Attenuation of Hyperinsulinemia by NN414, a SUR1/Kir6.2 Selective K^+-Adenosine Triphosphate Channel Opener, Improves Glucose Tolerance and Lipid Profile in Obese Zucker Rats

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Chronic attenuation of hyperinsulinemia by diazoxide (DZ), a K-adenosine triphosphate (ATP) channel opener and an inhibitor of glucose-mediated insulin secretion, improved glucose tolerance and lipid profile and decreased the rate of weight gain in obese Zucker rats. To determine whether suppression of hyperinsulinemia alters daily food consumption, rate of weight gain, glucose tolerance, and lipid profile, we compared the effects of NN414, a potent and SUR1/Kir6.2 selective K_{ATP} channel opener, with DZ in obese and lean Zucker rats. DZ (150 mg/kg/d), low-dose (LDNN414: 10 mg/kg/d), high-dose (HDNN414: 30 mg/kg/d), and vehicle (C) were administered to 7-week-old obese and lean female Zucker rats for a period of 6 weeks. Each animal underwent an intraperitoneal glucose tolerance test (IPGTT) at the end of study period. While NN414 treatment did not affect food intake and rate of weight gain in any of the strains, DZ treatment reduced food intake ($P < .001$) and rate of weight gain ($P < .001$) in obese rats. The fasting plasma insulin levels and area under the curve (AUC) insulin response to IPGTT were significantly attenuated in LDNN414 ($P < .05$), HDNN414 ($P < .01$), and DZ ($P < .01$) obese and lean rats compared with their controls. This was accompanied by a significant reduction in AUC glucose only in LDNN414 ($P < .05$), HDNN414 ($P < .01$), and DZ ($P < .01$) obese rats compared with controls. While hemoglobin A_{1c} (HbA_{1c}) was not affected in LDNN414 obese rats, it was higher in HDNN414 obese animals ($P < .001$), LD-, HDNN414 ($P < .001$), and DZ ($P < .005$) lean rats compared with their respective controls. DZ obese rats showed lower HbA_{1c} levels than C obese rats ($P < .02$). The plasma free fatty acid (FFA) levels were only decreased in HDNN414 ($P < .05$) and DZ ($P < .002$) obese rats, whereas plasma triglyceride (TG) levels were decreased in LDNN414 ($P < .05$), HDNN414 ($P < .001$), and DZ ($P < .001$) obese rats compared with controls. Finally, plasma leptin level was only decreased in DZ obese rats compared with controls ($P < .001$). The new SUR1/Kir6.2 selective K_{ATP} channel opener, NN414, reduced hyperinsulinemia in a dose-dependent manner without a significant effect on food consumption and rate of weight gain. NN414-induced β-cell rest in obese rats was associated with a significant improvement in glucose responsiveness, suggesting an increase in insulin sensitivity after its withdrawal. There was an overall deterioration in glycemic control at the high dose as measured by HbA_{1c}. There was a dose-dependent improvement in lipid profiles of obese Zucker rats. These results suggest that pharmacologic attenuation of hyperinsulinemic state by low-dose NN414 may be therapeutically beneficial in insulin-resistant states without any deterioration in overall glycemic control.

OBESITY HAS BECOME the single most prevalent health problem in the United States and type 2 diabetes its most common complication.[1,2] Obese humans and experimental animals characteristically manifest hyperinsulinemia, insulin resistance, and hyperlipidemia, which predispose to glucose intolerance and diabetes.[1] Hyperinsulinemia and insulin resistance are believed to cause preferential shunting of substrates to adipose tissue and conversion of preadipocytes to adipocytes. This is associated with hypertrophy and hyperplasia of fat cells, inducing an unabated lipogenic state and obesity.[3] Further, it has been demonstrated that insulin plays a major role in modulation of key genes in lipid metabolism and triglyceride (TG) storage, including fatty acid synthase (FAS),[4] lipoprotein lipase (LPL),[5] and leptin.[6] FAS catalyzes the synthesis of long chain fatty acids, palmitate from acetyl CoA, and malonyl CoA in the presence of nicotinamide adenine dinucleotide phosphate (NADPH).[7] FAS concentrations in hepatic and adipose tissues are highly sensitive to nutritional, hormonal, and developmental states.[7,8]

