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Glucose handling in streptozotocin-induced diabetic rats is improved by tyramine but not by the amine oxidase inhibitor semicarbazide

Virgile Visentin [a], Sandy Bour [a], Jérémie Boucher [a], Danielle Prévot [a], Philippe Valet [a], Catherine Ordener [b], Angelo Parini [b], Christian Carpéné [a,*]

[a] *Institut National de la Santé et de la Recherche Médicale, U586, IFR 31, Bat. L3, CHU Rangueil, BP 84225 31432 Toulouse Cedex 4, France*
[b] *U388, IFR 31, CHU Rangueil, Université Paul Sabatier, 31432 Toulouse, France*

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Abstract

A soluble form of semicarbazide-sensitive amine oxidase (SSAO) circulating in plasma is known to increase in type 1 and 2 diabetes. This cuproenzyme generates hydrogen peroxide, ammonia, and aldehydes when oxidizing circulating biogenic or exogenous amines. Based on the angiotoxicity of these products, inhibition of SSAO has been proposed to prevent vascular complications of diabetes. However, substrates of SSAO and monoamine oxidase (MAO) have been recently evidenced to activate glucose utilisation in insulin-sensitive tissues and to exhibit antihyperglycemic actions. To determine whether amine oxidase blockade or activation could be beneficial for diabetes, we aimed at comparing the influence of prolonged treatments with semicarbazide (SSAO-inhibitor), pargyline (MAO-inhibitor), or tyramine (amine oxidase substrate) on amine oxidase activities and glycemic control in streptozotocin-induced diabetic rats. The increase in plasma SSAO was confirmed in diabetic rats, while MAO and SSAO were decreased in subcutaneous adipose tissue when compared with normoglycemic controls. Among the diabetic rats, only those receiving tyramine exhibited slightly decreased hyperglycemia and improved glucose tolerance. Adipocytes from untreated or treated diabetic rats shared similar sensitivity to insulin. However glucose uptake activation and lipolysis inhibition in response to amine oxidase substrates combined with vanadate were impaired in rats treated with amine oxidase inhibitors. Thus, amine oxidase inhibition does not improve metabolic control while prolonged administration of tyramine slightly improves glucose disposal. It is therefore concluded that amine oxidase activation by increased substrate supply elicits insulin-like actions that may be more beneficial in diabetes than SSAO inhibition formerly proposed to prevent vascular complications.

Keywords: SSAO (Semicarbazide-sensitive amine oxidase); Monoamine oxidase; Diabetes; Insulin; Adipose tissue

1. Introduction

Biogenic amines and several xenobiotics can be metabolized by oxidative deamination, which is catalyzed in a broad range of mammalian tissues by both monoamine oxidases (MAO) and semicarbazide-sensitive amine oxidases (SSAO) (Strolin Benedetti, 2001). SSAO are cuproenzymes present as a tissue-bound form essentially in endothelial cells (Salmi et al., 2001), vascular smooth muscle cells (Conklin et al., 2004) and fat cells (Morris et al., 1997; Carpéné et al., 2003). SSAO also exists as a soluble form circulating in blood (for review, see: Boomsma et al., 2003). Although the exact origin of this soluble form is still a matter of debate, it has been repeatedly reported that soluble SSAO levels are increased in the plasma of diabetic patients, either in type 1 or in type 2 diabetes (Boomsma et al., 1999; Garpenstrand et al., 1999; Meszaros et al., 1999; Grönvall-Nordquist et al., 2001; Salmi et al., 2002; Boomsma et al., 2003). An increase in circulating SSAO activity has also been reported in streptozotocin-induced diabetic rats (Hayes and Clarke, 1990) or in alloxan-induced diabetic mice (Nordquist et al., 2002). Amine oxidase activity generates hydrogen peroxide, ammonia and aldehydes when oxidizing endogenous or exogenous amines. When catabolizing aminoacetone or methylamine, believed to be present in blood at micromolar range (Li et al., 2004), soluble SSAO can produce methylglyoxal or formaldehyde, respectively (Yu and Zuo, 1993). These highly reactive products contribute to the formation of

* Corresponding author. Fax: +33 5 61 33 17 21.
E-mail address: carpene@toulouse.inserm.fr (C. Carpéné).

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advanced-glycation end products (AGEs), which are associated with vascular complications of diabetes (Mathys et al., 2002). Therefore, it has been suggested that inhibition of circulating SSAO may reduce the production of angiotoxic agents and will limit the development of vascular complications of diabetes (Yu and Zuo, 1993; Ekblom, 1998; Yu, 1998; Kinemuchi et al., 2004). The fact that aminoguanidine, a compound that prevents AGEs formation and diabetic complications (Thornalley, 2003), is also able to inhibit SSAO (Yu and Zuo, 1997) strengthened this hypothesis.

