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Amine oxidase substrates for impaired glucose tolerance correction

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Amine oxidases are widely distributed from microorganisms to vertebrates and produce hydrogen peroxide plus aldehyde when catabolizing endogenous or xenobiotic amines. Novel roles have been attributed to several members of the amine oxidase families, which cannot be anymore considered as simple amine scavengers. Semicarbazide-sensitive amine oxidase (SSAO) is abundantly expressed in mammalian endothelial, smooth muscle, and fat cells, and plays a role in lymphocyte adhesion to vascular wall, arterial fiber elastic maturation, and glucose transport, respectively. This latter role was studied in detail and the perspectives of insulin-like actions of amine oxidase substrates are discussed in the present review. Independent studies have demonstrated that SSAO substrates and monoamine oxidase substrates mimic diverse insulin effects in adipocytes: glucose transport activation, lipogenesis stimulation and lipolysis inhibition. These substrates also stimulate *in vitro* adipogenesis. Acute *in vivo* administration of amine oxidase substrates improves glucose tolerance in rats, mice and rabbits, while chronic treatments with benzylamine plus vanadate exert an antihyperglycaemic effect in diabetic rats. Dietary supplementations with methylamine, benzylamine or tyramine have been proven to influence metabolic control in rodents by increasing glucose tolerance or decreasing lipid mobilisation, without noticeable changes in the plasma markers of lipid peroxidation or protein glycation, despite adverse effects on vasculature. Thus, the ingested amines are not totally metabolized at the intestinal level and can act on adipose and vascular tissues. In regard with this influence on metabolic control, more attention must be paid to the composition or supplementation in amines in foods and nutraceutics.

Key words: Insulin, Monoamine oxidase, Semicarbazide-sensitive amine oxidase, Adipocyte, Obesity.

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Amine oxidases (AOs) catalyze, in the presence of oxygen, a reaction by which an amine is converted into its corresponding aldehyde with concomitant production of hydrogen peroxide and ammonia. Until recently, amine oxidases were considered as scavengers of biogenic amines, but given to the highly reactive products they generate, these enzymes have been suspected to exert other functions. This review will focus on one of the novel roles of AOs, demonstrated to be dependent on the hydrogen peroxide generation in tissues which increase their glucose uptake in response to insulin stimulation: adipose tissues, skeletal and cardiac muscles.

The amine oxidase families

The nomenclature of deaminating oxidases is still under debate as a result of their wide distribution in nature. Amine oxidases are currently classified in two families, according to the cofactor they use for oxidative deamination: FAD-containing AO and copper-containing AO. The former family contains the polyamine oxidase (PAO) and the mitochondrial monoamine oxidases (MAO, EC 1.4.3.4.), mainly of the A and B form in mammals. The latter encircles all the enzymes containing a topaquinone, which is a modified tyrosine residue (EC 1.4.3.6.). Namely, they are the diamine oxidase (DAO), encoded by *AOC1* gene in man, the semicarbazide-sensitive amine oxidases (SSAO) produced by *AOC2* and *AOC3* genes, and the lysyl oxidase (LO) (for review, see 24). Despite their distinct cofactors, AOs share an overlaping selectivity towards substrates. In rodents, for instance, tyramine is a non-selective substrate for MAO-A, MAO-B and SSAO, while histamine can be oxidized by both DAO and SSAO. Regarding inhibitors, there are various agents more or less specific for a subset of oxidases. Thus, semicarbazide inhibits SSAO, and all the topaquinone-containing AOs, but not MAOs, while clorgyline and selegiline are selective blockers of MAO-A and MAO-B, respectively. Recent progress in Pharmacology and Molecular Biology have allowed to better distinguish each of the above-mentioned AOs, including their alternative splicing variants, but there is no specific inhibitor available for each type of AO (for recent reviews, see 24, 26, 45). Moreover, it is well recognized that there are large interspecies variations in the expression of AO proteins and in their relative affinities towards substrates. Therefore, further studies are still needed to obtain a complete characterization of all the members of the AO family in laboratory animals and man. This review will not examine the novel tools and findings that improve the current characterization of AOs, it will focus interest on novel functions of mamalian MAO and SSAO. A special attention will be given on the interplay between amine oxidation and glucose handling, and on its potential interest in future pharmacological treatments of obesity, diabetes, and related disorders.

