretion was significantly ($P < .005$) greater in SHR versus Wistar rats during second-phase (10 to 22 minutes) insulin secretion (Fig 1). However, there was no significant difference in the total amount of insulin secreted between SHR and Wistar rats (208.7 ± 19.6 ng/20 min). Insulin secretion induced by glucose was therefore significantly ($P < .001$) greater in Wistar compared with WKY rats.

On the other hand, when 19 mmol/L arginine was perfused in the presence of 4.4 mmol/L glucose, the magnitude of insulin release was equivalent among each group.

Figure 2 shows the dynamics of insulin secretion induced by 16.7 mmol/L glucose for 180 minutes in SHR (n = 8), WKY (n = 5), and Wistar (n = 4) rats. The peak level of insulin secretion appeared at 130 minutes, followed by the third phase of declining insulin secretion[13] in the three groups. All phases of insulin secretion were greater in SHR versus WKY rats. Although insulin secretion was greater in SHR compared with Wistar rats, statistical significance ($P < .01$) was only obtained at 110 and 130 minutes during glucose stimulation.

The insulin content of the unperfused pancreas and at termination of the perfusion was not significantly different between each group (Table 2). Total insulin secretion for 180 minutes was eight and seven times greater in SHR and Wistar rats versus WKY rats, respectively. However, insulin biosynthesis was not significantly different between each group.

DISCUSSION

Previous studies demonstrated that the SHR exhibited exaggerated insulin secretion in response to an oral or intravenous glucose challenge.[4,5] A decreased rate of insulin removal in the SHR was also suggested by Mondon and Reaven.[4] The exaggerated insulin secretion may be due to a larger islet area[14] and higher insulin content in the SHR relative to the WKY rat (Table 2). In addition, hyperinsulinemia may reflect insulin resistance in the euglycemic SHR.

The data presented here provide the first in vitro comparison of the insulin response to glucose and arginine in SHR, WKY, and Wistar rats. From the results shown in Figs 1 and 2, it is

