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Short- and long-term insulin-like effects of monoamine oxidases and semicarbazide-sensitive amine oxidase substrates in cultured adipocytes

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Abstract

Semicarbazide-sensitive amine oxidase (SSAO) is known to increase during in vitro adipogenesis and to be one of the most highly expressed membrane proteins of white adipocytes. Although less well documented, mitochondrial monoamine oxidases (MAOs) are also present in adipocytes and share with SSAO the capacity to generate hydrogen peroxide. This work therefore aimed to compare several biologic effects of MAO and SSAO substrates in 3T3-F442A adipocytes. In differentiated cells, tyramine oxidation was predominantly MAO dependent, whereas benzylamine oxidation was SSAO dependent. Both amines partially mimicked insulin actions, including stimulation of Akt phosphorylation and glucose uptake. In addition, tyramine and benzylamine impaired tumor necrosis factor α–dependent nitric oxide formation in a pargyline- and semicarbazide-sensitive manner, respectively. Various biogenic amines were tested in competition for tyramine or benzylamine oxidation and classified as MAO-preferring (methoxytyramine, tryptamine) or SSAO-preferring substrates (methylamine, octopamine). Short-term incubation with 1 mmol/L of all amines except histamine stimulated glucose uptake up to 20% to 50% of maximal insulin activation. One-week treatment with either MAO or SSAO substrates alone allowed postconfluent cells to differentiate into adipocytes, reproducing 60% of insulin-promoted lipid accumulation. All amines also exerted a slight improvement in the adipogenic action of insulin. Therefore, like SSAO, substrate activation of MAO can interact with adipocyte metabolism by mimicking diverse effects of insulin in addition to preventing tumor necrosis factor α–dependent responses.

1. Introduction

Adipocytes accumulate energy in the form of triacylglycerols, which, in turn, can be hydrolyzed and released as glycerol plus free fatty acids. When energy intake is dominant, excess lipid storage in white adipose tissue can be obtained via an increase in the size of mature adipocytes and/or via the recruitment of preadipocytes. This may lead not only to obesity but also to related pathologies such as type 2 diabetes mellitus because adipose tissue is not simply an insulin-responsive tissue that converts glucose into fat stores, but is also an endocrine organ participating in the regulation of energy balance [1,2]. Adipogenesis has been extensively studied in vitro through murine preadipocyte lineages. Adipose conversion from such fibroblastic precursors is divided into at least 3 steps: cell commitment, clonal amplification, and phenotype expression [3]. Initial events involve the induction of expression of several adipose-specific genes, whereas later events result in the appearance of functional characteristics of mature adipocytes such as increased glucose transport capacity, through expression of insulin-responsive glucose transporters, which represent a key step in lipogenesis, leading to progressive lipid accumulation.

In addition to the well-defined molecular events triggered by insulin [4], a strong increase in the expression of semicarbazide-sensitive amine oxidase (SSAO) has repeatedly

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been reported to occur during adipocyte differentiation [5-9]. SSAO has been considered as a late adipogenesis marker in 3T3 murine lineages owing to its impressive emergence during differentiation, initially detected at the messenger RNA level by a differential display technique [6]. Although this copper-containing amine oxidase is also expressed in endothelial cells (known as vascular adhesion protein [VAP-1]) [10] and in smooth muscle cells [11], SSAO/VAP-1 is particularly abundant in mature adipocytes [12]. Indeed, several independent investigations have observed a very high expression of SSAO in adipocytes in a variety of species [13-15], including man [16]. A small proportion of the adipose tissue–bound SSAO has been shown to reach the circulation under a soluble, truncated form and to contribute to the presence of SSAO activity in plasma, especially in diabetic conditions [17,18].

Although less well documented, monoamine oxidase (MAO) activity is also present in adipose tissue [19] and is predominantly expressed in human fat cells as MAO-A, with a minor amount of MAO-B [20]. MAO differs from SSAO regarding not only its subcellular localization (mitochondrial and cell surface, respectively) but also biochemically and pharmacologically. Despite clear differences in inhibitor specificity, these oxidases have a large number of substrates and exhibit partially overlapping selectivities toward exogenous or biogenic amines. Indeed, only few of the substrates can be considered as selective for MAO-A, MAO-B, or SSAO, and this depends on the animal species considered. Regardless of the model or the primary amine substrate considered, all amine oxidases catalyze a similar oxidative deamination reaction that produces hydrogen peroxide, aldehydes, and ammonia [21]. Hydrogen peroxide is not only involved in oxidative stress and cell signaling but is also a well-established insulin-mimicking agent, and adipose cells have been used as a convenient model to demonstrate that hydrogen peroxide enhances glucose transport and oxidation, inhibits hormone-stimulated lipolysis, and stimulates lipogenesis [22]. These insulin-like effects result from an increased tyrosine phosphorylation of intracellular proteins that is, in turn, mediated by the inhibition of protein tyrosine phosphatases via the oxidation of a unique cysteine residue of their active site [23]. We have already shown that a hydrogen peroxide–dependent mechanism is involved in the insulin-mimicking effects of an SSAO substrate of reference, benzylamine, which, in rat adipocytes and especially in the presence of vanadate, is able to increase insulin receptor substrate phosphorylation, phosphoinositide 3-kinase activity and glucose transporter translocation [24].