Old substrates and novel roles of amine oxidases

A quick review of AO substrates is necessary before exploring novel functions of these enzymes primarily considered only as amine metabolizing enzymes. In vertebrates, the known AO substrates belong to the broad family of biogenic amines, which encompasses various neurotransmitters (noradrenaline, adrenaline, dopamine, serotonin or 5-hydroxytryptamine), diamines (putrescine, histamine),

polyamines (spermine, spermidine...) and numerous trace amines such as β-phenethylamine (β-PEA), para-tyramine, tryptamine, and octopamine. Products of the protein catabolism also belong to the long list of AO substrates: polyamines, methylamine, aminoacetone, etc. In addition to these biogenic amines, which are found in peripheral tissues as well as in the central nervous system, diverse amines naturally occuring in plants or food are also substrates of mammalian amine oxidases, as in the case for benzylamine (44) or several polyamines. Synthetic products and xenobiotics bearing an amino group can also be oxidized by MAO and/or SSAO (57). The most widely mentioned example being 1-methyl-4-phenyl-1,2,3,4 -tetrahydropyridine (MPTP), known for its neurotoxity and Parkinson-like symptoms it provokes when oxidized by brain monoamine oxidases (27). Finally, amino group on the side chain of lysine or arginine residues have been proposed to easily reach the catalytic center of topaquinone containing AOs (at least LO and SSAO), making likely the presence of polypeptide chains in the growing list of AO substrates (53). The diverse heterocyclic amines that are produced during food processing and mainly found in fried meat can also be added to the list, since their carcinogenic effects are somewhat blunted by antioxidants (22) and several of them have been shown to be oxidized by AOs.

Any given member of the biogenic amine family cannot be considered solely as a substrate of amine oxidases, but generally exhibits other pharmacological properties. Thus, the diverse neurotransmitters display agonistic action to their respective receptors (catecholaminergic, serotoninergic, histaminergic), while trace amines are able to stimulate trace amine receptors (TAR), which represent a family of 15 members in rat, currently named rTAR1 to 15 (4). Moreover, many biogenic amines interact more or less with neurotransmitter membrane carriers or with catecholamine precursors and their metabolizing enzymes. Therefore, many biogenic amines can alter neurotransmitter synthesis, storage, or flux accross membranes. Indeed, tyramine and β-PEA are capable of producing robust sympathomimetic effects, described in pioneering studies at the beginning of the twentieth century, and responsible for the "cheese effect" corresponding to an hypertensive crisis provoked by ingestion of dietary amines in patients treated with irreversible MAO inhibitors. Besides these well-documented actions, other peripheral effects have been recently observed with amine oxidase substrates, independently of disturbance of neurotransmitter efflux. Most of them have been reviewed by TIPTON and coworkers (45). The most surprising function of SSAO, which has apparently no link with the scavenging of circulating amines, is endowed by the Vascular Adhesion Protein 1 (VAP1), the product of *AOC3* gene which exerts an amine oxidase activity (24). The group of JALKANEN has demonstrated that this ecto-enzyme SSAO/VAP-1, abundant on endothelial cells, particularly in lymphatic tissues, is involved in lymphocyte adhesion to vascular walls (53). A soluble form of SSAO/VAP1 exists which may interfere with this process and which is known to increase in diabetic states (52). SSAO/VAP1 is also present in vascular smooth muscle cells, and seems to interfere with extracellular matrix development (29). Interestingly, SSAO/VAP1 protein and activity are also abundant in adipocytes (43, 51) and were proposed to interact with the regulation of glucose utilisation

(10). The recent findings about this novel role will be summarized below, just before the presentation of current observations obtained on animal models after long-term treatment with amine oxidase-interacting drugs. Finally, the potential therapeutic interest of the interplay between amine oxidation and glucose metabolism will be discussed.

