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High Expression of Monoamine Oxidases in Human White Adipose Tissue: Evidence for Their Involvement in Noradrenaline Clearance

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ABSTRACT. The clearance of plasma adrenaline and noradrenaline by human adipose tissue suggests the expression of the catecholamine-degrading enzyme monoamine oxidases and of catecholamine transport systems in adipocytes. In the present study, we identified and characterized the monoamine oxidases and an extraneuronal noradrenaline transporter expressed in human adipocytes. Enzyme assays using the monoamine oxidase A/B substrate [^{14}C]tyramine showed that abdominal and mammary human adipocytes contain one of the highest monoamine oxidase activities in the body. Characterization of the enzyme isoforms by inhibition profiles of [^{14}C]tyramine oxidation and Western and Northern blot analyses showed that mRNAs and proteins related to both monoamine oxidases A and B were expressed in adipocytes. Quantification of each enzyme isoform performed by enzyme assay and Western blot showed that monoamine oxidase A was predominant, representing 70–80% of the total enzyme activity. In uptake experiments, the monoamine oxidase substrate [^{3}H]noradrenaline was transported into white adipocytes (V_{max} 0.81 ± 0.3 nmol/30 min/100 mg of lipid, K_m 235 ± 104 μM). The inhibition of [^{3}H]noradrenaline uptake by specific inhibitors indicated that white human adipocytes contain an extraneuronal-type noradrenaline transporter. Competition studies of [^{14}C]tyramine oxidation showed that noradrenaline is metabolized by monoamine oxidases in intact cells. In conclusion, the concomitant expression of monoamine oxidases and of a noradrenaline transporter in human white adipocytes supports the role of the adipose tissue in the clearance of peripheral catecholamines. These results suggest that adipocytes should be considered as a previously unknown potential target of drugs acting on monoamine oxidases and noradrenaline transporters. BIOCHEM PHARMACOL **58**;11:1735–1742, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. monoamine oxidases; adipocyte; human; noradrenaline transporter

The oxidative deamination catalyzed by the mitochondrial enzyme monoamine oxidases (MAO‖, EC 1.4.3.4) A and B represents one of the major metabolic pathways for degradation of neurotransmitters (i.e. noradrenaline, dopamine, and serotonin) and exogenous amines (e.g. tyramine [1]. These enzymes, which are encoded by separate genes sharing a common intron/exon organization, have been subdivided into A and B isoforms based on substrate specificity: MAO-A preferentially metabolizes serotonin and kynuramine, whilst MAO-B mainly deaminates phenylethylamine and benzylamine. The two MAO isoforms can also be differentiated according to their inhibition by synthetic compounds (clorgyline and moclobemide for MAO-A; selegiline and lazabemide for MAO-B) acting on the catalytic site or the flavin adenine dinucleotide prosthetic group of the enzyme [2]. Recently, we showed that MAO-A and MAO-B possess a previously unknown binding domain, the I_2-imidazoline binding site [3]. This binding site, which is distinct from the enzyme domains interacting with classical MAO inhibitors, is selectively recognized by a series of imidazoline and guanidinium compounds and may constitute a novel potential target for the pharmacological regulation of the enzyme function [4–6].

Although MAOs have been identified in brain and in peripheral organs, most of the studies concerning the structure, function, and involvement of these enzymes in pathological processes have been focused on the central nervous system. The relative density and total amount of MAO markedly differ depending upon the regional localization: MAO-A is localized in noradrenergic neurons, whilst MAO-B is preferentially expressed in serotoninergic and histaminergic neurons as well as in glial cells [7, 8]. Each of the two enzyme isoforms seems to be specifically

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‖ *Abbreviations:* MAO, monoamine oxidase; disprocynium24, 1,1′-diisopropyl-2,4′-cyanine; and SSAO, semicarbazide-sensitive amine oxidase.

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altered in some psychiatric and neurodegenerative disorders. The significance of MAO in central nervous system functions is demonstrated by the aggressive behavior associated with genetic deficiencies in MAO-A activity in man [9] and in transgenic mice [10]. In addition, a role for MAO-B in the development and/or progression of Parkinson's and Alzheimer's diseases has been proposed [11]. Three major observations indicate the potential relevance of peripheral MAOs: 1) these enzymes are widely distributed in peripheral organs and, for some (e.g. in liver and kidney), their level of expression is equivalent to or even higher than that in the central nervous system [12]; 2) monoamine transmitters regulate functions of a large variety of peripheral organs [13]; and 3) peripheral MAOs represent one of the major metabolic pathways of circulating catecholamines and exogenous amines [14].

