Reduction of Insulin Resistance in Obese and/or Diabetic Animals by 5-[4-(1-Methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, Ciglitazone), a New Antidiabetic Agent

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SUMMARY

Effects of 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, Ciglitazone) on glucose and lipid metabolism were examined in various animal models. ADD-3878, administered as a dietary admixture (30–186 mg/kg/day) to obese-diabetic yellow KK (KK-A^y) mice, markedly suppressed the diabetic syndromes (hyperglycemia, hypertriglyceridemia, and hyperinsulinemia), accompanied by the reduction of insulin resistance as manifested by improvement of overall insulin sensitivity in either the insulin tolerance test or the steady-state blood glucose test. Chronic administration of ADD-3878 for as long as 12 wk to young yellow KK mice, which were in the early stage of diabetes and obesity, depressed age-dependent rises in blood glucose, plasma triglyceride, and insulin without exerting any effect on obesity.

When orally administered to obese Zucker-fatty rats, ADD-3878 decreased plasma insulin and triglyceride in a dose-dependent manner (5–100 mg/kg/day). The treated rats showed increased tolerance and decreased insulin secretion in response to oral glucose. The glycemic response to insulin and the steady-state plasma glucose were also normalized in the treated rats. Chronic administration of ADD-3878 to young fatty rats for as long as 12 wk decreased the dose-dependent rises in blood glucose, plasma triglyceride, and insulin without exerting any effect on body weight.

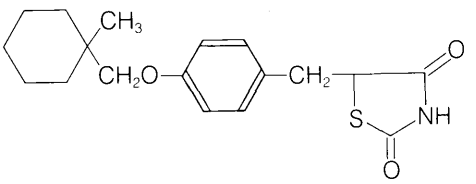
ADD-3878 had no effect on glucose and lipid metabolism of young Sprague-Dawley rats and mild streptozotocin-diabetic rats. However, in old Sprague-Dawley rats that were moderately insulin resistant and hyperlipidemic compared with young ones, ADD-3878 decreased plasma triglyceride and insulin and improved insulin sensitivity.

Five-day administration of ADD-3878 to beagle dogs with slightly impaired glucose tolerance increased glucose tolerance and suppressed postprandial rises in plasma glucose, insulin, and triglyceride.

Based on these results, ADD-3878 is effective on abnormal glucose and lipid metabolism associated with insulin resistance or obesity through reduction of peripheral insulin resistance. Therefore, ADD-3878 is expected to be useful in the treatment of hyperglycemia, hyperinsulinemia, and hyperlipemia in obese type II diabetes and obesity. DIABETES 32:804–810, September 1983.

Insulin resistance is a characteristic feature of both type II diabetes mellitus and obesity and is frequently associated with hyperinsulinemia and hyperlipidemia.[1–4] Although the mechanism of insulin resistance is still obscure, there is evidence that insulin resistance or decreased efficiency of insulin action is involved in the pathogenesis of type II diabetes and obesity in human subjects[5–8] and animal models.[9–11] However, few drugs are developed on the basis of this concept, although some evidence suggests that some sulfonylureas potentiate insulin action in peripheral tissue by increasing the number of insulin receptors.[12–14]

We have been looking for new compounds that decrease insulin resistance in diabetic and/or obese animals. On the basis of pharmacologic findings, ADD-3878 {5-[4-(1-methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione} has been selected as one of the promising candidates. The present article describes unique pharmacologic actions of this compound on insulin resistance and carbohydrate and lipid metabolism in various insulin-resistant animal models. (The chemical structure of ADD-3878 is given below.)



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MATERIALS AND METHODS

Animals. Yellow KK (KK-A^y/+) mice were bred by mating female KK mice with male yellow KK mice as reported previously.[10] Zucker-fatty (*fa/fa*) rats were bred by matings between heterozygous lean rats (*fa*/+). They were produced in our Drug Safety Evaluation Laboratories of this division. Sprague-Dawley rats were purchased from CLEA Japan Inc. These mice and rats were maintained on a laboratory chow, CE-2 (CLEA, Japan), consisting of 52.7% carbohydrate, 23.6% protein, 4.4% fat, 4.9% fiber, 6.6% minerals and vitamins, and water ad libitum. They were housed in individual metal cages in a room controlled for temperature (23 ± 1°C), humidity (55 ± 5%), and light (0800–2000 h).

Male Sprague-Dawley rats (6 wk old) were rendered diabetic by intravenous injection of streptozotocin (40 mg/kg × 2 with a 6-day interval) in citrated normal saline prepared immediately before injection. The diabetic rats, which exhibited hyperglycemia (>350 mg/dl) in the fed state, but normoglycemia in a 20-h fast, were selected for use in the experiment 20 days after the injection of streptozotocin.

