

Combined Treatment With Benzylamine and Low Dosages of Vanadate Enhances Glucose Tolerance and Reduces Hyperglycemia in Streptozotocin-Induced Diabetic Rats

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Semicarbazide-sensitive amine oxidase (SSAO) is highly expressed in adipose cells, and substrates of SSAO, such as benzylamine, in combination with low concentrations of vanadate strongly stimulate glucose transport and GLUT4 recruitment in 3T3-L1 and rat adipocytes. Here we examined whether acute and chronic administration of benzylamine and vanadate in vivo enhances glucose tolerance and reduces hyperglycemia in diabetic rats. Acute intravenous administration of these drugs enhanced glucose tolerance in nondiabetic rats and in streptozotocin (STZ)-induced diabetic rats. This occurred in the absence of changes in plasma insulin concentrations. However, the administration of benzylamine or vanadate alone did not improve glucose tolerance. The improvement caused by benzylamine plus vanadate was abolished when rats were pretreated with the SSAO-inhibitor semicarbazide. Chronic administration of benzylamine and vanadate exerted potent antidiabetic effects in STZ-induced diabetic rats. Although daily administration of vanadate alone (50 and 25 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ i.p.) for 2 weeks had little or no effect on glycemia, vanadate plus benzylamine reduced hyperglycemia in diabetic rats, enhanced basal and insulin-stimulated glucose transport, and upregulated GLUT4 expression in isolated adipocytes. In all, our results substantiated that acute and chronic administration of benzylamine with low dosages of vanadate have potent antidiabetic effects in rats. *Diabetes* 50:2061–2068, 2001

The semicarbazide-sensitive amine oxidase (SSAO) belongs to the large family of copper-containing amine oxidases (EC 1.4.3.6) that convert primary amines to aldehydes, with the concomitant production of hydrogen peroxide and ammonia. These pro-

teins are characterized by broad substrate selectivity among species, which makes it difficult to ascertain their biological function. The enzyme readily oxidizes exogenous (e.g., benzylamine, tyramine) or endogenous (e.g., phenylethylamine, histamine) aromatic primary amines, but also endogenous (e.g., methylamine, aminoacetone) aliphatic primary amines (1). More recently, a new function has been assigned to SSAO: the vascular adhesion protein-1 (VAP-1), found to be identical to SSAO, belongs to the family of adhesive proteins implicated in processes like inflammation or cell-to-cell interaction (2–4). The relation between the enzymatic and adhesive functions of SSAO/VAP-1 remains to be determined.

Adipose tissue contains high levels of SSAO (5–9), and an increase in the expression of the membrane-bound SSAO has been reported in adipocyte differentiation (8,10). As to the function of adipocyte SSAO, hydrogen peroxide, one of the reaction products, has insulinomimetic properties (11). Moreover, membrane fractionation and vesicle immunoisolation analysis have shown that a portion of the SSAO protein or enzymatic activity colocalizes with intracellular GLUT4-containing vesicles (5,12). We recently reported that substrates of SSAO can stimulate glucose transport in rat adipocytes (12–14). The amine-induced stimulation of glucose transport was observed in the presence of ineffective concentrations of vanadate and was abolished by semicarbazide and catalase (12,13). These observations suggested that the SSAO-dependent generation of hydrogen peroxide is responsible for the increased stimulation of glucose transport via a chemical interaction. The combination of vanadate and hydrogen peroxide can form peroxovanadate, a powerful insulinomimetic agent that may be partly responsible for this effect (13,15). In addition, the combination of SSAO substrates with vanadate stimulates glucose transport through translocation of GLUT4 to the adipocyte cell surface (12,13). Furthermore, the combination of SSAO substrates and vanadate stimulates phosphatidylinositol (PI) 3-kinase activity and tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and -3 in conditions in which the insulin receptors themselves are only slightly activated (14). All these data support the view that the combination of SSAO substrates and vanadate stimulates glucose transport via a pathway that does not involve the insulin receptor.

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2-DG, 2-deoxy-D-[1,2-³H]glucose; IRS, insulin receptor substrate; PBS, phosphate-buffered saline; PI, phosphatidylinositol; PTP, protein tyrosine phosphatase; SSAO, semicarbazide-sensitive amine oxidase; STZ, streptozotocin; VAP-1, vascular adhesion protein-1.

In this study, we examined the acute and chronic effects of benzylamine and vanadate on control and diabetic rats. We demonstrated potent antidiabetic properties of benzylamine and vanadate in streptozotocin (STZ)-induced diabetic rats.

RESEARCH DESIGN AND METHODS

Materials. We obtained 2-deoxy-D-[1,2-³H]glucose (2-DG; 26 Ci/mmol) from NEN Life Science Products and [¹⁴C]benzylamine (59 Ci/mmol) from Amersham Pharmacia Biotech (Arlington Heights, IL). Purified porcine insulin was a kind gift from Eli Lilly (Indianapolis, IN). STZ, semicarbazide hydrochloride, benzylamine hydrochloride, sodium orthovanadate, and other chemicals were purchased from Sigma Aldrich (St. Louis, MO). Ketamine was obtained from Mérieux (Imalgene, Mérieux, France) and collagenase type I from Worthington. The osmotic minipumps used in the chronic studies were from Alza. All electrophoresis reagents and molecular weight markers were obtained from Bio-Rad. Enhanced chemiluminescence reagents (super signal substrate) were obtained from Amersham. Anti-GLUT4 antibody (OSCRX) was produced from rabbit, as previously reported (15). Rabbit polyclonal antibodies against rat α_1 -integrin were kindly given by Dr. C. Enrich (Universitat de Barcelona). **Animals.** Male Wistar rats weighing 180–220 g were purchased from Harlan (Interfauna Ibérica S.A., Spain). The animals were housed in animal quarters at 22°C with a 12-h light/dark cycle and were fed ad libitum, unless otherwise stated. Type 1 diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (45–70 mg/kg body wt dissolved in 50 mmol/l citrate buffer [pH 4.5]). Only diabetic animals with glycemia >300 mg/dl were used. Inhibition of SSAO activity was obtained in vivo by administration of semicarbazide (5 mg · kg⁻¹ · day⁻¹ i.p.) for 3 days. All procedures used were approved by the Ethics Committee of the University of Barcelona.