Adipocyte amine oxidases and insulin mimicry

It has been originally reported in 1995 that 5-hydroxytryptamine was able to activate hexose uptake in cultured cardiomyocytes in a MAO- and hydrogen peroxide-dependent manner, and not via serotoninergic receptor activation (16). Then, tyramine and benzylamine were demonstrated to increase glucose uptake via MAO and/or SSAO activation in freshly isolated rat fat cells (15, 35), and in cultured preadipocytes (17). A similar activation of *in vitro* glucose utilisation was demonstrated with methylamine in preadipocytes (40) and smooth muscle cells (13) and with other amines in other models listed in Table I. It is important to note that such *in vitro* actions were prevented by amine oxidase inhibitors and therefore demonstrated to be direct consequences of oxidative deamination, whereas the *in vivo* hypertensive "cheese effect" was only detected in the presence of MAO inhibitors. Indeed, the "cheese effect"resulted from an enhanced interaction between the excess of dietary amines (mainly tyramine), protected from degradation by irreversible MAO blockade, and neurotransmitter transporters (27). On the contrary, generation of hydrogen peroxide during AO-catalyzed amine oxidation is a key element in the amine-induced stimulation of glucose utilization in insulin-sensitive cells, demonstrated to be sensitive to catalase or antioxidants by independent studies (35, 40, 66). In fact, hydrogen peroxide, well known as an oxidative stress agent, is also recognized to mimic diverse insulin actions in adipocytes (37). The presence of 0.1 mM vanadate, i.e. at concentrations without effect on basal or insulin-simulated glucose uptake, was able to potentiate the amine insulin-mimicking effects in almost all the models studied. The formation of peroxovanadate, obtained by chemical combination between hydrogen peroxide produced via AO activation and added orthovanadate has been observed in rat (1). This special oxidation state of vanadate has a powerful insulin mimicking effect (54) and is likely responsible for the synergism between amines and vanadate. Additional evidence for hydrogen peroxide involvement in the amine-induced stimulation of glucose transport is that neither aldehydes (benzaldehyde, formaldehyde, phenylacetaldehyde) nor ammonia were able to stimulate glucose uptake, even in the presence of vanadate (Carpéné C. *et al.*, unpublished observations). To date, benzylamine, well recognized as an investigational substrate of SSAO and MAO-B, is one of the most potent amines able to stimulate glucose uptake (14) and is proposed as the current reference agent for basic investigations on the insulin-like effects of AO substrates since it is unable to stimulate TARs in the pharmacological surveys tested so far (8). Histamine, the substrate of DAO is much less efficient in activating glucose transport in fat cells (30) while no specific substrate for LO or PAO has been reported to be active in this model. Of note, polyamines were reported in the seventies to stimulate glucose uptake in fat cells, but although mediated by hydrogen peroxide, this effect was

ascribed to a bovine amine oxidase which is a known contaminant of the bovine albumin present the incubation medium, and not to the fat cells themselves (32). Nowadays, commercial albumin preparations are much less contaminated by this soluble bovine amine oxidase and the stimulatory effect of amines on glucose uptake has been found even in fat cells incubated in albumin-free medium.

The insulin-signaling pathways activated by MAO and/or SSAO substrates have been recently reviewed (69). Briefly, they correspond to phosphorylation of insulin receptor substrates (IRS), phosphatidylinositol 3-kinase activation and protein kinase B activation. These events follow tyrosine phosphatase inhibition and lead to glucose transporter recruitment to the plasma membranes and increased hexose uptake. However, insulin not only activates glucose uptake, it also regulates lipid metabolism and influences cell growth and differentiation. Table I summarizes the diverse short and long-term insulin-like effects reported so far to be mimicked by MAO and/or SSAO substrates. These substrates can directly interfere not only with glucose metabolism, but also with lipid metabolism in fat cells, and an amine oxidase-mediated stimulation of lipogenesis and inhibition of lipolysis has been reported in rat, mouse and human adipocytes (9, 41, 64). The presence of vanadate is necessary to obtain a full insulin-mimicry with amines in rat adipocytes, but not in human fat cells where it is ineffective (41). Long-term actions of insulin on *in vitro* adipogenesis have also been reproduced, although to a lesser extent, with benzylamine or tyramine alone in murine as well as in human preadipocytes (Table I). The stimulation of glucose uptake was not limited to *in vitro* models since an improvement of glucose handling was also found in conscious or anesthetized rats treated by either tyramine (42) or benzylamine plus vanadate (33). Table II summarizes similar antihyperglycemic actions observed with benzylamine in rabbits but also in diabetic and obese rodents such as high-fat fed mice (23), streptozotocin-diabetic rats or Goto-Kakisaki diabetic rats (1, 61). An insulin-like inhibition of triacylglycerol breakdown was also observed *in vivo* since benzylamine counteracted the lipid-mobilizing effect of the β-adrenergic agonist isoprenaline in mice (23).

Repeated administration of AO-substrates and dietary amines

Since single injection or infusion of amine oxidase substrates acutely improved glucose tolerance, it is relevant to test whether repeated administration of amines could improve metabolic control, especially in glucose intolerant rodents. The blood glucose lowering effects obtained in diabetic rats with subcutaneously implanted osmotic minipumps delivering benzylamine and repeated injections of vanadate (1, 33) reinforced the hypothesis of insulin-like activity of AO substrates. However, it remains to be verified whether: 1) AO substrates could exert oral antidiabetic activity, and 2) exogenous vanadate could be omitted. Based on the "cheese effect" of foods rich in tyramine, it was believed that many dietary amines could not be easily uptaken through the intestinal barreer. Since dietary tyramine was able to produce pharmacological adverse effects only when central and peripheral MAO were irreversibly blocked, a great rate of amine degradation was attributed to the intestinal tract. This was in agreement with the lack of adverse effects of the dietary