Five male beagle dogs (13–14 kg, 1–3 yr old), whose oral glucose tolerance was slightly lower than the normal range, were selected from 24 dogs supplied by CLEA Japan Inc. They were fed a CD-5 diet (CLEA, Japan), consisting of 48.6% carbohydrate, 25.2% protein, 6.0% fat, 5.5% fiber, and 8.7% minerals and vitamins, at 0830–0930 h.

Drug administration. ADD-3878 was synthesized as reported previously.[15] To adult rats and dogs, the compound suspended in 5% gum arabic solution was orally administered by stomach tube. To mice and young fatty rats, the compound was given as a dietary admixture in CE-2 powdered diet. The dosages were estimated from the diet intake. In the case of yellow KK mice, the diet intake was determined as total food consumption of all mice in a group.

Glucose and insulin tolerance tests in rats and mice. In the oral glucose tolerance test, rats received a 40% glucose solution (2 g glucose/kg) after 20 h of fasting. In the insulin tolerance test, rats and mice were fasted for 20 h and received i.p. insulin (Novo, regular, 0.5 or 1.0 U/kg) (Novo Industries, Copenhagen, Denmark).

Intravenous glucose tolerance test (IV-GTT) in dogs. IV-GTT was performed according to the method of Kaneko et al.[16] After 24 h of fasting, dogs were subjected to IV-GTT with a rapid injection (20 s) of 0.5 g/kg glucose into the foreleg vein. Blood samples were drawn 5, 10, 20, and 30 min after glucose injection for measurement of plasma glucose and insulin.

Steady-state plasma glucose (SSPG). The SSPG of fatty rats was measured by the method of Shen et al.[17] After 20 h of fasting, the rats received a bolus injection of propranolol (700 μg/rat) followed by a constant infusion of glucose (29 mg/kg/min), insulin (20 mU/kg/min), epinephrine (0.4 μg/kg/min), and propranolol (20 μg/kg/min) into the jugular vein. The mean SSPG and steady-state plasma insulin (SSPI) were determined with the values obtained from the blood samples drawn 90, 120, and 150 min after the start of the infusion.

For the measurement of steady-state blood glucose (SSBG) of yellow KK mice, the simplified method previously reported by Taketomi et al.[18] was used. After 20 h of fasting, mice were subcutaneously injected with epinephrine (100 μg/kg), propranolol (5 mg/kg), glucose (3 g/kg, [3-^{3}H]-glucose, 1.36 μCi/mouse), and insulin (0, 1, or 2 U/kg). Blood samples were obtained from the orbital sinus 45, 60, and 75 min after the injection for determination of mean SSBG and SSPI values and specific activity of blood glucose.

Estimation of peripheral disposal of exogenous triglyceride. The removal rate of plasma triglyceride was determined according to the method of Boberg et al.[19] Fatty rats were injected with Intralipid emulsion (10%, 3 ml/kg) via the tail vein. Plasma triglyceride concentration was measured 10, 20, and 30 min after the injection to calculate the lipid disappearance rate.

Estimation of hepatic triglyceride secretion. The hepatic triglyceride secretion rate was determined according to the method of Otway and Robinson.[20] Fatty rats were injected with Triton WR-1339 (20% in saline, 500 mg/kg) via the tail vein. Plasma triglyceride concentration was measured 0, 15, 30, and 45 min after the injection. The rate of hepatic triglyceride output was manifested as the increment of plasma triglyceride concentration per minute.

Analytic methods. Blood samples were deproteinized with $Ba(OH)_2$ and $ZnSO_4$ solutions. After centrifugation, the resultant supernatant was used for the determination of glucose, which was determined by a glucose-oxidase method.[21] For determination of the specific activity of blood glucose, an aliquot of the supernatant was dried to remove tritiated water and the resultant precipitate was resolved in 0.5 ml of

TABLE 1
Effects of ADD-3878 on body weight, food intake, blood glucose, plasma insulin, and plasma lipids in male and female yellow KK mice

	Dosage (mg/kg/day)	Body weight (g)	Food intake (g/4 days)	Blood glucose (mg/dl)	Plasma insulin (μU/ml)	Plasma NEFA (μeq/L)	Plasma triglyceride (mg/dl)	Plasma cholesterol (mg/dl)	Plasma phospholipid (mg/dl)
Male									
Control		35.0 ± 0.8	26.3	429 ± 63	1268 ± 698	127 ± 13	512 ± 52	147 ± 22	310 ± 31
ADD-3878	39	35.4 ± 1.3	27.5	278 ± 104†	1098 ± 834	86 ± 29†	344 ± 164*	130 ± 12	290 ± 30
ADD-3878	95	35.6 ± 2.0	27.0	244 ± 62§	497 ± 274†	52 ± 17§	314 ± 79§	114 ± 16†	263 ± 33*
ADD-3878	186	36.4 ± 0.8	26.7	183 ± 17§	383 ± 229†	79 ± 31‡	206 ± 34§	96 ± 8§	235 ± 12§
Female									
Control		40.3 ± 2.1	25.7	415 ± 80	1303 ± 627	155 ± 37	539 ± 108	227 ± 37	394 ± 66
ADD-3878	30	39.8 ± 1.2	23.5	272 ± 64‡	942 ± 504	113 ± 44	373 ± 59‡	220 ± 37	393 ± 50
ADD-3878	74	40.2 ± 1.1	23.5	216 ± 32§	562 ± 534	63 ± 33‡	221 ± 70§	180 ± 36	357 ± 59
ADD-3878	155	39.9 ± 1.7	24.3	210 ± 13§	88 ± 62§	95 ± 37†	166 ± 65§	177 ± 21†	313 ± 48*

