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Inhibition of rat fat cell lipolysis by monoamine oxidase and semicarbazide-sensitive amine oxidase substrates

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Abstract

It has been demonstrated that amine oxidase substrates stimulate glucose transport in cardiomyocytes and adipocytes, promote adipogenesis in pre-adipose cell lines and lower blood glucose in diabetic rats. These insulin-like effects are dependent on amine oxidation by semicarbazide-sensitive amine oxidase or by monoamine oxidase. The present study aimed to investigate whether amine oxidase substrates also exhibit another insulin-like property, the inhibition of lipolysis. We therefore tested the influence of tyramine and benzylamine on lipolytic activity in rat adipocytes. These amines did not modify basal lipolysis but dose-dependently counteracted the stimulation induced by lipolytic agents. The response to 10 nM isoprenaline was totally inhibited by tyramine 1 mM. The blockade produced by inhibition of amine oxidase activity or by 1 mM glutathione suggested that the generation of oxidative species, which occurs during amine oxidation, was involved in tyramine antilipolytic effect. Among the products resulting from amine oxidation, only hydrogen peroxide was antilipolytic in a manner that was potentiated by vanadate, as for tyramine or benzylamine. Antilipolytic responses to tyramine and to insulin were sensitive to wortmannin. These data suggest that inhibition of lipolysis is a novel insulin-like effect of amine oxidase substrates which is mediated by hydrogen peroxide generated during amine oxidation.

Keywords: Semicarbazide-sensitive amine oxidase; Monoamine oxidase; Glutathione; Hydrogen peroxide; Isoprenaline; Tyramine; Adipose cell

1. Introduction

The presence of amine oxidase activity in adipose cells has been substantiated in different mammalian species (Barrand and Callingham, 1982; Raimondi et al., 1991; Tong et al., 1979) including humans (Morin et al., 2001; Pizzinat et al., 1999), but its biological role remains to be clarified. Two families of amine oxidases are involved in amine oxidation: the monoamine oxidases A and B (E.C. 1.4.3.4) (Bach et al., 1988), which are flavoproteins present in the outer mitochondrial membrane, and the copper-containing amine oxidases, among which the semicarbazide-sensitive amine oxidases (E.C. 1.4.3.6) are ectoenzymes located at the cell surface (Salminen et al., 1998). Despite their different subcellular location, monoamine oxidase and semicarbazide-sensitive amine oxidase exhibit only discrete differences regarding their substrate specificity. For instance, tyramine, which is present in food and beverages (Kopin, 1993), is a common substrate for monoamine oxidase A, monoamine oxidase B and semicarbazide-sensitive amine oxidase in rats but is only a monoamine oxidase substrate in humans, while benzylamine and methylamine can be considered as relatively selective substrates for semicarbazide-sensitive amine oxidase in both species (Lyles, 1996). The pharmacological distinction between mono- and semicarbazide-sensitive amine oxidases has been essentially established according to inhibitor selectivity: semicarbazide-sensitive amine oxidase is inhibited by carbonyl reagents like semicarbazide but is resistant to most of the selective monoamine oxidase inhibitors (pargyline, clorgyline, deprenyl).

In addition to the classical scavenger function attributed to the amine oxidases of different anatomical locations, the monoamine oxidase and semicarbazide-sensitive amine oxidase present on adipose cells have been recently suspected to participate in the regulation of carbohydrate metabolism on the basis of a growing number of observations. First, a stimulation of glucose transport by benzylamine or tyramine

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has been reported in rat fat cells (Marti et al., 1998; Enrique-Tarancon et al., 1998). Then, semicarbazide-sensitive amine oxidase activity has been proposed to mediate the stimulation of glucose uptake by benzylamine in human adipocytes (Morin et al., 2001), while monoamine oxidase is involved in the serotonin-induced activation of glucose uptake into rat cardiomyocytes (Fischer et al., 1995). Similarly, the insulin-like differentiating effects of benzylamine, tyramine (Fontana et al., 2001) or methylamine (Mercier et al., 2001) on the pre-adipose cells lines 3T3 F442A and 3T3 L1 have been demonstrated to be mediated via semicarbazide-sensitive amine oxidase-dependent oxidation. Finally, tyramine (Morin et al., 2002) and benzylamine (Marti et al., 2001) were found to enhance glucose disposal in vivo. One common mechanism in all these biological responses was the involvement of hydrogen peroxide generated during oxidative deamination, since all these responses were blocked by amine oxidase inhibitors or by catalase and/or glutathione as well as by other antioxidant systems. In rat adipocytes, the presence of vanadate at 0.1 mM allowed both monoamine oxidase and semicarbazide-sensitive amine oxidase substrates to activate the translocation of glucose transporters to the cell surface and to stimulate glucose uptake up to one half the maximal effect of insulin (Marti et al., 1998; Enrique-Tarancon et al., 1998). The potentiation of amine effects by vanadate was likely due to an interaction between the hydrogen peroxide resulting from amine oxidase activity and vanadate, and to the subsequent generation of peroxovanadate (Enrique-Tarancon et al., 2000), a powerful tyrosine phosphatase inhibitor (Huyer et al., 1997) and a potent insulin-mimicking agent (Lönnroth et al., 1993).

