Benzylamine Exhibits Insulin-Like Effects on Glucose Disposal, Glucose Transport, and Fat Cell Lipolysis in Rabbits and Diabetic Mice

Maria Carmen Iglesias-Osma, Maria José García-Barrado, Virgile Visentin, Maria Francisca Pastor-Mansilla, Sandy Bour, Danielle Prévot, Philippe Valet, Julio Moratinos, and Christian Carpenté

Unité de recherches sur les obésités, Institut National de la Santé et de la Recherche Médicale U586, Institut Fédératif de Recherche 31, Centre Hospitalier Universitaire de Toulouse, Université Paul Sabatier, Toulouse, France (V.V., S.B., D.P., P.V., C.C.); and Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Salamanca, Salamanca, Spain (M.C.I.-O., M.J.G.-B., M.F.P.-M., J.M.)

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ABSTRACT

Benzylamine, a substrate of semicarbazide-sensitive amine oxidase (SSAO), stimulates glucose transport in rat adipocytes and improves glucose disposal in diabetic rats only in the presence of vanadate. These effects have been described to result from a synergism between the hydrogen peroxide formed during amine oxidation and vanadate, via the generation of pervanadate, a powerful insulin mimicker. However, it has also been reported that benzylamine alone can stimulate glucose uptake and inhibit lipolysis in human fat cells. In this work, we therefore investigated whether benzylamine on its own was able to induce both in vivo and in vitro insulin-like responses in animal models other than rat. In rabbits, the i.v. infusion of 7 μmol/kg benzylamine before a glucose tolerance test resulted in a net reduction of the hyperglycemic response without a change in insulin secretion. Benzylamine also improved glucose tolerance and reduced lipid mobilization in hyperglycemic/obese mice. In vitro, 0.1 mM benzylamine stimulated glucose transport and inhibited lipolysis in mouse and rabbit adipocytes. These effects were blocked by previous treatments with semicarbazide, a SSAO inhibitor. Levels of benzylamine oxidation were more elevated in mouse than in rabbit adipose tissues, whereas the reverse was observed for skeletal muscles. Finally, benzylamine was unable to stimulate insulin secretion by isolated pancreatic islets from both species and SSAO activity was hardly detectable in pancreas. Together, our results bring evidence that benzylamine on its own can improve glucose tolerance in rabbit and mouse, likely by stimulating glucose uptake via amine oxidase activation in insulin-sensitive tissues.

Semicarbazide-sensitive amine oxidase (SSAO) is a widely expressed copper-containing enzyme (Lyles, 1996). However, SSAO is especially abundant in endothelial cells (Salmi et al., 2001), vascular smooth muscle cells (Langford et al., 2002), and adipocytes (Raimondi et al., 1991). SSAO was primarily defined as a scavenger of endogenous or exogenous amines (such as benzylamine, a non-naturally occurring amine) because this ecto-enzyme (Salminen et al., 1998) belongs to the large family of amine oxidases that deaminate mono-, di-, or polyamines into the corresponding aldehydes, with subsequent production of ammonia and hydrogen peroxide (Buffoni, 1995). Although the metabolic reactions catalyzed by diverse amine oxidase activities have been known for decades (Blaschko, 1952), their physiological and pathological implications are still under investigation (Yu et al., 2003b). In its vascular location, SSAO, also called vascular adhesion protein-1, seems to be involved in lymphocyte adhesion to the vascular walls (Salmi et al., 2001). In smooth muscle cells, SSAO seems to interfere with extracellular matrix development (Langford et al., 2002). Regarding adipocytes, it has been found that glucose transport can be enhanced in vitro when adding SSAO substrates (benzylamine and methyl-
amine) to these cells together with vanadate at a dose without effect on its own (Enrique-Tarancon et al., 1998). In fact, besides a weak insulin receptor phosphorylation, benzylamine plus vanadate promoted phosphorylation of insulin receptor substrates, activation of phosphoinositide 3-kinase, and translocation of the glucose transporters GLUT4 to the cell surface (Enrique-Tarancon et al., 2000). These effects could be blocked by amine oxidase inhibitors or antioxidants (catalase, glutathione, and N-acetylcysteine) (Marti et al., 1998). They have been shown to result from a chemical interaction between vanadate and hydrogen peroxide, one end-product of the SSAO-dependent amine oxidation (Abella et al., 2003). The complex generated, peroxovanadium, is known as a potent insulin mimicker (Nolte et al., 2003) and reproduced all the in vitro effects of the combination of amine plus vanadate. Insulin-like effects of the association SSAO substrates-vanadate were also observed in vivo: the amines plus vanadate. Insulin-like effects of the association SSAO substrates-vanadate were also observed in vivo: the combination of inactive doses of vanadate (20–50 μmol/kg) and benzylamine (7–84 μmol/kg) was able to enhance glucose tolerance and to reduce hyperglycemia in streptozotocin-induced diabetic rats (Marti et al., 2001) and in a rat model of type 2 diabetes (Abella et al., 2003). Meanwhile, in vitro observations reported that benzylamine on its own, was able to promote diverse insulin-like responses, because 0.1 to 1 mM benzylamine, as well as other SSAO substrates, promoted adipocyte differentiation of murine preadipocyte lineages (Fontana et al., 2001; Mercier et al., 2001), inhibited lipolytic activity, and stimulated heroxen uptake in human adipocytes (Morin et al., 2001). These differences in the vanadate requirement for the observation of insulin-mimicking effects with benzylamine led us to further study in vivo the putative antihyperglycemic properties of benzylamine in species other than rat. To this aim, we have studied the effects of benzylamine on glucose tolerance tests (GTTs) in the rabbit, an excellent model for the human glucose homeostasis (Moratínos et al., 1986, 1988), and in the mouse, which accounts for a growing number of either genetically or nutritionally induced models of diabetes and/or obesity (Valet et al., 2002). Then, we explored the mechanisms of the antihyperglycemic effects of benzylamine. Involvement of a direct stimulation of peripheral glucose uptake was assessed by measuring heroxen transport in isolated adipocytes, whereas benzylamine influence on insulin secretion was tested in isolated pancreatic islets. We have also tested whether benzylamine could inhibit lipolysis in fat cells and reduce lipid mobilization. Finally, the benzylamine oxidation was studied in muscles, adipose tissues, and pancreas.