We have previously demonstrated that attenuation of hyperinsulinemia in obese Zucker rats by diazoxide (DZ), a K-adenosine triphosphate (ATP) channel opener and an inhibitor of glucose-induced insulin secretion, resulted in decreased rate of weight gain, enhanced adipocyte insulin receptor binding, and improved glucose tolerance.[9,10] This was accompanied by decreased hepatic gluconeogenic activity and glycogen storage in obese Zucker rats.[11] Indeed, Carr et al[12] have shown that DZ treatment of obese Zucker rats results in significant reduction in hepatic glucose production. Recently, we have also demonstrated that DZ treatment of obese Zucker rats results in down-regulation of leptin and lipid metabolizing enzymes, FAS, and LPL in adipose tissue[13] and is accompanied by decreased plasma TG and FFA levels. These findings indicate that modification of disturbed insulin metabolism and insulin-sensitive pathways can be therapeutically beneficial in the management of hyperinsulinemic obesity and associated impaired glucose tolerance. While antiobesity effect of DZ is believed to be primarily due to its inhibition of hyperinsulinemia, it may, in part, be influenced by nonpancreatic actions or by its anorectic effect.

Recently, a new analogue of DZ, NN414, has been tested in experimental animals.[14,15] This compound acts as a potassium

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channel opener and demonstrates high selectivity for the SUR1/Kir6.2 subtype. It has been observed that NN414 inhibits insulin secretion, after oral administration, with a potency approximately 20 times higher than that seen with DZ treatment.[15] This compound has been given in high doses to rats with no effects on blood pressure and heart rate.[15]

To determine whether suppression of hyperinsulinemia alters daily food consumption, rate of weight gain, glucose tolerance, and lipid profile, we compared the effects of NN414, which selectively activates SUR1/Kir6.2 K_{ATP} channels present in the pancreatic β cells, with DZ in obese and lean Zucker rats.

MATERIALS AND METHODS

Experimental Animals, Dietary, and NN414 and DZ Treatment

Seven-week-old female Zucker obese (*fa/fa*) and lean (*Fa/?*) rats were obtained at 6 weeks of age from Charles River Laboratory (Wilmington, MA). The animals were housed in pairs in standard animal cages and were provided rat chow, PMI LabDiet 5001(Purina Mills, St Louis, MO), and water ad libitum. Seven-week-old obese and lean rats were divided into 4 subgroups (7 to 9 animals per subgroup): DZ (Proglycem suspension 50 mg/mL, Baker-Norton Pharmaceuticals, Miami, FL), low-dose (LD) and, high-dose (HD) NN414 (Novo Nordisk A/S, Bagsvaerd, Denmark) and control (C). DZ (150 mg/kg/d), LDNN414 (10 mg/kg/d), HDNN414 (30 mg/kg/d), and vehicle (C) were administered twice daily by gavage needles for 6 weeks. The control group was treated with an equivalent volume of vehicle suspension twice daily. Studies lasted for a period of 6 weeks.

Rats were weighed twice weekly to determine weight gain. Food consumption was measured while animals were in separate metabolic cages during the second and fifth weeks of treatment. Blood samples from tail vein were obtained for analysis of glucose after an 18-hour fast once weekly. When animals were fasted, doses of drugs (DZ and NN414) or vehicles were withheld for at least 18 hours. At the end of the 6-week period, intraperitoneal glucose tolerance tests (IPGTT; 1.0 g glucose/kg body weight [BW]) were performed after an overnight fast (18 hours). Blood for glucose and insulin was drawn into heparinized tubes from the supraorbital sinus 0, 15, 30, and 60 minutes after glucose administration under anesthesia by intramuscular ketamine (65 to 100 mg/kg BW). On a separate day, fasting blood samples were obtained for glucose, insulin, leptin, FFA, TG, and hemoglobinA_{1c} (HbA_{1c}). The animals were then euthanized by a terminal cardiac puncture and exsanguinations. The animal procedures were approved by the Medical College of Wisconsin Animal Resource Center.

Assays

Plasma glucose, insulin, and leptin assays. Glucose level was measured by the glucose oxidase method (Sigma Chemical, St Louis, MO). Insulin and leptin concentrations were determined by radioimmunoassay kits using a double-antibody method (LINCO Research, St Louis, MO).

Plasma TG and FFA. Cholesterol and TG levels were measured by an enzymatic method (Sigma Diagnostics, St Louis, MO). Plasma FFA was determined by an enzymatic colorimetric method (Wako Chemicals, Richmond, VA).

HbA_{1c}. Analysis of HbA_{1c} was performed using a Roche Cobas spectrophotometer (Roche Diagnostic Systems, Branchburg, NJ).