Since insulin not only stimulates glucose transport but also inhibits lipolysis, we asked ourselves whether tyramine or benzylamine is also able to inhibit lipolysis in a manner dependent on oxidation by monoamine oxidase or semicarbazide-sensitive amine oxidase. Because hydrogen peroxide inhibits lipolysis in rodent fat cells (Little and De Haën, 1980) and because all the metabolic effects of amine oxidase substrates described so far were somewhat insulin-like, an antilipolytic effect was expected. Although this was confirmed by our observation of an antilipolytic effect of benzylamine in human fat cells (Morin et al., 2001), the opposite was reported in the literature, where tyramine has been shown to increase the lipolytic response to isoprenaline in rat adipocytes (Raimondi et al., 2000b). This discrepancy led us to conduct a pharmacological analysis of the effects of tyramine and benzylamine on the lipolytic activity of rat adipocytes. To this end, we tested these monoamine oxidase and semicarbazide-sensitive amine oxidase substrates alone, and against different lipolytic agents. Since the oxidation of a given amine by either monoamine oxidase or semicarbazide-sensitive amine oxidase generates not only hydrogen peroxide but also the corresponding aldehyde and ammonia, we also tested these oxidation products.

The results indicate that 0.1–1 mM tyramine or benzylamine, which are known to activate glucose uptake (Enrique-Tarancon et al., 2000) and to produce hydrogen peroxide (Raimondi et al., 2000b) in rat fat cells, caused an inhibition of lipolysis which was reversed by amine oxidase blockers or by glutathione. Among the end-products of deaminative oxidation, only hydrogen peroxide was able to induce an antilipolytic response that was enhanced by vanadate.

2. Materials and methods

2.1. Animals

Male Wistar rats of 190–230 g (obtained from Harlan France, Gannat) were housed individually with free access to water and chow in accordance with the European Communities Council Directives for experimental animal care.

2.2. Adipocyte isolation

The epididymal and retroperitoneal fat pads were removed and minced with scissors in Krebs–Ringer containing 15 mM sodium bicarbonate, 10 mM HEPES,and bovine serum albumin (3.5% w/v) (KRBH buffer, pH 7.5). For each animal, the white adipose tissues were digested for 35–45 min at 37 °C with 1.5 mg/ml collagenase. Isolated fat cells were washed three times in a large amount (around 30 ml) of the same buffer without collagenase.

2.3. Lipolysis measurements

After being washed, the floating fat cells were diluted in around 10-fold their volume of KRBH, and 500 μl of the cell suspension was immediately distributed under shaking into plastic incubation vials containing 5 μl of drug dilutions at 100× the final concentration to be tested. The cellular lipid content of the vials was 20 ± 2 mg/500 μl, equivalent to around 100,000 cells. After a 90-min incubation, the glycerol released into the medium was enzymatically assayed as previously described (Carpéné et al., 1999) and used as an index of lipolytic activity, expressed as μmol of glycerol released/100 mg of cellular lipid/90 min.

2.4. Amine oxidase activity

Oxidation of [^{14}C]tyramine and [^{14}C]benzylamine by fat cell supensions or crude membrane preparations was conducted as previously described (Marti et al., 1998). Briefly, intact fat cells were incubated in KRBH buffer while membranes were incubated in 200 mM phosphate buffer, pH 7.4, in the presence of increasing concentrations of radioactive amine. Assays were conducted under conditions where oxidation increased linearly with time and protein concentration. Assays were stopped by adding 4 M HCl. Reaction products were extracted by addition of 1 ml of solvent (toluene/ethylacetate, v/v). Then, 0.7-ml aliquots of the organic phase were transferred to scintillation vials and

counted. Non-enzymatic oxidation was defined as that resistant to inhibition by 1 mM pargyline plus 1 mM semicarbazide: it accounted for less than 3% of total oxidation in all cases.

2.5. Drugs

[^{14}C]Tyramine and [^{14}C]benzylamine came from Perkin Elmer Life Sciences and from Amersham Pharmacia Biotech, respectively. Bovine insulin and serum albumin, quercetin, wortmannin, semicarbazide, pargyline, tyramine, benzylamine, sodium orthovanadate, hydrogen peroxide and most commonly used chemicals were from Sigma-Aldrich (St. Louis, MO). The *p*-tyramine form was used unless specified. Collagenase, enzymes and cofactors for the determination of glycerol levels were from Roche Molecular Biochemicals (Mannheim, Germany).

2.6. Statistical analyses

The number of experiments is given by *n*. Student's *t*-test for unpaired samples was used to detect significant differences between results, given as means ± S.E.M. NS corresponds to a nonsignificant difference. EC_{50} is the agent concentration eliciting half-maximal effect and was calculated by computer fitting of the concentration-dependent curves.