Glucose tolerance tests. Anesthetized (pentobarbital 80 mg/kg body wt) control rats or nonanesthetized diabetic rats were injected intravenously via the tail vein with vehicle (phosphate-buffered saline [PBS], pH 7.4) or with 7 μ mol/kg body wt benzylamine and/or 20 μ mol/kg sodium orthovanadate. In anesthetized control rats, an intravenous (saphenous vein) glucose tolerance test (0.8 g/kg body wt) (16) was carried out 30 min after administration of benzylamine, vanadate, or both. Blood samples were collected before (0 min) and 5, 10, 15, 20, 30, and 45 min after glucose administration. In other studies of diabetic rats, an oral glucose tolerance test (2 g/kg body wt) (17) was carried out 15 min after injection of benzylamine, vanadate, or both. Blood samples were collected before and 15, 30, 45, 60, 90, and 150 min after glucose administration. Plasma was obtained for the determination of glucose and insulin concentrations. Food was withdrawn at 8:00 A.M. to ensure a 3-h fast before the beginning of the study.

Chronic treatment of diabetic animals. Osmotic minipumps containing benzylamine (84 μ mol · kg⁻¹ body wt · day⁻¹) were implanted subcutaneously in STZ-induced diabetic rats anesthetized by ketamine hydrochloride (95 mg/kg) and xylazine (10 mg/kg). Animals that did not receive benzylamine were sham-operated. A single intraperitoneal injection of vanadate (25 or 50 μ mol/kg body wt) or the vehicle (PBS) was administered daily at 9:00 A.M. Food was removed after the administration of vanadate and replaced at 2:00 P.M. Glycemia was measured in arteriovenous blood collected from the tail vessels at 9:00 A.M., before the administration of vanadate, for 14 days. Insulin concentrations were determined before and after treatment.

Glucose transport measurements in isolated rat adipocytes and preparation of membrane extracts. Adipocytes were isolated from epididymal fat pads in nondiabetic and diabetic male Wistar rats (180–220 g), as previously reported (12,13). After a preincubation period of 45 min at 37°C, each vial, containing 400 μ l of cell suspension in Krebs-Ringer bicarbonate buffer and the drugs being tested, received an isotopic dilution of 2-DG in a final concentration of 0.1 mmol/l, equivalent to ~1,300,000 dpm/vial. 2-DG transport assays were performed as previously reported (12,13). Isolated fat cells from control or treated rats were disrupted for total membrane preparation by hypo-osmotic lysis in a 20 mmol/l HES buffer and an antiprotease cocktail, as previously reported (20). Protein concentrations were determined by the Bradford method (18), with γ -globulin as a standard.

Amine oxidase activity assays. The radiochemical determination of amine oxidase activity was performed basically as described by Fowler and Tipton (19), with slight modifications (13).

Electrophoresis and immunoblot analysis. SDS-PAGE was performed on membrane proteins following the method of Laemmli (20). Proteins were transferred to etorphine hydrochloride (Immobilon) in buffer consisting of 20% methanol, 200 mmol/l glycine, and 25 mmol/l Tris (pH 8.3). After transfer, the filters were blocked with 5% nonfat milk in PBS for 1 h at 37°C and then incubated with polyclonal antibodies raised against GLUT-4 or β_1 -integrin.

The immune complex was detected by enhanced chemiluminescence Western blot, in the linear response range.

Analytical methods. The glucose concentration in plasma or urine was determined by the glucose oxidase method (MPR-3 glucose/GOD-PAP Method; Boehringer) in glucose-tolerance experiments and with a rapid glucose analyzer (Accutrend Sensor Comfort; Roche) in chronic treatments. The plasma immunoreactive insulin concentration was determined with a sensitive rat insulin radioimmunoassay kit (Linco Research, St. Charles, MO).

Calculations and statistical analysis. Insulin and glucose responses during the glucose tolerance tests were calculated as the incremental plasma values integrated over a 45-min period in control animals and 150-min period in diabetic animals after the injection of glucose. Areas under the curve for insulin and glucose responses were calculated using the Graph Prism program (GraphPad Software). Data are presented as means \pm SE; an unpaired Student's *t* test was used to compare two groups. When experimental series involved more than two groups, statistical analysis was done by one-way analysis of variance and further post hoc Dunnett's or Tukey's *t* tests.

RESULTS

Acute administration of benzylamine and vanadate enhanced glucose tolerance in nondiabetic rats. We have previously reported that in vitro substrates of SSAO, such as benzylamine or tyramine, in combination with low concentrations of vanadate strongly stimulate glucose transport and GLUT4 recruitment in 3T3-L1 and rat adipocytes (12,13). In this study, we examined whether the combination of SSAO substrates and vanadate exhibit insulin-like effects in vivo.

Control anesthetized nondiabetic rats were injected via the tail vein with vehicle (PBS) or 7 μ mol/kg benzylamine and/or 20 μ mol/kg sodium orthovanadate. Glucose load (0.8 g/kg body wt by saphenous vein) was carried out 30 min after administration of benzylamine, vanadate, or both. Acute intravenous administration of vanadate did not alter glucose tolerance (Fig. 1A), and acute administration of benzylamine only caused reduced glycemia values 5 min after glucose injection, but the integrated glucose area under the curve remained unaltered (Fig. 1B and D). In the same conditions, administration of benzylamine plus vanadate reduced glycemia at different times after glucose injection (Fig. 1C) and increased glucose tolerance by 35% (Fig. 1D). No changes in glucose concentrations in urine were detected between the control and benzylamine + vanadate groups (0.38 \pm 0.07 vs. 0.42 \pm 0.09 mmol/l, respectively) during the glucose tolerance test (data not shown).