Since the study of Stock and Westermann in 1963 showing that rodent fat depots are able to oxidize serotonin and noradrenaline [15], the expression of MAO in white adipose tissue has not been clearly defined. Recent studies in humans have suggested that adipose tissue may be involved in the clearance and metabolism of circulating catecholamines. Indeed, it has been shown that plasma adrenaline and noradrenaline were partially extracted after the passage across the adipose tissue [16, 17]. These data supplied indirect evidence for the expression of monoamine oxidases and extraneuronal noradrenaline transporters in human adipocytes. The presence of MAO in human fat cells has been also supported by the demonstration that fat cells from various animal species contain a large number of I_2-binding sites [18] and, in rat white adipocytes, MAO activity is inhibited by a series of imidazoline compounds [19].

In the present study, we show that: 1) human white adipose tissue contains one of the highest MAO activities in the body; 2) both MAO isoforms are expressed in adipocytes but MAO-A is predominant; and 3) noradrenaline can be transported into white adipocytes by an extraneuronal-type uptake system and metabolized by MAOs. These results further support the role of the adipose tissue in the clearance of peripheral catecholamines and suggests that adipocytes may be a previously unknown potential target of drugs acting on monoamine oxidases and noradrenaline transporters.

MATERIALS AND METHODS

Materials

[^{14}C]Tyramine (45.2 Ci/mol) and polyvinylidene difluoride membranes were purchased from DuPont NEN, [^{125}I]protein A, [^{14}C]mannitol, and nylon membranes from Amersham International, and α[^{32}P]-dCTP was purchased from ICN. Acrylamide, bisacrylamide, and Tween 20 were purchased from Bio-Rad. Disprocynium24 was a generous gift from Dr. Edgard Schömig (Heidelberg, Germany). Selegiline was provided by Research Biochemicals Int. All remaining drugs and chemicals were purchased from Sigma.

Adipocyte and Liver Membrane Preparation

Adipocytes were isolated from adipose depots obtained from women undergoing abdominal or mammary dermolipectomy (abdominal lipectomy group: mean age, 42 ± 5 years, body mass index 24.9 ± 1.2, N = 11; mammary lipectomy group: mean age, 36 ± 4 years, body mass index 26.2 ± 1.9, N = 11). The protocol was accepted by the Ethical Committee of the Hospital and all the subjects gave their consent to use the removed fat depots for research. Adipocytes were isolated as previously described [18]. Briefly, adipocytes were obtained by collagenase (1 mg/mL) digestion in a Krebs–Ringer bicarbonate buffer containing 3.5% BSA and 6 mM glucose adjusted to pH 7.4 with 10 mM HEPES and 15 mM bicarbonate just before use. Collagenase digestion varied from 30 to 60 min according to the anatomic origin of the fat pad. All digestion and cell isolation procedures were carried out at 37°. After washing, isolated adipocytes were used in enzyme assays and [^{3}H]noradrenaline uptake or resuspended in a hypotonic lysing medium for preparation of fat cell ghosts. The lysing medium was composed of 3.5 mM $MgCl_2$, 1 mM $KHCO_3$, 3 mM EGTA, 2 mM Tris–HCl, pH 7.5, and the following protease inhibitors: leupeptin (5 μg/mL), benzamidine (10 μM), and phenylmethylsulfonyl fluoride (100 μM). Cell lysis was performed at 20–22° under vigorous shaking. Crude adipocyte ghosts were pelleted by centrifugation at 40,000 *g* for 15 min at 15°, after which the pellets were suspended in the lysis buffer and immediately frozen, and then stored at −80° and generally used within 1 month.

For human liver, experiments were performed on organs not suitable for transplantation or on biopsies. Samples were frozen and stored at −80°. After thawing, samples were washed in ice-cold buffer containing 1 mM $MgCl_2$, 2 mM EDTA, 0.1 mM phenyline thylsulfonyl fluoride and 50 mM Tris–HCl, pH 7.4, minced and disrupted in a Dounce homogenizer (pestle A). The homogenate was filtered through two layers of cheesecloth mesh and centrifuged at 600 *g* for 10 min at 4°. Then, the supernatant was decanted and centrifuged at 28,000 *g* for 30 min, and the pellet was resuspended in the same buffer and passed five times in a Dounce homogenizer (Teflon/glass). This homogenate was centrifuged at 28,000 *g* for 30 min at 4°. The pellet was then resuspended in the appropriate buffer.