ADD-3878 was given to yellow KK mice (9 wk old) as a dietary admixture for 4 days. Body weight, food intake, blood glucose, and plasma lipids were determined in the morning on day 5. *$P < 0.05$, †$P < 0.02$, ‡$P < 0.01$, §$P < 0.001$ versus control. Mean ± SD ($N = 6$).

TABLE 2
Effects of chronic administration of ADD-3878 on body weight, blood glucose, plasma insulin, and plasma triglyceride in young yellow KK mice and fatty rats

		Yellow KK mice (no. of animals)		Fatty rats (no. of animals)	
	Weeks of treatment	Control 10	ADD-3878 10	Control 8	ADD-3878 8
Body weight (g)	2	29.0 ± 1.2	28.6 ± 1.5	211 ± 18	204 ± 26
	4	34.1 ± 1.0	34.4 ± 1.3	314 ± 21	311 ± 35
	8	39.7 ± 0.6	39.5 ± 1.6	448 ± 25	466 ± 41
	12	42.0 ± 1.3	42.7 ± 2.0	517 ± 27	550 ± 48
Food intake (g/wk)	2	42.8	41.4	181 ± 15	185 ± 20
	4	36.0	38.2	215 ± 23	232 ± 22
	8	40.2	38.9	211 ± 28	238 ± 28
	12	36.4	39.7	175 ± 20	175 ± 18
Blood glucose (mg/dl)	2	191 ± 58	171 ± 10	91 ± 7	87 ± 9
	4	327 ± 73	198 ± 53‡	105 ± 41	81 ± 9
	8	429 ± 35	312 ± 59‡	136 ± 51	90 ± 8*
	12	442 ± 68	311 ± 94†	136 ± 51	98 ± 20
Plasma insulin (μU/ml)	2	—	—	510 ± 304	153 ± 65†
	4	1250 ± 421	347 ± 216‡	1029 ± 560	305 ± 110†
	8	4330 ± 2020	2180 ± 759†	2103 ± 599	631 ± 142‡
	12	6520 ± 2030	4290 ± 2130*	2550 ± 588	790 ± 419‡
Plasma triglyceride (mg/dl)	2	—	—	197 ± 40	137 ± 39†
	4	—	—	384 ± 129	106 ± 34‡
	8	—	—	667 ± 223	300 ± 183†
	12	449 ± 129	322 ± 51†	592 ± 332	282 ± 146*

Female yellow KK mice (4 wk old) and male fatty rats (4 wk old) were kept on the CE-2 diet with or without ADD-3878 (0.05%) for 12 wk. Body weight, food intake, blood glucose, and plasma insulin and triglyceride were determined the next morning after each dosing.
*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ versus control. Mean ± SD.

water. Radioactivity of glucose was determined in a liquid scintillation spectrometer (Aloka LSC-903, Aloka Co. Ltd., Japan) using Bray's cocktail.[22] Plasma immunoreactive insulin was measured by the double-antibody method based on Hales and Randle[23] using a commercial kit (Amersham, England) with human insulin as a standard. Plasma triglyceride, cholesterol, phospholipid, and NEFA were determined using commercially available assay kits (Wako Chemicals, Japan).

Reagents. L-Epinephrine from E. Merck, A. G. (Darmstadt, Germany), DL-propranolol hydrochloride from Sigma Chemical Company (St. Louis, Missouri), and Novo regular insulin from Novo Laboratories were used. [3-^{3}H]Glucose was purchased from New England Nuclear Corp. (Boston, Massachusetts).

Evaluation of the data. The data were presented by mean ± SD and statistically analyzed by Student's *t* test. The minimum effective dose of ADD-3878 was defined as the smallest dose to produce a statistically significant decrease ($P < 0.05$, Student's *t* test) in blood glucose and lipid concentration as compared with those of the control group.