However, it has been recently observed that SSAO substrates exhibit in vitro insulin-like properties (Zorzano et al., 2003) and that chronic administration of the SSAO substrate benzylamine reduces the hyperglycemia of experimental models of type 1 (streptozotocin-induced) or type 2 diabetes (Marti et al., 2001; Abella et al., 2003). Mitochondrial MAO has also been reported to interact with the regulation of glucose utilisation. In fact, either tyramine or serotonin elicited, in a MAO- and SSAO-dependent manner, a stimulation of hexose transport in rat cardiomyocytes (Fischer et al., 1995), fat cells (Carpéné et al., 2003), skeletal muscles (Morin et al., 2002), and cultured murine preadipocytes (Zorzano et al., 2003). In addition, other insulin-like effects of tyramine have been described in rat fat cells, such as inhibition of lipolysis (Visentin et al., 2003b) or increase in lactate production (Bairras et al., 2003), and it has been recently reported that acute treatments with tyramine improve glucose tolerance in rats, even when rendered insulin-deficient (Morin et al., 2002). Moreover, scarce reports on a putative link between MAO activity and glucose homeostasis argue that MAO may be involved in insulin secretion by pancreatic islets (Pizzinat et al., 1999; Adeghate and Parvez, 2004). These findings, showing that MAO and SSAO substrates induce to some extent antihyperglycemic effects, are in apparent contradiction with the suggestion of a protective role of SSAO inhibitors against diabetes-associated vascular damages (Yu and Zuo, 1997; Ekblom, 1998). Hence, our objective was to study the influence of chronic SSAO- or MAO-inhibition on glucose disposal in diabetic rats and to compare it with the influence of prolonged administration of the MAO/SSAO substrate tyramine.

The following results will show that the changes in MAO and SSAO activities associated with streptozotocin-induced diabetes were not limited to the well-recognized increase in circulating SSAO since they were also characterized by a quantitatively more important decrease of both MAO and SSAO activities in subcutaneous white adipose tissue. As expected, prolonged treatment with semicarbazide or pargyline further inhibited SSAO or MAO activities in white adipose tissue of diabetic rats. However, this prolonged inhibition could not relieve the impaired glucose disposal, while it reduced the in vitro pharmacological capacity of amines to stimulate glucose uptake or to inhibit lipolysis in adipocytes. On the contrary, repeated administration of MAO/SSAO substrate alone or in combination with vanadate improved glucose disposal, as already reported (Morin et al., 2002; Visentin et al., 2003a). Thus, we propose that, although presumed to limit vascular diabetic complications (Yu and Zuo, 1997; Ekblom, 1998) and to exhibit renal protective effects (Bianchi et al., 2003), amine oxidase inhibitors have only a very limited interest in diabetes, since they do not correct the defective glucose handling.

2. Materials and methods

2.1. Chemicals

[^{14}C]benzylamine (57 mCi/mmol) came from Amersham Biosciences (Buckinghamshire, UK). 2-[1,2-^{3}H]Deoxyglucose (2-DG, 26 Ci/mmol) was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). [^{14}C]tyramine (7.5 mCi/mmol), semicarbazide, pargyline, tyramine, collagenase, cytochalasin B, fatty-acid-free bovine serum albumin, and other reagents were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France).

2.2. Animals and prolonged treatment

Male Wistar rats from Harlan (Gannat, France) were individually housed with free access to food and water in accordance with the European Communities Council Directives for experimental animal care. They were rendered hyperglycemic and insulin-deficient by single injection of streptozotocin (65 mg/kg) at 5 weeks of age. Two weeks after streptozotocin challenge, a total of forty polyuric and hyperglycemic rats (mean glycemia 23.8±0.8 mM) was distributed, in different sets of experiments, into untreated or treated groups, receiving during three weeks, by i.p. injection: semicarbazide (45 μmol/kg/day), pargyline (100 μmol/kg every two days), or sodium orthovanadate (4.6 mg/kg/day). In two groups, tyramine (116 μmol/kg/day) was delivered by osmotic minipumps (Alzet, Palo Alto, CA) implanted in the dorsal region every two weeks under pentobarbital anaesthesia. Blood glucose, plasma insulin and malondialdehyde levels were determined as previously described (Visentin et al., 2003a).