Statistical Analysis

The reported values represent the mean ± SEM. Statistical comparisons between subgroups were assessed by 1-way analysis of variance (ANOVA) and Dunnett's and Tukey's tests. *P* less than .05 was considered statistically significant.

RESULTS

Effect of NN414 and DZ on Food Intake and BW

Table 1 shows weight and food intake in obese and lean Zucker rats. Figure 1 illustrates the progression of BW among different all subgroups of rats over the 6-week period. Control obese rats exhibited higher initial weight and greater weight gain over the 6-week observation period than lean animals ($P < .001$). The final BW and average weight gain among the DZ obese animals were reduced compared with control obese rats ($P < .001$), whereas NN414 treatment had no effect on the

Table 1. Weight and Food Intake in Obese and Lean Zucker Rats

Subgroup	No.	Initial Weight (g)	Final Weight (g)	Weight Gain (per 100 g BW)	Calories/d	
					2nd Week	5th Week
DZ-Ob	8	216 ± 8	327 ± 9*	34 ± 2*	80 ± 4	73 ± 7
LDNN414-Ob	8	220 ± 12	385 ± 11	44 ± 2	121 ± 4	106 ± 2
HDNN414-Ob	9	218 ± 11	391 ± 13	44 ± 1	114 ± 4	113 ± 4
C-Ob	9	212 ± 5	413 ± 13	48 ± 1	115 ± 6	112 ± 5
DZ-Ln	7	137 ± 7	212 ± 6	35 ± 3	53 ± 7	58 ± 5
LDNN414-Ln	8	131 ± 4	200 ± 5	34 ± 1	65 ± 2	60 ± 2
HDNN414-Ln	8	128 ± 2	212 ± 6	40 ± 1	61 ± 2	59 ± 4
C-Ln	7	132 ± 6	224 ± 6	41 ± 1	71 ± 2	66 ± 1
P						
DZ *v* C		NS	<.001*	<.001*	<.001*	<.001*
DZ *v* LDNN414		NS	<.005*	<.005*	<.001*	<.001*
DZ *v* HDNN414		NS	<.005*	<.005*	<.001*	<.001*
LDNN414 *v* C		NS	NS	NS	NS	NS
HDNN414 *v* C		NS	NS	NS	NS	NS
C-Ob *v* C-Ln		<.001	<.001	<.005	<.001	<.001

NOTE. Data are the mean ± SEM and were analyzed by 1-way analysis of variance.

Abbreviation: NS, not significant.

*Only DZ-Ob *v* C, LDNNC-Ob and HDNNC-Ob.

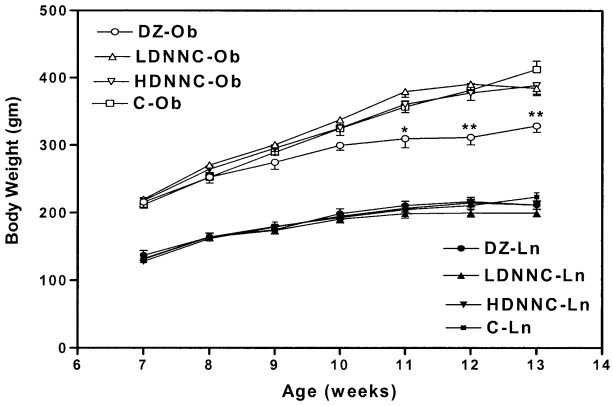

Fig 1. BW progression (mean ± SEM) in NN414, DZ, and C obese and lean rats. *$P < .01$; **$P < .001$.

rate of weight gain in obese animals. Further, DZ and NN414 had no effect on the rate of weight gain in lean animals.

When food intake was measured during the treatment period (second and fifth weeks), control obese rats consumed larger amounts of food than control lean animals ($P < .001$). While DZ obese rats demonstrated a significant reduction in food intake compared with control obese ($P < .001$), the LD- and HDNN414 subgroups of lean and obese animals did not shown any significant change in daily food consumption compared with their respective controls.

Effect of NN414 and DZ on Plasma Glucose, Lipids, Insulin, and Leptin Concentrations and HbA_{1c} Levels

Figure 2 illustrates weekly fasting blood glucose levels in obese and lean Zucker rats. Both DZ and HDNN414 obese rats demonstrated a significant decrease in fasting blood glucose levels between 9 and 13 weeks of age compared with obese controls ($P < .001$). Also, DZ and HDNN414 lean rats showed a significant reduction in fasting blood glucose at 11 weeks of age compared with lean controls ($P < .001$). Table 2 shows fasting plasma levels of glucose, insulin, leptin, FFA, and TG following 6 weeks of DZ or vehicle treatment. Fasting plasma glucose and insulin concentrations were significantly higher among control obese animals compared with lean rats ($P < .001$). Fasting plasma insulin levels were significantly decreased in LDNN414 ($P < .05$), HDNN414 ($P < .001$), and DZ ($P < .001$) obese and lean rats compared with their respective control. This was associated with a significant reduction in fasting plasma glucose concentrations in HDNN414 ($P < .02$) and DZ ($P < .005$) obese animals compared with controls. DZ and LDNN414 did not significantly affect fasting plasma glucose levels in lean animals.