Our goal was therefore to verify whether stimulation of MAO activity was also able to mimic insulin effects. To this aim, we strove to compare the influence of various MAO and SSAO substrates on the metabolism of cultured murine preadipocytes. Interestingly, MAO has already been reported to be present at the preadipocyte stage in the 3T3-F442A cell line [7], whereas SSAO emerges only during the adipose differentiation process [6]. We have carried out a preliminary investigation of the effects of tyramine and benzylamine in this cell line [7]; we have completed this study and extended it to the characterization of the effects of more than half a dozen amines on glucose transport and adipogenesis. Because amine oxidases have been recently shown to mediate the modulation of inducible nitric oxide synthase (NOS2) in macrophages [25], we also studied a putative role of MAO and SSAO in the control of NOS2 induction in adipocytes. We therefore tested whether MAO or SSAO activity could modulate the tumor necrosis factor α (TNF-α)–dependent induction of NOS2 activity previously described in 3T3-F442A cells [26].

The following results show that tyramine is predominantly oxidized by MAO and, like the SSAO substrate benzylamine, partially reproduces the stimulatory effects of insulin on PKB/Akt phosphorylation, glucose transport, and lipid accumulation. Tyramine and benzylamine also inhibit lipolytic activity and counteract TNF-α action in 3T3-F442A cells. All the other amines shown to be substrates of either MAO or SSAO exhibited equivalent insulin mimicry, with the exception of histamine, which was found to be a weak substrate for both oxidases. Taken together, these findings indicate that amine oxidation favors adipocyte differentiation and suggest both MAO and SSAO as possible novel targets for therapeutic approaches for obesity and insulin-resistant states.

2. Materials and methods

2.1. Cell culture and adipocyte differentiation assessment

3T3-F442A cells were seeded (1500 cells/cm^2) and grown at 37°C under 7% CO_2 atmosphere. Cells were kept in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% donor calf serum and antibiotic mixture (100 U/mL penicillin + 100 μg/mL streptomycin) until confluence. When confluent cells were induced to differentiate by 50 nmol/L insulin or otherwise stated treatments, fresh medium containing the tested compounds was changed daily during 8 days. Postconfluent cells kept for the same period in DMEM with 5% fetal calf serum only served as negative controls. The determinations of cell number, differentiation state, and triacylglycerol and protein contents have been previously described [7].

2.2. Amine oxidase assays

With the radiometric method, amine oxidase activity was measured with cell extracts and [^{14}C]benzylamine or [^{14}C]tyramine as previously described [7]. With the fluorometric method, the oxidation of the Amplex Red probe (Interchim-Molecular Probes, Montluçon, France) was followed according to the already described procedure [10] with minor modifications. Cell extracts (50 μL) were mixed in 96-well black microplates with 50 μL of substrates and 50 μL of 200 mmol/L phosphate buffer

with or without enzyme inhibitor. Then, 50 μL of the chromogenic mixture containing Amplex Red (100 μmol/L) and horseradish peroxidase (1 U/mL) was added. Fluorescence was measured with a microplate reader set at 544/590 nm (Fluoroskan Ascent, Labsystems, Helsinki, Finland). For all oxidase assays, MAO activity was defined as the oxidation sensitive to 0.1 mmol/L pargyline, whereas SSAO activity was that inhibited by 1 mmol/L semicarbazide. When using increasing doses of substrates, the double reciprocal plot was used to determine kinetic parameters by linear regression.

2.3. PKB/Akt phosphorylation

Overnight-starved cells were incubated without (basal) or with the tested agents for 45 minutes, then cells extracts were prepared in lysing medium (TRIS, 10 mmol/L; NaCl, 150 mmol/L; sodium vanadate, 2 mmol/L; sodium dodecyl sulfate, 0.1%; NP40, 1%; sodium deoxycholate, 1%; antiprotease cocktail, 1%). Cell extracts, the protein concentration of which was determined by using the DC protein kit (BioRad), were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The blots were soaked for 1 hour at room temperature in 10 mmol/L TRIS, 150 mmol/L NaCl (pH 8) containing Tween 0.01% and 5% dried milk, and incubated overnight at 4°C in the same solution supplemented with a 1/1000 dilution of the monoclonal antibody antiphosho-Akt (ser 473) from Cell Signalling Technologies (Beverly, MA, www.cellsignal.com). An immunoreactive band of 60 kDa was detected by using horseradish peroxidase–linked secondary antibodies and enhanced chemiluminescence detection (Amersham Pharmacia Biotech, Orsay, France). The total amount of Akt was detected on destripped membranes with the polyclonal antibody anti–mouse Akt proteins from Cell Signalling Technologies.

2.4. Hexose transport and lipolysis

Cells were starved from fetal calf serum by being kept overnight in DMEM containing only 0.1% bovine serum albumin (fraction V, Sigma-Aldrich, St Quent Fallavier, France) before glucose transport assay, which consisted in a 45-minute preincubation with the tested compounds followed by a 10-minute incubation with a 2-deoxyglucose (2-DG) isotopic dilution (corresponding to 140 nmol of tritiated 2-DG and around 500 000 dpm) and a determination of the uptake radioactivity as previously defined [7]. Lipolytic activity was defined under similar conditions by determining the glycerol released after 45 minutes incubation as already described [16].

2.5. TNF-α treatment and nitrite production

Ten days after confluence in 12-well plates, cells were maintained overnight in a serum-deprived medium containing 0.1% albumin and were then treated with TNF-α (10 ng/mL for 24 hours) with or without tyramine or benzylamine (0.5 mmol/L) and the indicated inhibitors (pargyline 0.5 mmol/L or semicarbazide 1 mmol/L) in phenol-free DMEM. Nitrite accumulation was spectrophotometrically determined at 540 nm in cell supernatants by using Griess reagent (5.4% sulfanylamide and 0.1% *N*-[1-naphthyl]ethylenediamine in 0.5 mol/L HCl) as previously described [26].

2.6. Chemicals

[^{14}C]Benzylamine was from Amersham Biosciences (Buckinghamshire, UK), [^{14}C]tyramine and [2-^{3}H]DG were from Perkin Elmer (Boston, MA). All other reagents or chemicals were obtained from Sigma-Aldrich unless otherwise stated.