As expected, plasma insulin was maximal 5 min after glucose injection (Table 1), and returned to basal levels 30 min later (data not shown). The acute administration of benzylamine, vanadate, or both had no effect on plasma insulin levels (Table 1).

Semicarbazide treatment prevented the effects of benzylamine and vanadate on glucose tolerance in nondiabetic rats. To determine whether the effect of benzylamine plus vanadate on glucose tolerance was a consequence of the SSAO activity, rats previously treated for 3 days with the SSAO inhibitor semicarbazide (5 mg · kg⁻¹ · day⁻¹ i.p.) were subjected to a glucose tolerance test. Semicarbazide treatment caused >90% inhibition of SSAO activity in adipose tissue extracts (data not shown). In keeping with the inhibition of SSAO activity, isolated rat adipocytes from semicarbazide-treated rats did not respond to the combination of 0.1 mmol/l benzylamine plus 0.1 mmol/l vanadate by stimulating glucose transport (data not shown).

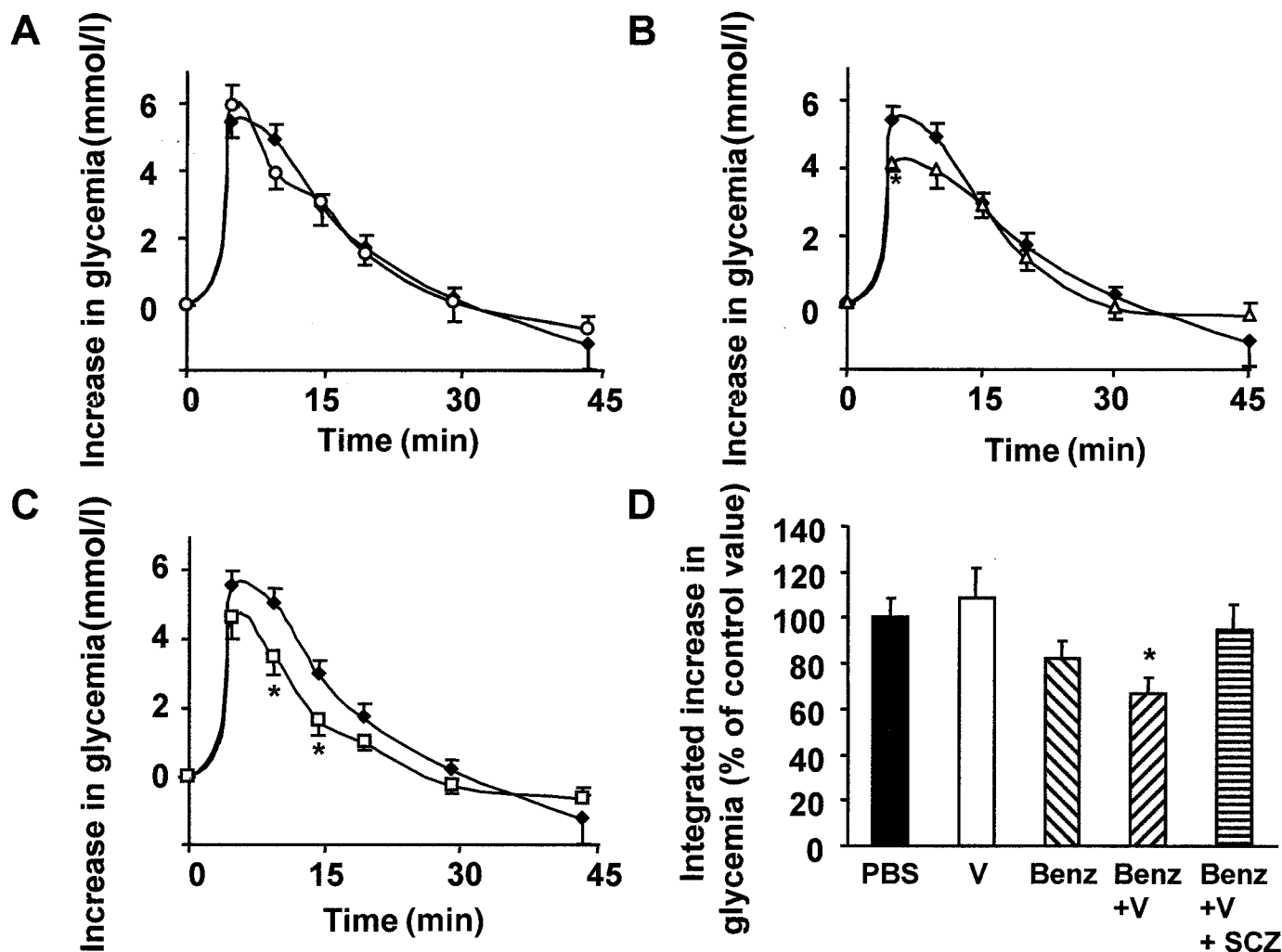


FIG. 1. Effect of acute administration of benzylamine and vanadate on glucose tolerance in control rats. Glucose was injected in the saphenous vein (0.8 g/kg body wt) 30 min after intravenous administration of vehicle (A, B, and C; ◆), 20 $\mu\text{mol/kg}$ vanadate (A; ○), 7 $\mu\text{mol/kg}$ benzylamine (B; △), or vanadate plus benzylamine (C; □). Results are expressed as increments in plasma glucose. Plasma glucose concentrations at time zero were 5.5 ± 0.2 , 5.1 ± 0.4 , 5.4 ± 0.2 , and 4.6 ± 0.3 $\mu\text{mol/l}$ in control, vanadate, benzylamine, and benzylamine plus vanadate groups, respectively. D: Integrated increase in glycemia. Data are means \pm SE of six to nine observations presented in A, B, and C and are expressed as a percentage of the control (PBS) group. Last bar corresponds to the increase in glycemia from rats pretreated with semicarbazide (SCZ; 5 mg/kg) for 3 days and receiving benzylamine (Benz) plus vanadate (V) just before the glucose tolerance test. *Different from vehicle control at $P < 0.05$.