The following results show that in vivo benzylamine improved by its own the glucose disposal and attenuated lipid mobilization. In vitro, benzylamine was able to stimulate glucose uptake and to reduce lipolytic activity in fat cells in a semicarbazide-sensitive manner, whereas it was unable to increase insulin secretion in vivo or in isolated pancreatic islets. Accordingly, benzylamine was much more oxidized by adipose tissues and muscles than by pancreas. These observations allow us to consider that, in rabbits as well as in mice, the non-naturally occurring amine oxidase substrate benzylamine is an insulin-mimicking rather than an insulin-releasing drug.

### Materials and Methods

**Chemicals.** 2-[1,2-3H]Deoxyglucose (2-DG, 26 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA) and [14C]benzylamine (57 mCi/mmol) came from Amersham Biosciences UK Ltd. (Buckinghamshire, Little Chalfont, UK). Collagenase, cytochalasin B, fatty acid-free bovine serum albumin, hydrogen peroxide, sodium orthovanadate, semicarbazide, benzylamine, isobutyrylthiophenanthrene, human and bovine insulin, and other chemicals were from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

**Animals and Tissue Sampling.** Male New Zealand White rabbits aged 7 to 12 months (body weight between 2.8 and 3.5 kg) were individually housed at 22°C with free access to food and water. The 20- to 30-week-old mice used in this study were of both sexes from the C57BL/6J strain. They were grown under rodent standard chow diet or submitted for 3 months to a very high fat diet containing, as percentage of energy content, 72% fat (corn oil and lard), 28% protein, and <1% carbohydrate (Burcelin et al., 2002). Drug administration and blood sampling necessary for GTTs were performed in conscious animals, whereas in vitro experiments were performed with tissues removed from euthanized animals and immediately used for pancreatic islet or fat cell isolation and subsequent determinations of insulin secretion, glucose transport, or lipolysis. Samples of plasma, pancreas, adipose, or muscular tissues were frozen until determination of amine oxidase activity.

**Intravenous Glucose Tolerance Tests.** The experimental design carried out in 24-h fasted rabbits has been fully described in previous publications (Moratínos et al., 1988; García-Barrado et al., 2004). Arterial blood was sampled by means of an indwelling cannula placed in the central artery of one ear. Two control samples separated by an interval of 30 min were taken before drug infusion. Drug solutions were infused at a constant rate (0.15 ml/min) for 30 min through an indwelling cannula in the marginal vein of the contralateral ear. The arterial cannula was kept functional by a slow constant infusion of physiological saline (0.07 ml/min). Plasma glucose was estimated by means of the glucose oxidase procedure using a commercial kit (Roche Diagnostics, Mannheim, Germany); immunoreactive insulin was determined by using a radioimmunooassay kit (CIS Radioquimica-Scherer, Madrid, Spain).

**Intraperitoneal Glucose Tolerance and Lipid-Mobilizing Tests.** Mice of 25 to 30 g were fasted during 6 h (from 8:00 AM to 2:00 PM) before GTT. A bolus of benzylamine hydrochloride at 1 mg/kg (7 μmol/kg) or vehicle (0.9% NaCl, saline) was i.p. administered before a glucose load. Blood samples were drawn from tail vein of conscious animals at the indicated times before and after the glucose load (i.p. bolus of 1 g/kg injected in saline at time 0). Blood glucose was determined with Glucotrend II glucometer (Roche Diagnostics). Similarly, lipid mobilization was tested in response to isoproterenol (0.4 μmol/kg i.p.), and plasma free fatty acids (FFA) was determined using a spectrophotometric kit (NEFA C; Wako Chemicals, Neuss, Germany).

**Insulin Secretion in Pancreatic Islets.** Islets were isolated by collagenase digestion of rabbit or mouse pancreas. After isolation, islets were first preincubated for 60 min at 37°C in a bicarbonate buffer medium, pH 7.4, containing 15 mM glucose and supplemented with 1 μmol/l bovine serum albumin fraction V (Roche Diagnostics). Batches of three islets were then incubated for 60 min in 1 ml of medium containing appropriate concentrations of glucose and test substances, as detailed in Jonas et al. (1994). At the end of the incubation, a portion of the medium was withdrawn for insulin determination with a radioimmunoassay kit (CIS Radioquimica-Scherer).

**Lipolytic Activity in Isolated Adipocytes.** The epididymal and perirenal fat pads (intra-abdominal white adipose tissue, INWAT) were removed and minced with scissors in Krebs-Ringer containing 15 mM sodium bicarbonate, 10 mM HEPES, and bovine serum albumin (3.5% w/v) (pH 7.4). The white adipose tissues were digested for 35 to 45 min at 37°C with 1.5 mg/ml collagenase. Isolated
fat cells were washed three times in a large amount (around 20 ml) of the same buffer without collagenase. After being washed, the floating fat cells were diluted in around 10-fold their volume of Krebs-Ringer containing 15 mM sodium bicarbonate, 10 mM HEPES, and bovine serum albumin, and 400 µl of the cell suspension was immediately distributed under shaking into plastic incubation vials containing 4 µl of drug dilutions at 100 µM Final concentration to be tested. After a 90-min incubation, the glycerol released into the medium was enzymatically assayed as described previously (Morin et al., 2001).