Amine Oxidase Activity

MAO activity was measured by the method of Yu [20] using [^{14}C]tyramine as substrate. Thawed membranes were centrifuged at 40,000 *g* for 15 min at 4° in a large volume of phosphate buffer (0.2 M), pH 7.4. Incubation was carried out at 37° in a final volume of 200 μL in the same buffer. The protein content varied between 50 to 100 μg/assay tube. No difference in the amount of tyramine oxidized per mg of protein was found when the protein concentration ranged between 10 and 100 μg/100 μL. For enzyme assays in intact cells, adipocytes were incubated under gentle

shaking at 37° in plastic tubes containing 500 μL of cell suspension (approximately 100,000 cells representing around 20 mg of cellular lipids) in the Krebs–Ringer bicarbonate buffer. After 30 min, reactions were stopped by adding 200 μL of 2 M HCl and 1 mL of extraction solvent (toluene/ethyl acetate, v/v). After vigorous mixing and centrifugation, a 0.7-mL aliquot of the organic phase was counted in a liquid scintillation counter (Packard 1900 TR). Approximately 90% of the deanimated products were recovered after extraction. This yield was taken into account for the calculation of MAO activity as previously reported [21]. Blank values were measured in assays immediately stopped by 2 M HCl after addition of cellular extracts and represented less than 1% of the total radioactivity added to the incubation vials.

Western Blot Analyses

Membrane proteins were solubilized in loading buffer (60 mM Tris–HCl, pH 6.8 containing 2% SDS, 10% glycerol, 1% β-mercaptoethanol, and 0.05% bromophenol blue) at 100° for 5 min and subjected to 9% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes with a semidry electroblotter (Trans-Blot, Bio-Rad) for 1 hr at 450 mA. The blots were blocked with 5% non-fat dried milk in washing buffer (PBS, pH 7.5 and 0.1% Tween 20) overnight at 4°, washed twice, and incubated for 1 hr at room temperature with a rabbit polyclonal antiserum obtained from rabbits immunized with the peptide corresponding to amino acids 211–225 in MAO-A and 202–216 in MAO-B. Blots were incubated with [^{125}I]-protein A (0.14 μCi/mL) in washing buffer for 30 min. After extensive washing, the radioactivity was visualized using a 445 SI phosphorimager and quantified using the ImageQuant software (Molecular Dynamics).

Reverse Transcription-Polymerase Chain Reaction and Northern Blot Analyses

Total adipocyte RNA was extracted according to the protocol of Chomczynski and Sacchi [22]. Poly A^+ RNA was purified using Dynabeads (Dynal). Specific cDNA probes for MAO-A (corresponding to nucleotides 1116 to 1724) and MAO-B (corresponding to nucleotides 1037 to 1645) were obtained by reverse transcription-polymerase chain reaction of adipocyte poly A^+ RNA using the following primers: 5′-GGAAAGCTGATCGACTGACTTGCCTAAGC-3′ (sense), 5′-GTACCGGGTTACTGTGTCGG-3′ (antisense) for MAO-A, and 5′-AGAAGCTCCAGTTGCCTACACG-3′ (sense), 5′-AGAGAAATCTGAGAGTGTTCATCGGG-3′ (antisense) for MAO-B.

The amplified cDNA fragments were cloned into pBluescript vector (Stratagene). The orientation and the nature of the cloned fragments were confirmed by dideoxy sequencing. Separation of mRNA was achieved by electrophoresis on 1.2% agarose gels containing 2% formaldehyde with 1% MOPS (3-(*N*-morpholino)propanesulfonic acid) as gel running buffer. RNA was transferred to nylon membrane by capillarity blotting and fixed by UV cross-linker (Stratagene). Hybridization was performed with ^{32}P-labeled cDNA probes. Hybridization was carried out in hybridization buffer containing 45% formamide, 4X saline sodium citrate (SSC), 0.1% SDS, 0.1 M sodium phosphate buffer pH 6.5 at 42°, and the membrane was washed with 0.1X SSC containing 0.1% SDS at 65°.