RESULTS

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN GENETICALLY DIABETIC AND OBESE MICE, YELLOW KK

Yellow KK mice have genetically determined obese and diabetic syndromes such as hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and severe insulin resistance, all of which increase with age.[10]

Effects on hyperglycemia and hyperlipidemia. When ADD-3878 was given to male or female yellow KK mice (9 wk old) as a dietary admixture (30–186 mg/kg/day, calculated from food intake) for 4 days, nonfasting blood glucose, plasma insulin, and plasma lipids, especially triglyceride, were decreased in a dose-dependent manner, but neither

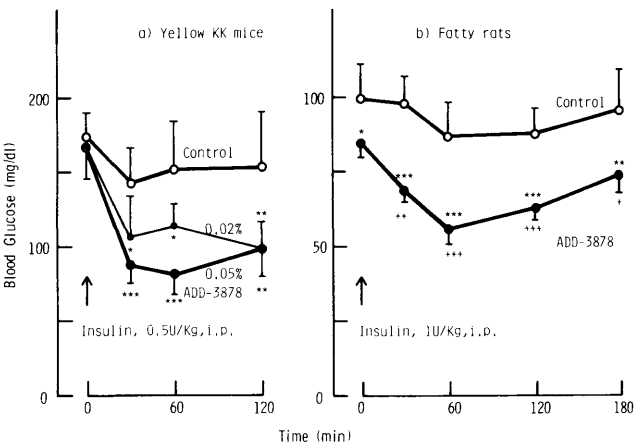

FIGURE 1. Effects of ADD-3878 on insulin tolerance in yellow KK mice and fatty rats. (a) Male yellow KK mice (9 wk old) were kept on the CE-2 diet with ADD-3878 (0.02% or 0.05%) for 4 days. They were given insulin (0.5 U/kg, i.p.) on day 5 after 20 h of fasting. (b) ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (12 wk old) for 8 days. The rats were given insulin (1 U/kg, i.p.) on day 9 after 20 h of fasting. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus control. $^+P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ versus initial. Mean ± SD ($N = 5$).

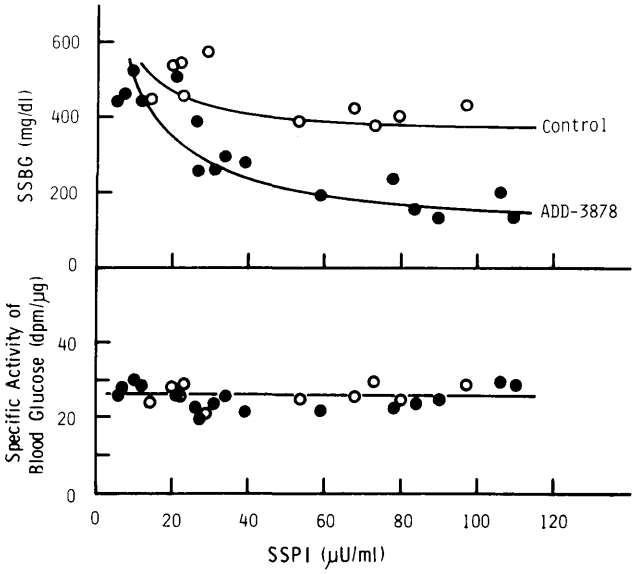

FIGURE 2. Relationship between SSPI and SSBG or the specific activity of blood glucose in yellow KK mice. Male yellow KK mice (12 wk old) were kept on the CE-2 diet with or without ADD-3878 (0.15%) for 4 days. After 20 h of fasting, they were subcutaneously injected with epinephrine (100 μg/kg), propranolol (5 mg/kg), glucose (3 g/kg, [3-^{3}H]glucose, 1.36 μCi/mouse), and insulin (0, 1, or 2 U/kg). Details of procedures are described in MATERIALS AND METHODS.

food intake nor body weight was altered (Table 1). A significant fall of blood glucose (by more than 10%) could be detected at a dose as low as 7 mg/kg/day (data not shown). When ADD-3878 was chronically administered (12 wk) to young yellow KK mice (4 wk old), which were in the early phase of obesity and diabetes, age-dependent increases in blood glucose, plasma insulin, and triglyceride were markedly suppressed (Table 2). However, body weight and food intake were not affected throughout the experiment. A single oral administration of ADD-3878 (100 mg/kg) resulted in no changes in blood glucose, plasma insulin, and triglyceride (data not shown).