**Deoxyglucose Transport in Isolated Adipocytes.** Fat cell suspensions were prepared as described above in the presence of pyruvate 2 mM instead of glucose 6 mM. Incubations with the tested drugs lasted 45 min at 37°C in a final volume of 400 µl. Then, 2-DG (0.4 µCi) was added at a final concentration of 0.1 mM for 10 min. Assays were stopped with 100 µl of 100 µM cytochalasin B, and aliquots of the cell suspension were centrifuged in microtubes containing di-isononyl phthalate (density 0.974 g/ml), which allowed to separate adipocytes from the buffer and to count the radioactive intracellular 2-DG as described previously (Morin et al., 2002).

**Amine Oxidase Activity.** Oxidase activity was measured using [14C]benzylamine according to the radiochemical method developed by Fowler and Tipton (1981) with slight modifications described previously (Morin et al., 2002). Briefly, homogenates of thawed tissues were incubated for 30 min at 37°C in 200 µl phosphate buffer in the presence of 0.1 mM of an isotopic dilution of [14C]benzylamine (approx. 220,000 dpm) after a 15-min preincubation without (total oxidation) or with either 0.5 mM pargyline or 1 mM semicarbazide to delineate the respective proportions of MAO and SSAO activity.

**Statistical Analyses.** Results are given as mean ± S.E.M. Statistical significance was assessed by use of Student’s t test.

**Results**

**Effect of Benzylamine Infusion on Intravenous Glucose Tolerance Test and Plasma Insulin Levels in Conscious Fasted Rabbits.** The i.v. perfusion of benzylamine alone at the dose of 0.23 µmol/kg/min (0.033 mg/kg/min) or its vehicle (saline) did not produce any significant change in the fasting levels of plasma glucose (Fig. 1A) or insulin (Fig. 1B). When benzylamine perfusion was conducted just before an i.v. glucose tolerance test (IVGTT), consisting in a 30-min glucose load of 55.5 µmol/kg/min (10 mg/kg/min), the overall hyperglycemic response was clearly reduced (increase at 30 min in animals infused with glucose alone or after benzylamine infusion was 3.55 ± 0.30 versus 1.42 ± 0.24 mM, n = 10, respectively, p < 0.001; Fig. 1A). The benzylamine effect was quickly observed because the increase in plasma glucose found during the first 15 min of glucose perfusion was attenuated and did not remain significant in benzylamine-
treated rabbits. At the end of the glucose perfusion, a significantly lower glucose plasma level was observed in the benzylamine-treated animals, whereas there was no significant change in the levels of circulating insulin (Fig. 1, A and B). In all tested rabbits, the increased plasma glucose and insulin levels returned toward basal values in approximately 60 min after the end of hexose load. The effect of a higher i.v. benzylamine dose of 2.3 μmol/kg/min (0.33 mg/kg/min) was similar on glycemia and insulinemia in conscious fasted rabbits: clear reduction of the hyperglycemic response without net changes in plasma insulin (data not shown).

**Influence of a Chronic Treatment with Semicarbazide on the Benzylamine-Dependent Improvement of Glucose Tolerance in the Conscious Rabbit.** In rabbits previously treated for 3 weeks (45 μmol/kg/d) with semicarbazide, an SSAO inhibitor of reference, the IVGTT induced the same hyperglycemic response than in nonsemicarbazide-treated animals. However, benzylamine showed no evident antihyperglycemic effect (Fig. 1C). Although in these animals the insulin levels found when infusing benzylamine + glucose were higher than those registered in nonsemicarbazide-treated rabbits, again these values were not statistically significant compared with the response attained with glucose alone (Fig. 1D). Interestingly, semicarbazide-treated rabbits showed basal insulin levels that were significantly higher than mean preinfusion values found in non semicarbazide-treated animals (22.5 ± 2.5 μU/ml, n = 17 versus 10.7 ± 0.5 μU/ml, n = 31, p < 0.05; data not shown), without any change in mean basal glycemia.

Benzylamine oxidation thus seemed to improve glucose disposal in rabbits in an SSAO-dependent manner. Although convenient for in vivo studies, the rabbit is not commonly used as a model of diabetes; therefore, we investigated whether the antihyperglycemic effect of benzylamine was also found in a more widely used model of glucose intolerance, the mouse.