Acute administration of benzylamine (7 $\mu\text{mol/kg}$ body wt) with vanadate (20 $\mu\text{mol/kg}$ body wt) in semicarbazide-treated rats had no effect on glucose tolerance (Fig. 1D). In these conditions, plasma insulin levels were normal (data not shown).

Acute administration of benzylamine plus vanadate enhanced glucose tolerance in STZ-induced diabetic rats. Next, we tested the in vitro and in vivo effects of the combination of benzylamine and vanadate in STZ-induced

diabetic rats. Adipose tissue from diabetic rats shows normal SSAO activity (21; G. Enrique-Tarancón, unpublished observations); however, we evaluated whether adipose cells from diabetic rats respond to SSAO substrates and vanadate by stimulating glucose transport.

As expected from previous reports (22,23), isolated adipocytes from diabetic rats showed a reduced maximal stimulation of glucose transport in response to insulin (Table 2). Interestingly, the combination of 0.1 mmol/l

TABLE 1
Effect of acute administration of benzylamine and vanadate on plasma insulin concentrations in control rats

	5 min	10 min	15 min	20 min	ΔI ($\text{ng} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$)
PBS treated	5.4 ± 1	3.8 ± 0.8	2.6 ± 0.5	1.7 ± 0.3	78.9 ± 9.6
Benzylamine treated	4.2 ± 1.2	3.8 ± 1.3	2.2 ± 0.2	1.0 ± 0.4	63.3 ± 10.6
Vanadate treated	5.3 ± 2	2.6 ± 1	1.9 ± 1	1.2 ± 0.4	66.6 ± 19.1
Benzylamine + vanadate treated	5.9 ± 0.5	3.7 ± 0.9	2.6 ± 0.7	1.1 ± 0.3	65.8 ± 8.11

Data are means \pm SE of four to eight rats per group and are given as nanogram of immunoreactive insulin per milliliter of plasma at different time periods or integrated increase in plasma insulin (ΔI) obtained during the glucose tolerance tests presented in Fig. 1 (see legend for experimental details). Plasma insulin concentrations at time zero were 0.8 ± 0.17 , 0.52 ± 0.12 , 1.1 ± 0.39 , and 1.0 ± 0.1 mmol/l in the control (PBS-treated), vanadate, benzylamine, and benzylamine plus vanadate groups, respectively.

TABLE 2

Effect of benzylamine and vanadate on glucose transport in isolated adipocytes from control or STZ-induced diabetic rats, as measured by 2-DG uptake ($\text{nmol} \cdot 100 \text{ mg}^{-1} \text{ lipid} \cdot 5 \text{ min}^{-1}$)

	Nondiabetic rat	STZ-induced diabetic rat
Basal	0.48 ± 0.15	0.57 ± 0.08
Insulin	4.58 ± 0.88*	2.97 ± 0.33*
Vanadate	0.83 ± 0.31	0.80 ± 0.13
Benzylamine	0.58 ± 0.18	0.91 ± 0.14
Benzylamine + vanadate	3.25 ± 0.45*	2.57 ± 0.28*

Data are means ± SE of 2-DG transport. Isolated adipocytes from control rats ($n = 5$) or STZ-induced diabetic rats ($n = 11$) were incubated for 45 min in basal conditions or in the presence of 100 nmol/l insulin, 100 μmol/l vanadate, 100 μmol/l benzylamine, or benzylamine plus vanadate. Subsequently, 2-DG transport was measured over 5 min as described in RESEARCH DESIGN AND METHODS. *A significant stimulation of 2-DG uptake compared with basal transport value at $P < 0.01$.

benzylamine and 0.1 mmol/l vanadate stimulated glucose transport to a similar extent in adipocytes from diabetic and nondiabetic rats (Table 2). Moreover, in diabetic rats, this stimulation was comparable to that of insulin, although it reached only 70–80% of insulin's effect in control animals (Table 2).

Nonanesthetized diabetic rats were injected via the tail vein with vehicle or 7 μmol/kg body wt benzylamine and/or 20 μmol/kg sodium orthovanadate. An oral glucose tolerance test (2 g/kg body wt) was carried out 15 min after drug injection (Fig. 2). The acute intravenous administration of benzylamine plus vanadate reduced glycemia (Fig. 2A) and enhanced glucose tolerance in the STZ-induced diabetic rats (42% increase), but benzylamine or vanadate alone did not alter glucose tolerance (Fig. 2B). The effect of benzylamine plus vanadate on glucose tolerance in vivo was independent of changes in plasma insulin concentrations (Table 3). In addition, the improvement in

TABLE 3

Effect of the acute administration of benzylamine and vanadate on plasma insulin concentrations in diabetic rats

	Before glucose administration	At glucose peak
Nondiabetic rats—PBS	1.1 ± 0.3	6.2 ± 1.0*
Diabetic rats		
PBS	0.44 ± 0.26	0.28 ± 0.21
Benzylamine + vanadate	0.33 ± 0.05	0.56 ± 0.13

Data are means ± SE of three to six rats per group and are given as nanograms of insulin per milliliter of plasma. *A significant difference ($P < 0.05$) between insulin levels before glucose administration and at the peak of hyperglycemia (5 min in nondiabetic rats and 30 min in diabetic rats).

glucose tolerance induced by benzylamine plus vanadate was abolished in semicarbazide-treated diabetic rats, indicating that intact SSAO activity was required to improve glucose tolerance (Fig. 2B). No changes in glycosuria were detected in control or benzylamine + vanadate groups (515 ± 55 vs. 447 ± 43 mmol/l, respectively) during the tolerance test.