2.3. Intraperitoneal glucose tolerance tests

During the last week of treatment, rats were fasted during 6 h before glucose load (i.p. bolus of 2 g/kg at time 0). At the indicated times, blood samples were drawn from tail vein of conscious animals for immediate glucose determination with Glucotrend II glucometer (Roche Diagnostics, Mannheim, D), as already detailed (Morin et al., 2002). Blood glucose was determined every 15 min on a 2-h period. Data are expressed as arbitrary units of the area under the curve (AUC) of the hyperglycemic response, taking as baseline the mean of the glycemia 15 min and immediately before glucose load.

2.4. Glucose uptake and glycerol release by isolated adipocytes

Intra-abdominal (epididymal, retroperitoneal and perirenal) white adipose tissues were removed after sacrifice and immediately used for adipocyte isolation and subsequent determinations of glucose uptake and lipolysis activities as previously described (Morin et al., 2002).

2.5. Amine oxidase activities

Subcutaneous (inguinal) adipose tissue, kidney, heart, and skeletal muscles of the hindlimb were removed and immediately frozen at −80 °C until determinations of amine oxidase activities as already detailed (Morin et al., 2002). Blood aliquots were also frozen, as well as plasma samples. For both [^{14}C]benzylamine and [^{14}C]tyramine, MAO-dependent oxidation was defined as sensitive to inhibition by pargyline 0.5 mM and SSAO-dependent oxidation was inhibited by 1 mM semicarbazide.

2.6. Statistical analyses

Results are given as mean±S.E.M. Statistical significance was assessed by use of Student's *t*-test. NS means no significant difference between the compared samples.

3. Results

3.1. Biological parameters, MAO and SSAO activities in control and diabetic rats

Body weight, adiposity and diverse biological parameters of streptozotocin-induced diabetic rats are reported in Table 1. Five weeks after single streptozotocin injection, insulin-deficiency was associated with an increase of blood glucose and plasma oxidative stress marker, while body weight gain and fat deposition were dramatically hampered. Maximal oxidation of tyramine or benzylamine was determined in different tissue homogenates to compare MAO and SSAO activities in streptozotocin-induced diabetic rats and their normoglycemic controls. MAO activities were unchanged in liver, heart, kidney and skeletal muscles (Fig. 1A). However, MAO activity was decreased in subcutaneous white adipose tissue of rats rendered hyperglycemic and hypoinsulinemic. MAO-dependent oxidation of tyramine was at the limit of detection in blood. Regarding SSAO, there was, as expected, practically no activity in hepatic extracts since hepatocytes express only MAO and since SSAO is limited to sinusoid cells that are minority in liver (Lalor et al., 2002). A weak SSAO activity was also found in heart, kidney and skeletal muscles, in agreement with previous anatomical distribution of SSAO in rat (Morin et al., 2002). SSAO activity was barely higher in blood than in the above-mentioned tissues

Table 1
Body weight, adiposity and circulating parameters in control and diabetic rats

Group of rats:	Control	Diabetic
Body weight, g	289±9	186±12^{c}
Intra-abdominal WAT weight, g	8.5±1.2	1.8±0.3^{c}
Subcutaneous WAT weight, g	5.2±0.3	1.6±0.2^{c}
Blood glucose, mM	4.5±0.2	24.8±1.6^{c}
Plasma insulin, ng/ml	3.9±0.5	0.6±0.1^{c}
Plasma malondialdehyde, μM	2.4±0.1	4.9±0.3^{c}

Mean±S.E.M. of 15 normoglycemic and 14 diabetic rats. Different from control at: $^{c}P<0.001$. WAT : white adipose tissue.