**Influence of an Acute Administration of Benzylamine on Glucose Tolerance and Lipid Mobilization in Conscious Mice.** The intraperitoneal glucose tolerance tests (IPGTTs) were conducted on mice rendered obese and diabetic by 3 months of feeding with very high fat diet, as tests (IPGTTs) were conducted on mice rendered obese and diabetic by 3 months of feeding with very high fat diet, as described previously (Burrellin et al., 2002). Table 1 shows the characteristics of the animals used for IPGTTs to substantiate our hypothesis: they showed increased fatness and moderate fasting hyperglycemia together with a relative glucose intolerance, assessed by increased areas under the curve (AUC) of the glycemia in response to IPGTT. When benzylamine was administered in the hyperglycemic/obese mice 15 min before the glucose load via an i.p bolus of 7 μmol/kg, (corresponding to the total amount of benzylamine infused during 30 min in rabbits), it provoked a significant reduction of the hyperglycemic response (Fig. 2A). Single administration of the same dose of benzylamine did not modify the elevated fasting blood glucose level of VHFD mice (data not shown). A higher dose of benzylamine (700 μmol/kg) was not more effective in reducing the hyperglycemic response of the hyperglycemic/obese mice: mean AUC values of the integrated increase in blood glucose were, in arbitrary units, 830 ± 52 for saline and 624 ± 62 for benzylamine 700 μmol/kg, respectively (n = 4, p < 0.05). As in rabbits, a previous treatment with semicarbazide (27 μmol/kg/d i.p. for 3 days) did not modify the IPGTT-provoked hyperglycemia but abolished the benzylamine antihyperglycemic effect (Fig. 2B). The semicarbazide-treated mice exhibited unchanged fasting blood glucose (9.9 ± 0.3 versus 9.4 ± 0.2 mM in untreated mice, n = 22, N.S.) and insulin levels (28.2 ± 5.3 versus 24.9 ± 1.0 μIU/ml, N.S.).

The capacity to reduce lipid mobilization, another possible insulin-like effect of benzylamine, was evaluated in fasted VHFD mice. Intraperitoneal administration of benzylamine at 7 μmol/kg did not modify on its own the circulating levels of FFAs, but partially counteracted the lipid-mobilizing effect of isoproterenol, a lipolytic β-adrenergic agonist of reference (Fig. 3).

**Insulin-Like Effects of Benzylamine on Isolated Adipocytes.** Because benzylamine administration resulted in a net improvement of glycemic control and a limitation of lipid mobilization, we tested the in vitro capacities of this amine to stimulate glucose uptake or to reduce lipolysis in rabbit and mouse fat cells. A significant enhancement of 2-deoxyglucose transport was found with 0.1 mM benzylamine in both rabbit and mouse adipocytes; it represented a 2-fold increase over basal uptake (Fig. 4). The stimulation of glucose uptake by benzylamine did not reach the maximal effect of insulin even when the amine was tested at 1 mM. At this dose, the small but significant effect of benzylamine reproduced only 12 ± 2, 25 ± 5, and 21 ± 4% of the maximal insulin effect in rabbits, high fat diet-fed, and control mice (data not shown). Although moderate, compared with the maximal effect of 100 nM insulin, the benzylamine-induced stimulation of hexose uptake was similar to that obtained with physiological doses of the hormone, i.e., ranging between 1 and 10 nM (Fig. 4). Vanadate (0.1 mM), which was without any effect on basal or on insulin-dependent uptake, was unable to potentiate the partial effect of benzylamine in rabbit. However, the combination of vanadate with benzylamine 0.1 and 1 mM increased uptake in murine adipocytes up to 46 ± 8 and 59 ± 10% of the maximal insulin effect (n = 5, p < 0.01). Semicarbazide 1 mM inhibited almost totally the effect of 0.1 mM benzylamine alone or in combination with vanadate, the remaining stimulation representing only 0 ± 1 and 11 ± 2% of the maximal insulin effect (n = 5; data not shown).

Antilipolytic capacity of benzylamine was then tested on isoproterenol-induced lipolysis. Figure 5 shows that, in fat cells from both species, 1 mM benzylamine induced a rightward shift of the isoproterenol dose-response curve. Again, this partial antilipolytic effect was weaker than that of insulin. For instance, in mouse adipocytes, the submaximal stimulation induced by 0.1 μM isoproterenol was inhibited by only 18 ± 4% with 1 mM benzylamine alone and by 58 ± 8% with 100 nM insulin (data not shown). Vanadate (0.1 mM)
was unable to modify benzylamine antilipolytic effect in rabbit (data not shown), whereas it slightly improved it in mouse (reaching 33 ± 2% of inhibition of 0.1 μM isoproterenol, n = 6, p < 0.01). This partial antilipolytic action of benzylamine plus vanadate was totally prevented by 1 mM semicarbazide because inhibition returned to 4 ± 3% (n = 4). Benzylamine was also able to counteract other lipolytic agents. Table 2 shows that benzylamine inhibited in a dose-dependent manner the lipolytic action of forskolin (direct activator of adenylyl cyclase) and 3-isobutyl-1-methylxanthine (inhibitor of phosphodiesterases). Therefore, partial inhibition of the lipolytic action of isoproterenol by benzylamine was not resulting from an impairment of β-adrenoceptor activation, but was rather the consequence of postreceptor antilipolytic actions.

**Fig. 2.** Antihyperglycemic effect of benzylamine in the conscious mouse and its blockade by previous semicarbazide treatment. A, VHFD-fed mice of around 28 g received benzylamine (7 μmol/kg) or its vehicle (saline) by i.p bolus 15 min before the i.p. administration of glucose at 55.5 μmol/kg, and then blood samples were taken at the indicated times for the determination of blood glucose. Mean ± S.E.M. of 14 (benzylamine) to 20 (saline) IPGTT experiments. Different from respective control (saline) at ***, p < 0.01. B, similar IPGTT was conducted in mice previously treated with semicarbazide (27 μmol/kg/d i.p.) for 3 days. Data are given as changes in blood glucose, which averaged 9.9 ± 0.3 mM at t = 15 (22 determinations). Each point is the mean ± S.E.M. of 11 experiments.