2.7. Statistical analysis

Data are presented as means ± SEM of n observations, where n represents the number of different replicates. Statistical differences between the groups were evaluated by using Student *t* test.

3. Results

3.1. MAO and SSAO activity in 3T3-F442A adipocytes

In differentiated 3T3-F442A adipocytes, tyramine oxidation was mainly MAO dependent as shown by its almost complete inhibition by 0.1 mmol/L pargyline (Fig. 1). Conversely, benzylamine oxidation was only sensitive to 1 mmol/L semicarbazide, indicating it to be catalyzed by SSAO. No amine oxidation was detected when pargyline and semicarbazide were both present in the assays. The maximal oxidation of labeled tyramine was similar between intact and homogenized cells, indicating that the mitochondrial location of MAO was apparently not limiting in substrate accessibility (Fig. 1). Similarly, SSAO activity was unaltered after cell disruption. Notably, the level of [^{14}C]tyramine oxidation was about 3-fold higher than the SSAO-dependent oxidation of [^{14}C]benzylamine. This was confirmed by kinetic analyses of tyramine and benzylamine oxidation by homogenates of differentiated 3T3-F442A adipocytes, conducted at 37°C and with amine concentrations ranging from 0.02 to 1 mmol/L. MAO-dependent oxidation of tyramine (resistant to 1 mmol/L semicarbazide) was characterized by a K_m of 125 μmol/L and a V_{max} of 1.54 ± 0.27 nmol (mg protein)$^{-1}$ min^{-1}. Under the same conditions, benzylamine was oxidized, in the presence of 0.1 mmol/L pargyline (MAO-A and MAO-B inhibitor), by SSAO with a K_m of 18 μmol/L and a V_{max} of 0.54 ± 0.04 nmol (mg protein)$^{-1}$ min^{-1} (n = 4, $P < .02$).

When amine oxidase activity was determined via the fluorometric detection of the hydrogen peroxide produced, tyramine oxidation was again greater than that of benzylamine at all doses studied (at 1 mmol/L, it was 0.32 ± 0.03 vs 0.14 ± 0.02 nmol [mg protein]$^{-1}$ min^{-1}, $P < .01$, n = 3). Again, tyramine oxidation was largely inhibited by 0.1 mmol/L pargyline, and therefore predominantly MAO dependent, whereas benzylamine-induced production of

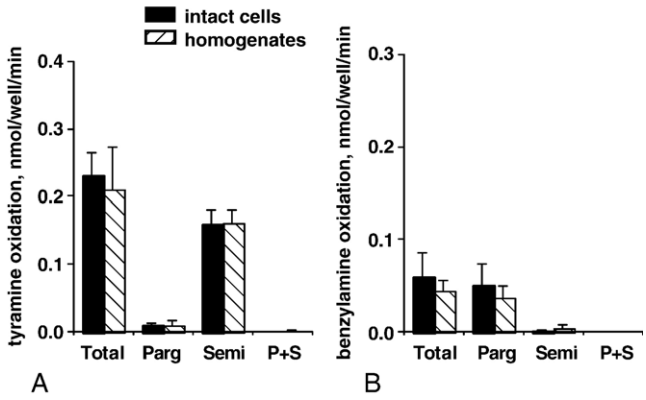

Fig. 1. MAO and SSAO activities in intact and disrupted 3T3-F442A adipocytes. Homogenized or intact cells were incubated with 0.5 mmol/L tyramine (A) or 0.1 mmol/L benzylamine (B) for 30 minutes. Total oxidation corresponds to the oxidase activity without any inhibitor. MAO and SSAO activities were determined as resistant to 1 mmol/L semicarbazide (Semi) or 100 μmol/L pargyline (Parg), respectively. P + S corresponds to the non-MAO-non-SSAO residual oxidation in the presence of both inhibitors. Mean ± SEM of 3 different experiments.

hydrogen peroxide was completely blocked by 1 mmol/L semicarbazide. Neither methylhistamine nor histamine was able to produce detectable amounts of hydrogen peroxide, even at 1 mmol/L (not shown). Because it has already been reported that tyramine and benzylamine activate glucose uptake in 3T3-F442A adipocytes in a hydrogen peroxide–dependent manner [7], we further compared their mechanism of action on this cell function and extended our comparison to their effects on other biologic responses.

3.2. Activation of glucose uptake and PKB/Akt phosphorylation, inhibition of lipolysis and counterregulation of TNF by tyramine and benzylamine

Neither the amines nor insulin was able to stimulate hexose uptake in undifferentiated preadipocytes that do not express GLUT4. However, tyramine and benzylamine activated glucose uptake in differentiated 3T3-F442A cells. This stimulation was roughly equivalent to one third of the maximal response found with 100 nmol/L insulin and was observed only at concentrations higher than the K_m of the amines toward MAO and SSAO, that is, between 0.1 and 1 mmol/L. The partial stimulation induced by tyramine or benzylamine was additive to that of the submaximal concentration of insulin: the effect of 10 nmol/L insulin represented 71% ± 8% of maximal hexose uptake and was increased up to 91% ± 6% or 92% ± 6% in the presence of 0.75 mmol/L tyramine or benzylamine (n = 4, $P < .05$, not shown). Although the amine-induced stimulations were lower than that promoted by 100 nmol/L insulin, they were completely blocked by 100 nmol/L wortmannin (Fig. 2A). It can therefore be suggested that phosphatidylinositol 3-kinase activation was involved in the observed stimulations of hexose uptake because wortmannin did not interact with MAO or SSAO activity (amine oxidation in its presence was equivalent to 96% ± 5% and 99% ± 5% of