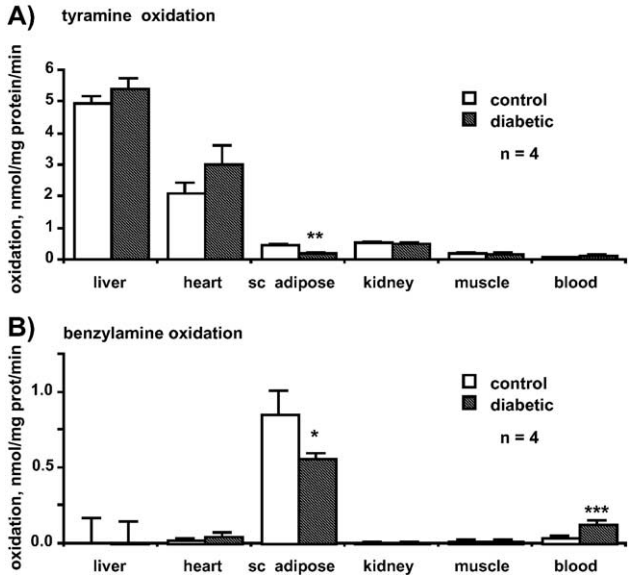

Fig. 1. MAO and SSAO activities in different tissues of control and streptozotocin-induced diabetic rats. Amine oxidation by crude homogenates of the indicated anatomical locations was measured on a 30-min period. A) Maximal MAO activity was defined as oxidation of 0.5 mM tyramine sensitive to inhibition by pargyline 0.5 mM as described in Materials and methods. B) SSAO activity was defined as oxidation of 0.1 mM benzylamine sensitive to inhibition by semicarbazide 1 mM. Mean±S.E.M. of four control (white bars) and diabetic rats (black bars). Different from control at *$P<0.05$, **$P<0.01$, ***$P<0.001$.

but was significantly increased in diabetic rats (Fig. 1B). When soluble SSAO activity was measured in the plasma fraction, it increased from 0.12±0.05 in controls to 0.46±0.02 pmol/mg protein/min in diabetics ($n=5$, $P<0.001$) in accordance with previous observations (Hayes and Clarke, 1990). This increase in SSAO plasma levels was not due to a change in plasma protein content (not shown). The most substantial change was the decrease of SSAO activity in subcutaneous white adipose tissue of diabetic rats. Of note, the SSAO activity found in this tissue was much more larger than that found in blood or other peripheral tissues (Fig. 1B). Amine oxidase activities were even higher in intra-abdominal than in subcutaneous white adipose tissue homogenates but were much less altered with diabetes: maximal activities were 0.43±0.02 vs. 0.37±0.01 nmol/mg protein/min for MAO, and 3.27±0.15 vs. 2.71±0.30 nmol/mg protein/min for SSAO, in control and diabetic rats, respectively ($n=4$, NS).

A more complete analysis of the dose-dependent oxidation of labeled tyramine and benzylamine was conducted on subcutaneous white adipose tissue homogenates. Double-reciprocal plot analysis of the saturation curves gave a V_{max} value of 0.22±0.01 and 0.12±0.02 nmol/mg protein/min ($n=4$ $P<0.001$) for the maximal velocity of MAO-dependent [^{14}C] tyramine oxidation, while K_m was 73.6±9.4 and 81.0±8.4 μM (NS) in control and diabetic groups, respectively. V_{max} of SSAO-dependent [^{14}C]benzylamine oxidation also fell from 0.64±0.06 to 0.31±0.05 nmol/mg protein/min ($n=5$, $P<0.001$), while K_m remained unchanged (46.9±4.9 and 59.4±5.1 μM, NS).

Table 2
Amine oxidase activities in subcutaneous adipose tissue of diabetic rats treated with semicarbazide, pargyline, or tyramine

Experimental group:	MAO	SSAO
	nmol of deaminated substrate/g of tissue/min	
Untreated diabetic	17.0±2.7	50.0±10.2
Semicarbazide-treated (45 μmol/kg/d)	19.6±2.2	6.0±1.5[c]
Pargyline-treated (100 μmol/kg/2d)	5.9±0.9[b]	59.7±8.2
Tyramine-treated (116 μmol/kg/d)	24.3±3.7	44.2±4.8

Mean±S.E.M. of 4 determinations. Different from untreated diabetic at: [b]$P<0.01$, [c]$P<0.001$.

3.2. Effect of repeated administration of semicarbazide and pargyline on amine oxidase activities in subcutaneous white adipose tissue

To obtain a sustained inhibition or activation of MAO and SSAO activities, diabetic rats were submitted during three weeks to treatments with the following drugs: the irreversible SSAO-inhibitor semicarbazide (45 μmol/kg/day), the irreversible MAO-inhibitor pargyline (100 μmol/kg/every two days), and the MAO/SSAO substrate tyramine (116 μmol/kg/day). An almost complete and selective inhibition of SSAO was observed in subcutaneous white adipose tissue of diabetic rats after 21 days of semicarbazide treatment, as evidenced by a severe decrease of SSAO-dependent oxidation of [^{14}C]benzylamine, while MAO-dependent oxidation of [^{14}C]tyramine was unaffected, whatever the expression of activity: per milligram protein (not shown) or per gram of tissue (Table 2). Only one-third of MAO-dependent oxidation of tyramine resisted to prolonged treatment with pargyline, whereas SSAO was unaltered. Continuous administration of tyramine did not alter the maximal in vitro activities of MAO and SSAO. Similar results were found in intra-abdominal fat depots (not shown).