[^{3}H] Noradrenaline Uptake

Isolated adipocytes were preincubated at 37° for 30 min in the Krebs–Ringer bicarbonate buffer with 50 μM pargyline and 5 μM tolcapone to prevent degradation of noradrenaline by monoamine oxidases and catechol-*O*-methyltransferase, respectively. Subsequently, adipocytes were incubated with [^{3}H]noradrenaline (10 to 1000 μM) at 37° for 30 min. In additional experiments, monoamine uptake inhibitors were added during both the preincubation and incubation periods. Incubation was stopped by brief centrifugation of adipocyte suspension in Beckman microtubes containing 200 μL of dinonyl phthalate (d = 0.98). This allowed us to separate intact adipocytes from incubation medium. This separation procedure, based on the flotation properties of the fat cells, was slightly modified from the original method of Olefsky [23]. The upper part of the microtubes containing adipocytes was cut and radioactivity was measured by liquid scintillation counting. Contamination by extracellular medium was calculated using [^{14}C]mannitol as a non-permeant marker and accounted for less than 0.1% of the total radioactivity present in the incubation medium. Consequently, uptake values were corrected according to the amount of radioactivity related to the presence of extracellular medium.

Statistical Analyses

Monoamine oxidase steady-state kinetic parameters and the IC_{50}s of inhibition of enzyme activity were evaluated using non-linear least square curve-fitting procedures (Prism™ GraphPad). Student's *t*-test was used for the statistical comparison of the results. Results are expressed as means ± SEM.

RESULTS

Monoamine oxidase isoforms were characterized by enzyme assays and Western and Northern blot analyses. A first series of experiments was carried out on membrane preparations and intact adipocytes from abdominal adipose tissue. Enzyme assays were performed using the common MAO-A/MAO-B substrate tyramine. A common substrate was chosen in order to quantify each MAO isoform by using subtype-specific MAO inhibitors. As shown in Fig. 1, velocity versus substrate concentration curves were constructed with [^{14}C]tyramine concentrations ranging from 0.01 to 1 mM. Computer-assisted analyses of the individual

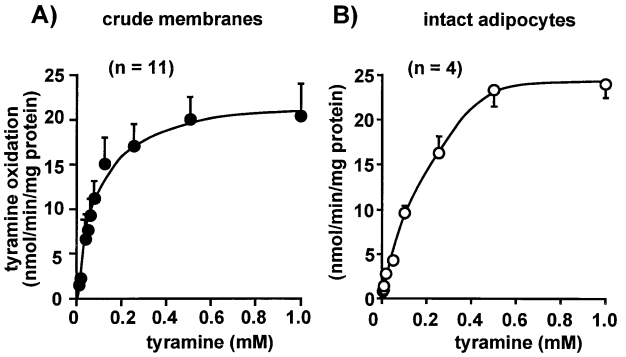

FIG. 1. Monoamine oxidase activity in human adipocytes. (A) [^{14}C]tyramine oxidation by crude adipocyte membranes. Mean protein concentration was 34 ± 5 μg/100 μL of membrane suspension. Each point is the mean ± SEM of 11 independent determinations where each preparation was tested with all the tyramine concentrations for 30 min. The K_m was 94 ± 6 μM and the V_{max} 23.7 ± 2.7 nmol of oxidized tyramine/min/mg membrane protein. (B) [^{14}C]tyramine oxidation by intact adipocytes. Mean cellular lipid content was 18.5 ± 1.9 mg/500 μL in adipocyte suspensions. The membrane protein concentration was determined on 10 mL of the same adipocyte preparation subjected to cell subfractionation as described in Materials and Methods, and the results were then expressed as nmol of tyramine oxidized per min per mg membrane protein. Each point is the mean ± SEM of 4 determinations. Estimated K_m and V_{max} values were 208 ± 24 μM and 29.1 ± 2.2 nmol tyramine/min/mg protein.