Chronic administration of benzylamine plus vanadate reduced hyperglycemia in diabetic rats. Based on the finding of potent insulin-like effects of acute administration of benzylamine plus vanadate, we tested chronic administration of these compounds in diabetic rats. To this end, STZ-induced diabetic rats were implanted subcutaneously with osmotic minipumps releasing benzylamine ($84 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or were sham-operated. Preliminary studies have indicated that benzylamine is stable for 2 weeks in implanted osmotic minipumps, based on its capacity to further stimulate glucose transport in isolated adipocytes after this period (data not shown). Another group of diabetic animals was subjected to daily intraperitoneal injection of vanadate (50 μmol/kg body wt) for 2 weeks, as compared with a group of diabetic rats that

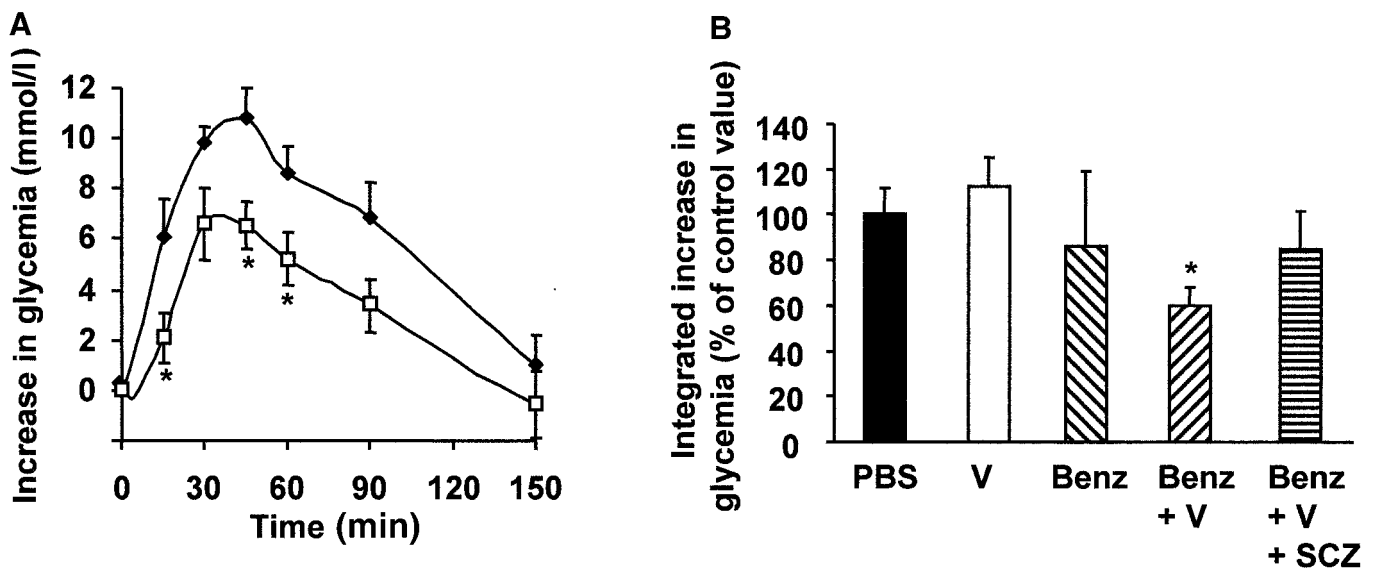


FIG. 2. Effect of the acute administration of benzylamine plus vanadate on oral glucose tolerance test in STZ-induced diabetic rats. A: Relative changes in glycemia with an oral glucose load (2 g/kg) starting 15 min after intravenous injection of vehicle (◆) or 20 μmol/kg vanadate + 7 μmol/kg benzylamine (□). Results are expressed as increments in plasma glucose. B: Integrated increase in glycemia. Concentrations of plasma glucose at time zero were 21.1 ± 1.2, 23.1 ± 2.3, 23.2 ± 3.9, 21.2 ± 1.3, and 26.2 ± 1.1 mmol/l in diabetic rats untreated (PBS) or treated with vanadate (V), benzylamine (Benz), Benz + V, and Benz + V + semicarbazide (SCZ), respectively. Data are means ± SE of 4–15 observations expressed as percentage, with 100% corresponding to the PBS group. *A significant difference versus PBS group at $P < 0.05$.