Fasting plasma leptin levels were significantly higher in obese than lean animals ($P < .001$). While DZ treatment resulted in significant reduction of plasma leptin in obese animals, LD- and HDNN414 did not alter plasma leptin concentrations in obese and lean rats.

Fasting plasma levels of FFA and TG were significantly higher in obese than lean animals ($P < .001$). The plasma FFA levels were only decreased in HDNN414 ($P < .05$) and DZ ($P < .002$) obese rats, whereas plasma TG levels were decreased in LDNN414 ($P < .05$), HDNN414 ($P < .001$), and DZ ($P < .001$) obese rats compared with controls.

Control obese animals had higher HbA_{1c} levels compared with lean controls ($P < .001$). While the HbA_{1c} level was not significantly affected in LDNN414 obese rats compared with control obese, it was significantly higher in HDNN414 animals ($P < .001$). DZ obese demonstrated a significantly lower HbA_{1c} level compared with C obese ($P < .02$). Compared with control lean rats, LDNN414 ($P < .005$), HDNN414 ($P < .001$), and DZ ($P < .01$) had significantly higher HbA_{1c} levels.

Effect of NN414 and DZ on Glucose Tolerance

Figure 3 illustrates the glucose and insulin responses to IPGTT. Figure 4 depicts the bar graphs representing areas under the curve (AUC) for glucose (Fig 4A and C) and insulin (Fig 4B and D) in obese and lean animals. The AUC glucose levels (979 ± 50 *v* 695 ± 48 mmol/L · min, $P < .001$) and AUC insulin concentrations (166 ± 8 *v* 60 ± 7 pmol · min, $P < .0001$) in obese animals were significantly higher than lean animals. Compared with the obese controls (166 ± 8 pmol · min), the AUC insulin was significantly attenuated in LDNN414 (141 ± 15 pmol · min, $P < .05$), HDNN414 (91 ± 6 pmol · min, $P < .001$) and DZ (70 ± 3, $P < .001$) obese animals. Similarly, the AUC insulin was significantly decreased in LDNN414 (41 ± 5 pmol · min, $P < .05$), HDNN414 (16 ± 2 pmol · min, $P < .001$) and DZ (18 ± 3 pmol · min, $P < .001$) lean compared with control lean animals (60 ± 7 pmol · min). This was accompanied by a significant reduction in AUC

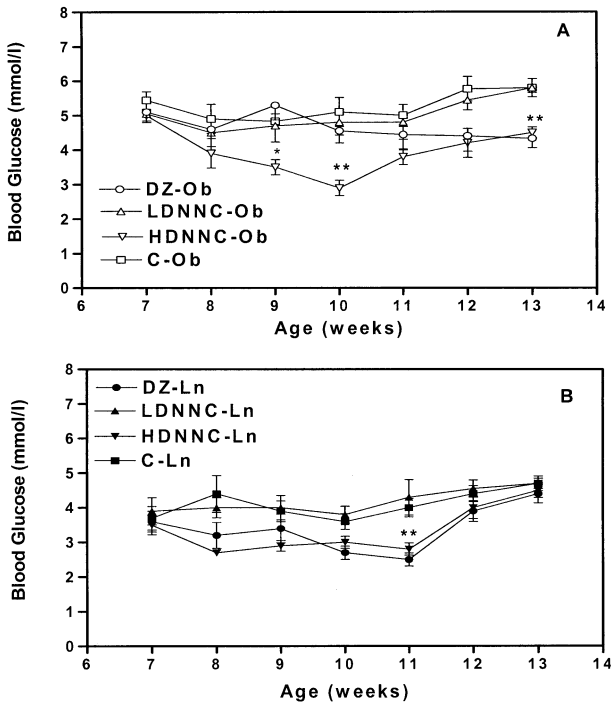

Fig 2. Weekly fasting blood glucose levels (mean ± SEM) in (A) obese and (B) lean Zucker rats. *$P < .01$; **$P < .001$.