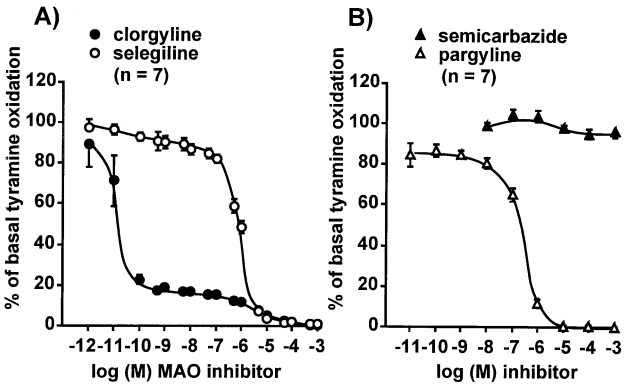

FIG. 2. Inhibition of MAO activity in human adipocyte membranes. (A) MAO inhibition by increasing concentrations of clorgyline (MAO-A inhibitor) and selegiline (MAO-B inhibitor). (B) MAO inhibition by pargyline (MAO inhibitor) or semicarbazide (SSAO inhibitor). Thawed membrane preparations were preincubated for 30 min at 37° without (basal = 100%) or with increasing concentrations of the tested compounds. Then, [^{14}C]tyramine was added at a final concentration of 0.5 mM and the oxidation reaction was stopped 30 min later by acidification. Basal tyramine oxidation averaged 20.9 ± 3.1 nmol/min/mg protein. Each point is mean ± SEM of 7 preparations.

curves were used to estimate K_m and V_{max} values. In 11 membrane preparations of different abdominal fat depots, mean K_m and V_{max} values were 94 ± 6 μM and 23.7 ± 2.7 nmol of oxidized tyramine/min/mg protein, respectively (Fig. 1A). Similar V_{max} values were obtained in intact adipocytes (29.1 ± 2.2 nmol tyramine/min/mg protein, N = 4, not significant). In contrast, K_m values were significantly higher than those obtained in membrane preparations (208 ± 24 μM, N = 4, $P < 0.001$) (Fig. 1B), suggesting that tyramine oxidation may depend, in part, on its uptake rate into the fat cells.

In order to identify the MAO isoforms expressed in adipose tissue, we tested the effect of clorgyline and selegiline, specific inhibitors of MAO-A and MAO-B respectively, on [^{14}C]tyramine oxidation. As shown in Fig. 2A, inhibition curves were biphasic with high- and low-affinity components (clorgyline, high-affinity component: IC_{50} 0.03 ± 0.004 nM and 80% inhibition; low-affinity component: IC_{50} 5.7 ± 1.3 μM and 20% inhibition; selegiline, high-affinity component: IC_{50} 0.3 ± 0.2 nM and 20% inhibition; low-affinity component: IC_{50} 0.8 ± 0.15 μM and 80% inhibition), suggesting the presence of the two MAO isoforms in adipocyte membranes. Simultaneous addition of 10 nM clorgyline and 10 nM selegiline totally inhibited tyramine oxidation (N = 11). Tyramine oxidation was also fully prevented by the MAO inhibitors phenelzine (not shown) and pargyline but not by semicarbazide, an inhibitor of the semicarbazide-sensitive oxidase (SSAO) (Fig. 2B). These data indicate that, in contrast to that observed in rat adipocytes [24, 25], SSAO does not account for tyramine degradation in human adipose tissue.

Previous studies showed that enzyme assays may not provide an accurate quantification of the MAO isoforms expressed in a given tissue [26]. In order to determine the relative expression of MAO-A and -B in human adipocytes, we performed Western blot analyses using an antiserum obtained from rabbit immunized with a peptide common to both MAO-A and -B [6]. This antiserum allowed the identification and relative quantification of the two MAO isoforms in various human, rabbit, and rat tissues [6, 27, 28]. Mitochondria recovery, determined by Western blot using an antibody directed against the β subunit of the mitochondrial ATPase, was identical for liver and adipocyte membrane preparations (data not shown). As shown in Fig. 3A, Western blot analyses of adipocyte membranes revealed two peptides with the apparent molecular weight expected for MAO-A and -B (61 and 55 kDa respectively) at relative abundance of 70 ± 2.5% (MAO-A) and 30 ± 2.5% (MAO-B) (N = 7). In contrast, in human liver membranes, which also express both MAO isoforms, MAO-B was predominant (approximately 90% of MAO-B and 10% of MAO-A) (Fig. 3A). However, the total amount of immunoreactive proteins was equivalent in membrane preparations from liver and adipocytes (Fig. 3A). According to these results, these tissues demonstrated similar MAO activity measured as [^{14}C]tyramine oxidation (adipocytes: 23.7 ± 2.7 nmol/min/mg protein, N = 11; liver: 15.1 ± 2.7 nmol/min/mg protein, N = 3, not significant).