3.3. Effect of semicarbazide, pargyline, tyramine and vanadate on glycemic control of diabetic rats

None of the treatments improved the reduced weight gain and adiposity of streptozotocin-treated rats, which remained polyuric and insulinopenic (not shown). Although hyperglycemic, the rats responded to intraperitoneal glucose tolerance tests by a further increase in blood glucose. Prolonged treatment with semicarbazide modified neither fasting hyperglycemia nor area under the curve of the hyperglycemic excursion during glucose tolerance test (Table 3). In diabetic rats treated with pargyline, blood glucose levels were higher than the maximal limit of detection of the portable glucometers we used; therefore glucose tolerance tests could not be completed. In a complementary set of experiments with different animals and different glucose assay (hexokinase method on Olympus AU2700 apparatus, linear up to 45 mM), pargyline treatment did not correct plasma glucose of streptozotocin-treated rats: 39.1±1.5 vs. 38.4±2.3 mM, for untreated and treated diabetic group ($n=4$, NS). Fasting blood glucose exhibited a tendency to decrease in rats receiving tyramine and was significantly reduced in rats receiving tyramine together with sodium orthovanadate (Table 3), previously shown to improve in vitro and in vivo insulin-like effects of SSAO substrates (Enrique-Tarancon et al., 2000; Abella et al., 2003). Similarly, the areas under the curve (AUC) of hyperglycemic response during glucose tolerance test showed a tendency to be lower in rats treated by tyramine alone or by tyramine plus vanadate. AUC was significantly reduced when comparing all the rats receiving tyramine to the untreated diabetic: mean was 4601±636 vs. 6785±721 ($n=10$ and 14, respectively, $P<0.05$).

3.4. Influence of chronic treatment with amine oxidase inhibitors or tyramine on glucose uptake into adipocytes

It was then tested whether the treatments with amine oxidase inhibitors could alter the insulin-like effects of MAO and SSAO substrates on glucose transport in adipocytes. Fig. 2A shows that the dose-dependent curves of insulin stimulation of hexose uptake into intra-abdominal fat cells were superimposable in control and diabetic rats. When 1 mM tyramine, methylamine or 0.1 mM benzylamine were tested, they totally reproduced the maximal insulin action only in the presence of 0.1 mM vanadate (Fig. 2B). At this dose, vanadate did not stimulate basal or insulin-stimulated uptake but potentiated the stimulatory action of amines from two-fold to eight-fold increase over basal uptake, whatever the insulinic status of the rats (Fig. 2B). Vanadate also acted in synergism with hydrogen peroxide in adipocytes from both control and diabetic rats (not shown). This synergism between vanadate and amine oxidase substrates or hydrogen peroxide has been already demonstrated to result from the generation of peroxovanadate, a powerful insulin-mimicking agent (Enrique-Tarancon et al., 2000). In adipocytes from semicarbazide-treated diabetic rats, the effect of benzylamine, methylamine and tyramine was deeply depressed (Fig. 2C) while insulin-dependent activation remained unaltered (not shown). In pargyline-treated rats, the tyramine-induced activation of hexose uptake was slightly reduced while the

Table 3
Glycemic control in diabetic rats treated with semicarbazide, pargyline, or tyramine

Experimental group	Blood glucose, mM	AUC in IPGTT
Untreated diabetic	24.8±1.6 (14)	6785±721 (14)
Semicarbazide-treated	30.4±2.5 (5)	6768±1593 (5)
Pargyline-treated	>33.3 (5)	ND
Tyramine-treated	19.9±2.4 (5)	4900±913 (5)
Tyramine+vanadate-treated	17.8±2.6 (5)*	4226±976 (5)

Two weeks after streptozotocin challenge, the hyperglycemic and insulinopenic rats were subjected to the indicated treatments for 21 days. Fasting blood glucose were determined at the time of sacrifice. During the last week of treatment, rats were fasted for 6 h before receiving an i.p. glucose load of 2 g/kg. Data are expressed as arbitrary units of the area under the curve (AUC) of the hyperglycemic response, measured during intraperitoneal glucose tolerance test (IPGTT) on a 120-min period after glucose load. Mean±S.E.M. of (n) determinations. Different from untreated diabetic at: * $P<0.05$. ND: not determined.

SSAO substrates benzylamine and methylamine remained efficient (Fig. 2C). This could be explained by the remaining MAO and unaltered SSAO activity found in adipocytes from pargyline-treated rats. Interestingly, adipocytes from rats receiving tyramine alone or together with vanadate exhibited unchanged responsiveness to the tested amines (Fig. 2C).