**Fig. 3.** Time-dependent changes in circulating free fatty acids of fasted mice in response to benzylamine and isoproterenol alone or in combination. Benzylamine (7 μmol/kg) or saline was i.p injected at t = 15, whereas isoproterenol was injected at time 0 (0.4 μmol/kg, arrow) in fasted conscious mice, and blood samples were withdrawn from tail vein at the indicated times. Each point of the time-response curves of plasma FFA is the mean ± S.E.M. of four animals. Different from isoproterenol alone at *, p < 0.05.

**Fig. 4.** Stimulation of hexose uptake by benzylamine and insulin in rabbit or mouse fat cells. White adipocytes isolated from INWAT were incubated 45 min with 0.1 and 1 mM benzylamine or with increasing doses of bovine insulin before a 10-min 2-DG uptake assay. Data are expressed as relative to basal 2-DG uptake, which was set at 1 (dotted line) and accounted for 0.32 ± 0.07 and 1.98 ± 0.25 nmol of 2-DG uptake/100 mg of lipid/10 min in rabbit and mouse adipocytes, respectively. Mean ± S.E.M. of four (rabbit, white columns) or 14 (mouse, shaded columns) determinations.
Insulin-Mimicking Effects of Benzylamine

The present work brings evidence that benzylamine exerts antihyperglycemic properties in rabbit and mouse. Stimulation of glucose uptake into adipocytes is one of the mechanisms likely implicated in the blood glucose-lowering effect of benzylamine, which seemed to result from beneficial extrapancreatic actions on glucose clearance rather than from enhanced insulin release. Our observations agree with previous reports describing in vitro and in vivo insulin-mimicking actions of benzylamine or tyramine. However, the benzylamine effects observed in rabbits and mice were obtained without exogenous vanadate, which was required to observe benzylamine insulin-like actions in rats. This will be discussed below, together with the arguments demonstrating the involvement of amine-oxidases in the observed effects.

Benzylamine administration did not change fasting plasma glucose levels either in rabbits or mice. Thus, the synthetic amine seemed to be devoid of a strong hypoglycemic activity. Nevertheless, it provoked clear-cut antihyperglycemic response at the dose of 7 μmol/kg in both species. Blockade of this response in rabbits and in mice treated with the SSAO inhibitor semicarbazide is in complete agreement with former observations made in normoglycemic and streptozotocin-induced diabetic rats, which demonstrated that benzylamine antihyperglycemic effect was dependent on its oxidation (Marti et al., 2001). It can be supposed that the in vitro effects of benzylamine observed in rabbit adipocytes were also semicarbazide-sensitive, because this SSAO inhibitor blocked lipolysis inhibition and glucose transport stimulation induced by benzylamine alone or in combination with vanadate in mouse (this report; Yu et al., 2004) and human and rat adipocytes (Enrique-Tarancon et al., 1998; Morin et al., 2001; Visentin et al., 2003). We report here that regardless of its mode of administration (in vitro or in vivo), semicarbazide inhibits SSAO activity and benzylamine, but not insulin, effects. However, chronic semicarbazide treatment induced an increase in basal insulin levels without changing the glycemia in fasting rabbits. Considering that the non-naturally occurring SSAO substrate benzylamine improves glucose clearance, it could be hypothesized that SSAO inhibitors could impair a putative action of biogenic amines and may predispose to insulin resistance, therefore explaining the hyperinsulinism observed in treated rabbits, or provoke glucose intolerance, as very recently evidenced in another model of obese diabetic mice (Yu et al., 2004). Thus, the proposed use of SSAO inhibitors in diabetic states, assumed to limit the deleterious effects on vasculature resulting from SSAO-catalyzed methylamine oxidation (Yu et al., 2003), seems less useful at present and deserves further consideration.

Discussion

The comparison of the capacity of insulin-sensitive tissues (i.e., skeletal muscles and adipose tissues) to oxidize benzylamine is presented in Fig. 7. In rabbits and in obese diabetic mice, benzylamine was mainly oxidized by SSAO in the adipose tissues and by MAO in muscles. Noteworthy, the benzylamine oxidation was surprisingly higher in mouse than in rabbit adipose tissues, whereas the reverse was observed for muscles. In the rabbit, the amount of benzylamine oxidized per gram of tissue was almost similar in skeletal muscles and in adipose tissues. Thus, it can be assumed that in rabbits, the capacity of benzylamine to activate glucose uptake was comparable in these two insulin-sensitive tissues. In the mouse, benzylamine was probably more prone to be oxidized, and to exert insulin-like effects, in adipose tissues than in muscles, as a consequence of both the richness in SSAO and the enlargement of the fat depots. In both species, the anatomical location of the fat depots (intra-abdominal white adipose tissue) did not influence its content in MAO or SSAO activities. Finally, pancreas represented a quantitatively minor site of benzylamine oxidation: although reaching 1.8 ± 0.4 nmol/g tissue/min in mice (n = 15; data not shown) and being predominantly due to MAO, the total capacity of this organ to oxidize benzylamine was limited compared with the overall mass and the amine oxidase activities of muscular and adipose tissues.

Influence of Benzylamine on Insulin Secretion by Isolated Pancreatic Islets from Rabbit and Mouse.

Alongside to its insulin-like properties, benzylamine could have increased glucose tolerance in rabbit and mouse via an enhanced insulin secretion. To verify this hypothesis, benzylamine was tested on insulin release by isolated pancreatic islets. In the presence of glucose 8 mM, isolated islets responded to the secretagogue agent forskolin (1 μmol/L) with increased glucose uptake and inhibition of lipolysis (Table 3). Inhibition of SSAO activity was not followed by a clear inhibition of the insulin-dependent activation of glucose and inhibition of lipolysis. The insulin-like effect of hydrogen peroxide on glucose uptake was also unchanged in adipocytes from semicarbazide-treated mice.