Reverse transcription-polymerase chain reaction experiments using specific primers for MAO-A and MAO-B gave products of expected size (608 bp for both MAO isoforms). Identity of the products to MAO-A and MAO-B cDNA sequences was confirmed by dideoxysequencing (data not

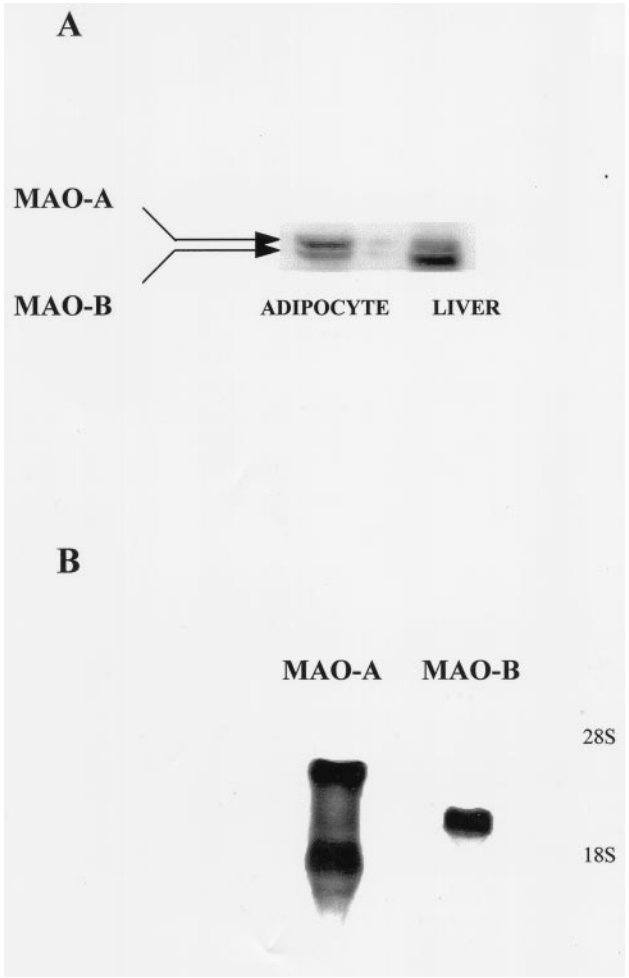

FIG. 3. (A) Typical Western blot analyses of MAO-A and MAO-B in human adipocyte and liver membranes. Membranes of these tissues (50 μg) were electrophoresed on a 9% SDS-PAGE gel and transferred to polyvinylidene difluoride membranes. The blot was incubated with a rabbit polyclonal antiserum to both MAO isoforms. Immunoreactive proteins were identified and then quantified using an ImageQuant Software as described in the experimental procedures. (B) MAO-A and MAO-B mRNA in adipose tissue. Polyadenylated RNA from human adipocytes (1 μg) were hybridized with a MAO-A or MAO-B cDNA subfragment. The arrows indicate the size of MAO mRNA calculated by comparison with rRNA.

shown). Northern blot was performed on 1 μg of polyA$^+$ RNA. When the MAO-A cDNA probe was used, two bands were observed: a predominant band of ≈2 kb and another dense band of ≈5 kb (Fig. 3B). The hybridization of the MAO-B cDNA probe revealed the presence of a single 3 kb transcript (Fig. 3B). Taken together, these data indicate that abdominal adipose tissue express both MAO-A and MAO-B mRNAs.

It has been shown previously that the expression and function of various adipocyte proteins may depend upon the regional localization of the fat depot [18, 29, 30]. In order to determine whether the high level of MAO-A and MAO-B was restricted to abdominal subcutaneous adipocytes, we compared the expression of the two MAO isoforms in abdominal and mammary adipose tissues. The apparent kinetic constants found in membranes from mammary adipocytes were not significantly different from those reported above for abdominal adipose depots (V_{max}, 33.7 ± 4.5 nmol/min/mg protein, K_m 107 ± 10 μM, N = 11, not significant). In addition, Western blot analyses and inhibition of [^{14}C]tyramine oxidation by subtype-specific MAO inhibitors showed that, as observed for abdominal adipocytes, MAO-A is the enzyme isoform largely predominant in mammary adipocytes (data not shown). These data indicate that adipocytes from the two different regions express high amounts of MAOs and similar proportions of the MAO-A and MAO-B isoforms.