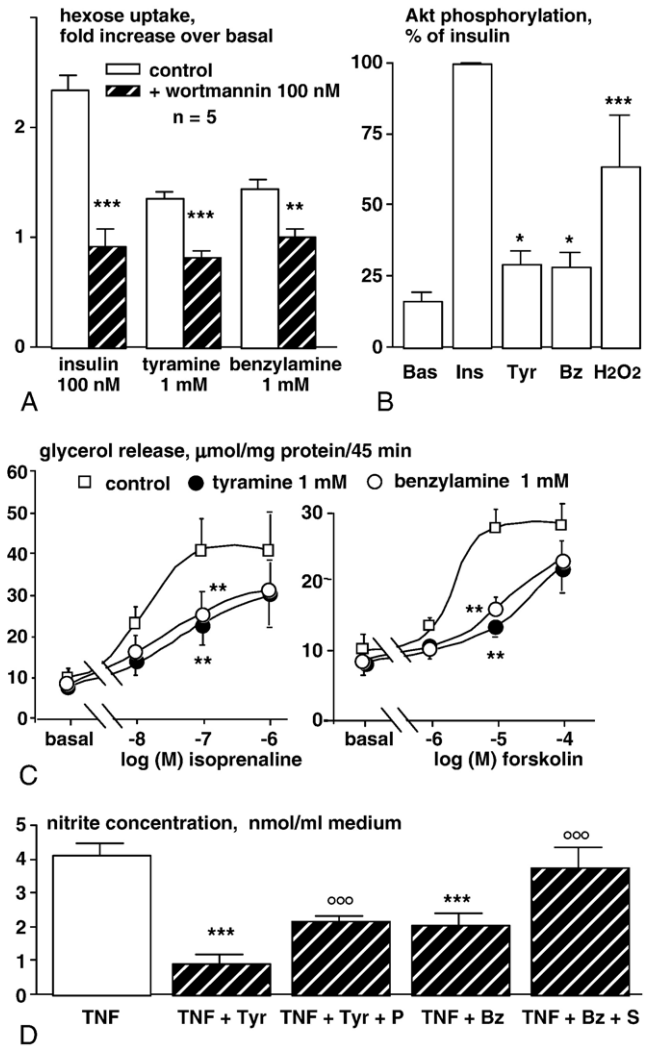

Fig. 2. Activation by tyramine and benzylamine of hexose uptake and PKB/Akt phosphorylation, and inhibition of lipolysis and of NOS2 activity. Differentiated 3T3-F442A adipocytes (around 34 000 cells/cm^2) were serum deprived overnight before exposure to the indicated agents. A, 2-Deoxyglucose uptake (2-DG) was assayed after 45 minutes incubation in the absence (set at 1) or the presence of 100 nmol/L insulin or 1 mmol/L amines, without (white columns) or with 100 nmol/L wortmannin (shaded columns). Different from corresponding control at $^{**}P < .02$; $^{***}P < .01$. B, Phosphorylation of PKB/Akt was determined by Western blot analysis performed with antibody against phospho-Akt. Cells were incubated 45 minutes without (Bas) or with 100 nmol/L insulin (Ins), 1 mmol/L tyramine, benzylamine, or hydrogen peroxide. Results are expressed as percentage of the 60-kDa band density found in insulin-treated cells and presented as mean ± SEM of 8 replicates. Different from basal at $^{*}P < .05$; $^{***}P < .01$. C, Lipolysis was stimulated with increasing concentrations of isoprenaline or forskolin (squares) in the presence of 1 mmol/L tyramine (closed circles) or benzylamine (open circles). Mean ± SEM of 4 determinations. Different from respective control at $^{**}P < .02$. D, Total nitrite production was determined in cell supernatants after 24-h exposure to 10 ng/mL TNF-α (TNF, 0.6 nmol/L) and 0.5 mmol/L amine oxidase substrates (tyramine, benzylamine) or inhibitors (pargyline, P; semicarbazide, S) as indicated. Mean ± SEM of 10 to 15 determinations. Different from TNF-α alone at $^{***}P < .001$. Different from TNF-α plus amine at $^{\circ\circ\circ}P < .001$.

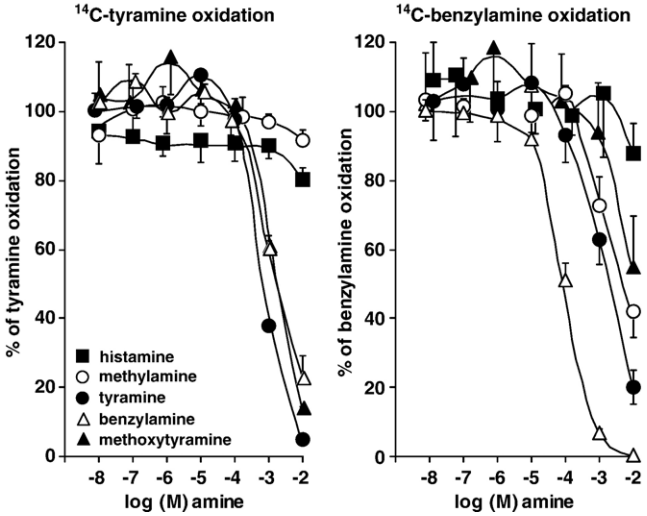

Fig. 3. Inhibition by various biogenic amines of MAO dependent oxidation of [^{14}C]tyramine in 3T3-F442A adipocytes. Homogenates were incubated for 30 minutes with 0.5 mmol/L [^{14}C]tyramine or 0.1 mmol/L [^{14}C]benzylamine and in the presence of increasing concentrations of amines. Results are expressed as percentage of oxidation, which was equivalent to 19.1 ± 6.0 and 4.4 ± 1.5 nmol/mg protein per 30 minutes for labeled tyramine and benzylamine, respectively (n = 4-6).

control, respectively). The phosphorylation of Akt 1/2, a couple of serine/threonine kinases (alternatively referred to as PKB) which signals downstream of phosphatidylinositol 3-kinase, was also partially activated by benzylamine or tyramine (Fig. 2B). PKB/Akt phosphorylation was lower in response to 1 mmol/L amines than that seen in response to 100 nmol/L insulin or 1 mmol/L hydrogen peroxide, as was also the case for glucose uptake activation.