Table I. In vitro *short and long-term insulin-like effects of amine oxidase substrates.*

Model	Insulin-like effect	Substrates	Reference
rat adipocytes	glucose uptake stimulation	tyramine +/- Van	(35)
		benzylamine + Van	(15, 30, 66)
		methylamine + Van	(14)
		n-decylamine, β-PEA +/- Van	(14)
		tryptamine, acetyl putrescine + Van	(14)
		histamine + Van	(30)
	IRS tyrosine phosphorylation	benzylamine, tyramine +/- Van	(14)
	GLUT4 translocation	benzylamine + Van	(14)
	glucose incorporation into lipids	benzylamine +/- Van	(9)
	lipolysis inhibition	benzylamine, tyramine +/- Van	(64)
		histamine, methylamine	(38)
	lactate release	tyramine	(2)
mouse adipocytes	glucose uptake stimulation	benzylamine, tyramine +/- Van	(62)
		methylamine, amino acetone + Van	(66)
	lipolysis inhibition	benzylamine, methylamine	(23)
rabbit adipocytes	glucose uptake stimulation	benzylamine	(23)
	lipolysis inhibition	benzylamine	(23)
human adipocytes	glucose uptake stimulation	benzylamine, methylamine	(10, 41)
		tryptamine, β-PEA	(41)
		octopamine	(62)
	lipolysis inhibition	benzylamine, methylamine	(41)
3T3 preadipocytes (murine)	glucose uptake stimulation	benzylamine, tyramine	(17)
		benzylamine + Van	(40)
	adipogenesis promotion	benzylamine, tyramine, methylamine	(17, 39, 58)
	GLUT4 translocation	benzylamine + Van	(14)
	GPDH activation	methylamine, benzylamine, tyramine	(39)
SGBS preadipocytes (human)	adipogenesis promotion	benzylamine, tyramine,	(5)
		histamine	(5)
rat cardiomyocytes	glucose uptake stimulation	5-hydroxytryptamine	(16)
		tyramine +/- Van	(42)
rat soleus muscle	glucose uptake stimulation	tyramine	(42)

+ Van: amines efficient only in the presence of vanadate; +/- Van: amine effect improved by 0.1 mM vanadate; GPDH: glycerol-3-phosphate dehydrogenase; GLUT4: insulin-responsive glucose transporter.

Table II. In vivo *short-term improvement of glucose tolerance by amine oxidase substrates.*

Animal model	Compound	Reference
normoglycemic rats	tyramine +/-Van	(42)
	benzylamine + Van	(33)
STZ diabetic rats	benzylamine + Van	(33)
	tyramine	(42)
GK diabetic rats	benzylamine + Van	(1)
obese diabetic mice	benzylamine	(23)
rabbits	benzylamine	(23)

+ Van: amines tested in the presence of vanadate.

amines in healthy individuals without defectuous intestinal tract or without AO blockade (27). Conversely, several observations indicated that dietary amine intake could induce changes within the organism other than modification of gastrointestinal mucosa (12). Indeed, mice invalidated for the histidine decarboxylase gene, cannot synthesize their own histamine, but become clearly obese only when fed with histamine-free chow (18). Changes in cancer development observed in animals fed with different supply in polyamines also argue in favour of a substantial entry of dietary amines into the organism (49). An estimation of the daily global amount of dietary amines spontaneously ingested by laboratory rodents is therefore necessary before choosing the doses for oral administration of each tested amine, but is not available in the literature, at least to our knowledge.

Chronic treatments with AO-interacting agents: metabolic control *vs.* vasculotoxicity