3. Results

3.1. Oxidation of tyramine and benzylamine by rat fat cells

Tyramine was readily oxidized by both intact and broken fat cell preparations. In both cases, [^{14}C]tyramine oxidation was totally prevented in the presence of 1 mM semicarbazide plus pargyline. Around one half of the oxidation was resistant to semicarbazide and could be attributed to monoamine oxidase, while the other half was semicarbazide sensitive (Table 1). These data suggest that tyramine can not only be oxidized by semicarbazide-sensitive amine oxidase at the cell surface but also be taken up into fat cells and oxidized by mitochondrial monoamine oxidase. [^{14}C]Benzylamine was oxidized by isolated adipocytes and by ghosts, in a manner that was totally inhibited by semicarbazide and which could be entirely attributed to semicarbazide-sensitive amine oxidase activity.

Table 1
Oxidation of tyramine and benzylamine by crude membranes and intact adipocyte preparations

Biological preparation	Crude membranes		Intact adipocytes	
Amine oxidized	Tyramine	Benzylamine	Tyramine	Benzylamine
V_m, nmol/mg protein/min	12 ± 1	21 ± 5	13 ± 3	21 ± 2
K_m, μM	85 ± 18	42 ± 7	158 ± 40	29 ± 8
% resistant to pargyline	54 ± 2	97 ± 2	57 ± 4	98 ± 5
% resistant to semicarbazide	45 ± 2	3 ± 0	45 ± 4	3 ± 5

Amine oxidation was measured after 30 min of incubation with increasing concentrations of substrates (up to 1 and 0.2 mM for [^{14}C]tyramine and [^{14}C]benzylamine, respectively) and crude membranes or adipocyte suspensions. The oxidation sensitive to pargyline plus semicarbazide 1 mM is expressed as nanomoles of amine oxidized per milligram of membrane proteins (present in the membrane assays or recovered after centrifugation in the case of fat cell suspensions). The residual oxidation of 0.5 mM tyramine or 0.1 mM benzylamine resistant to pargyline or to semicarbazide is reported as percentage of total oxidation. Results are means ± S.E.M. of four observations.

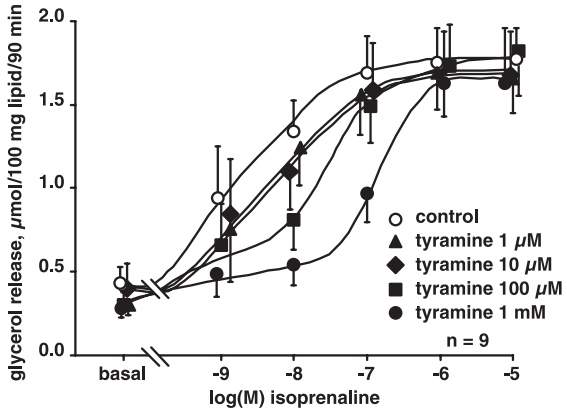

Fig. 1. Inhibitory effect of tyramine on isoprenaline-dependent lipolysis. Lipolysis was stimulated by isoprenaline without (control, open circles) or with the indicated concentrations of tyramine (black symbols). Basal glycerol release was measured without isoprenaline. The EC_{50} values for isoprenaline-dependent stimulation were (in nM) 7.4 ± 1.2, 14.9 ± 4.8, 15.6 ± 4.0, 30.5 ± 6.7 and 202.5 ± 43.0 for control and in the presence of 1, 10, 100 and 1000 μM tyramine, respectively. Mean ± S.E.M. of nine experiments.

3.2. Antilipolytic effects of tyramine, benzylamine and hydrogen peroxide

When present alone, tyramine did not stimulate lipolysis whatever the concentration tested, ranging from 10^{-6} to 10^{-3} M (Fig. 1). Tyramine was also unable to inhibit the maximal lipolytic effect of 1–10 μM of the mixed β-adrenoceptor agonist isoprenaline. However, tyramine provoked a marked shift to the right of the dose–response curve of isoprenaline: the EC_{50} values increased from 7.4 ± 1.2 nM for isoprenaline alone to 30.5 ± 6.7 and 203 ± 43 nM with 100 μM or 1 mM tyramine ($P < 0.01$ and $P < 0.001$, respectively, $n = 9$). Fig. 2 shows that the semicarbazide-sensitive amine oxidase substrate benzylamine also inhibited the lipolytic response to isoprenaline. When added at 1 mM, benzylamine increased the EC_{50} value for isoprenaline up to 18 ± 2 nM ($P < 0.05$, $n = 9$).

Hydrogen peroxide, which is a common product of amine oxidation whatever the substrate or enzyme in-

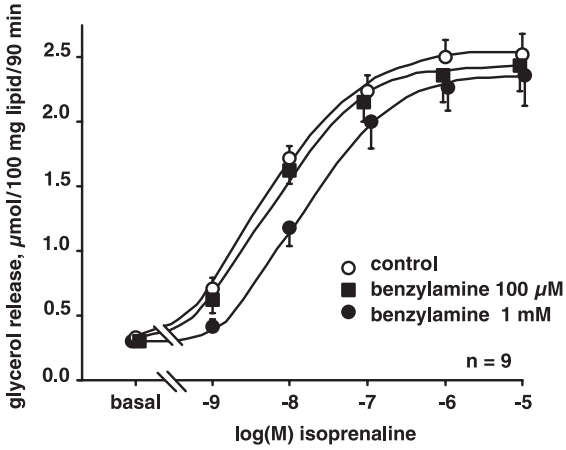

Fig. 2. Inhibitory effect of benzylamine on isoprenaline-dependent lipolysis. Increasing concentrations of isoprenaline were tested alone (control, open circles), or with benzylamine (black symbols). The EC_{50} values for isoprenaline-dependent lipolysis were (in nM) 8.7 ± 2.7, 8.9 ± 2.3, and 17.8 ± 2.3 for control and in the presence of 100 and 1000 μM benzylamine, respectively.