3.5. Influence of chronic treatments on adipocyte lipolysis

In diabetic rats, insulin inhibited the lipolytic effect of adenosine deaminase (8 IU/ml). The antilipolytic effect of 100 nM insulin was not altered whatever the treatment (Fig. 3). Tyramine and benzylamine were also able to inhibit adenosine deaminase-stimulated lipolysis, essentially in the presence of vanadate and in a MAO- and SSAO-dependent manner, as previously reported (Visentin et al., 2003b). Adipocytes from semicarbazide-treated diabetic rats did not exhibit any anti-lipolytic response to benzylamine. Tyramine antilipolytic action completely disappeared in fat cells from pargyline-treated rats, as a result of MAO inhibition, while benzylamine effect was also hampered despite an unchanged SSAO activity (Fig. 3). Methylamine also was antilipolytic at 1 mM in combination with vanadate: in its presence, the remaining lipolytic activation represented 67%±10%, 42%±17% and 50%±14% of the response to adenosine deaminase alone in normoglycemic, untreated and tyramine-treated diabetic rats, respectively ($n=4$). No methylamine-induced inhibition was detected in semi-carbazide-treated rats. Finally, 1 mM hydrogen peroxide plus 0.1 mM vanadate was always partially antilipolytic, whatever the group studied (not shown).

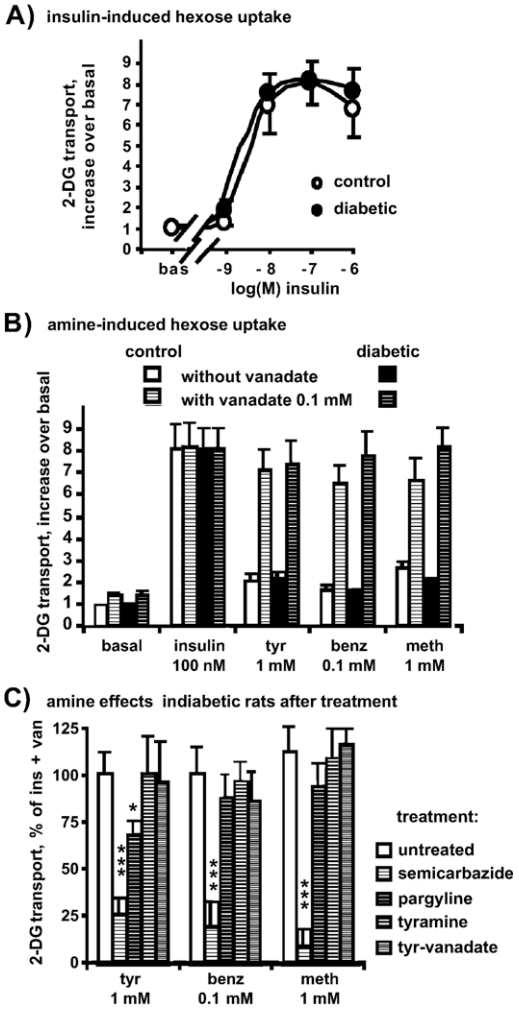

Fig. 2. Stimulation of glucose uptake by insulin (A), amines and vanadate (B) in adipocytes: influence of treatment of diabetic rats with semicarbazide, pargyline or tyramine plus vanadate (C). Adipocytes isolated from intra-abdominal white adipose tissue were incubated for 45 min with insulin (ins), 0.1 mM benzylamine (benz), 1 mM tyramine (tyr) or methylamine (meth) immediately before 2-DG uptake assay. A and B) Hexose transport was expressed as increase over basal uptake, without any addition. Mean+S.E.M. of 10–15 determinations. No significant difference was found between diabetic and normoglycemic (control) groups. C) Hexose transport was assayed in the presence of 0.1 mM vanadate on adipocytes from diabetic rats subjected to the indicated prolonged treatments. Data as percentage of maximal response to 100 nM insulin. Mean+S.E.M. of 5 treated rats in semicarbazide, pargyline, tyramine, and tyramine plus vanadate groups. Different from untreated diabetic (white bars, $n=14$) at: $^{*}P<0.05$, $^{***}P<0.01$.