The limited results published until now on chronic administration of drugs interacting with AOs are presented in Table III. Many of them show a sustained insulin-like effect of orally administered amine oxidase substrates. By chronically treating transgenic mice expressing human VAP-1/SSAO and their wild type controls for 15 months with water containing methylamine at 4 mg/ml a somewhat insulin-like efficacy was observed (55). No change in fasting blood glucose was observed between control and methylamine-treated mice since all were normoglycaemic. However, in response to an i.p. glucose challenge, the methylamine-treated mice exhibited enhanced glucose tolerance, especially the transgenic ones, overexpressing SSAO (55). This beneficial effect on glucose disposal could be detected after only 16 days of oral methylamine administration and was reversed by an SSAO-inhibitor. Under similar conditions, an increased response to insulin was found with regard to the stimulation of hexose uptake into skeletal muscle. After more than one year of methylamine supplementation, mice did not exhibit changes in fasting blood glucose or plasma insulin but showed decreased levels of glycosylated hemoglobin A1c (HbA1c) whatever the genotype. Methylamine treatment did not increase circulating SSAO activity, serum advanced glycation end-products (AGE), or even body weight gain and calorie intake. Of note, the epididymal fat pads of treated transgenic mice were heavier than in treated non-transgenic mice (55). However, this treatment with pharmacological doses of methylamine (estimated to lead to a daily oral intake of 7000 µmol/kg) produced various adverse effects: elevated blood pressure, accelerated atherosclerosis progression, and nephropathy (55). Therefore, methylamine supplementation had a dual influence. On one hand, the increased fat deposition induced by methylamine was probably a consequence of the adipogenic, lipogenic and antilipolytic properties demonstrated *in vitro* (Table I). On the other hand, methylamine facilitated the emergence of vascular damages, mainly in mice overexpressing circulating SSAO, but did not increase mortality at 15 months of age. Both murine adipose and vascular tissues were affected by methylamine supplementation, an observation which makes this model very valuable since methylamine effects have been previously described on these tissues in man (11, 21, 41). Despite being present at high concentration in

human plasma (31.8 ng/ml or 1 µM, according to LI and coworkers) (31), and being proposed as a physiological SSAO substrate (45), methylamine forms a peculiar aldehyde during its oxidative deamination: formaldehyde, which posseses an oral LD_{50} of around 300 mg/kg in rodents, and which was likely responsible for all diabetes-like complications reported above. As a consequence of the potential vasculotoxicity of methylamine oxidation products, SSAO inhibitors have been repeatedly proposed to prevent vascular diabetic complications, but have never been demonstrated to reduce hyperglycaemia (67, 68). By contrast to the insulin-like effects of pharmacological doses of AO substrates, the blockade of SSAO activity has been reported to alter metabolic control. In a study on obese KKAy mice chronically treated with SSAO inhibitor (E)-2-(4-fluorophenetyl)-3-fluoroallylamine (FPFA), YU *et al.* serendipitously observed a slimming effect of FPFA (66). They also demonstrated that this inhibitor counteracts the *in vitro* stimulation of hexose uptake by benzylamine, methylamine and amino-acetone, and induces an *in vivo* alteration of glucose tolerance. In agreement with these observations is the slimming effect obtained by the combination of pargyline plus semicarbazide in Zucker obese rats mainly due to a diminished fat deposition (CARPÉNÉ *et al.* unpublished data). As with dietary methylamine, chronic treatment with SSAO blockers is expected not only to influence adipose tissue development and glucose disposal but also to affect vasculature (Table III). In this view, SSAO inhibition seemed to reduce atherogenesis in KKAy diabetic mice fed with high-cholesterol diet (67). However, SSAO inhibition also exerts deleterious effects such as aortic dilation and disturbances in arterial wall structure, as already reported in rats (28).

Thus, any treatment aiming at modifying SSAO activity is expected to produce at least dual effects, the balance of which will depend on the interaction with soluble/ vascular vs adipose SSAO. Under these conditions, methylamine must therefore be considered as a prototypical SSAO substrate that does not deserve to be further studied in pharmacological approaches, except as a reference agent. Other AO substrates with more beneficial effect on metabolic control have to be detected by pharmacological screening. These drugs could be designated to influence differently adipose tissue-bound SSAO or MAO and vascular/circulating SSAO/VAP-1. Indeed, a drug which could quickly escape from the blood stream and could be readily oxidized within the adipose tissue will probably have less adverse effects than methylamine or aminoacetone and their toxic oxidation products, formaldehyde and methylglyoxal (36). It is therefore conceivable to compete for methylamine and aminoacetone oxidation via soluble/vascular SSAO by using other substrates with high affinity for SSAO which generate aldehydes less toxic than formaldehyde and methylglyoxal themselves, which are readily able to promote cross-linking reactions on circulating and vascular proteins. This approach will have the advantage to prevent methylamine and aminoacetone oxidation at the vascular level without limiting the insulin-like action of AOs in the insulin-sensitive tissues. The transgenic mice overexpressing SSAO in vascular endothelial or smooth muscle cells, or in fat cells will be valuable tools for studying this aspect (20, 55, 56). Oral administration of benzylamine and tyramine are therefore under study to verify whether

Table III. *Influence of prolonged treaments with drugs interacting with amine oxidases.*