Benzyalmine Oxidation in Rabbit and Mouse Tissues.

The comparison of the capacity of insulin-sensitive tissues (i.e., skeletal muscles and adipose tissues) to oxidize benzylamine is presented in Fig. 7. In rabbits and in obese diabetic mice, benzylamine was mainly oxidized by SSAO in the adipose tissues and by MAO in muscles. Noteworthy, the benzylamine oxidation was surprisingly higher in mouse than in rabbit adipose tissues, whereas the reverse was observed for muscles. In the rabbit, the amount of benzylamine oxidized per gram of tissue was almost similar in skeletal muscles and in adipose tissues. Thus, it can be assumed that in rabbits, the capacity of benzylamine to activate glucose uptake was comparable in these two insulin-sensitive tissues. In the mouse, benzylamine was probably more prone to be oxidized, and to exert insulin-like effects, in adipose tissues than in muscles, as a consequence of both the richness in SSAO and the enlargement of the fat depots. In both species, the anatomical location of the fat depots (intra-abdominal white adipose tissue) did not influence its content in MAO or SSAO activities. Finally, pancreas represented a quantitatively minor site of benzylamine oxidation: although reaching 1.8 ± 0.4 nmol/g tissue/min in mice (n = 15; data not shown) and being predominantly due to MAO, the total capacity of this organ to oxidize benzylamine was limited compared with the overall mass and the amine oxidase activities of muscular and adipose tissues.
and repeatedly treated mice, respectively. Antilipolytic effects were expressed as percentage of inhibition of 10 nM isoproterenol-induced glycerol release, which represented using as 100% reference the response to 100 nM insulin, which corresponded to an increase of basal uptake by 5.1

SSAO-dependent, and that sensitive to 0.5 mM pargyline was MAO-dependent. Effects of tested agents on 2-DG uptake into adipocytes isolated from INWAT were expressed

hyperglycemia found in benzylamine-treated animals. On

tained at 100 nM), it can explain, at least partly, the reduced amplitude compared with insulin maximal effect (ob-

small amplitude compared with insulin maximal effect (ob-

TABLE 2

Inhibitory effect of benzylamine on different lipolytic stimulations in rabbit and mouse adipocytes

Data are expressed as relative to basal lipolysis set at 100%, which was 0.28 ± 0.01 and 0.21 ± 0.03 μmol of glycerol released/100 mg of lipid/90 min in adipocytes from rabbits (n = 6–16) or mice (n = 4–10), respectively.

<table>
<thead>
<tr>
<th>Glycerol Release</th>
<th>Basal</th>
<th>Forskolin 10 μM</th>
<th>IBMX 1 mM</th>
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<tbody>
<tr>
<td>Rabbit adipocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100.0 ± 1.2</td>
<td>485.1 ± 25.3</td>
<td>237.7 ± 21.0</td>
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<tr>
<td>+ Benzylamine 0.1 mM</td>
<td>105.7 ± 3.9</td>
<td>444.5 ± 13.6</td>
<td>N.D.</td>
</tr>
<tr>
<td>+ Benzylamine 1 mM</td>
<td>122.1 ± 6.2*</td>
<td>328.4 ± 62.7*</td>
<td>172.4 ± 8.1**</td>
</tr>
<tr>
<td>Mouse adipocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100.0 ± 1.5</td>
<td>444.6 ± 19.9</td>
<td>374.2 ± 10.7</td>
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<tr>
<td>+ Benzylamine 0.1 mM</td>
<td>90.3 ± 14.0</td>
<td>393.2 ± 14.3*</td>
<td>N.D.</td>
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<tr>
<td>+ Benzylamine 1 mM</td>
<td>94.0 ± 3.8</td>
<td>302.5 ± 15.8**</td>
<td>213.3 ± 8.6***</td>
</tr>
</tbody>
</table>

IBMX, 3-isobutyl-1-methylxanthine; N.D., not determined.

Different from corresponding condition without benzylamine at *p < 0.05, **p < 0.01, and ***p < 0.001.

TABLE 3

Inhibition of adipose SSAO activity and benzylamine-induced metabolic responses in mouse adipocytes by single or repeated semicarbazide administration

Adipose tissues were obtained from untreated VHFD-mice, 2 h after single semicarbazide administration (81 μmol/kg i.p.), or after repeated treatment (27 μmol/kg i.p. daily during 1 week, last injection being 12 h before sacrifice). The part of benzylamine oxidation by SCWAT homogenates inhibited by 1 mM semicarbazide was SSAO-dependent, and that sensitive to 0.5 mM pargyline was MAO-dependent. Effects of tested agents on 2-DG uptake into adipocytes isolated from INWAT were expressed using as 100% reference the response to 100 nM insulin, which corresponded to an increase of basal uptake by 5.1 ± 0.7, 4.1 ± 0.5, and 3.6 ± 0.4-fold in untreated and single and repeatedly treated mice, respectively. Antilipolytic effects were expressed as percentage of inhibition of 10 nM isoproterenol-induced glycerol release, which represented 1.18 ± 0.18, 0.99 ± 0.20, and 1.13 ± 0.19 μmol/100 mg of lipid/90 min in the respective groups. Mean ± S.E.M. of 15 control or six semicarbazide-treated mice.