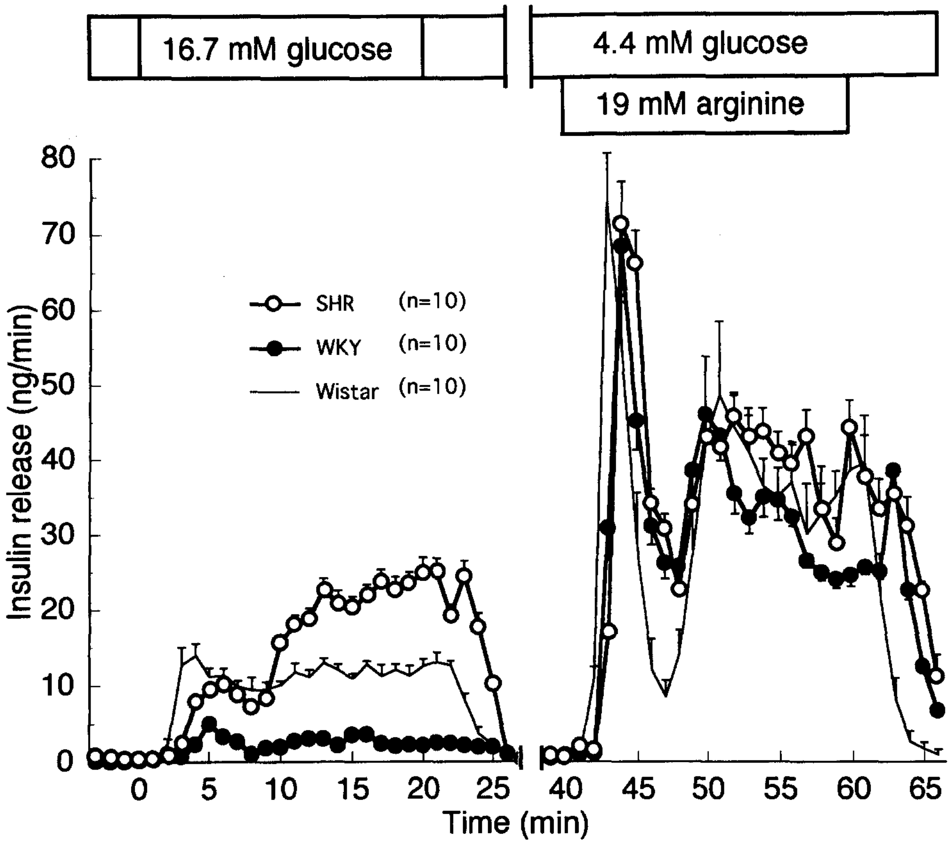

Fig 1. Effect of 16.7 mmol/L glucose and 19 mmol/L arginine on insulin release from the perfused pancreas of SHR, WKY, and Wistar rats. Experiments were performed in the presence of 4.4 mmol/L glucose in the prestimulatory and poststimulatory periods. Values are the mean ± SE of 10 experiments in each group.

clear that the SHR secretes much more insulin in response to glucose than the WKY rat. A previous study[15] revealed that the pancreatic islet had a lower set-point for glucose-induced insulin secretion in the SHR compared with the WKY rat, indicating higher β-cell sensitivity to glucose in SHR versus WKY rats. The investigators explained the difference by implicating the enhanced activity of glucokinase and greater expression of glucose transporter (GLUT 2) in pancreatic islets. However, it remains unclear whether these changes observed in the SHR correspond to a genetic predisposition or to an increase in the demand on the islet due to persistent insulin resistance.

Since there is approximately a 20% difference in body weight between the SHR and Wistar rat (Table 1), it is possible to speculate that the pancreata of these animals most likely differ in weight, too. Thus, the insulin content of the pancreas would be much greater in SHR versus Wistar rats, and this would favor a greater difference in insulin release between SHR and Wistar rats than the difference obtained in the present study.

It is of special interest that insulin secretion in response to arginine was not different for each group. These findings are similar to observations made in the diabetic pancreas, in which glucose-induced insulin secretion is impaired but arginine-induced insulin secretion is intact.[16,17] In addition, this study clearly showed that glucose-induced insulin release was unusually low in WKY compared with Wistar rats, although the mechanisms for the impaired insulin stimulus-secretion coupling in WKY rats remain unclear. One possible explanation is that the chronic hyperglycemia in WKY compared with SHR rats may influence, to some extent, glucose-induced insulin release, because chronic hyperglycemia desensitizes pancreatic β cells to glucose.[18-20] Despite showing decreased insulin secretion, WKY rats show a normal plasma glucose response to the oral or intravenous glucose tolerance test.[4,5] From the present study, it should be clarified as to whether the WKY rat is an appropriate control for the SHR in the investigation of hypertension.

The fact that de novo insulin synthesis and conversion of existing preproinsulin and proinsulin to insulin can be determined using long-term perfusion was first described by Curry.[10] Since preproinsulin mRNA is expressed within 2 hours of the start of perfusion with glucose and arginine,[21] it is possible that newly synthesized insulin can be measured following 3 hours of perfusion with glucose alone. We found a tendency for insulin biosynthesis to be greater in SHR versus WKY rats, but there was no statistical significance. However, persistent insulin resistance in the SHR may cause increasing secretory demand for insulin, resulting in greater insulin biosynthesis in SHR versus WKY rats. Direct measurement of the preproinsulin mRNA level in the perfused pancreas is necessary to test this hypothesis.

Table 2. Insulin Content, Release, and Biosynthesis in WKY, SHR, and Wistar Rats

Rat Group	ICb (μg)	ICe (μg)	IR (μg)	IB (μg)
WKY	115.0 ± 13.5 (8)	142.5 ± 7.0 (5)	0.8 ± 0.0 (5)	28.2 ± 15.2 (5)
SHR	130.2 ± 7.8 (10)	160.6 ± 8.2 (8)	6.4 ± 0.1 (8)*	37.3 ± 10.9 (8)
Wistar	120.0 ± 8.0 (6)	148.8 ± 8.0 (4)	5.6 ± 0.6 (4)*	34.7 ± 11.3 (4)

NOTE. Values are the mean ± SE, with the number of animals in parentheses. IB = ICe + IR − ICb.