Compound	Model	Route	Effect	Ref.
methylamine	FVB/n mice	po	decreased glycosylated hemoglobin	(55)
methylamine	tg mice expressing human SSAO	po	increased adiposity improved glucose tolerance	(55)
benzylamine	Wistar rats	po	decreased plasma FFA improved glucose tolerance	(7)
tyramine	streptozotocin-diabetic rats	ip	improved glucose tolerance	(61)
benz + vanadate	streptozotocin-diabetic rats	ip	improved glucose tolerance decreased hyperglycemia increased GLUT4 expression	(33)
benz + vanadate	Goto-Kakisaki diabetic rats	ip	improved glucose tolerance decreased hyperglycemia	(1)
octopamine	Zucker obese rats	ip	slight weight gain reduction	(6)
aminoacetone	Sprague Dawley rats	iv	increased vascular glycation	(36)
FPFA	KKAy mice fed atherogenic diet	po	weight gain reduction decreased adiposity	(66)
semicarbazide	Sprague Dawley rats	ip	vasculotoxicity	(28)

po, per os administration; ip, daily intraperitoneal injections; iv,daily injection in caudal vein; tg, transgenic; benz, benzylamine; FPFA, (E)-2-(4-fluorophenetyl)-3-fluoroallylamine.

any amine substrate other than methylamine, capable to generate less toxic aldehydes, would possess a better risk/benefit ratio regarding to the improvement of glucose disposal.

Effects of oral administration of AO substrates other than methylamine

Our current findings obtained with benzylamine supplementation in rats are summarized in Table III. The improvement in glucose tolerance and the decrease in circulating free fatty acids are detailed in a companion research article to the present review (7). Tyramine supplementation was also tested in normoglycaemic rats, based on our previous estimation of the spontaneous daily tyramine intake in laboratory rats with free access to standard pellets: approximatively 26 µmol/kg body weight (61). The chosen dose was tyramine at 1.38 mg/ml in the drinking water, resulting in a daily intake between 500 and 900 µmol/kg in male Wistar rats, which represented a large increase over control conditions. Then, analyses of the stability of tyramine solution and of the amount of tyramine found in the urines of animals subjected to such drinking solution were carried out using an HPLC determination developed for amine detection (47). The amount of tyramine detected in the drinking water was almost identical in a freshly prepared solution and four days later in the feeding-bottles given to the rats, indicating that tyramine solution was stable at room temperature: 1.40 ± 0.01 *vs.* 1.38 ± 0.01 mg/ml (n = 3). Tyramine concentration in urines was 0.006 ± 0.003 mg/ml (n = 4) after 4-day treatment, indicating that tyramine ingestion was followed by, at least, a detectable absorption and a metabolism which leaves a small proportion of the unchanged product to be excreted in urines. Although the relative proportion of each

phase of tyramine absorption/ metabolism/excretion remains to be determined, the presence of tyramine at low concentration in urines indicated that not all amine was degraded at intestinal level. Thereafter, rats were treated for 7 weeks with tyramine at 1.38 mg/ml and several biological parameters were measured at the end of treatment. No change in body weight gain or food intake was observed despite a weakly decreased water consumption (from 27 to 22 ml/rat/day), possibly due to an aversion for the amine solution. Tyramine-treated animals did not shown any change in their adiposity, fasting blood glucose or plasma insulin (Table IV). No significant difference was found for the lipid peroxidation products, 4-hydroxynonenal and malondialdehyde, used as blood markers of oxidative stress. However, rats administered with tyramine exhibited reduced plasma free fatty acids under fasting conditions (Table IV). This reduction was probably the result of a blunted lipid mobilization that could reflect the tyramine antilipolytic effect previously demonstrated *in vitro* (64). This antilipolytic effect was confirmed in adipocytes isolated from tyramine-treated rats since the *in vitro* lipolytic stimulation of 10 nM isoprenaline was dose-dependently counteracted by tyramine: it decreased from 0.50 ± 0.08 to 0.31 ± 0.08 and 0.25 ± 0.08 µmol glycerol released/ 100 mg cell lipid /90 min in the presence of 0.1 and 1 mM tyramine, respectively ($n = 3$, $P < 0.05$). Moreover, the antilipolytic effect of insulin was not altered in adipocytes from tyramine-treated rats (not shown), suggesting that the metabolism of tyramine, even if in part due to its oxidation, was not an early instigator of insulin resistance. Taken together, these findings do not support that tyramine supplementation is able to produce an oxidative stress deleterious for vascular physiology and metabolic control. On the contrary, the tyramine-induced reduction of circulating fatty acids can favour peripheral glucose utilisation and probably could limit the onset of insulin resistance.