<table>
<thead>
<tr>
<th>Semicarbazide Treatment</th>
<th>Untreated</th>
<th>Single</th>
<th>Repeated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyalmine oxidation (nmol/mg protein/min)</td>
<td>0.91 ± 0.12</td>
<td>0.13 ± 0.03***</td>
<td>0.39 ± 0.02**</td>
</tr>
<tr>
<td>MAO-dependent</td>
<td>0.04 ± 0.02</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Hexose uptake stimulation (% of 100 nM insulin effect)</td>
<td>21.0 ± 2.7</td>
<td>-1.9 ± 3.9***</td>
<td>6.7 ± 4.4*</td>
</tr>
<tr>
<td>1 mM benzylamine</td>
<td>28.2 ± 4.1</td>
<td>27.8 ± 4.0</td>
<td>42.7 ± 4.3</td>
</tr>
<tr>
<td>1 mM H₂O₂</td>
<td>87.9 ± 8.4</td>
<td>88.8 ± 18.4</td>
<td>91.4 ± 5.4</td>
</tr>
<tr>
<td>Lipolysis inhibition (% inhibition of 10 nM isoproterenol-dependent glycerol release)</td>
<td>28.4 ± 4.4</td>
<td>11.0 ± 6.9*</td>
<td>4.7 ± 5.8**</td>
</tr>
<tr>
<td>1 mM benzylamine</td>
<td>55.1 ± 4.7</td>
<td>50.4 ± 7.1</td>
<td>39.7 ± 11</td>
</tr>
</tbody>
</table>

Different from untreated condition at *p < 0.05, **p < 0.01, and ***p < 0.001.

Fig. 6. Effect of benzylamine on insulin secretion by islets of Langerhans.

Islets were isolated from rabbit (open bars) or mouse (cross-hatched bars) and incubated for 1 h with 8 mM glucose (Glc), in the absence or presence of the indicated concentrations of benzylamine (Bza). Values represent mean ± S.E.M. of insulin release relative to basal (Glc 8 mM set at 100%), which was equivalent to 16.5 ± 1.2 and 13.9 ± 1.6 μIU/ml/islet/h in rabbit (n = 20) and mouse (n = 9), respectively.

Because benzylamine improved glucose clearance without enhancing insulin circulating levels, we focused our interest in testing a direct stimulatory action on glucose uptake in peripheral tissues. Although the hexose uptake stimulation induced by benzylamine alone in isolated adipocytes was of small amplitude compared with insulin maximal effect (obtained at 100 nM), it can explain, at least partly, the reduced hyperglycemia found in benzylamine-treated animals. On the one hand, a parallelism was noticed between the plateau obtained with 0.1–1 mM benzylamine on hexose uptake and the plateau of the antihyperglycemic effect of benzylamine, found between 7 and 70 to 700 μmol/kg. On the other hand, the 2-fold increase in glucose uptake provoked by benzylamine was comparable with the effect of physiological concentrations of insulin, that should be lower than 10 nM (Fig. 5) because, during IVGTT in rabbits, plasma insulin levels varied from around 10 μIU/ml to less than 100 μIU/ml, corresponding to around 0.07 to 0.7 nM (approximating that for insulin: 1 μIU/ml = 0.04 ng/ml = 0.007 pmol/ml). Accordingly, benzylamine plus vanadate has also been reported to induce a 2-fold increase in glucose uptake into fat cells, but not muscles, of rat (Yu et al., 2004).

In addition to glucose uptake activation, benzylamine exerted another insulin-like action: antilipolysis. Again, this effect was limited compared with the insulin maximal effect, as described previously in human and rat fat cells (Morin et al., 2001; Visentin et al., 2003). Reduction of triacylglycerol breakdown in adipose tissue is likely the mechanism by which benzylamine counteracts the lipid-mobilizing effect of isoproterenol. Moreover, this inhibition of lipolysis can also contribute to the glucose lowering action of benzylamine by limiting the detrimental effect of FFA on glucose disposal in peripheral tissues (Randle et al., 1963, 1988; Randle, 1998).

It is known that hydrogen peroxide and vanadate could, like insulin, stimulate glucose utilization and inhibit lipoly-
Insulin-Mimicking Effects of Benzylamine

Fig. 7. Comparison of the amine oxidase-dependent oxidation of benzylamine in adipose tissues and skeletal muscles. Oxidation is expressed as nanomoles of [14C]benzylamine oxidized per gram of tissue in 1 min. Total oxidation was measured in the absence of inhibitor; MAO-dependent oxidation corresponded to proportion that was inhibited by 0.5 mM pargyline, whereas SSAAO-dependent oxidation was the part that was inhibited by 1 mM semicarbazide. Mean ± S.E.M. of eight (rabbit, top) or 14 to 20 (mouse, bottom) determinations. Different from respective condition in muscle at *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

Comparisons of the amine oxidase-dependent oxidation of benzylamine in adipose tissues and skeletal muscles. Oxidation is expressed as nanomoles of [14C]benzylamine oxidized per gram of tissue in 1 min. Total oxidation was measured in the absence of inhibitor; MAO-dependent oxidation corresponded to proportion that was inhibited by 0.5 mM pargyline, whereas SSAAO-dependent oxidation was the part that was inhibited by 1 mM semicarbazide. Mean ± S.E.M. of eight (rabbit, top) or 14 to 20 (mouse, bottom) determinations. Different from respective condition in muscle at *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