Both tyramine and benzylamine were able to inhibit lipolysis. At 1 mmol/L, they provoked a marked shift to the right of the dose-response curve of isoprenaline or forskolin (Fig. 2C). The production of nitrites induced by 24-hour exposure to 10 ng/mL TNF-α, which results from NOS2 induction in 3T3-F442A [26], was impaired by a cotreatment with either tyramine or benzylamine (Fig. 2D). The inhibition induced by tyramine appeared stronger than that induced by benzylamine. However, it was only partially reversed by pargyline, whereas the benzylamine effect was totally abolished by semicarbazide.

3.3. Interaction of different biogenic amines with MAO and SSAO in 3T3-F442A cells

To further compare the influence of MAO or SSAO activation on adipocyte biology, we strove to reproduce the effects of tyramine and benzylamine with other biogenic amines behaving as selective MAO or SSAO substrates. However, MAO and SSAO substrate specificities greatly differ depending on the animal species and the cell type considered [21]. It was therefore necessary to redefine the selectivity ratio for the MAO and SSAO expressed in the murine 3T3-F442A cells. A selection of more than a dozen biogenic amines and their derivatives was tested in competition assays with increasing doses from 10 nmol/L to 10 mmol/L, against 0.5 mmol/L [^{14}C]tyramine or 0.1 mmol/L [^{14}C]benzylamine oxidation in 3T3-F442A homogenates. The competition assays were performed in the presence of 1 mmol/L semicarbazide to prevent participation of SSAO in [^{14}C]tyramine oxidation, or with 0.1 mmol/L pargyline to block possible MAO-dependent metabolism of benzylamine. As illustrated in Fig. 3 for several amines only, there was great variation in the maximal capacity of the tested agents to inhibit oxidation. Indeed, histamine was unable to inhibit either [^{14}C]tyramine or [^{14}C]benzylamine oxidation and could not be considered as being readily oxidized by MAO or SSAO. Methylamine behaved as an "SSAO-preferring" substrate because it inhibited [^{14}C]benzylamine, whereas it did not impair [^{14}C]tyramine oxidation (Fig. 3). To better delineate the MAO/SSAO selectivity ratio of the tested amines, their capacity to inhibit [^{14}C]tyramine oxidation was plotted against their capacity to inhibit [^{14}C]benzylamine oxidation (Fig. 4). Based on this analysis, amines were classified into 4 groups: (1) agents that were virtually unable to interact with both MAO and SSAO (histamine, methylhistamine, phenylephrine, adrenaline); (2) compounds that interacted more readily with MAO than with SSAO (tyramine, 3-methoxytyramine, tryptamine, dopamine); (3) those that behaved more as SSAO substrates than MAO substrates (benzylamine, methylamine, octopamine, β-phenylethylamine); and (4) the nonselective agents that interacted similarly with SSAO and MAO (normetanephrine and serotonin). Despite the absence of a highly MAO-selective substrate, several amines from the 3 former groups were further tested for immediate (glucose uptake) or long-term effects (triacylglycerol accumulation) on 3T3-F442A cells.

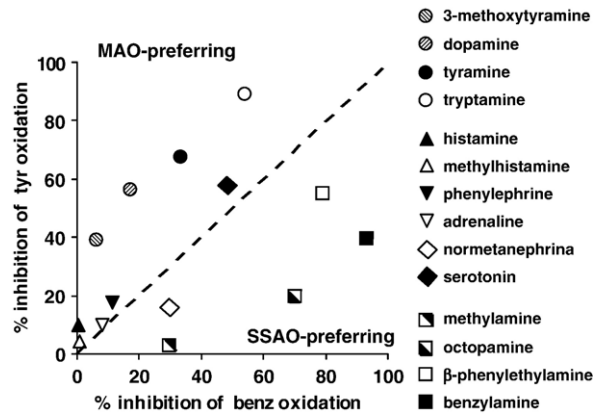

Fig. 4. Comparative plot of the affinity of amines for MAO vs SSAO interaction. Abscissa values designate the percent inhibition by 1 mmol/L amine on [^{14}C]benzylamine oxidation in the presence of pargyline, considered as SSAO dependent. Ordinate values designate the percent inhibition of 1 mmol/L of the same amines tested in competition with [^{14}C]tyramine oxidation in the presence of semicarbazide, representative of MAO activity. In the legend, amines are presented in increasing rank of inhibition of either SSAO or MAO oxidation. Each point is the mean of at least 3 determinations.