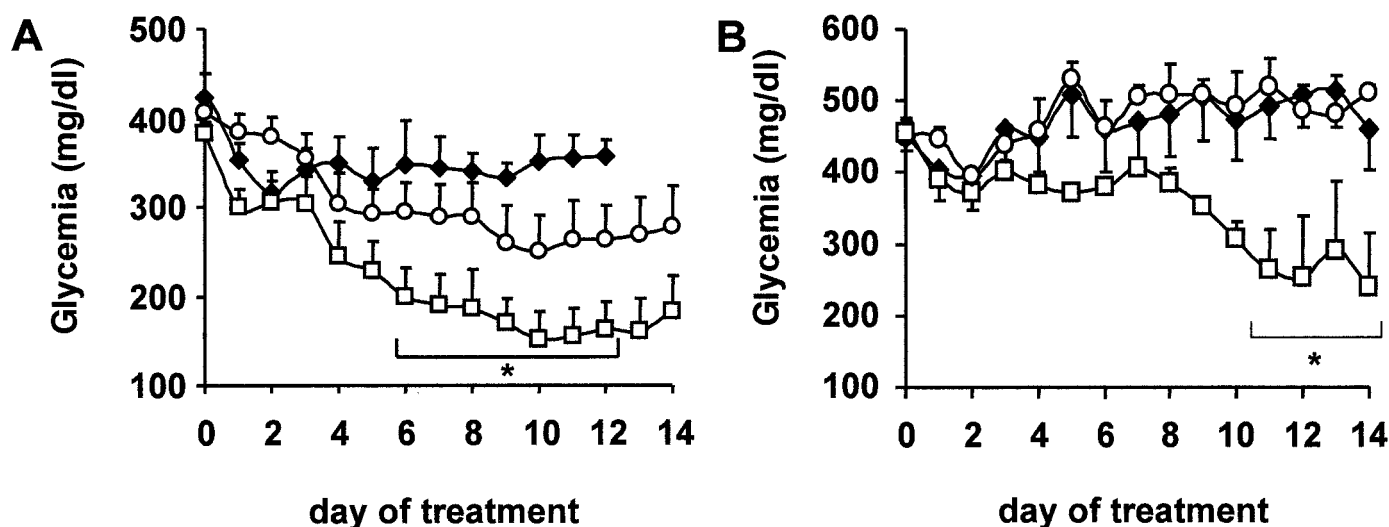


FIG. 3. Chronic treatment with benzylamine and vanadate ameliorates glycemia in STZ-induced diabetic rats. **A:** Diabetic rats received daily intraperitoneal injection of vanadate ($50 \mu\text{mol/kg}$) alone (\circ) or in combination with benzylamine ($84 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (\square) delivered by osmotic minipumps. Control animals (\blacklozenge) were sham-operated diabetic rats that received a daily injection of the vehicle (PBS). Data are means \pm SE of 4–12 observations. *Significantly different from sham-operated group from day 6 at $P < 0.05$. **B:** Rats received daily intraperitoneal injections of vanadate at 25 and $50 \mu\text{mol/kg}$ body wt for the 1st and 2nd weeks, respectively. Glycemia of diabetic rats untreated (PBS; \blacklozenge) or treated with vanadate (\circ) or benzylamine plus vanadate (\square) is presented. Data are means \pm SE of three to six observations per group. *Significantly different ($P < 0.05$) versus PBS group from day 11.

received both benzylamine and vanadate. Although treatment with vanadate caused a moderate reduction of glycemia, only the combination of benzylamine plus vanadate normalized glycemia after 1 week of treatment (Fig. 3A). Administration of benzylamine plus vanadate for 2 weeks did not alter SSAO activity in extracts from adipose cells (data not shown).

In other studies, diabetic rats were daily injected intraperitoneally with $25 \mu\text{mol/kg}$ vanadate for the 1st week and $50 \mu\text{mol/kg}$ vanadate for the 2nd week. These rats, as well as diabetic rats treated with $84 \mu\text{mol/kg}$ benzylamine alone, remained hyperglycemic (Fig. 3B; data not shown). However, the combination of benzylamine plus vanadate reduced hyperglycemia in diabetic rats from day 10 of treatment (Fig. 3B).

The chronic treatment with benzylamine plus vanadate caused a substantial decrease in food and water consumption to normal levels, and a 45% increase in the weight of epididymal adipose tissue (Table 4). All these variations occurred in the absence of changes in body weight. The effects on food and water intake were not detected when benzylamine or vanadate was administered alone (Table 4).

The normalization of glycemia caused by chronic treat-

ment with both benzylamine and vanadate took place in the absence of changes in plasma insulin concentrations (Table 4).

Chronic administration of benzylamine plus vanadate stimulated glucose transport and GLUT4 expression in adipocytes from diabetic rats. To determine whether the antidiabetic effects caused by chronic treatment with benzylamine plus vanadate were a consequence of stimulation of peripheral glucose utilization, we analyzed glucose transport and glucose transporter expression in adipose cells obtained from rats treated for 2 weeks with different combinations of benzylamine and/or vanadate. Treatment for 2 weeks with benzylamine plus vanadate stimulated basal glucose transport in isolated adipocytes and normalized insulin-stimulated glucose transport (Fig. 4A). These effects were not detected in adipose cells obtained from rats treated with benzylamine or vanadate alone. Indeed, in diabetic rats there was a negative correlation between glycemia at the end of the experiment and basal glucose transport in adipose cells (Fig. 4B).

Treatment with benzylamine plus vanadate increased GLUT4 expression (by sevenfold) more than vanadate

TABLE 4

Effect of chronic treatment with benzylamine and vanadate on body weight, epididymal adipose tissue weight, food and water intake, and plasma insulin

	Body weight (g)	Adipose tissue weight (g)	Food intake (g)	Water intake (ml)	Insulin (ng/ml)
Diabetic rats					
PBS treated	235 ± 10	1.33 ± 0.31	40 ± 2	176 ± 29	0.45 ± 0.07
Vanadate treated	261 ± 11	1.42 ± 0.19	36 ± 7	158 ± 42	0.56 ± 0.08
Benzylamine treated	250 ± 10	1.41 ± 0.15	39 ± 3	154 ± 18	ND
Benzylamine + vanadate treated	259 ± 16	1.93 ± 0.13	$22 \pm 3^\dagger$	$50 \pm 15^\dagger$	0.59 ± 0.07
Nondiabetic rats	$278 \pm 2^\dagger$	$2.83 \pm 0.21^\dagger$	$21 \pm 1^\dagger$	$31 \pm 2^\dagger$	$1.10 \pm 0.30^*$

Data are means \pm SE of three to seven rats per group. Diabetic rats correspond to groups presented in Fig. 3B. All groups were age-matched. * $P < 0.05$, $^\dagger P < 0.01$ vs. PBS group. ND, not determined.

