Disappearance of Glibenclamide-Induced Hypoglycemia in Wistar-Kyoto Rats

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Received October 17, 2005; accepted December 2, 2005; published online December 6, 2005

The aim of the present study is to investigate difference in sensitivity to glibenclamide, a sulfonylurea oral antidiabetic agent, among Wistar rats, Spontaneously Hypertensive rats (SHR/Izm) and Wistar-Kyoto rats (WKY/Izm). We examined the effect of glibenclamide on blood levels of glucose and insulin in these rat strains. Under anesthesia with pentobarbital sodium (50 mg/kg, i.p.), blood samples were collected before and 5—120 min after administration of glibenclamide (10 mg/kg, i.p.). Blood levels of glucose and insulin in each sample were measured by glucose oxidase method and radioimmunoassay, respectively. In 8 week-old rats of all strains tested, blood levels of glucose were decreased by glibenclamide. In 12—20-week-old rats, although blood levels of glucose in Wistar and SHR/Izm were decreased after glibenclamide administration, those of WKY/Izm were not decreased. In rats of this age, time-course and extent of increases in blood insulin levels observed after administration of glibenclamide in WKY/Izm was almost the same as that of SHR/Izm, however, smaller than that of Wistar. Both insulin secretions induced *via* inactivation of ATP-sensitive K^+ channel and sensitivity of pancreatic β-cells to insulin seems to be decreased in WKY/Izm after 12 weeks of age. This phenomenon may explain the mechanism of glucose intolerance previously reported in WKY/Izm.

Key words glibenclamide; blood glucose; wistar-Kyoto rats (WKY)/Izm; spontaneously hypertensive rats (SHR)/Izm; insulin

Insulin resistance, defined as a reduced sensitivity to the effect of insulin action, is a major metabolic abnormality that causes hyperglycemia in patients with non-insulin-dependent diabetes mellitus (NIDDM). It has been reported that glucose intolerance, hyperinsulinemia, or both, are present in many patients with essential hypertension.[1—5] In spite of great scientific efforts, relationship between high blood pressure and these metabolic abnormalities is still unclear.

Spontaneously hypertensive rat (SHR) is thought to be the best animal model of human essential hypertension. In contrast to other rat hypertension models which are induced by surgical intervention,[6] hypertension in SHR is genetically determined, as it is in human. Many studies about relationship between hypertension and metabolic abnormality have been previously examined using SHR. However, they yielded contradictory results. Reduction of glucose tolerance and insulin action in SHR compared to its control strain Wistar-Kyoto rat (WKY) was demonstrated in some studies.[7—10] In contrast, increased insulin sensitivity[11,12] and no evidence of insulin resistance[12—14] was reported in SHR. In addition, glucose tolerance was reported to be found in not only SHR but also WKY.[15] These contradictory results suggest some heterogeneity with respect to glucose metabolism among SHRs and/or among WKYs originated from different vendors. There are little genetically difference between SHR/Izm and WKY/Izm, the original strains of SHR and WKY respectively. For example, WKY/Izm strain has the histocompatible antigen, k type, as well as SHR and SHRSP strains, but other WKY strains such as WKY/N and WKY/Crj obtained from the National Institute of Health, Maryland, U.S.A., and from commercial breeders have one type.[16] In addition, though Sa gene expression in the kidneys was obviously decreased in WKY/Crj strain compared with that of SHR/Crj and SHRSP/Crj strains as previously reported, the expression in WKY/Izm strain was identical or very similar to that of SHR/Izm and SHRSP/Izm strains.[17] Not only SHR/Izm but also WKY/Izm was reported to be glucose intolerant.[18] However, the mechanism that WKY/Izm also shows glucose intorerance has not been clarified yet.

Sulfonylurea oral antidiabetic agents are widely used for treatment of NIDDM. These drugs have been known to inhibit ATP-sensitive K^+ channels, increase insulin secretion from pancreatic β-cells, and decrease blood level of glucose. Although there are many reports about glucose tolerance in SHR and WKY, the differences in sensitivity to sulfonylurea agents among Wistar, SHR and WKY has not been clarified yet.

The aim of the present study is to investigate differences in sensitivity to glibenclamide, a sulfonylurea oral antidiabetic agent, among Wistar rats, SHR/Izm and WKY/Izm. We measure the changes of blood concentration of glucose and insulin induced by administration of glibenclamide in Wistar rats, SHR and WKY.