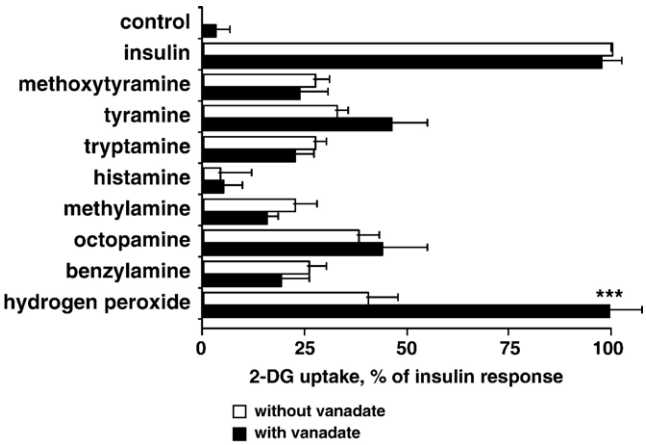

Fig. 5. Stimulation of 2-DG uptake by biogenic amines in 3T3-F442A adipocytes. Cells were incubated during 45 minutes without (open columns) or with 0.1 mmol/L vanadate (black columns) in the absence (control) or in the presence of 100 nmol/L insulin or 1 mmol/L of the indicated amines or hydrogen peroxide (5). Results are expressed as percent of the maximal response to insulin, which was 12.7 ± 1.7 nmol 2-DG/10^6 cells per 10 minutes (n = 36). Mean ± SEM, number of replicates is given between brackets. Amines were ranked as "MAO preferring" (methoxytyramine [5], tyramine [11], tryptamine [5]), weak MAO/SSAO substrate (histamine [5]) and SSAO-preferring substrates (methylamine [7], octopamine [5], benzylamine [13]). Significantly different from corresponding condition without vanadate at ***$P < .01$.

3.4. Effect of MAO and SSAO substrates on glucose uptake

Fig. 5 illustrates that, with the exception of histamine, all the amines tested at 1 mmol/L were able to stimulate glucose transport in 3T3-F442A cells as did 1 mmol/L hydrogen peroxide. In fact, both SSAO- and MAO-preferring amines stimulated hexose transport to approximately 25% to 50% of the maximal insulin effect. The presence of 0.1 mmol/L vanadate did not enhance the effect of tested amines on glucose transport. This is in accordance with our previous findings on this cell line [7], but remains in contrast to the observations made in rat adipocytes for tyramine [27] or benzylamine [28]. Nevertheless, 0.1 mmol/L vanadate exerted an expected synergistic effect with exogenously added hydrogen peroxide, without modifying basal or insulin-stimulated glucose transport. Increasing the dose or time of exposure to vanadate did not result in the development of a synergism between this transition metal and amines (not shown).

The blockade of glucose uptake by MAO and SSAO inhibitors was tested by incubating the 3T3-F442A cells with 100 μmol/L pargyline or 1 mmol/L semicarbazide 45 minutes before hexose uptake assays. This treatment did not affect insulin stimulation of glucose transport (Fig. 6) but inhibited the effects of biogenic amines, with pargyline being more effective than semicarbazide against the MAO-preferring substrates. Conversely, benzylamine- and methylamine-dependent effects were more greatly inhibited by semicarbazide than by pargyline (Fig. 6). The effect of octopamine was partially inhibited by MAO or SSAO blockade. None of the amines attenuated the effect of 100 nmol/L insulin, taken as 100% reference of maximal hexose uptake. On the contrary, many of them slightly improved this maximal effect, with 141% ± 13% and 123% ± 17% activation for 1 mmol/L tyramine or benzylamine, respectively (not shown).

Because both MAO and SSAO substrates mimicked several short-term effects of insulin, we investigated whether they could also mimic long-term insulin-like effect such as the stimulation of adipogenesis.

3.5. Effect of chronic treatment with different biogenic amines on lipid accumulation

Fig. 7 shows the adipogenic response in intracellular triacylglycerol accumulation in 3T3-F442A cells treated for 8 days with 1 mmol/L amine, or with 50 nmol/L insulin, taken as reference of adipocyte differentiation. Only histamine-treated cells did not show any increase compared with untreated control cells (cells kept in medium supplemented with 5% calf serum alone). The lipid accumulation induced by the MAO-preferring substrates methoxytyramine and tryptamine reached 76% ± 11% and 85% ± 9% of the maximal insulin effect, respectively. Similarly, 70% ± 8% and 66% ± 6% of the maximal insulin effect were obtained with the SSAO-preferring substrates, methylamine and benzylamine. This indicated that both MAO and SSAO substrates were able to activate lipid accumulation and therefore to partially mimic the adipogenic effect of insulin. When 1 mmol/L semicarbazide was present during the 8-day treatment, it significantly reduced the effects of SSAO-preferring substrates and of tyramine, whereas it did not impair the effects of methoxytyramine or tryptamine (Fig. 7). Semicarbazide was without any effect on triglyceride accumulation under control, insulin-stimulated, or histamine conditions.

Although daily addition of 1 mmol/L pargyline did not modify insulin-dependent adipogenesis, it enhanced spontaneous lipid deposition. This adipogenic effect complicated

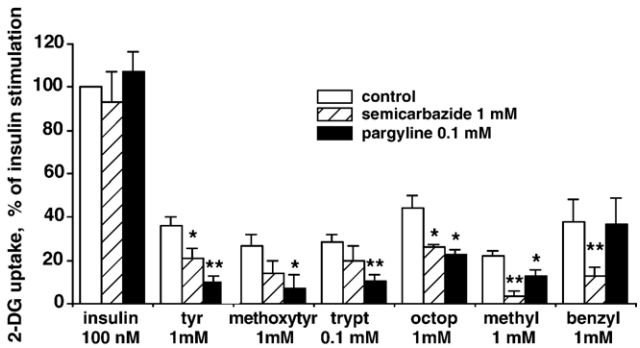

Fig. 6. Inhibition by semicarbazide or pargyline of amine-induced glucose transport. 3T3-F442A adipocytes were incubated for 45 minutes with the indicated compounds without (white columns) or with 1 mmol/L semicarbazide (shaded columns) or 0.1 mmol/L pargyline (dark columns). 2-DG uptake is expressed as percentage of insulin-stimulated response, with basal set at 0% and response to insulin 100 nmol/L set at 100%. Mean ± SEM of 4 determinations (7 for octopamine). Different from respective control at *$P < .05$; **$P < .02$.