Table 2. Biochemical Data in Obese and Lean Zucker Rats

Subgroup	No.	Plasma FFA (mmol/L)	Plasma Triglycerides (mmol/L)	Plasma Glucose (mmol/L)	Plasma Insulin (pmol/mL)	Plasma Leptin (ng/mL)	HbA_{1c} (%)
DZ-Ob	7	0.51 ± 0.11	1.6 ± 0.30	4.3 ± 0.3	0.82 ± 0.08	11.1 ± 2.1	2.8 ± 0.1
LDNN414-Ob	8	0.99 ± 0.08	3.4 ± 0.6	5.8 ± 0.1	1.32 ± 0.10	23.0 ± 2.9	3.5 ± 0.1
HDN414-Ob	8	0.61 ± 0.08	3.2 ± 0.3	4.5 ± 0.2	0.92 ± 0.11	21.1 ± 3.2	5.2 ± 0.3
C-Ob	7	0.95 ± 0.11	5.0 ± 0.4	5.8 ± 0.30	1.95 ± 0.10	25.0 ± 3.5	3.2 ± 0.1
DZ-Ln	7	0.43 ± 0.03	0.51 ± 0.06	4.6 ± 0.23	0.15 ± 0.02	1.30 ± 0.20	3.1 ± 0.2
LDNN414-Ln	8	0.56 ± 0.05	0.34 ± 0.03	4.7 ± 0.21	0.25 ± 0.01	1.21 ± 0.23	2.9 ± 0.1
HDNN414-Ln	8	0.39 ± 0.03	0.63 ± 0.09	3.6 ± 0.35	0.12 ± 0.01	1.10 ± 0.27	5.0 ± 0.2
C-Ln	7	0.46 ± 0.05	0.51 ± 0.02	4.9 ± 0.27	0.40 ± 0.06	2.00 ± 0.35	2.4 ± 0.1
P							
DZ *v* C		<.02*	<.001	<.005*	<.001	<.01*	<.02
DZ *v* LDNN414		<.005*	<.02*	<.001*	<.05	<.02*	<.001*
DZ *v* HDNN414		NS	<.05*	NS	NS	<.05*	<.001
LDNN414 *v* C		NS	<.05†	NS	<.05	NS	<.005§
HDNN414 *v* C		<.05‡	<.002‡	<.02	<.001	NS	<.001
C-Ob *v* C-Ln		<.002	<.001	<.01	<.001	<.001	<.001

NOTE. Data are the mean ± SEM and were analyzed by 1-way analysis of variance.
*Only DZ-Ob *v* C-Ob, LDNN414-Ob and HDNN414-Ob.
†Only LDNN414-Ob *v* C-Ob.
‡Only HDNN414-Ob *v* C-Ob.
§Only LDNN414-Ln *v* C-Ln.

glucose in LDNN414 (833 ± 43 mmol/L · min, $P < .05$), HDNN414 (731 ± 14 mmol/L · min, $P < .01$) and DZ (606 ± 52, $P < .001$) only in obese rats compared with controls (979 ± 50 mmol/L · min) without a significant change in AUC glucose among LDNN414 (649 ± 34 mmol/L · min), HDNN414 (790 ± 52 mmol/L · min) and DZ (631 ± 68 mmol/L · min) lean rats compared with control lean (695 ± 48 mmol/L · min) animals.