volved, was tested and clearly counteracted the effect of isoprenaline (Fig. 3), resulting in an increase of the EC_{50} value from 7.7 ± 1.6 (isoprenaline alone, $n = 21$) to 18.2 ± 4.3, 130 ± 64 and 533 ± 200 nM when added at a final concentration of 10, 100 and 1000 μM (different from control at $P < 0.01$ for the lower dose and at $P < 0.001$, for the two higher, $n = 8–9$). Since the maximal stimulation of lipolysis obtained with 1–10 μM isoprenaline was resistant to inhibition by 1 mM amines, hydrogen peroxide or 100 nM insulin (not shown), the submaximal stimulation elicited by 10 nM isoprenaline (see Figs. 1–3) was used to further study the antilipolytic action of amines.

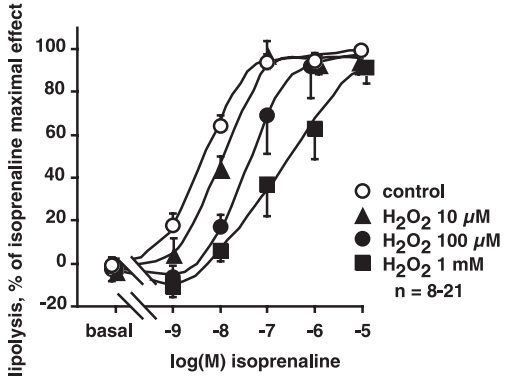

Fig. 3. Influence of hydrogen peroxide on isoprenaline-dependent lipolysis. Stimulation of lipolysis was determined with isoprenaline alone (control, open circles) or in the presence of hydrogen peroxide at the indicated doses (H_2O_2, black symbols). Results are expressed as percentage of maximal lipolysis, which with 10 μM isoprenaline was 2.1 ± 0.2 μmol of glycerol released/100 mg lipid/90 min ($n = 21$), with basal lipolysis set at 0%. Each point is the mean ± S.E.M. of 8–21 determinations.

3.3. Blockade of the antilipolytic effects of tyramine and benzylamine by glutathione and influence of amine oxidation products on lipolysis

The antilipolytic effect of tyramine, which was not influenced by the hydroxyl position on the benzene ring, was reversed by the reduced form of glutathione (GSH) (Table 2). GSH was, at 1 mM, without noticeable effect on basal or 10 nM isoprenaline-stimulated lipolysis (0.30 ± 0.03 vs. 0.32 ± 0.05 and 1.72 ± 0.10 vs. 1.74 ± 0.15 μmol of glycerol released/100 mg lipid/90 min, NS, $n = 9$). Glutathione also reversed the antilipolytic effect of benzylamine and hydrogen peroxide (Table 2). Since glutathione also inhibited the effect of hydrogen peroxide, as well as that of amines, on glucose transport in rat cardiomyocytes (Fischer et al., 1995) or human adipocytes (Morin et al., 2001), it seems likely that the hydrogen peroxide produced during oxidative deamination was responsible for the antilipolytic effect of amine oxidase substrates. However, we tested *p*- and *m*-hydroxyphenylacetic acid and benzaldehyde, which are respective end-products of *p*- and *m*-tyramine and benzylamine oxidation. These compounds were unable to activate lipolysis (not

Table 2
Antilipolytic effects of tyramine or benzylamine are blocked by glutathione and mimicked by hydrogen peroxide but not by other end-products of amine oxidation

Agent added to isoprenaline at	Lipolysis, % of 10 nM isoprenaline-induced effect	
	0.1 mM	1 mM
p-Tyramine	68.2 ± 6.1^a	19.3 ± 5.4^a
m-Tyramine	63.4 ± 9.8^b	8.8 ± 6.6^a
Benzylamine	84.0 ± 7.4	59.2 ± 5.2^a
Hydrogen peroxide	27.9 ± 6.6^a	0.7 ± 6.4^a
p-Tyramine + GSH 1 mM	77.8 ± 17.2	80.8 ± 9.7
Benzylamine + GSH 1 mM	96.5 ± 6.9	84.3 ± 6.1
Hydrogen peroxide + GSH 1 mM	98.2 ± 17.1	74.5 ± 9.0
p-HPAA	90.8 ± 6.7	85.9 ± 9.5
m-HPAA	80.7 ± 10.6	89.0 ± 10.8
Benzaldehyde	94.7 ± 9.1	88.4 ± 25.0
Ammonium	92.9 ± 13.2	85.3 ± 10.0
Hydrogen peroxide + *p*-HPAA 0.1mM	19.3 ± 4.6^a	-11.6 ± 5.9^a
Hydrogen peroxide + benzaldehyde 0.1mM	29.8 ± 3.4^a	-4.1 ± 3.8^a
Hydrogen peroxide + ammonium 1mM	34.7 ± 4.5^a	0.2 ± 6.1^a

Lipolysis was stimulated with 10 nM isoprenaline alone (1.47 ± 0.10 μmol/100 mg lipid/90 min, taken as 100% reference) or with the indicated compounds. Negative percentages correspond to values below basal glycerol release. GSH: reduced glutathione; HPAA: hydroxyphenylacetic acid. Means ± S.E.M. for 6–13 rats.