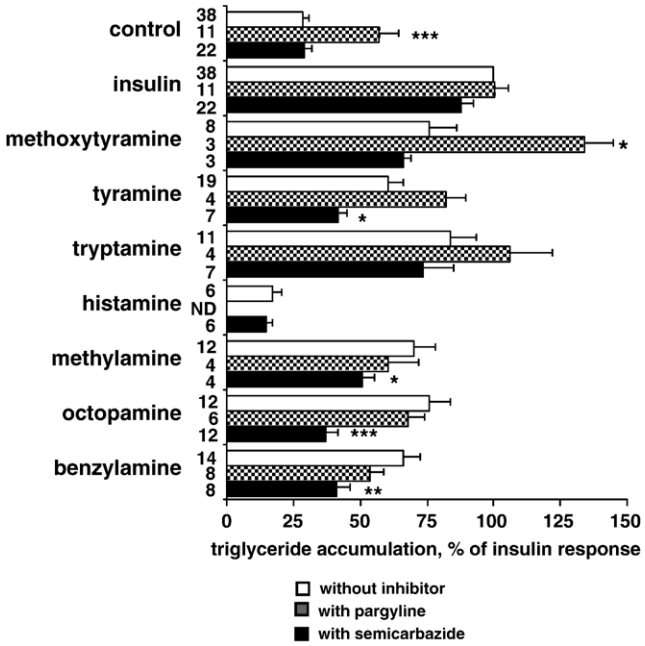

Fig. 7. Effect of an 8-day treatment with different biogenic amines on triacylglycerol accumulation. Postconfluent 3T3-F442A cells were kept for 8 days in culture medium alone (control) or containing 50 nmol/L insulin as a reference condition for optimal differentiation (insulin), or 1 mmol/L of the indicated amine. Semicarbazide (black columns) or pargyline (striped columns) was, similar to the other agents, daily added to reach a final concentration of 1 or 0.1 mmol/L, respectively. Results are expressed as percentage of the triacylglycerol content of the cells maintained in medium containing insulin alone (which led to a content of 174 ± 15 nmol triacylglycerol/10^6 cells, n = 22). Mean ± SEM of the number of observations indicated for each bar. Different from respective condition without inhibitor at $^*P < .05$; $^{**}P < .02$; $^{***}P < .01$.

interpretation of its lack of blockade or even its potentiation of amine-induced adipogenesis (Fig. 7). Conversely, sodium orthovanadate at 1 μmol/L did not significantly enhance lipid accumulation neither in the amine-treated cells nor in the insulin-treated cells (not shown).

Daily addition of 1 mmol/L amine in supplement to insulin tended to increase hormone-stimulated lipid accumulation: triacylglycerol content of cells treated with insulin plus tyramine, methylamine, or octopamine, respectively, represented 115% ± 11%, 122% ± 12%, and 123% ± 17% of lipid stored in cells differentiated under insulin alone (n = 4-8, not significant). Only the combination of 1 mmol/L benzylamine plus 50 nmol/L insulin resulted in a significantly greater lipid accumulation, as it was equivalent to 152% ± 13% of that obtained with insulin alone (n = 8, $P < .01$). Simultaneous treatment with insulin plus benzylamine was also the most efficient regarding protein anabolism, resulting in 131% of the total protein content found in insulin-treated cells.

4. Discussion

The goal of this work was to determine whether MAO substrates, like substrates of SSAO, were able to mimic or to favor insulin actions in cultured adipocytes. We hypothesized that increasing the number of substrates tested would strengthen the comparison initiated with tyramine and benzylamine only [7]. Therefore, we tested whether several biogenic amines could be considered as highly selective substrates for MAO and compared their short-term effects on glucose uptake in fully differentiated 3T3-F442A cells, or their long-term action on adipogenesis in postconfluent cells. Regardless of the predominant oxidase responsible for their oxidation, almost all biogenic amines tested were shown to influence adipocyte metabolism, suggesting that MAO activation also exhibits the insulin-mimicking properties already reported for SSAO in fat cells.

It has been documented that SSAO activity is practically absent in rodent preadipocytes and that SSAO messenger RNAs, protein, and activity dramatically increase during the adipose differentiation process [5-9]. The abundant expression of SSAO found in adipocytes has prompted many investigators to reevaluate the regulation and function of this copper-containing amine oxidase [16-18,29]. A result of this increasing interest in the role of SSAO/VAP-1 in metabolic control may lead to an underestimation of the putative role of MAOs, which are also abundantly expressed in fat cells [20]. When compared with freshly isolated fat cells, the 3T3-F442A murine lineage constitutes a convenient model to study both short- and long-term effects of amine oxidase activation because it expresses not only SSAO but also MAO (predominantly the A form) [7]. The use of this model allowed us to demonstrate that, like SSAO, MAO activation mimics the actions of insulin with regard to glucose uptake or antilipolysis and modulates the response to the proinflammatory cytokine TNF-α. This last point extends to adipocytes the hydrogen peroxide–dependent mechanism that was recently attributed to MAO and SSAO in macrophages [25]. This mechanism is responsible for the inhibition of lipopolysaccharide or interferon-γ induction of NOS2 by substrate-activated MAO and SSAO. This novel effect of amine oxidases not only offers a new insight into the control of inducible NOS2, but also reinforces the growing similarity found between fat cells and cells from immune system [30].