Effects on insulin resistance. Insulin resistance of yellow KK mice was manifested by an impaired glycemic response to exogenous insulin as high as 0.5 U/kg. The glycemic response was improved dose-dependently after a 4-day treatment with this compound (Figure 1a). To clarify the effect of ADD-3878 on peripheral insulin resistance, the steady-state blood glucose (SSBG) was determined at different steady-state plasma insulin (SSPI) levels (Figure 2). In accordance with results obtained in KK and C57BL/6 mice as previously reported,[18] specific activity of blood glucose of control and treated mice did not change with increasing SSPI, suggesting no differences in exogenous glucose influx and hepatic glucose output among the animals. Therefore, SSBG could indicate relative impedance of glucose metabolism of the peripheral tissue under this condition. The SSBG at 20 μU/ml of SSPI was about 500 mg/dl and this value indicated severe insulin resistance of these mice (cf. 160 mg/dl for C57BL mice[18]). SSBG of control and treated groups decreased gradually with increasing levels of SSPI. The SSBG response to SSPI was exaggerated in ADD-3878-treated mice (Figure 2). These findings indicate that ADD-3878 decreased peripheral insulin resistance in yellow KK mice.

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN GENETICALLY OBESE RATS, FATTY

Fatty rats develop obesity-associated syndromes such as hyperinsulinemia, hypertriglyceridemia, and insulin resistance as their genetic traits.[9]

Effects on hyperlipemia and hyperinsulinemia. When orally administered to fatty rats for 7 days, ADD-3878 (50 mg/kg/day) decreased nonfasting plasma insulin and triglyceride without changing body weight and food intake (expt. 1 in Table 3). Blood glucose, plasma cholesterol, and NEFA were also decreased by ADD-3878. In the dose-finding study, a significant reduction of plasma triglyceride (by 30%) was observed at a dose as low as 10 mg/kg/day (data not shown).

To examine the lipid-lowering mechanism of ADD-3878, the rates of lipid removal and hepatic triglyceride output were calculated by measuring plasma triglyceride after Intralipid injection and after Triton injection, respectively. In fatty rats treated with ADD-3878, the removal rate of plasma triglyceride was markedly elevated, but the hepatic output of triglyceride was not altered (expt. 3 in Table 3).

When the compound was chronically administered (12 wk) to young fatty rats (4 wk old) as a dietary admixture, the age-dependent elevation of plasma triglyceride and insulin levels was markedly suppressed (Table 2). A slight elevation of

TABLE 3
Effects of ADD-3878 on body weight, food intake, blood glucose, plasma insulin (IRI), plasma lipids, steady-state plasma glucose (SSPG), plasma triglyceride removal, and hepatic triglyceride output in fatty rats

	Control	ADD-3878
Expt. 1		
Body weight (g)	505 ± 24	522 ± 34
Food intake (g/day)	23 ± 3	25 ± 2
Blood glucose (mg/dl)	111 ± 11	79 ± 10‡
Plasma IRI (μU/ml)	2110 ± 243	897 ± 210§
Plasma triglyceride (mg/dl)	872 ± 379	286 ± 85‡
Plasma cholesterol (mg/dl)	165 ± 28	120 ± 26*
Plasma NEFA (μeq/L)	458 ± 100	249 ± 35‡
Expt. 2		
SSPG (mg/dl)	400 ± 31	325 ± 37‡
SSPI (μU/ml)	980 ± 155	785 ± 227
Expt. 3		
Plasma triglyceride removal (%/min)	3.62 ± 0.61	4.85 ± 0.63†
Hepatic triglyceride output (mg/dl/min)	21.7 ± 7	20.0 ± 3.7

Expt. 1: ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (12–14 wk old) for 7 days. Blood samples were taken 20 h after the last dosing.
Expt. 2: ADD-3878 (100 mg/kg/day) was orally administered to male fatty rats (12 wk old) for 10 days. After 20 h fasting, SSPG was determined as described in MATERIALS AND METHODS.
Expt. 3: ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (7 wk old) for 8 days. The removal rate of plasma triglyceride on day 5 was estimated from the disappearance of plasma triglyceride after injection of Intralipid. The rate of hepatic triglyceride output on day 9 was determined from increments of plasma triglyceride concentration after injection of Triton WR-1339.
*$P < 0.05$, †$P < 0.02$, ‡$P < 0.01$, §$P < 0.001$ versus control. Mean ± SD ($N = 5$).