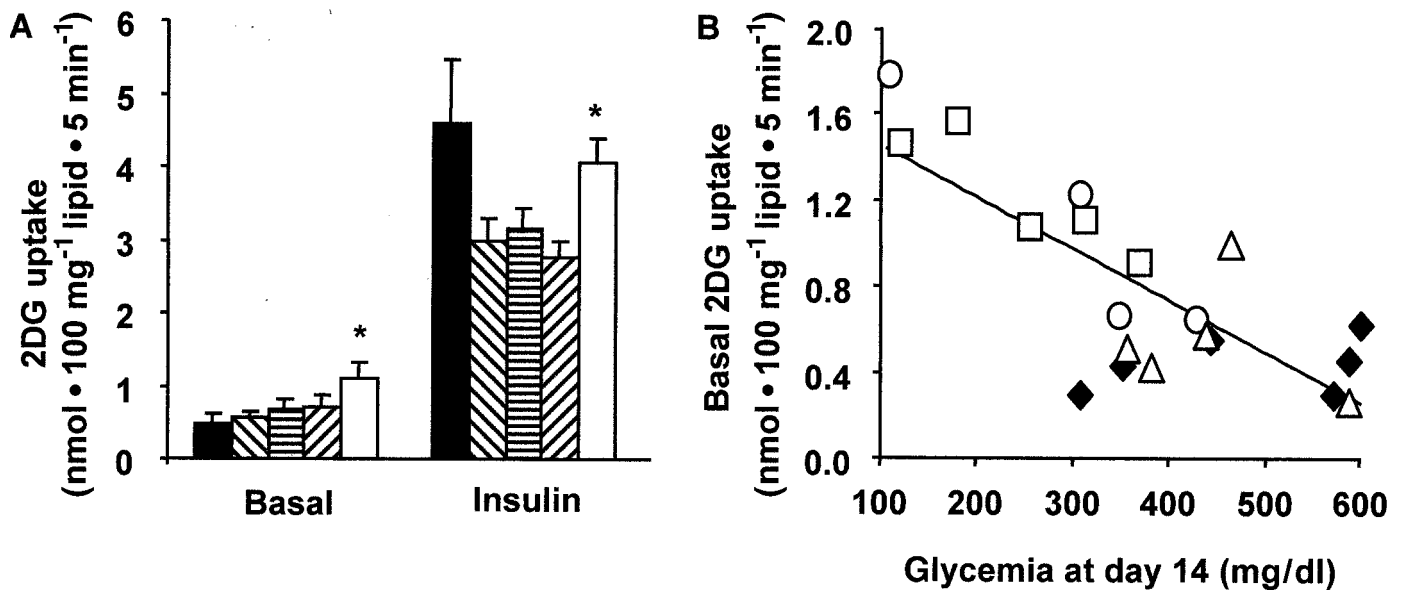


FIG. 4. Chronic treatment with benzylamine plus vanadate stimulates glucose transport in adipocytes from diabetic rats. *A*: STZ-induced diabetic rats were implanted subcutaneously with an osmotic pump containing benzylamine ($84 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or sham-operated. Rats received daily intraperitoneal injections of vanadate at 25 and $50 \mu\text{mol/kg}$ body wt for the 1st and 2nd weeks, respectively. After 2 weeks of treatment, isolated adipocytes from nondiabetic rats (■) and untreated (▨), vanadate-treated (▩), benzylamine-treated (▩), and benzylamine plus vanadate-treated diabetic rats (□) were obtained from epididymal adipose tissue. Adipose cells were incubated for 30 min in basal conditions or in the presence of 100 nmol/l insulin. Subsequently, 2-DG transport was measured for 5 min (expressed as $\text{nmol} \cdot 5 \text{ min}^{-1} \cdot 100 \text{ mg}^{-1}$ lipid). Data are means \pm SE of four to five observations per group. * $P < 0.05$. *B*: Correlation between basal glucose transport in isolated rat adipocytes and plasma glycemia of the rats ($r = 0.77$, $P < 0.01$). Data from rats treated with PBS (◆), vanadate (○), benzylamine (△), and benzylamine plus vanadate (□) are shown.

alone (Fig. 5). In addition, there was a correlation between the amount of GLUT4 and basal glucose transport in adipocytes (data not shown). No changes in GLUT4 expression were detected in soleus or extensor digitorum longus muscles in response to chronic treatment with benzylamine plus vanadate (data not shown).

DISCUSSION

Previous studies have demonstrated that many SSAO substrates, in combination with low vanadate concentrations, stimulate glucose transport in rat and 3T3-L1 adipose cells (14). The stimulation of glucose transport is dependent on SSAO activity and hydrogen peroxide production rather than on aldehyde production (13). The enhanced glucose transport induced by SSAO substrates plus vanadate is characterized by the recruitment of GLUT4 glucose transporters to the cell surface, as demonstrated by subcellular fractionation and plasma membrane lawn techniques (12,13). As to the nature of the mechanisms involved, SSAO substrates plus vanadate markedly stimulate tyrosine phosphorylation of IRS-1 and -3 as well as PI 3-kinase activity (14). On the basis of these data, we proposed that SSAO substrates and vanadate synergistically stimulate one or several tyrosine protein kinases or inhibit protein tyrosine phosphatases, leading to activation of an intracellular pathway similar to that triggered by insulin (14).

In keeping with these observations in vitro, we found that acute administration of an SSAO substrate, benzylamine, together with a low dosage of vanadate, enhances glucose tolerance in nondiabetic and diabetic rats. This effect did not alter the profile of plasma insulin concentrations after glucose challenge, was not associated with alterations in renal glucose reabsorption, and re-

quired SSAO activity. These data indicate that the combination of benzylamine and vanadate stimulates glucose disposal after a glucose challenge in vivo.

Because the benzylamine plus vanadate combinations stimulated glucose transport in isolated rat adipocytes from nondiabetic and diabetic rats, we suggest that adipose tissue participates in this response. In addition, given the low concentrations of insulin in diabetic rats, we favor the view that benzylamine and vanadate have insulin-like effects in adipose tissue.