[a] $P < 0.001$ in comparison with isoprenaline alone.

[b] $P < 0.01$ in comparison with isoprenaline alone.

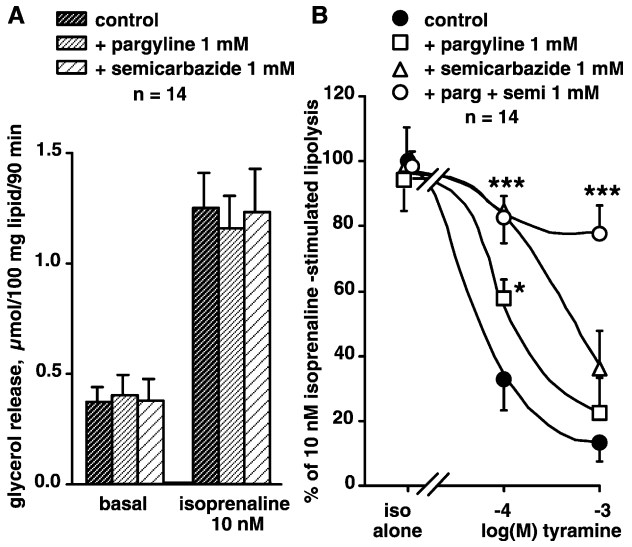

Fig. 4. Influence of pargyline and semicarbazide on lipolysis in rat adipocytes. (A) Basal lipolysis was stimulated by 10 nM isoprenaline in the absence (control) or the presence of 1 mM amine oxidase inhibitors. (B) Lipolysis is expressed as a percentage of the isoprenaline-induced stimulation, with basal lipolysis set at 0% and response to 10 nM isoprenaline (iso alone) set at 100% for each of the 14 experiments. Different from the corresponding lipolytic activity found with tyramine in the absence of inhibitor (black circles) at $*P<0.05$, $***P<0.001$.

shown), to counteract the effect of isoprenaline, or to hamper the antilipolytic effect of hydrogen peroxide (Table 2). Similar observations were obtained with ammonium, another product of amine oxidation, at least when increasing concentrations of ammonium chloride up to 1 mM were tested (Table 2).

3.4. Reversal of the tyramine antilipolytic action by amine oxidase inhibitors

The antilipolytic effect of tyramine was studied in the presence of pargyline (monoamine oxidase A/B inhibitor) or semicarbazide (Lyles, 1996). The rightward shift induced by tyramine 100 µM of the isoprenaline dose–response curve was less pronounced with 1 mM semicarbazide than with 1 mM pargyline (not shown). Fig. 4A shows that neither semicarbazide nor pargyline significantly modified basal or 10 nM isoprenaline-stimulated lipolysis. The blockade of tyramine-induced antilipolysis was weaker with pargyline than with semicarbazide and complete only with the combination of both inhibitors (Fig. 4B). Taken together, these data suggest that the metabolism of tyramine by semicarbazide-sensitive amine oxidase, and to a lesser extent by monoamine oxidase, was involved in its antilipolytic action. In the same way, the antilipolytic effect of benzylamine disappeared under blockade of semicarbazide-sensitive amine oxidase (not shown).

3.5. Potentiation by vanadate of the antilipolytic effects of tyramine, benzylamine and hydrogen peroxide

Vanadate potentiates the effect of hydrogen peroxide or amines on glucose uptake, when used at 0.1 mM, a dose that has no significant effect on its own (Enrique-Tarancon et al., 1998; Marti et al., 1998). We tested whether vanadate could improve the antilipolytic effect of tyramine, benzylamine, or their common oxidation product, hydrogen peroxide. At 0.1 mM, sodium orthovanadate did not inhibit the lipolytic effect of 10 nM isoprenaline but reinforced the tyramine action by improving significantly its maximal antilipolytic effect (Fig. 5A). The partial inhibition of isoprenaline-stimulated lipolysis observed with benzylamine was dramatically enhanced in both potency and efficacy by vanadate (Fig. 5B). Vanadate also potentiated the antilipolytic effect of hydrogen peroxide (Fig. 5C), while it did not allow hydroxyphenylacetic acid, benzaldehyde or ammonium to exert any antilipolytic action (not shown).