MATERIALS AND METHODS

In the present study, experimental procedures conformed to the Guiding Principles for the Care and Use of Laboratory Animals, approved by the Japanese Pharmacological Society. Male Wistar rats, SHR/Izm and WKY/Izm, which are 8—20 weeks old (Japan SLC, Hamamatsu, Japan), were anesthetized with pentobarbital sodium (50 mg/kg i.p.; Nembutal® injection, Abbott Laboratories, North Chicago, IL, U.S.A.), and the rectal temperature of animals was maintained at 37 °C during experiment using heating pad and heating lamp. The femoral veins were cannulated for administration of the drug. Blood samples were collected *via* tail vein before and 5—120 min after administration of glibenclamide (10 mg/kg, i.p.; Nacalai Tesque, Kyoto, Japan), and centrifuged to obtain plasma samples. Plasma levels of glu-

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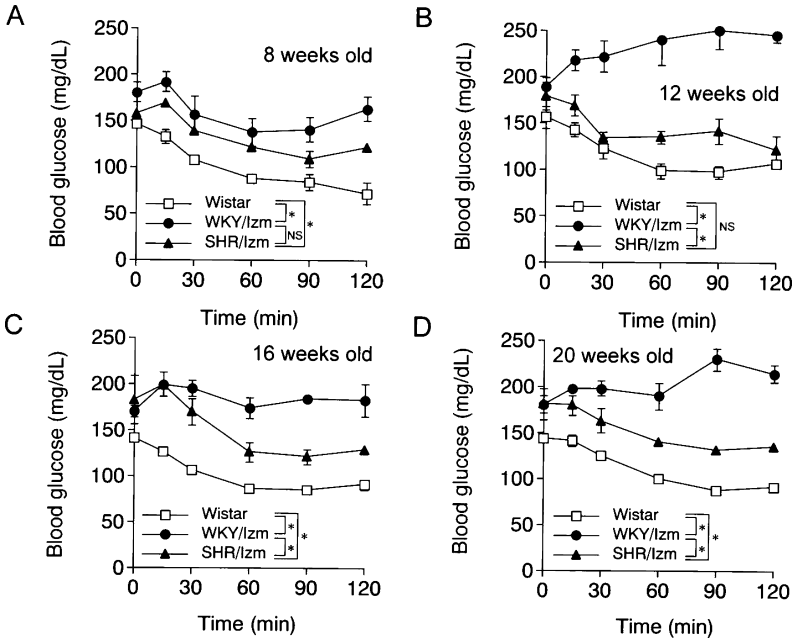

Fig. 1. Effect of Glibenclamide (10 mg/kg, i.p.) on the Level of Blood Glucose in 8- (A), 12- (B), 16- (C) and 20-Week (D) Old Wistar, WKY/Izm and SHR/Izm Rats

Each datum is expressed as a mean±S.E.M. of 3 independent experiments. * $p<0.05$, between the indicated pair. NS, non-significance.

cose and insulin in each sample were measured by glucose oxidase method (Glucose C-II Test Wako, Wako Pure Chemical, Osaka, Japan) and radioimmunoassay (Rat Insulin [^{125}I] RIA System, Amersham Biosciences, Piscataway, NJ, U.S.A.), respectively.

Each datum is expressed as a mean±S.E.M. The statistical differences among groups were analyzed by repeated measures two-way ANOVA followed by Fisher's least square difference test. Differences were considered significant when p values were less than 0.05.

RESULTS

Systolic blood pressure was higher in SHR/Izm aged 8, 12, 16 and 20 weeks than age-matched Wistar and WKY/Izm (data not shown). At all the ages that we tested, SHR/Izm and WKY/Izm tended to have higher levels of plasma glucose than Wistar before treatment with glibenclamide (at 0 min in Fig. 1). Levels of plasma glucose were decreased after treatment of glibenclamide in SHR/Izm and Wistar at all the ages that we tested and WKY/Izm aged 8 weeks (Fig. 1). At 8 weeks old, the extent of the decreases in WKY/Izm and SHR/Izm was smaller than that in Wistar. Surprisingly, level of plasma glucose was not decreased after treatment with glibenclamide in WKY/Izm aged 12, 16 and 20 weeks (Figs. 1B—D). At all the ages that we tested, levels of plasma insulin are almost the same among all of the strain (at 0 min in Fig. 2). Plasma insulin levels increased after treatment of glibenclamide. At 8 weeks old, the change in the plasma insulin levels induced by glibenclamide was almost the same among Wistar, SHR/Izm and WKY/Izm. At 12, 16 and 20 weeks old, the plasma insulin levels in SHR/Izm and WKY/Izm were almost the same after administration of glibenclamide, and lower than those in Wistar (Fig. 2).