Adipose tissue-bound AO in obesity

Further investigations are necessary to complete the study of putative insulin-like properties of tyramine, especially in obese and diabetic models. The considerable enlargement of fat stores found in obesity may facilitate the oxidation of substrates in this tissue by increasing the ratio of the amount of adipose-bound SSAO/soluble

Table IV. *Biological parameters of male rats after 7 weeks of oral treament with tyramine.*

	Control	Tyramine-treated
body weight (g)	503 ± 16	491 ± 8
visceral WAT weight (g)	20.3 ± 1.8	20.5 ± 1.3
blood glucose (mg/dl)	86 ± 2	86 ± 2
plasma insulin (µIU/ml)	18.4 ± 2.0	19.9 ± 2.0
plasma HNE + MDA (µM)	3.9 ± 0.7	5.2 ± 1.4
plasma free fatty acids (mM)	1.33 ± 0.04	1.11 ± 0.06**

Circulating parameters were measured after overnight fasting. HNE + MDA: 4-hydroxynonenal + malondialdehyde; WAT: white adipose tissue. Mean ± SEM of 5 control and 7 treated rats. Difference between control and treated at $p < 0.02$.

SSAO. In a recent clinical approach, no change of SSAO activity has been found in the adipose tissue of obese humans (63), at least when activity is expressed per milligram of proteins. Moreover, an increased amount of adipose proteins becomes evident in obesity when considering that enlarged fat depots consist in massive lipid accumulation accompanied by a net hypertrophy and/or hyperplasia of adipocytes. As a consequence, the total amount of SSAO/VAP-1 present in fat stores is larger in obesity, as recently reported in transgenic mice submitted to high fat diet (60). In addition, human adipose tissue is considerably more rich in SSAO and MAO activity than blood (Vmax of SSAO-dependent oxidation of benzylamine is 50.1 = 4.9 nmol/min per mg of adipose tissue, and 0.27 ± 0.02 nmol/min per ml of blood) (63), and has therefore a large capacity to metabolise AO substrates. The exact amount of AO contained in the adipose tissues from different anatomical regions remains to be established for healthy subjects and obese and/or diabetic patients, the only available information being that SSAO activity is unchanged and MAO is decreased in the subcutaneous abdominal fat depot of young obese males when compared to age-matched lean controls (63). On the contrary, an elevation of circulating SSAO/VAP-1, the role of which remains to be established, is widely recognized to occur in diverse pathologies (for a review, see: 3). Although the increase in soluble SSAO has been repeatedly reported by independent studies in diabetic patients, the small increase (17 %) reported in one study on massively obese subjects with probably several of the complications of the metabolic syndrome (65), remains controversial, since not confirmed in a comparison between young obese individuals and their age-matched controls (63). Therefore, an increased circulating SSAO, if any, cannot disagree with the present hypothesis of a predominent amine oxidation at the level of fat depots in obese subjects. In keeping with this, an atherogenic diet conducted by STOLEN and coworkers in parallel to methylamine treatment (55), induced an increased body weight, adiposity, circulating glucose, insulin, AGE, together with a high mortality rate in FVB/n mice at 15 months of age, without modifying plasma SSAO activity, demonstrating that increased soluble SSAO is not the sole instigator of cardiovascular complications accompanying metabolic disorders. At last, it must be mentioned that adipose tissue has been proposed as one of the sources of the circulating SSAO (56), which is another confirmation of the quantitative importance of adipose SSAO in obesity.

Whether both adipose and plasma AOs could participate to the generation of oxidative stress in diabetes and/or obesity remains a subject of debate, and future pharmacological trials with AO-interacting drugs will shed more light on this topic. Increased oxidative stress seems to occur in obesity and, according to recent findings obtained in obese KKAy mice by FURUKAWA and coworkers, the oxidative stress in accumulated fat may have a deleterious impact on metabolic syndrome (19). However, such alterations of the redox state of adipose tissue in obesity are far from being established, since we did not found any sign of increased oxidative stress in young obese men, already hyperleptinemic and hyperinsulinemic (63). Despite this current discrepancy, it remains likely that any change in the level of oxidative stress in fat cells may influence the obesity-associated metabolic syndrome. Therefore, establishing mark-

ers of oxidative injury must be included in future investigations aiming at stimulating amine oxidase activity in order to improve glucose tolerance. This could be achieved by assessing lipid peroxidation via measurement of thiobarbituric acid reactive substances in plasma and in adipose tissue. Levels of circulating advanced glycation end products should also be monitored in the future long-term administrations of amine oxidase interacting agents.