3.6. Tyramine-, benzylamine- and insulin-induced antilipolysis counteract different lipolytic stimulants

The antilipolytic responses to tyramine or benzylamine were then compared to that of insulin, regarding the nature of the lipolytic stimulation. The three compounds partially inhibited the submaximal stimulation of lipolysis evoked by the selective β_3-adrenoceptor agonist CL 16243: at 1 nM, this agonist stimulated glycerol release by up to 83% of the maximal response, and this was reduced to 46%, 53% and 59% by 100 nM insulin, 1 mM tyramine and 0.1 mM

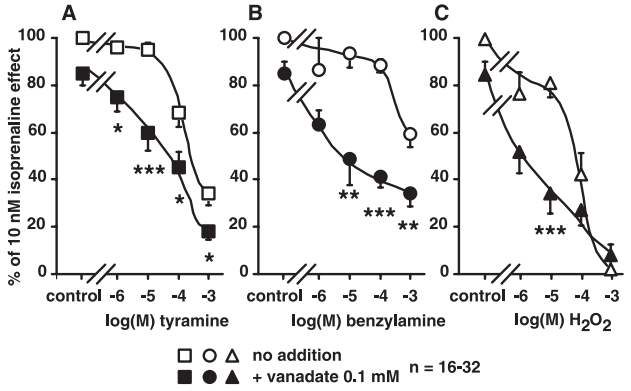

Fig. 5. Influence of vanadate on the dose-dependent antilipolytic effect of tyramine (A, left panel, squares), benzylamine (B, middle panel, circles) or hydrogen peroxide (C, right panel, triangles). Glycerol release was measured after incubation with 10 nM isoprenaline alone (control) or in the presence of the indicated agents without (no addition, open symbols) or with vanadate at 0.1 mM (closed symbols). Results are expressed as percentage of isoprenaline-induced stimulation. Basal and 10 nM isoprenaline-stimulated lipolysis (0.31 ± 0.04 and 1.38 ± 0.10 µmol glycerol/100 mg lipid/90 min, $n=24$) was set at 0% and 100%, respectively. Mean ± S.E.M. from 8 to 16 experiments. Different from corresponding condition without vanadate at $*P<0.05$, $**P<0.01$, $***P<0.001$.

benzylamine plus vanadate, respectively ($n=6$, $P<0.05$). Similar results were obtained against the β_1-adrenoceptor agonist dobutamine (not shown). Amines were also tested against non-adrenergic lipolytic agents, such as isobutylmethylxanthine (10 μM) and adenosine deaminase (4 IU/ml). Isobutylmethylxanthine is a phosphodiesterase inhibitor and an adenosine receptor antagonist; adenosine deaminase removes the adenosine produced by rat fat cells and thus activates lipolysis by inactivating an inhibitory tone mediated by adenosine A_1 receptors negatively coupled to adenylyl cyclase. The antilipolytic effects of tyramine and benzylamine were significant only in the presence of vanadate, while the effects of insulin and of a non-hydrolysable adenosine analogue were detectable even without vanadate (Table 3). These data suggest that the antilipolytic effects of tyramine and benzylamine cannot be exclusively attributed to an adenosine-mediated inhibition of adenylyl cyclase. Of note, tyramine and benzylamine were, like insulin, unable to counteract the maximal stimulation of lipolysis obtained with β-adrenoceptor agonists or with isobutylmethylxanthine (not shown).

3.7. Tyramine- and insulin-induced antilipolysis: additivity and sensitivity to kinase inhibitors

Wortmannin, a relatively selective inhibitor of phosphatidylinositol 3-kinase, abolished at 100 nM the antilipolytic effect of 0.1 mM tyramine as well as that of 100 nM insulin (Fig. 6A). Quercetin, a nonselective inhibitor of tyrosine kinases, also blocked at 1 μM the antilipolytic effects of both tyramine and insulin. Therefore, tyrosine phosphorylation of intracellular targets appears to be involved in the insulin-like antilipolytic action of amine oxidase substrates.

Table 3
Influence of vanadate on the inhibitory effects of tyramine, benzylamine and insulin on isobutylmethylxanthine and adenosine deaminase-stimulated lipolysis

Lipolytic agent	Glycerol release, % of maximal lipolysis			
	IBMX 10 μM		ADA 4 IU/ml	
Vanadate	No	0.1 mM	No	0.1 mM
Control	44.7 ± 5.7	49.0 ± 7.5	54.9 ± 3.4	46.1 ± 3.8
Tyramine 1 mM	24.9 ± 8.9	21.3 ± 2.7[a]	45.2 ± 4.4	31.5 ± 3.0[a]
Benzylamine 0.1 mM	38.5 ± 8.1	14.0 ± 4.1[a]	50.7 ± 3.8	33.1 ± 3.0[a]
Insulin 100 nM	2.5 ± 3.4[b]	3.1 ± 0.5[b]	19.7 ± 4.2[b]	20.3 ± 3.4[b]
PIA 1 μM[c]	0.0 ± 1.0[b]	0.6 ± 1.4[b]	0.3 ± 1.5[b]	1.0 ± 1.7[b]

Glycerol release was measured under stimulation with isobutylmethylxanthine (IBMX) or adenosine deaminase (ADA) without (control) or with the indicated compounds. Results are expressed as percentages of maximal lipolysis (reached with 10 μM isoprenaline: 1.74 ± 0.15 μmol/100 mg lipid/90 min, $n=30$). Each value is the mean ± S.E.M. for 5 rats for IBMX and for 15–30 rats for ADA.

[a] $P<0.01$ in comparison with respective control.

[b] $P<0.001$ in comparison with respective control.