Abbreviations: ICb, insulin content of pancreases that were not perfused; ICe, insulin content at termination of the perfusion; IR, amount of insulin released throughout the entire stimulatory period; IB, insulin biosynthesis.

$*P < .001$ v WKY.

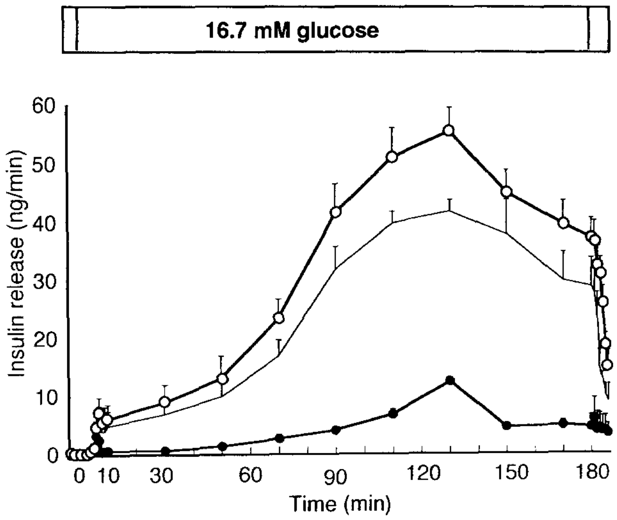

Fig 2. Dynamic insulin secretory response to 16.7 mmol/L glucose perfusion of the pancreas from SHR (○), WKY (●), and Wistar rats (—). Glucose concentration in the basal perfusate was 4.4 mmol/L.

REFERENCES

1. Modan M, Halkin H, Almog S, et al: Hyperinsulinemia: A link between hypertension, obesity and glucose intolerance. J Clin Invest 75:809-817, 1985

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

3. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

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

5. Gaboury CL, 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

6. Xu G, Tanigawa K, Nakamura S, et al: β-Cell function and replication in spontaneously hypertensive rats. Metabolism 44:1360-1364, 1995

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

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

9. Goto Y, Seino Y, Taminato T, et al: Modulation by alloxan of glucagon and insulin secretion in the isolated perfused rat pancreas. Endocrinology 102:1496-1500, 1978

10. Curry DL: Insulin content and insulinogenesis by the perfused

rat pancreas: Effects of long term glucose stimulation. Endocrinology 18:170-175, 1986

11. 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

12. 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

13. Grodsky GM: Perspectives in diabetes. A new phase of insulin secretion. How will it contribute to our understanding of β-cell function? Diabetes 38:673-678, 1989

14. 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

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

16. Weir GC, Leahy JC, Bonner-Weir S: Experimental reduction of B-cell mass: Implications for the pathogenesis of diabetes. Diabetes Metab Rev 2:125-161, 1986

17. Portha B, Blondel O, Serradas P, et al: The rat models of non–insulin dependent diabetes induced by neonatal streptozotocin. Diabetes Metab 15:61-75, 1989

18. Leahy J, Cooper H, Deal D, et al: Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion. A study in normal rats using chronic in vivo glucose infusions. J Clin Invest 77:908-915, 1986

19. Grill V, Westberg M, Ostenson C-G: B-cell insensitivity in a rat model of non–insulin-dependent diabetes: Evidence for a rapidly reversible effect of previous hyperglycemia. J Clin Invest 80:664-669, 1987

20. Rosetti L, Shulman GI, Zawalich W, et al: Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomized rats. J Clin Invest 80:1037-1044, 1987

21. Koh G, Seino Y, Takeda J, et al: Short term effects of glucose and arginine on the preproinsulin messenger ribonucleic acid level in the perfused rat pancreas: Comparison with insulin secretion. Endocrinology 124:707-711, 1989