Interestingly, the chronic administration of benzylamine plus vanadate reduced glycemia in STZ-induced diabetic rats, whereas in some experimental protocols, this combination normalized glycemia. As to the time-dependence of these effects, a moderate reduction of glycemia was already noted at day 5–6 after the onset of treatment, which is similar to the time-dependence shown by vanadate or peroxovanadate treatments (24,25). This antihyperglycemic effect of benzylamine plus vanadate was also accompanied by normalization in food and water intake and, as reported for vanadate alone (26), was not a consequence of any increase in plasma insulin.

The chronic administration of benzylamine plus vanadate stimulated glucose uptake in adipose cells. Thus, cells obtained after combined benzylamine and vanadate treatment showed enhanced rates of basal and insulin-stimulated glucose transport and GLUT4 expression. These effects were observed only when both compounds were given in combination but not when they were given separately. The enhanced basal glucose transport may have been a result of the acute effects of benzylamine and vanadate in promoting GLUT4 recruitment to the cell surface or the presence of a larger GLUT4 population. However, the enhanced glucose uptake by adipose cells

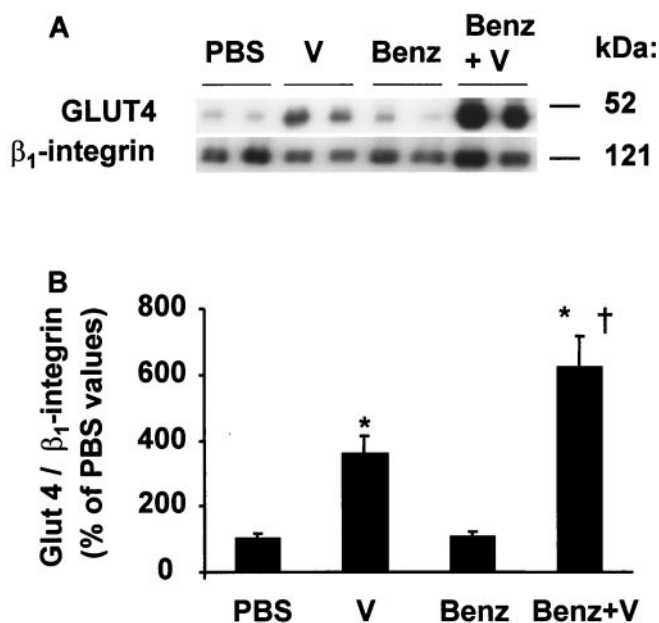


FIG. 5. Chronic treatment with benzylamine plus vanadate stimulates GLUT4 glucose transporter expression in adipocytes from diabetic rats. STZ-induced diabetic rats were implanted subcutaneously with an osmotic pump containing benzylamine ($84 \mu\text{mol} \cdot \text{kg}^{-1} \text{body wt} \cdot \text{day}^{-1}$) or were sham-operated (\blacklozenge). Rats received daily intraperitoneal injections of vanadate at 25 and $50 \mu\text{mol/kg body wt}$ for the 1st and 2nd weeks, respectively. After 2 weeks of treatment, isolated adipocytes from untreated diabetic rats and diabetic rats treated with vanadate, benzylamine, and benzylamine plus vanadate were obtained from epididymal adipose tissue. Total adipocyte membranes were obtained and subjected to SDS-PAGE and further immunoblotting using specific antibodies directed against GLUT4 or β_1 -integrin. **A:** Representative autoradiogram. **B:** GLUT4 abundance corrected by β_1 -integrin. Data are means \pm SE from six independent experiments. *Significant differences with the PBS group at $P < 0.05$; †significant difference with the vanadate-treated group at $P < 0.05$.

and the normalization of glycemia may also have been consequences of enhanced insulin sensitivity.

Our data indicate that the combination of benzylamine and vanadate was required for both acute and chronic effects; as previously observed in vitro (12,13), benzylamine or vanadate alone had little or no effect. Studies of adipose cells in vitro have suggested that hydrogen peroxide production is crucial for triggering the stimulation of glucose transport and GLUT4 recruitment to the cell surface, as catalase blocks the effects (12,13). Given that peroxovanadate is a potent insulin-like agent (27–31), it may be formed either inside or outside the cell and may be responsible for the effects of the combination of vanadate and SSAO substrates. Based on the fact that peroxovanadate inhibition of protein tyrosine phosphatases (PTPs) is irreversible, whereas the effect of vanadate is reversible (32), we have indirectly evaluated the generation of peroxovanadate by assessing PTP activity in the presence of 1 mmol/l EDTA and using extracts from adipose cells obtained from diabetic rats chronically treated with different compounds. Chronic vanadate treatment inhibited PTP activity, a result that is in keeping with prior observations (33); the administration of vanadate plus benzylamine also inhibited PTP activity, which was comparable to the effects of vanadate alone (data not shown). These data indicate that there is no correlation between total PTP activity and activation of basal glucose transport in adipose cells or reduction of hyperglycemia in

diabetic rats treated with vanadate or benzylamine plus vanadate. Thus, although peroxovanadate may explain the effects of benzylamine plus vanadate, our data do not justify any conclusion on whether peroxovanadate is the only signal generated in adipose cells in response to benzylamine plus vanadate treatment.

Restoration of adipose glucose transport activity may be insufficient to normalize glucose levels in diabetic rats after benzylamine plus vanadate treatment. In adipose cells, benzylamine plus vanadate may generate molecules that have insulin-like effects in other insulin-sensitive tissues. In addition, amelioration of adipose tissue metabolism subsequent to benzylamine plus vanadate treatment, via molecules or hormones released from adipose cells, such as leptin, tumor necrosis factor- α , or resistin (34–36), may have a profound impact on muscle metabolism. It is important to determine whether skeletal muscle increases glucose disposal in response to benzylamine and vanadate.

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