Conclusions and plea for future treatments with AO-interacting agents

The occurrence of adverse effects resembling to vascular complications of diabetes observed during chronic treatment of transgenic mice with methylamine (55) cannot impede furthers treatments with AO-interacting drugs. In this view, physical exercise is another phenomenon which provokes transient oxidative stress in skeletal muscles but which also dramatically alleviates insulin resistance. One can therefore imagine that the insulin mimicry of AO substrates can overpass the putative oxidative stress caused in the vasculature by increasing the former or decreasing the latter component of this balance. The synergism between AO substrates and vanadate regarding glucose disposal has perhaps no deleterious effects on vasculature, and after verification, could constitute a good alternative for the generation of oral antiabetic drugs. Nevertheless, searching for amine oxidase substrates generating less toxic aldehydes than methylamine remains mandatory. Mixing amine oxidase substrate properties to other relevant pharmacological efficacy is another alternative than can allow to detect novel antidiabetic agents. In this view, benzylamine has been described to exert hypophagic actions, independently from interaction with brain AO (50) that could be useful for treating obesity and diabetes. However, benzylamine is rather a relatively simple molecule (alpha-amino-toluene) naturally present in medicinal plants, such as *Moringa oleifera* (48), and is better known for its use in chemical organic synthesis than in therapeutic applications. Therefore, benzylamine analogs or derivatives could be expected to have higher therapeutic value. In order to detect other primary amines with more or less chemical similarity with benzylamine, recent studies aimed at better define the catalytic centre of SSAO (34). In a first subset of molecules fiting with the established pharmacophore, an antimicrobial sulfonamide, namely mafenide (alpha-amino-p-toluene sulfonamide) focused our interest. However, although being efficient in animal models, this compound was a low affinity substrate for human SSAO and was not a more efficient insulin-mimicker than benzylamine itself (Iglesias-Osma et al, personal communication). Many benzylamine derivatives are known to exhibit distinct pharmacological properties, such as inhibition of poly(ADP-ribose)polymerase (46), or inhibition of nitric oxide synthase (25), as does aminoguanidine , a SSAO inhibitor proposed to limit diabetic complications (59). Whether mixing these properties with the interaction with AO can improve the antidiabetic actions reported so far for classical substrates of MAO and SSAO deserves to be studied in future pharmacological approaches of obesity, diabetes, and related disorders.

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C. CARPÉNÉ, S. BOUR, V. VISENTIN, F. PELLATI, S. BENVENUTI, M. C. IGLESIAS-OSMA, J. MORATINOS y P. VALET. *Sustratos de amino oxidasas para el tratamiento de trastornos de la tolerancia a la glucosa* (minirrevisión). J. Physiol. Biochem., **61** (2), 405-420, 2005.

Las amino-oxidasas (AO) están ampliamente distribuidas, desde microorganismos hasta vertebrados, y producen peróxido de hidrógeno y aldehído al catabolizar aminas biógenas o exógenas. Datos recientes ponen de manifiesto que las AO no pueden considerarse exclusivamente como depuradoras de aminas. La amino-oxidasa sensible a semicarbazida (SSAO) es muy abundante en ciertas células de mamíferos como las endoteliales, las células musculares lisas y los adipocitos, donde desempeña un papel importante en la adhesión de los linfocitos a las paredes vasculares, la maduración de las fibras elásticas arteriales, y el transporte de glucosa, respectivamente. Este último efecto es el que se presenta en esta revisión donde, además, se discuten las perspectivas abiertas como consecuencia de sus acciones insulino-miméticas. Los sustratos de SSAO y de monoamino-oxidasa mimetizan varios efectos de la insulina en los adipocitos: activación del transporte de glucosa y de la lipogénesis e inhibición de la lipólisis. También estimulan *in vitro* la adipogénesis. La administración aguda *in vivo* de sustratos de AO mejora la tolerancia a la glucosa en ratas, ratones y conejos, y los tratamientos crónicos con benzilamina y vanadato tienen un efecto antihiperglucemiante en ratas diabéticas. Suplementos en la dieta con metilamina, benzilamina o tiramina han puesto de manifiesto una acción beneficiosa sobre el control metabólico en roedores a través de un aumento en la tolerancia a la glucosa o una disminución de la movilización lipídica, sin cambios notables en los marcadores plasmáticos de peroxidación lipídica o de glicación proteica, a pesar de unos efectos aterogénicos indeseables. Por tanto, las aminas ingeridas no se degradan totalmente a nivel de la barrera intestinal y pueden actuar sobre el tejido adiposo y vascular. En relación con esta influencia sobre el control metabólico, se concluye que es necesario prestar atención a la composición o adición como suplementos de estas aminas a los alimentos y medicamentos.

Palabras clave: Insulina, Monoamino-oxidasa, Lipólisis, Lipogénesis, Adipocitos, Obesidad.

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