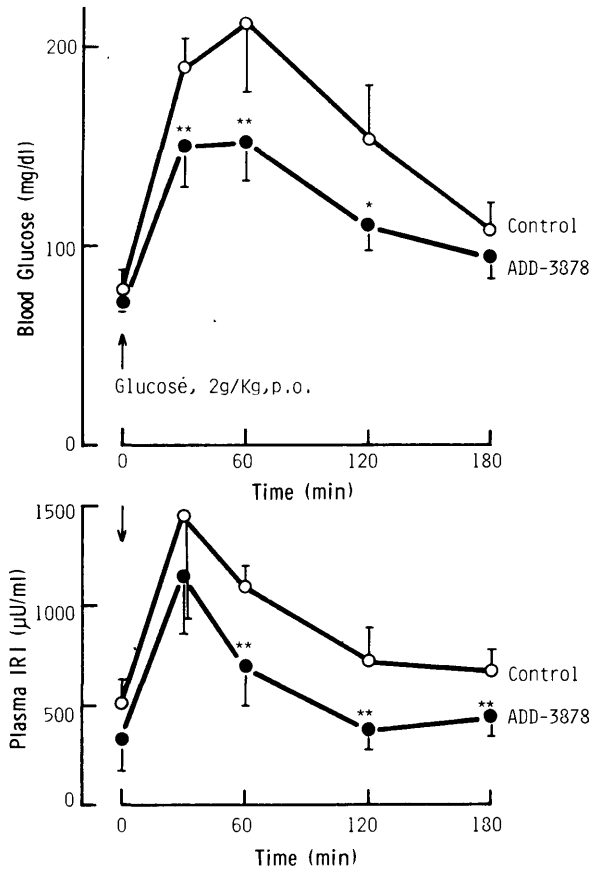

FIGURE 3. Effect of ADD-3878 on oral glucose tolerance in fatty rats. ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (14 wk old) for 8 days. After the last administration, rats were fasted for 20 h and subjected to an oral glucose tolerance test. *P < 0.02, **P < 0.01 versus control. Mean ± SD (N = 5).

blood glucose with advancing age was also depressed by the treatment (Table 2).

Effects on glucose tolerance and insulin resistance. To orally administered glucose, fatty rats exhibited marked responses of blood glucose and plasma insulin, which indicated glucose intolerance and insulin resistance (Figure 3). ADD-3878 administration resulted in marked normalization of the abnormal responses of blood glucose and plasma insulin in this test.

In the insulin tolerance test, fatty rats showed no significant change in blood glucose in response to a dose of insulin as high as 1 U/kg (Figure 1b). ADD-3878 administration decreased fasting blood glucose and increased the glycemic response to insulin.

In the SSPG study, the glucose level was significantly lower in ADD-3878-treated rats while the insulin level was somehow higher in control rats in spite of the same insulin infusion rate carried out in the both groups (expt. 2 in Table 3). If SSPI had been made the same in both groups by regulating insulin infusion, SSPG would have been much lower in the treated group.

All of these results indicate that ADD-3878 decreases the obesity-associated insulin resistance of fatty rats.

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN SPRAGUE-DAWLEY (SD) RATS AND STREPTOZOTOCIN-INDUCED-DIABETIC RATS

ADD-3878 (100 mg/kg/day, p.o.) showed no effects on blood glucose and plasma lipid in young SD rats (5 wk old), although the administration period was extended as long as 16 days (Table 4). Old SD rats (20 wk old), as compared with young ones, were moderately obese, hyperinsulinemic, and hypertriglyceridemic as described in many papers,[24,25] and had decreased plasma insulin and triglyceride levels in response to ADD-3878 administration (Table 4). Furthermore, a single administration of ADD-3878 to fasted young and old rats altered neither blood glucose nor plasma insulin (data not shown).

The same extent of glycemic response to insulin was obtained in the young and old rats by using a higher dose of the hormone with old rats, indicating insulin resistance of the old rats (Figure 4). ADD-3878 administration (100 mg/kg/day) increased the response to insulin in the old rats but not in the young rats (Figure 4).

In mildly streptozotocin-diabetic rats, oral ADD-3878 administration (100 mg/kg/day, for 12 days) neither decreased blood glucose and plasma triglyceride levels nor improved glucose tolerance (data not shown).

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN BEAGLE DOGS

Beagle dogs used in the experiment were selected from normal dogs based on glucose tolerance and insulin response. They were normal in plasma glucose, triglyceride, and insulin but slightly intolerant and hyperinsulinemic in response to oral glucose. When ADD-3878 (100 mg/kg/day) was orally administered to these dogs for 5 days, the mean k values (plasma glucose clearance rate) of IV-GTT were increased from 3.8 to 4.73%/min without a significant increment of plasma insulin area, indicating increased efficiency of insulin action in glucose metabolism by the treatment with this compound. The treated dogs showed no change in fasting levels of plasma components except decreasing plasma cholesterol, but significant decreases in postprandial plasma glucose, insulin, and triglyceride levels (Table 5). The lowering effect on the postprandial plasma triglyceride was dose-dependent, with a significant marginal effect (by 20%) observed at a dose as low as 5 mg/kg body wt (data not shown).