Unexpectedly, fluorometric detection of hydrogen peroxide resulted in lower enzymatic activities than the determination of radiolabeled aldehyde production, whereas, according to the stoichiometry of oxidative deamination, similar amounts of aldehyde and hydrogen peroxide were expected to be generated [10]. This discrepancy is likely to be due to the fate of hydrogen peroxide, which can react readily within adipocytes [31], and so a part of its production cannot be measured using enzymatic determination driven by horseradish peroxidase in the fluorometric technique [10]. Alternatively, it has been proposed that the trapping of hydrogen peroxide, which occurs only with the latter technique, may limit oxidase activity. Regardless of the technique used for measuring oxidative deamination, the maximal velocity of MAO-dependent oxidation of tyramine

was higher than that of the SSAO-dependent oxidation of benzylamine in differentiated 3T3-F442A adipocytes. Despite this difference, tyramine and benzylamine exerted comparable partial activation of glucose uptake, as also observed with the other biogenic amines tested. It was therefore difficult to correlate hydrogen peroxide production with the intensity of amine-induced stimulation of glucose uptake. However, it is important to note that direct addition of 1 mmol/L hydrogen peroxide also resulted in only a partial activation of hexose uptake, suggesting that an unknown limiting factor may prevent this reactive oxygen species to fully activate glucose uptake. Indeed, hydrogen peroxide reactivity remains poorly defined in adipocytes [31]. The conditions required for the interaction of hydrogen peroxide with different cell components (belonging to the insulin signaling cascade or to the antioxidant defenses) or even with exogenous vanadium to chemically produce oxovanadium compounds having insulin-mimetic effects [28,32] need to be further investigated and characterized. Despite this, it is important to note that the lack of synergism between vanadate and amine oxidase substrates regarding the stimulation of glucose transport in 3T3-F442A cells has already been observed in human adipocytes [16]. Similarly, in vivo improvement of glucose tolerance has been observed with benzylamine alone in rabbits [15] or with methylamine alone in transgenic mice [33]. Although the mechanism of action of hydrogen peroxide is far from being defined, and despite the differences observed between endogenously generated and exogenously added hydrogen peroxide regarding the synergism with vanadate, this reactive oxygen species is likely to be a key factor involved in the observed effects of amines. In fact, hydrogen peroxide is the common end product of oxidation of all amines that partially activate glucose uptake in 3T3 cells. Likewise, hydrogen peroxide has been directly shown to promote Akt phosphorylation, to activate glucose uptake, to inhibit lipolysis [34], and to be involved in the limitation of NOS2 induction by inflammatory stimuli in macrophages [25]. The other products of amine oxidation, namely, ammonia and the different aldehydes generated during amine oxidation, are unlikely to be involved in the partial activation of hexose uptake induced by the tested biogenic amines because ammonia and benzaldehyde, which are produced during benzylamine oxidation, are inactive on glucose transport, even in the presence of vanadate, in rat adipocytes [15].

Our pharmacologic analysis of 14 amines failed to detect highly specific substrates for MAO or SSAO but showed that various SSAO- or MAO-preferring substrates activate glucose uptake and lipid deposition to the same extent. As a consequence of the overlapping substrate selectivity found between MAO and SSAO, the blockade of amine-induced glucose uptake or adipogenesis was complex. Nevertheless, our data showed that the effects of 3-methoxytyramine on glucose uptake were more sensitive to pargyline than to semicarbazide inhibition, indicating that MAO was the amine oxidase predominantly involved. Pargyline also attenuated the stimulations of hexose uptake by tyramine or tryptamine, which were almost resistant to semicarbazide. Conversely, this MAO-A/MAO-B inhibitor was unable to block the effect of benzylamine on hexose uptake, only impaired by SSAO inhibition. Prolonged exposure to high concentrations of pargyline unexpectedly promoted lipid accumulation without modifying the adipogenic effect of insulin. This complicated the interpretation of lipid accumulation found in response to amines plus pargyline, especially with MAO-preferring substrates. Nevertheless, the effects of SSAO substrates were inhibited more by semicarbazide than by pargyline, as previously reported for benzylamine or methylamine [7,8].

In addition to their oxidation by amine oxidases, several biogenic amines interact with their own receptors and make more complex the interpretation of their effects. Serotonin, adrenaline, and dopamine were discarded from a complete study for this reason and because they exhibited poor MAO/SSAO selectivity ratios in 3T3-F442A cells. In keeping with this, the effect of octopamine on glucose uptake was particularly resistant to both pargyline and semicarbazide. An already reported interaction of this compound (which corresponds to dehydroxylated noradrenaline) with β_3-adrenergic receptors [35], abundant in differentiated 3T3-F442A cells [36] and shown to interact with glucose uptake [37], may be involved in this complex behavior.

Notably, when SSAO or MAO substrates were administered together with insulin to the cells, they did not inhibit the adipogenic effect of the hormone but exerted slight additive effects, especially in the case of benzylamine. This is in contrast to many other insulin-mimicking drugs that impair insulin action when mixed with the hormone. Finally, histamine, which was poorly oxidized, did not generate hydrogen peroxide and was unable to activate glucose uptake or adipogenesis. The lack of effect of this diamine oxidase substrate is in agreement with the low expression of *AOC1* gene in adipocytes [38] and indicates that not any given amine can mimic the effects of insulin in adipocytes: it has first to be oxidized by MAO or SSAO.

Taken together, our data indicate that high concentrations of both SSAO and MAO subtrates can partially mimic short- and long-term insulin actions in cultured fat cells. Although the mechanisms involved resulting in glucose uptake activation, lipolysis inhibition, and reduction of proinflammatory effects remain to be elucidated, it would be important to determine whether these experimental pharmacologic properties could be extrapolated in vivo, especially in obese or insulin-resistant conditions. It is likely that the current structure-activity analyses of the active sites of both MAO-A and MAO-B and SSAO may result in the design of novel substrates or inhibitors with higher affinity and selectivity, useful to be tested both in vitro and in vivo. Furthermore, future investigations aiming to demonstrate whether alimentary amines can exert similar influences at physiologic concentrations, especially in abdominal fat

depots under postprandial conditions, may bring novel functional significance for peripheral amine oxidases.

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