[c] PIA is the non-metabolizable purinergic analogue phenylisopropyladenosine.

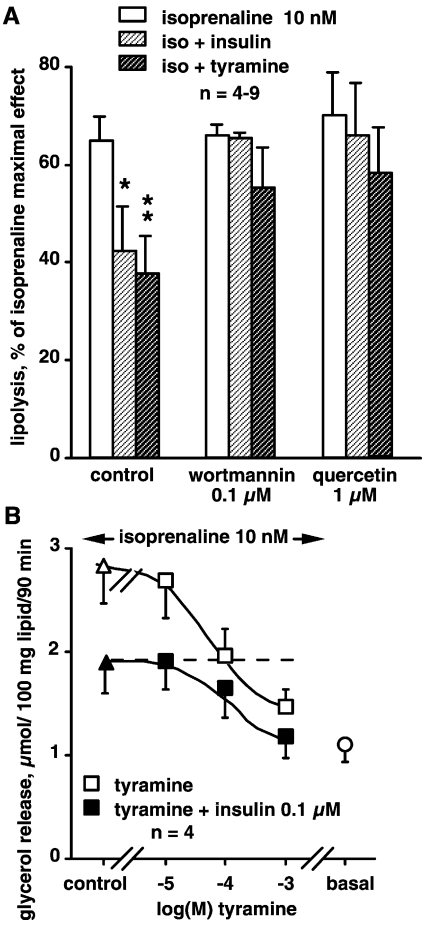

Fig. 6. Sensitivity to kinase inhibitors and additivity of tyramine- and insulin-induced antilipolysis. (A) A 100-μM sample of tyramine or 100 nM insulin was tested on 10 nM isoprenaline-stimulated lipolysis without and with 0.1 μM wortmannin or 1 μM quercetin. Results are expressed as percentage of maximal lipolysis. Mean ± S.E.M. of four to nine experiments. *$P<0.02$, **$P<0.01$ in comparison with 10 nM isoprenaline alone. (B) Antilipolytic effect of tyramine alone (open symbols) or in combination with insulin (closed symbols) was measured against the effect of 10 nM isoprenaline (control). Dotted line shows the maximal inhibitory effect of insulin alone. Mean ± S.E.M. of four experiments.

Finally, a complete inhibition of isoprenaline-stimulated lipolysis was obtained with the combination of 100 nM insulin plus 1 mM tyramine (Fig. 6B). Taken together, these data indicate that tyramine shares with insulin the activation of several common steps of signal transduction, but not all, in a manner that does not hamper—but is additive to—the action of insulin.

4. Discussion

The present study demonstrates that, in rat fat cells, tyramine and benzylamine were neither lipolytic nor able to improve the lipolytic action of isoprenaline while they exhibited clear-cut antilipolytic properties which were (i) mimicked by hydrogen peroxide, (ii) sensitive to inhibition

of amine oxidases or intracellular kinases and (iii) potentiated by vanadate. These characteristics concur with those reported for both amines regarding their activation of glucose transport (Enrique-Tarancon et al., 2000). Therefore, inhibition of lipolysis is a novel effect of amine oxidase substrates and totally agrees with their insulin-mimicking properties on mature adipocytes (Marti et al., 1998) and on cultured pre-adipocytes (Fontana et al., 2001; Mercier et al., 2001). These findings reinforce our observations previously made with human adipocytes, in which benzylamine, an exclusive semicarbazide-sensitive amine oxidase substrate, was antilipolytic in a manner that was not dependent or enhanced by vanadate (Morin et al., 2001).

Pharmacological analysis of the inhibition of isoprenaline- or CL 316243-dependent lipolysis does not suggest that tyramine exhibits affinity for β-adrenoceptors with a low intrinsic activity: maximal β-adrenergic activation was unaffected, while its potency was altered. This form of inhibition is not in agreement with partial agonism at β-adrenoceptors. Therefore, although structurally related to catecholamines, tyramine cannot be considered as a partial agonist or an antagonist of β-adrenoceptors. Similarly, benzylamine has been reported to be unable to activate lipolysis or to promote other signs of β-adrenoceptor activation in adipocytes from mice, even those with genetically modified adrenoceptor expression (Visentin et al., 2001). In addition, tyramine and benzylamine also counteracted the non-adrenergic activation of lipolysis by isobutylmethylxanthine and adenosine deaminase. Another putative mechanism involved in the antilipolytic action of tyramine or benzylamine could be a direct stimulation of antilipolytic α_2-adrenoceptors. This looks unlikely since the α_2-adrenergic antilipolytic responses are of minor importance in rat adipocytes, in which the full α_2-adrenoceptor agonist brimonidine (UK 14,304) maximally inhibits lipolysis by 20–30% (Carpéné et al., 1990). Thus, the antilipolytic actions of tyramine and benzylamine were more likely related to their oxidation by monoamine oxidase and/or semicarbazide-sensitive amine oxidase, characterized by K_m values ranging between 20 and 200 μM (Lyles, 1996).