DISCUSSION

Type II diabetes and obesity are very frequently accompanied by insulin resistance, which appears to be a direct or indirect cause for metabolic and endocrinologic abnormalities, such as hyperglycemia, hyperlipemia, and hyperinsulinemia, although its mechanism is still obscure.[1-8] In genetically obese and/or diabetic animals, including yellow (obese) KK mice and fatty rats used in the present studies, obese and diabetic syndromes are accompanied with insulin resistance.[9-11]

ADD-3878, a new compound with the thiazolidine ring differing from the sulfonylureas and biguanides in its chemical structure, had a marked effect on abnormalities of glucose and lipid metabolism in yellow KK mice and fatty rats, such as hyperinsulinemia, hypertriglyceridemia, hyperglycemia,

TABLE 4
Effects of ADD-3878 on body weight, blood glucose, plasma triglyceride (TG), plasma NEFA, and plasma insulin (IRI) in normal rats

Days on treatment of ADD-3878	Young rats		Old rats	
	Control	ADD-3878	Control	ADD-3878
3				
Body weight (g)	202 ± 8	200 ± 9	635 ± 43	632 ± 38
Blood glucose (mg/dl)	108 ± 12	102 ± 5	97 ± 4	105 ± 5
Plasma TG (mg/dl)	111 ± 15	100 ± 31	324 ± 119	143 ± 45†
Plasma NEFA (μeq/L)	300 ± 61	223 ± 64	316 ± 89	577 ± 201
Plasma IRI (μU/ml)	46 ± 9	25 ± 10‡	64 ± 16	40 ± 9
8				
Body weight (g)	234 ± 9	232 ± 10	623 ± 40	615 ± 33
Blood glucose (mg/dl)	91 ± 6	92 ± 1	83 ± 2	92 ± 5
Plasma TG (mg/dl)	83 ± 8	93 ± 9	201 ± 38	109 ± 13§
Plasma NEFA (μeq/L)	151 ± 29	123 ± 33	259 ± 77	214 ± 111
Plasma IRI (μU/ml)	31 ± 6	22 ± 5	80 ± 35	38 ± 12*
16				
Body weight (g)	284 ± 11	279 ± 14	636 ± 43	585 ± 62
Blood glucose (mg/dl)	90 ± 5	86 ± 5	89 ± 7	83 ± 32
Plasma TG (mg/dl)	131 ± 15	129 ± 26	270 ± 146	85 ± 35*
Plasma NEFA (μeq/L)	205 ± 49	150 ± 69	231 ± 80	339 ± 340
Plasma IRI (μU/ml)	46 ± 20	54 ± 16	58 ± 21	31 ± 12*

ADD-3878 (100 mg/kg/day) was orally administered to young (5 wk old) and old Sprague-Dawley rats (20 wk old) for 16 days. Blood samples were taken 20 h after administration of ADD-3878.
N = 5. Mean ± SD. *P < 0.05, †P < 0.02, ‡P < 0.01, §P < 0.001 versus control.

and glucose intolerance. ADD-3878 was also effective on glucose and lipid metabolism in aged (old) rats, which were moderately insulin resistant and hyperlipidemic compared with young rats as reported elsewhere,[24,25] but not on the metabolism in young normal rats or streptozotocin-diabetic rats. Furthermore, in beagle dogs with mild intolerance to glucose, ADD-3878 ameliorated glucose tolerance and suppressed postprandial rises in plasma triglyceride and insulin. Therefore, ADD-3878 is more likely to be effective on the metabolism of carbohydrate and lipid in insulin-resistant or obese animals than in normal or insulin-deficient diabetic animals.

ADD-3878 markedly exaggerated glycemic response to injected insulin and also decreased the SSPG, which was in a reversed relationship to overall insulin sensitivity of the peripheral tissue[17] in the insulin-resistant animals. These results suggest that the primary action of ADD-3878 is to decrease insulin resistance of peripheral tissue. The above postulation concerning the mechanism of actions of ADD-3878 may be supported by our preliminary studies. ADD-3878 administration to yellow KK mice decreased insulin resistance of peripheral tissue manifested by increased insulin sensitivity and insulin responsiveness of adipocytes and soleus muscles (unpublished data).

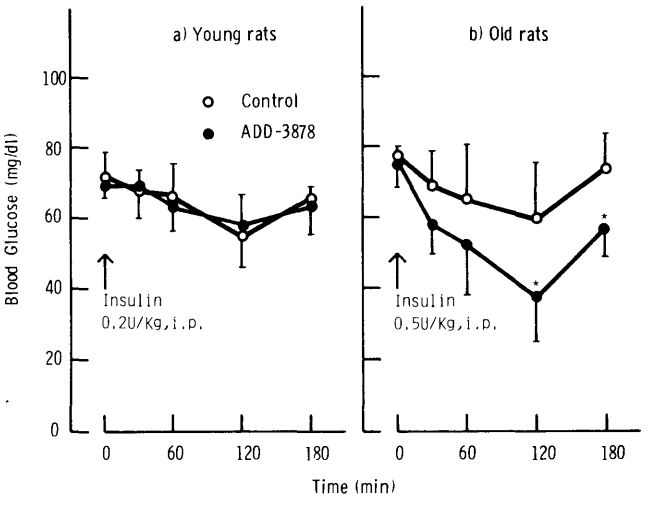

FIGURE 4. Effect of ADD-3878 on insulin tolerance in normal rats. Male Sprague-Dawley rats were used. ADD-3878 (100 mg/kg/day) was orally administered to young (6 wk old) and old (20 wk old) rats for 4 days. After the last administration, rats were fasted for 20 h and received an injection of insulin (0.2 or 0.5 U/kg, i.p.). *P < 0.05 versus control. Mean ± SD (N = 5).