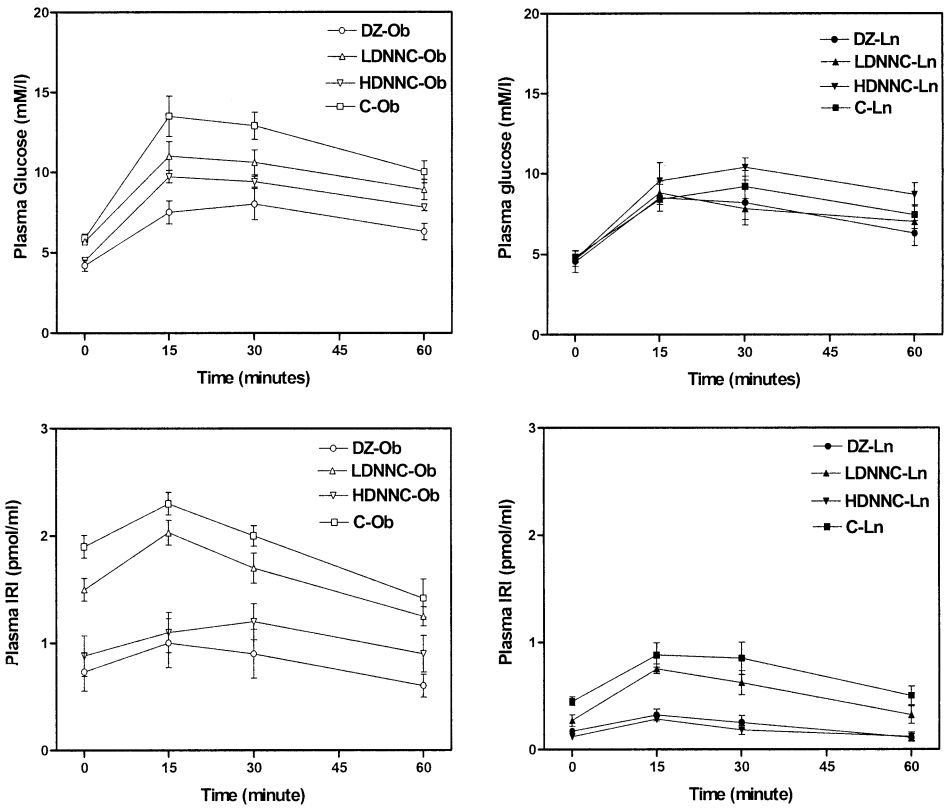

Fig 3. Plasma glucose and immunoreactive insulin (IRI) response (mean ± SEM) to IPGTT (1 g/kg) in NN414, DZ, and C obese and lean rats after an overnight fast.

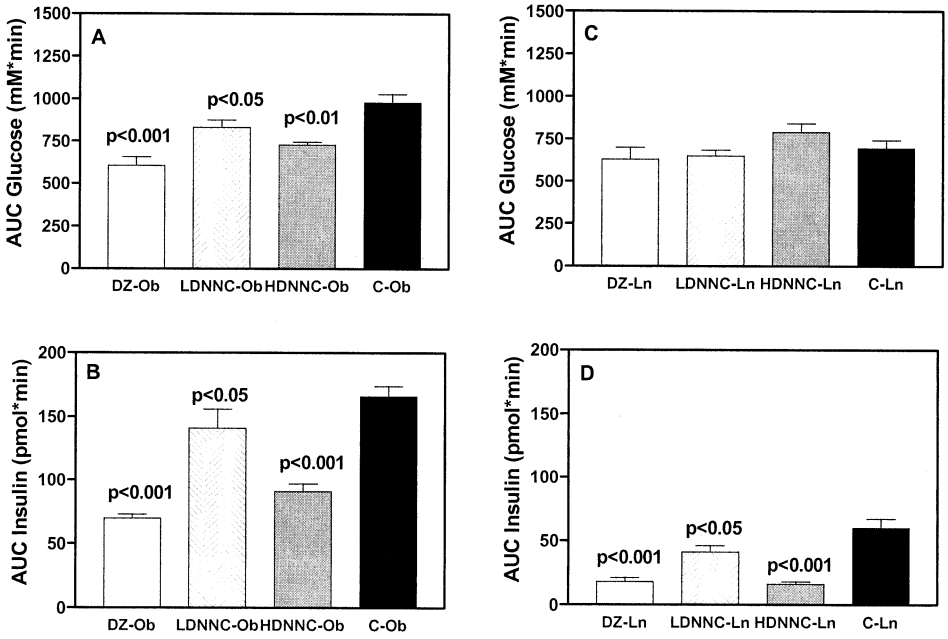

Fig 4. AUC glucose and AUC insulin (mean ± SEM) in response to IPGTT (1 g/kg) in (A and B) NN414, DZ, and C obese and (C and D) lean rats.

DISCUSSION

The present study demonstrated that the new DZ analogue, NN414, reduced hyperinsulinemia in a dose-dependent manner without a significant effect on food consumption. NN414 treatment caused significant improvement in glucose tolerance in obese rats, suggesting an increase in insulin sensitivity. This was associated with a dose-dependent improvement in lipid profiles of obese Zucker rats. Consistent with our previous studies, DZ treatment of obese Zucker rats decreased insulin secretion, food intake, and rate of weight gain, and this was accompanied by improved glucose tolerance and decreased plasma concentrations of FFA and TG.