When a given amine is oxidized by either mono- or semicarbazide-sensitive amine oxidase, it is converted into an aldehyde with the concomitant production of ammonia and H_2O_2 (Lyles, 1996). In isolated adipocytes as well as in ghost preparations, benzylamine is an exclusive semicarbazide-sensitive amine oxidase substrate, whereas tyramine is degraded by semicarbazide-sensitive amine oxidase located at the cell surface and by mitochondrial monoamine oxidase in equivalent proportions. The dual origin of hydrogen peroxide generation produced by tyramine likely explains why its inhibition of isoprenaline-stimulated lipolysis is stronger than that of benzylamine. Hydrogen peroxide can reproduce several actions of insulin (Little and De Haën, 1980; May and De Haën, 1979; Mukherjee, 1980), and it has been reported that the effects of insulin on glucose transport can be mimicked not only by the addition of 0.01–1 mM H_2O_2 but also by that produced during amine oxidase activation in rat cardiomyocytes (Fischer et al., 1995) or adipocytes (Marti et al., 1998). The involvement of hydrogen peroxide in the amine-induced antilipolytic response is also supported by the finding that other products of amine oxidation, ammonia, acids and aldehydes, were without an effect on lipolysis, like the amines themselves, when their oxidation was abolished after blockade of amine oxidases. Recently, it has been shown that hydrogen peroxide activates Gi proteins (Nishida et al., 2000) and that its increased production under insulin challenge leads to tyrosine phosphatase inhibition and improvement of the early insulin-stimulated cascade of tyrosine phosphorylations (Mahadev et al., 2001). In keeping with these recent findings, future investigations of the intracellular mechanisms involved in the effects of tyramine need to further quantify intra- and extracellular hydrogen peroxide formation by amine oxidases since only preliminary observations with fat cells are currently available (Raimondi et al., 2000a). This kind of investigation could also explain why our present observation, that tyramine inhibits lipolysis, is in contradiction with findings of Raimondi et al. (2000b), who reported that tyramine potentiated the lipolytic action of isoprenaline by an oxidation-dependent mechanism, implying an increase in cAMP accumulation. In this previous study, isoprenaline was tested at 50 nM, and the range of tyramine concentrations was limited between 10 and 50 μM. Slight modifications in our lipolysis assays (such as changes in the duration of stimulation and in the composition of the incubation medium) did not allow us to detect any stimulatory effect of tyramine alone or in combination with isoprenaline. Another important difference between the two studies is that the permissive effect of tyramine on lipolysis was solely blocked by pargyline (Raimondi et al., 2000b), while in our hands, semicarbazide was better than pargyline in reversing the antilipolytic effect of tyramine and benzylamine.

The antilipolytic action of tyramine was sensitive to wortmannin, as is the effect of insulin and the activation of glucose transport promoted by benzylamine in human adipocytes (Morin et al., 2001). These results suggest that phosphatidylinositol 3-kinase may be activated by amine oxidase substrates as well as by insulin. Tyrosine phosphorylation of proteins related to (or identical to) key enzymes involved in insulin signaling also appeared to occur, based on the sensitivity to quercetin. However, insulin-independent pathways were also activated by amine oxidation, as suggested by the partial additivity of the antilipolytic effects of tyramine and insulin. Obviously, further investigations are needed to describe the complete cascade of events involved in the insulin-like effects of tyramine and benzylamine, and to focus attention on the cellular targets of reactive oxygen species.

Although tyramine and benzylamine were found to be antilipolytic per se, their actions were improved when vanadate was added at 100 μM. Both amines induce

tyrosine phosphorylation of phosphatidylinositol 3-kinase-associated proteins, phosphatidylinositol 3-kinase activation, recruitment of glucose transporters to the surface and stimulation of glucose transport in the presence of a low concentration of vanadate that is ineffective in stimulating such events on its own (Enrique-Tarancon et al., 2000). The antilipolytic effect of hydrogen peroxide at 0.01–1 mM was also potentiated by vanadate. Therefore, we propose that the marked inhibition of lipolysis produced by the combination of amine and vanadate depends on hydrogen peroxide generation and subsequent peroxovanadate formation, as already suggested for glucose uptake (Enrique-Tarancon et al., 2000). This probably involves the activation of signaling pathways already described for antilipolytic (Castan et al., 1999) and other insulin-like effects of peroxovanadate (Bevan et al., 1995; Lönnroth et al., 1993), including irreversible inhibition of tyrosine phosphatases (Huyer et al., 1997).

Although the complete mechanisms of action of tyramine and benzylamine remain to be elucidated, it can be concluded from the present observations that the inhibition of lipolysis is a novel action to be added to the list of the numerous in vitro insulin-mimicking properties of these amines. All the responses observed in vitro suggest a key role for hydrogen peroxide as a signal molecule. These hydrogen peroxide-mediated actions also could be involved in the in vivo influence of tyramine on glucose disposal (Morin et al., 2002) or in the antihyperglycemic action of a treatment with benzylamine plus vanadate in streptozotocin-induced diabetic rats (Marti et al., 2001). Based on these findings, it appears necessary to define the respective role of monoamine oxidase and semicarbazide-sensitive amine oxidase in the insulin-mimicking properties of their diverse substrates and to assess whether these substrates are of potential interest as pharmacological treatment of insulin-resistant states.

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