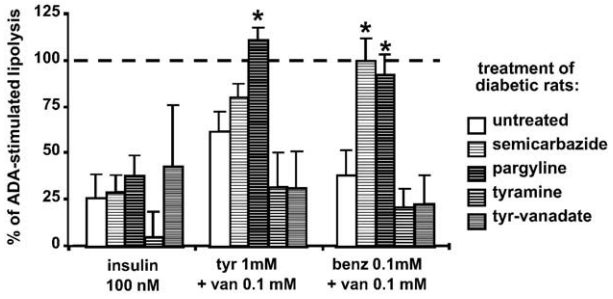

Fig. 3. Antilipolytic effect of insulin and amines plus vanadate in adipocytes from diabetic rats after treatment with amine oxidase inhibitors or with tyramine. Adipocytes were incubated for 90 min without any addition or in the presence of adenosine deaminase (ADA, 8 IU/ml) which stimulated basal glycerol release by three- to five-fold in the different groups of diabetic rats. Data are expressed as percentage of the lipolytic response to adenosine deaminase, taken as 100% (dotted line) with basal lipolysis set at 0%. Insulin, tyramine (tyr) benzylamine (benz) or vanadate (van) present when indicated. Mean+S.E.M. of 4–5 determinations. Different from untreated diabetic (white columns, $n=14$) at: $^{*}P<0.05$.

4. Discussion

Taken together, our results show that administration of SSAO inhibitor, proposed to limit vascular complications of diabetes (Ekblom, 1998), did not alleviate glucose intolerance or hyperglycemia in insulin-deficient rats. On the contrary, administration of the substrate tyramine slightly improved glucose tolerance in diabetic rats. This difference is probably the consequence of an opposite action on the amine oxidases of insulin-sensitive tissues, already reported to interact with glucose handling (Zorzano et al., 2003).

The most noticeable change in MAO or SSAO activity found in the present study between control and diabetic rats was not the well-established increase in plasma SSAO (Hayes and Clarke, 1990) but the unexpected decrease of both MAO and SSAO activities in subcutaneous adipose depots. Semicarbazide or pargyline treatment further inhibited these blunted oxidase

activities, without any detectable improvement of glucose tolerance in diabetic rats. This lack of advantageous effect of amine oxidase inhibition on glycemic control will be discussed in touch with the somewhat beneficial effects of tyramine treatment, pointing out the importance of the balance between tissue-bound and circulating amine oxidase activities.

So, our comparative approach confirms the well-known increase of circulating SSAO activity in diabetic models (Hayes and Clarke, 1990; Nordquist et al., 2002; Göktürk et al., 2004) or diabetic patients (Boomsma et al., 1999; Garpenstrand et al., 1999; Meszaros et al., 1999; Grönvall-Nordquist et al., 2001; Salmi et al., 2002; Boomsma et al., 2003). More importantly, it demonstrates that oxidative capacity of SSAO is indisputably weaker in plasma than in adipose tissue. This was not a consequence of a defect in our determination of soluble SSAO activity since our reported levels are in the range of values found in the literature: for instance a similar increase from 0.11 ± 0.01 to 0.31 ± 0.04 pmol benzylamine deaminated/mg protein/min has been already reported in the same model (Hayes and Clarke, 1990). Although being readily feasible in clinical studies, the determination of circulating SSAO may represent a surrogate marker of metabolic disturbances, but is not at all predictive of the physiopathological changes in amine oxidase activity occurring in tissues. Therefore, measuring SSAO plasma activity in future clinical trials should be associated with the determination of tissue-bound enzyme activity, which has been shown to be feasible in man, at least on needle biopsies of subcutaneous fat depots (Visentin et al., 2004).

The already proposed benefit of inhibiting the increased soluble SSAO to prevent the diabetes-associated vascular damages (Yu and Zuo, 1993, 1997; Ekblom, 1998) will be just summarized here before focusing attention on the balance between soluble and adipose tissue-bound amine oxidase activities. Although the oxidation of any given amine generates hydrogen peroxide and ammonia, the use of SSAO blockade in diabetes essentially aims at limiting oxidation of aminoacetone and methylamine and subsequent production in blood of the very reactive methylglyoxal and formaldehyde, which favour protein glycation, lipid peroxidation, and generate highly toxic stress conditions in the vicinity of endothelial cells. In this view, it has been demonstrated that administration of exogenous methylamine increases urinary levels of malondialdehyde, an end product of lipid peroxidation (Deng et al., 1998), and vascular damages in rodents (Stolen et al., 2004a). However, SSAO inhibitors are not so selective towards the soluble form, despite its increase in diabetes, and also block SSAO in other anatomical locations. Indeed, impaired vascular development can be produced by SSAO inhibitors in animal models (Langford et al., 1999), as a result of an altered involvement of the smooth muscle cell SSAO in the regulation of arterial wall elasticity (Sibon et al., 2004) or blockade of the vasoactive effects of methylamine (Conklin et al., 2004). In view of the insulin-like effects of amine oxidase substrates on adipocytes, one could ask whether SSAO blockade is beneficial or deleterious for metabolic control. In touch with this, it has been recently reported that a novel SSAO inhibitor acutely increases blood glucose, reduces glucose tolerance and can limit, via chronic treatment, adipose tissue deposition in mice (Yu et al., 2004).