Because insulin acts centrally to reduce BW,[16] a plausible hypothesis is that the obesity state and hyperphagia observed in Zucker rats are, in part, due to insulin resistance in the brain, as manifested by reduced capillary insulin binding, which is thought to mediate the transport of insulin into the brain. In our previous studies, we demonstrated that DZ treatment had significant anorectic and antiobesity effects in obese hyperphagic Zucker rats.[9,10,17] These effects were, in part, attributed to the attenuation of hyperinsulinemia and consequent enhancement of central nervous system (CNS) insulin sensitivity and uptake, as well as reduction of lipogenesis, leading to a decrease in food intake and rate of weight gain. Attenuation of hyperinsulinemia by the SUR1/Kir6.2 selective K^+_{ATP} channel opener, NN414, however, did not decrease food consumption and rate of weight gain in obese or lean rats, suggesting that antiobesity effects of DZ may, in part, be due to its direct extrapancreatic effects in CNS or some other unspecific effect on food intake. Because there were no measurements of CNS insulin sensitivity in this study, we cannot rule out a similar CNS insulin-sensitizing effect in NN414-treated animals.

It has been shown that the liver of obese Zucker rats exhibits an abnormal regulation of glucose production, together with a glycolysis that is continuously overstimulated by hyperinsulinemia and never becomes insulin resistant.[18] This results in a significant increase in hepatic lipid synthesis and an increase in very–low-density lipoprotein secretion in obese animals.[19] NN414 attenuation of hyperinsulinemia resulted in a dose-dependent reduction of plasma lipids similar to DZ's effects on lipid profile. However, the lipid-lowering effect of DZ has been attributed to its effect on the rate of weight gain and on the activities of FAS and LPL, key enzymes regulating lipogenesis in adipose tissue.[20] The DZ effect on FAS activity is believed to be, in part, due to its direct modulation of intracellular Ca^{2+} through K_{ATP} channels in adipocytes.[21] On the other hand, lipid-lowering effect of NN414, a SUR1/Kir6.2 selective β-cell K_{ATP} channel opener, appears to be primarily due its attenuation of hyperinsulinemic state and consequent reduction of hepatic lipid synthesis.[11] Although we did not determine the effect of NN414 on adipose tissue FAS activity, we speculate that it can modulate intracellular Ca^{2+} through its effect on K_{ATP} channel. Therefore, the lipid-lowering effects of DZ and NN414 may be through similar molecular mechanisms.

The dose-dependent decrease in plasma insulin concentrations in NN414-treated obese rats was accompanied by improved glucose tolerance, consistent with enhanced insulin sensitivity and responsiveness. Similar to our previous observations,[9,10] DZ-treated obese rats showed improvement in glucose tolerance and insulin sensitivity, but these effects were markedly greater than those observed in NN414-treated obese rats compared with controls. These treatment effect differences are believed to be primarily due to the lower BW observed in DZ rats compared with NN414 obese animals. In contrast, NN414 and DZ-treated lean animals did not display any significant change in their response to glucose load compared with control lean animals despite significant attenuation of plasma insulin concentrations. The difference between NN414 and DZ

obese and NN414 and DZ lean rats is believed to be due to the difference in their initial and subsequent insulin concentrations. In the obese rats, basal hyperinsulinemia was lowered by NN414 and DZ, but hyperinsulinemia persisted (compared with lean animals), while in the lean animals, basal normoinsulinemia was reduced to low normoinsulinemic to hypoinsulinemic range. Consequently, in the NN414 and DZ obese rats, the continuing hyperinsulinemia could be expected to use the enhanced peripheral insulin sensitivity to produce a more normoglycemic response, whereas in NN414 and DZ lean animals, the hypoinsulinemic state was accompanied by enhanced insulin sensitivity, with no significant change in glucose response.

On the other hand, it is interesting to note that the glycemic control as measured by HbA_{1c} in NN414- and DZ-treated obese and lean animals was markedly affected by the degree of insulin suppression. While LDNN414 obese rats did not show a significant change in HbA_{1c} levels, HDNN414 obese showed a marked increase in HbA_{1c} compared with obese controls, most probably due to increased ambient glucose levels in the free-fed state. It is likely that this increase in ambient glucose levels may be in conjunction with NN414 dosing, especially because HDNN414 obese rats displayed lower fasting blood glucose levels than obese controls. However, HbA_{1c} levels were significantly reduced in DZ obese rats primarily due to lower BW compared with NN414 and control obese rats. HbA_{1c} levels were significantly increased in NN414 and DZ lean rats compared with control lean animals, most likely due to higher plasma glucose concentrations in the free-fed state. The deterioration in HbA_{1c} is not surprising considering the fact that NN414 acutely and very potently reduces insulin release.[14,15]