DISCUSSION

The present study clearly demonstrated that blood levels of glucose in WKY/Izm were not decreased after glibenclamide administration in 12—20-week-old rats. Because plasma insulin levels after glibenclamide administration in WKY/Izm were almost the same as those in SHR/Izm, where hypoglycemia induced by glibenclamide was seen, decrease of sensitivity to insulin is thought to be involved in the mechanism of the age-dependent disappearance of glibenclamide-induced hypoglycemia in WKY/Izm. Glucose intolerance seen in WKY/Izm[18] may be caused by a low sensitivity to insulin. Since not only SHR/Izm but also WKY/Izm was reported to be glucose intolerant,[18] the mechanism of glucose intolerance seen in SHR/Izm may be different from that in WKY/Izm.

At all the ages that we tested, the plasma insulin levels in SHR/Izm and WKY/Izm were lower than those in Wistar. The reason for the reduction is currently unclear. It is possible that the sensitivity to sulfonylurea and/or the expression of ATP-sensitive K^+ channel in pancreatic β-cells is decreased. Further work is needed to elucidate the underlying mechanisms.

Although there is no significant difference in the insulin secretion caused by glibenclamide among all of the strains at 8 weeks old, the smaller decreases of blood glucose level were seen in WKY/Izm and SHR/Izm at the age. Therefore, the sensitivity to insulin in WKY/Izm and SHR/Izm before 8 weeks old may be lower than that in Wistar. Although the plasma insulin level after treatment with glibenclamide in SHR/Izm was lower than those in Wistar at 16 and 20 weeks old, the variation of blood glucose level induced by glibenclamide in SHR/Izm is almost the same as that in Wistar at the age. Therefore, the sensitivity to insulin in SHR/Izm after 16 weeks old may be higher than that in Wistar.

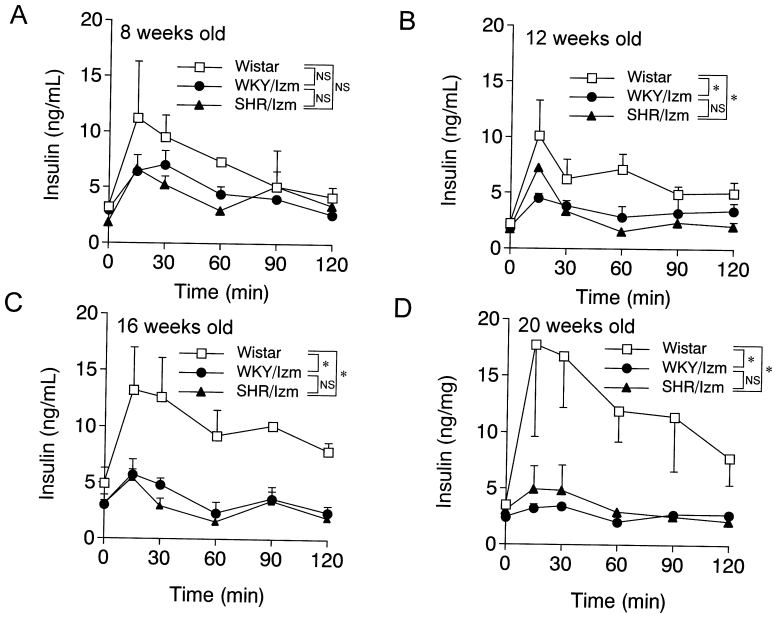

Fig. 2. Effect of Glibenclamide (10 mg/kg, i.p.) on the Level of Blood Insulin in 8- (A), 12- (B), 16- (C) and 20-Week (D) Old Wistar, WKY/Izm and SHR/Izm Rats

Each datum is expressed as a mean±S.E.M. of 3 independent experiments. * $p<0.05$, between the indicated pair. NS, non-significance.

Surprisingly, level of plasma glucose was significantly increased by glibenclamide in WKY/Izm aged 12 and 20 weeks. However, the mechanism of this phenomenon is still unclear. Further study is needed to clarify the underlying mechanisms.

In conclusion, we demonstrated for the first time that glibenclamide-induced hypoglycemia disappears in Wistar-Kyoto rats at 12—20 weeks old. Both insulin secretions induced *via* inactivation of ATP-sensitive K^+ channel and sensitivity of pancreatic β-cells to insulin seems to be decreased in WKY/Izm after 12 weeks of age. This phenomenon may explain the mechanism of glucose intolerance previously reported in WKY/Izm.

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