skeletale (May and De Haën, 1979; Little and De Haën, 1980; Castan et al., 1999). It has also been reported that a relatively low dose of vanadate, without effect on its own, acts in synergism with benzylamine or tyramine to improve glucose tolerance in streptozotocin-induced diabetic rats (Marti et al., 2001; Morin et al., 2002), to inhibit lipolysis (Visentin et al., 2003), or to increase glucose transport in rat adipocytes (Enrique-Tarancon et al., 1998; Marti et al., 1998). Vanadate has been demonstrated to chemically interact with the hydrogen peroxide formed by amine oxidases, resulting in the generation of peroxovanadium. This compound, which is one of the most powerful insulin mimickers (Nolte et al., 2003), is at the origin of the synergism between amines, or hydrogen peroxide, and vanadate. Although the combination of vanadate plus benzylamine was required in rodent adipocytes to observe activation of intracellular events belonging to insulin signaling (Enrique-Tarancon et al., 2000), benzylamine alone stimulated glucose uptake in human adipocytes, without need for exogenous vanadate (Morin et al., 2001). Therefore, our study extends the insulin-mimicking properties of benzylamine per se found in human cells to rabbits and obese diabetic mice. However, it cannot be concluded that the vanadate requirement for the occurrence of an amine-induced insulin-like effect shows only interspecific differences. In a given species, there are also unexplained variations, because 1) vanadate was required to observe benzylamine stimulation of glucose uptake in 3T3-L1 (Mercier et al., 2003), but not 3T3 F442A (Fontana et al., 2001) murine preadipocyte lineages; and 2) in rat, 0.1 mM vanadate was necessary for the tyramine stimulation of hexose uptake into adipocytes but not into cardiomyocytes (Morin et al., 2002). Because tyrosine phosphatases are key elements in insulin signaling and because their inhibition by hydrogen peroxide, vanadate, or by peroxovanadium is mediated by different mechanisms (Huyer et al., 1997), they may play a role in the vanadate requirement for benzylamine action. The different fates of hydrogen peroxide (oxidation of proteins and lipids, degradation by scavenger enzymes) may also vary according to the cell model and may be involved in these unexplained differences. Although a clearer characterization of the impact of SSAAO end products on glycemic control is needed, the improvement of glucose tolerance by benzylamine in rabbit and mouse favors further studies on these models. The former will allow to follow functional changes that may occur during amine administration (cardiovascular parameters, neuroendocrine functions, or lipid metabolism), whereas the latter will allow to test the influence of chronic treatments, including amine supplementation in food, on the control of glucose utilization in genetically or nutritionally induced insulin-resistant states.

To further assess the hypothesis of an amine oxidase-mediated improvement of glucose disposal in response to benzylamine, we have evaluated benzylamine oxidation by adipose tissues, skeletal muscles, and pancreas. We showed that, in rabbit, adipose tissues and skeletal muscles shared the same overall oxidative activity toward the synthetic amine, when data of oxidation were expressed per gram of tissue. Because skeletal muscles, which account for the most quantitatively important insulin-sensitive tissue, regarding glucose utilization, are also very important in term of mass, one could expect that a non-negligible proportion of the administered benzylamine is oxidized at the muscular level. Of note, SSAAO was predominantly involved in fat depots, whereas MAO was mostly in muscles. A different pattern was observed in mice because adipose tissues were able to oxidize a greater amount of benzylamine than muscles. The remarkably high benzylamine oxidation of murine adipose tissue is mainly due to an SSAAO activity and is in agreement with the extremely elevated SSAAO expression found in rat adipocytes (Mortis et al., 1997) and in fat cells from obese diabetic mice (Yu et al., 2004). The limited SSAAO activity of endocrine pancreas is in agreement with the lack of benzylamine effect on insulin secretion. Therefore, one could expect that, in obese diabetic mice, the considerably developed fat stores metabolize a substantial part of the administered benzylamine, increase their glucose uptake, and then facilitate the reduction of hyperglycemia independently from changes in plasma insulin.

Summarizing, our observations show that benzylamine administration can influence glucose homeostasis. Although antidiabetic actions of the combination of benzylamine plus vanadate has been already reported in rat (Marti et al., 2001; Abella et al., 2003), the present study shows that in other animal species, benzylamine on its own can increase glucose clearance independently from variations in plasma insulin levels. These data have to be considered together with our former demonstration of a benzylamine-induced activation of glucose transport in human subcutaneous fat cells (Morin et al., 2001). Although it remains to be demonstrated in vivo that benzylamine really produces hydrogen peroxide at a
sufficient level to activate glucose uptake in different anatomical locations, it can already be proposed that administration of any amine oxidase substrate may improve glucose clearance. Improvement of the benzylamine properties by drug design could generate amine derivatives with remarkable antihyperglycemic actions that will deserve to be tested in mouse, rabbit, or human when possible, to assess the risks/benefits ratio of such promising antidiabetic treatments.

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References


Good clinical response is an important endpoint that reflects the effectiveness of the treatment. The clinical response is determined based on the symptoms and signs that are present in patients who receive the treatment. The clinical response may vary depending on the disease being treated and the stage of the disease.

The clinical response is often assessed through various methods, such as physical examination, laboratory tests, imaging studies, and patient-reported outcomes. The clinical response may be measured using different scales or criteria, such as the Response Evaluation Criteria in Solid Tumors (RECIST) or the Progression-Free Survival (PFS) criteria.

The clinical response is an important aspect of the effectiveness of a treatment, as it provides a measure of how well the treatment is working in patients. The clinical response may also be used to determine if a treatment is effective enough to warrant further investigation or to be considered for routine clinical use.

The clinical response is also important for determining the appropriate dosage and frequency of treatment. The clinical response may be used to adjust the dosage or frequency of treatment if the response is not optimal.

The clinical response is an important aspect of the evaluation of the effectiveness of a treatment, and it is used to determine if a treatment is effective enough to warrant further investigation or to be considered for routine clinical use.