In the present study, a reduction of SSAO activity was evidenced in subcutaneous adipose tissue of diabetic rats by a decrease in [^{14}C]benzylamine oxidation but also by a decrease in the SSAO-dependent component of [^{14}C]tyramine oxidation (not shown), while a significant decrease of MAO activity was also observed. Nevertheless, only a faint tendency to decrease was observed in intra-abdominal fat depots, already shown to express more MAO and SSAO (Morin et al., 2002). Since type 1 diabetes is characterized by a severe fat depletion (see Table 1), the overall amine oxidase activity of both fat depots is reduced, whatever the anatomical location, as a result of diminished expression and/or decreased fat pad size. Moreover, the alteration of SSAO activity found in subcutaneous fat depots consisted in decreased V_{max}, rather than increased K_m as previously reported for histamine oxidation (Conforti et al., 1995). Another different situation was observed for alloxan-induced diabetes in mice since SSAO gene expression, but not activity, was decreased in this model (Nordquist et al., 2002). Likewise, the increase in kidney SSAO already reported in diabetic rats (Hayes and Clarke, 1990), was not observed in our study. All these differences impede to have a clear overview of the changes in amine oxidase activities occurring during diabetes, and make difficult the understanding of the determinism and consequences of amine oxidase alterations. Although the mechanisms leading to the reduction of amine oxidase activities in fat depots remain unclear, the hypothesis of an enhanced shedding of the tissue-bound SSAO and its release into the blood stream (Abella et al., 2004; Stolen et al., 2004b) conciliate the reduction of adipose SSAO together with the increase its circulating truncated form.

Amine oxidase substrates were able to completely mimic insulin effect on hexose uptake, at least in vitro in the presence of vanadate on intra-abdominal fat cells. Although this functional aspect has not been tested in inguinal adipocytes, well-known to be less metabolically active, it appeared clearly that treatment with amine oxidase inhibitors (especially semicarbazide) impaired the amine-induced glucose uptake stimulation and lipolysis inhibition. Since adipocytes of insulin-deficient diabetic rats respond very well to exogenous insulin, amines and peroxovanadate, it is therefore difficult to understand the advantage of inhibiting in vivo the MAO or SSAO activities which can potentially generate insulin-like effects. Our findings indicate that the use of amine oxidase inhibitors in diabetes appears questionable regarding metabolic control, while chronic exposure to tyramine, an amine oxidase substrate, led to an improvement of glucose tolerance in diabetic rats.

Even though plasma insulin was not measured during our glucose tolerance tests, it can be supposed that stimulation of insulin secretion was not primarily involved in the anti-hyperglycemic action of tyramine in the streptozotocin-treated rats which were insulin-depleted. A direct stimulation of glucose utilisation by peripheral tissues, as evidenced in vitro on fat cells, is more likely to explain the tyramine-induced increase in glucose tolerance and its tendency to lower blood glucose.

The improvement of glucose handling induced by chronic treatment with tyramine plus vanadate is in agreement with our previous studies in which an acute administration of tyramine, just before a glucose load, was found to exert antihyperglycemic properties in a manner that was inhibited by semicarbazide as well as by pargyline (Morin et al., 2002). Likewise, inhibition of adipocyte lipolysis is another tyramine action (Visentin et al., 2003b) that may improve glucose tolerance, and which was impaired by semicarbazide treatment.

Based on our observations, it appears more favourable to limit methylamine oxidation in diabetes via a supplementation with a competing amine oxidase substrate which will restrain the appearance of the angiotoxic formaldehyde and which may produce, via its oxidation in insulin-sensitive tissues, a valuable action on metabolic control. We thus conclude that, although previously recommended to exert cytoprotective action in diabetic vessels (Ekblom, 1998) and kidney (Bianchi et al., 2003), prolonged administration of amine oxidase inhibitors has only a very limited interest for glucose handling under diabetic states. Novel approaches have to be tested, taking into account the balance between insulin mimicry obtained with amine oxidase substrates in adipose tissues and limitation of vascular damages putatively obtained by SSAO-dependent oxidation of methylamine and aminoacetone in blood vessels. Chronic administration of amines, being partial substrates of the truncated form or producing aldehydes which will be less reactive than formaldehyde or methylglyoxal may combine these two goals.

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