TABLE 5
Effects of ADD-3878 on plasma glucose, insulin, triglyceride, and cholesterol in dogs

		ADD-3878 treatment		
		Before	After	(% initial)
Fasting plasma				
Glucose (mg/dl)		101 ± 4	92 ± 6	(92 ± 8)
IRI (μU/ml)		7.5 ± 1.8	7.6 ± 0.6	(105 ± 24)
Triglyceride (mg/dl)		25 ± 6	19 ± 7	(78 ± 27)
Cholesterol (mg/dl)		125 ± 26	93 ± 20	(74 ± 5‡)
Postprandial plasma				
Glucose (mg/dl)	2 h	98 ± 3	96 ± 2	(98 ± 3)
	3 h	108 ± 6	100 ± 4	(93 ± 2†)
IRI (μU/ml)	2 h	14.6 ± 5.3	12.5 ± 6.1	(107 ± 85)
	3 h	24.8 ± 8.3	13.5 ± 2.7	(58 ± 21*)
Triglyceride (mg/dl)	2 h	76 ± 39	43 ± 16	(61 ± 17†)
	3 h	79 ± 40	51 ± 25	(72 ± 43)

ADD-3878 (100 mg/kg/day) was orally administered to male beagle dogs after meals for 4 days. Values are represented as means ± SD of five dogs.
Fasting plasma determinations were obtained before the last administration of ADD-3878.
*P < 0.02, †P < 0.01, ‡P < 0.001 versus initial.

Insulin sensitivity is decreased with the advance of age in normal and genetically obese animals as well as in human subjects.[9,10,26] The age-dependent elevation of plasma glucose, insulin, and triglyceride in yellow KK mice and fatty rats appears to be closely related to increasing peripheral insulin resistance. The chronic administration of ADD-3878 for as long as 12 wk suppressed the age-dependent rises of the three parameters in yellow KK mice and fatty rats. These results also suggest the inhibitory action of ADD-3878 on insulin resistance.

From the pathogenic point of hyperglycemia and hyperinsulinemia, the effects of ADD-3878 on plasma glucose and insulin are clearly explained. Insulin resistance is assumed to lead to a compensatory adaptation of pancreatic B-cells, which results in hyperinsulinemia, although the feedback mechanism between insulin-resistant tissue and pancreatic B-cells remains unclear. In regard to impaired glucose metabolism acompanied by hyperinsulinemia, insulin resistance and the pancreatic compensation are the main determinants of glucose intolerance and/or hyperglycemia. Based on these concepts, the reduction of insulin resistance by ADD-3878 probably results in normalization of hyperglycemia and hyperinsulinemia of the insulin-resistant animal models.

Hyperinsulinemia and hyperlipemia are very common syndromes in obesity and diabetes. However, less information on the pathogenic action of insulin resistance has been presented for hyperlipemia than for hyperglycemia. Hypertriglyceridemia due to increased hepatic triglyceride output may be caused by hyperinsulinemia in the insulin-resistant state, because insulin is one of the potent stimulators of lipid synthesis and, furthermore, hepatic lipid synthesis is still sensitive to insulin in obese and diabetic animals and humans.[9,10,27] On the other hand, insulin resistance may decrease lipid uptake through decreasing insulin actions on lipoprotein lipase induction or glucose metabolism, which supplied substrates for esterification of fatty acids. The present studies clearly demonstrated that ADD-3878 administration to fatty rats increased the rate of lipid removal but did not affect hepatic triglyceride output. The insulin-resistance decreasing mechanism may be involved in the lipid-lowering action of ADD-3878.

ADD-3878 has neither acute hypoglycemic action nor stimulatory action on insulin secretion in the animals. In this regard, it can be distinguished from sulfonylureas. In other insulin-resistant mice such as the db/db mice, tolbutamide failed to decrease blood glucose,[28] but ADD-3878 decreased both hyperglycemia and hyperinsulinemia (unpublished data). Furthermore, in our preliminary study, yellow KK mice did not show any response to tolbutamide (unpublished data). Although extrapancreatic actions of sulfonylureas have been proposed,[12–14] so far, as examined in experimental animals, ADD-3878 is quite different from sulfonylureas in the effects on insulin-resistant or obese animals. It is postulated that ADD-3878 is more beneficial to obese and/or type II diabetes than sulfonylureas by its specific action, reduction of insulin resistance.

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