The discrepancy between plasma glucose levels during IPGTT in HDNN414 obese and NN414 and DZ lean rats and their HbA_{1c} levels may be due to augmented β-cell insulin content and glucose responsiveness after the direct insulin suppressive effect of the compound has disappeared, especially 18 hours after the last dose of NN414 administration. It is well recognized that the plasma glucose concentration controls insulin secretion through its actions to directly stimulate insulin release and also to modulate insulin output from other secretagogues.[22] Chronic hyperglycemia disrupts this relationship by impairing β-cell function and survival.[23] The mechanisms for these negative effects are multifactorial. However, it seems that overstimulation is an important factor.[24] In a previous study in which nondiabetic rats were made hyperglycemic for 48 hours by glucose infusion, Sako and Grill[25] demonstrated that hyperglycemia-induced attenuation of insulin secretion could be avoided by coinfusion of DZ. Also, Leahy et al[26] studied the effects of removal of excess glucose stimulation by DZ on β-cell function in 90% pancreatectomized rats. In these experiments, rats were treated with DZ for 5 days, and pancreas perfusion experiments were performed for 2 days after the last administration of DZ. DZ-treated pancreatectomized rats demonstrated increased islet insulin content and recovery of β-cell glucose responsiveness. In addition, Aizawa et al[27] demonstrated that chronic suppression of hyperinsulinemia by DZ in an animal model of type 2 DM, Otsuka Long-Evans Tokushima Fatty (OLETF) rats, resulted in marked improvement of glucose tolerance and disappearance of exaggerated β-cell insulin response to glucose in vitro. They suggested that the use of DZ is beneficial in pharmacologic prevention of obesity-related diabetes. Furthermore, Hiramatsu et al[28] showed that chronic DZ treatment caused prolonged improvement of β-cell function in rat islets transplanted to the diabetic environment, and that the β-cell function in transplanted islets was improved by DZ long after the end of treatment, presumably due to the removal of hyperglycemia-induced overstimulation. Induction of β-cell rest by short-term DZ dosing in insulin-treated patients with type 2 DM improved β-cell function without a significant change in mean plasma glucose levels and BW.[29] Song et al[30] demonstrated that in vitro DZ treatment of human islets resulted in attenuation of glucose-induced defects in first-phase insulin release and pulsatile insulin secretion. In our study, LDNN414 treatment did not cause any change in overall glycemia and improved glucose responsiveness and insulin sensitivity after drug withdrawal. On the other hand, HDNN414 (at 3.3 times the LDNN414 dose) caused a significant deterioration of glycemic control despite improvement in glucose responsiveness and insulin sensitivity after its discontinuation. Our data suggests that chronic suppression of hyperinsulinemia by NN414 in obese Zucker rats appears to have beneficial effect on glucose-induced insulin response, insulin sensitivity, and overall glucose tolerance only at a low-dosage level.

Insulin stimulates the synthesis of leptin, an *Ob* gene product, in adipose tissue,[31,32] while leptin inhibits the production of insulin in β cells by modulation of K_{ATP} channel[2] and activation of cyclic nucleotide phophodiestrase 3B and subsequent suppression of cyclic adenosine monophosphate (cAMP) levels.[33] Leptin receptor mutation in obese Zucker and Zucker diabetic fatty rats has been proposed to impair suppression of insulin secretion by leptin and play a major role in the development of adipogenic diabetes.[34-36] In our study, the plasma leptin levels were significantly higher than lean rats as previously shown.[31] While DZ treatment resulted in significant suppression of leptin levels only in obese rats, NN414-treated rats did not display any reduction in plasma leptin concentrations. The observed plasma leptin reduction in DZ obese rats appeared to parallel reduction in BW in these animals, because insulin suppression by NN414 without a change in the rate of weight gain was not associated with reduction in plasma leptin concentrations in either strain, suggesting a significant role of body fat and adipose tissue leptin production on plasma leptin levels.[37]

In conclusion, the new SUR1/Kir6.2 selective K_{ATP} channel opener, NN414, reduced hyperinsulinemia in a dose-dependent manner without a significant effect on food consumption and rate of weight gain. NN414-induced β-cell rest in obese rats was associated with a significant improvement in glucose responsiveness, suggesting an increase in insulin sensitivity after its withdrawal. There was an overall deterioration in glycemic control at the high dose as measured by HbA_{1c}. There was a dose-dependent improvement in lipid profiles of obese Zucker rats. These results suggest that pharmacologic attenuation of hyperinsulinemic state by low-dose NN414 may be therapeutically beneficial in insulin-resistant states without any deterioration in overall glycemic control.

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