Previous studies suggested that involvement of the adipose tissue in catecholamine clearance. As catecholamine metabolism by mitochondrial MAOs requires the substrate uptake into the cell, we next investigated whether a noradrenaline transporter was expressed in intact adipocytes. We focused our studies on noradrenaline for two reasons: 1) a previous report showed that noradrenaline can be removed from plasma after the passage across the adipose tissue [16] and 2) this catecholamine plays a critical role in regulation of adipocyte function. As shown in Fig. 4, after 30 min incubation, [^{3}H]noradrenaline was transported into intact adipocytes. Specific transport, as defined in the presence of the noradrenaline uptake inhibitor disprocynium24 (1 μM), was dose-dependent and reached its maximum at a [^{3}H]noradrenaline concentration between 500 and 1000 μM (V_{max} 0.81 ± 0.3 nmol/30 min/100 mg of lipid, K_m 235 ± 104 μM, N = 5). Total [^{3}H]noradrenaline uptake was inhibited to a similar extent (50%) by the noradrenaline uptake 2 inhibitors disprocynium24 (1 μM) and corticosterone (50 μM). In contrast, it was insensitive to the noradrenaline uptake 1 inhibitors imipramine and desipramine at a 100-μM concentration (Fig. 4B) [31].

Next, we determined whether, after internalization, noradrenaline was metabolized by adipocyte MAO, thereby testing the ability of noradrenaline to compete for [^{14}C]tyramine oxidation in intact fat cells. As shown in Fig. 5, [^{14}C]tyramine oxidation was inhibited in a dose-dependent manner by noradrenaline. The fact that the inhibition curves were similar in the absence and presence of the alpha-(phentolamine) and beta-adrenergic (bupranolol) receptor antagonists indicates that, under our experimental conditions, stimulation of adrenergic receptors did not affect noradrenaline uptake and oxidation. These data indicate that, in intact human white adipocytes, noradrenaline 1) is internalized into the cells through a transport system with pharmacological properties compatible with those reported for the extraneuronal noradrenaline transporter and 2) is metabolized by MAOs.

DISCUSSION

In the present study, we identified and characterized for the first time the MAO isoforms expressed in human abdominal and mammary adipose tissues. We also showed that

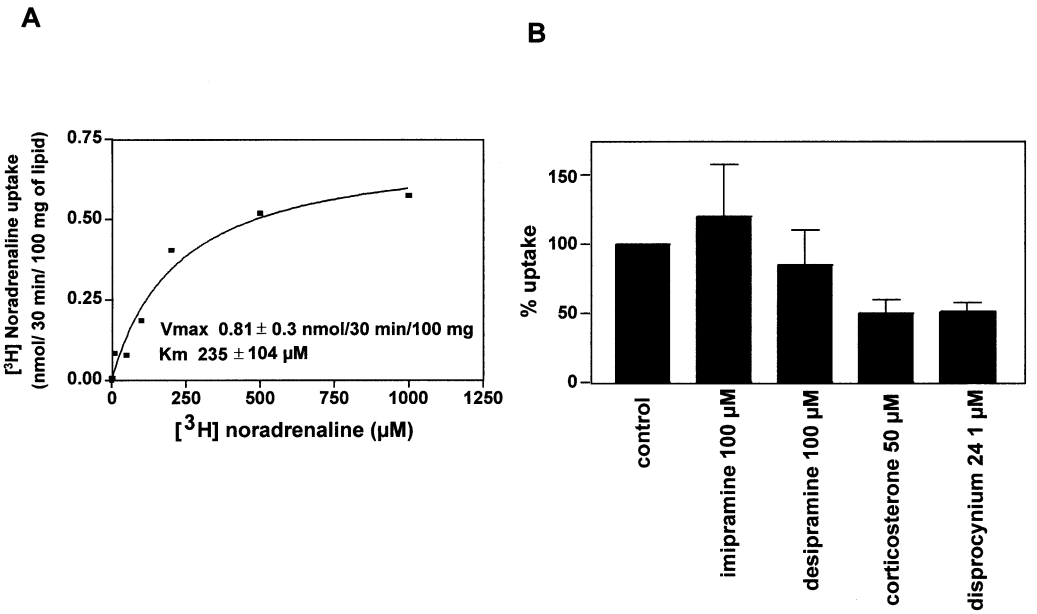

FIG. 4. (A) [^{3}H]noradrenaline uptake in isolated adipocytes. Cells were incubated with various concentrations of [^{3}H]noradrenaline at 37° for 30 min. Specific uptake was defined as that fraction of total uptake which is inhibited in the presence of 1 µM of disprocynium24. The curve is representative of five separate experiments. (B) Inhibition of [^{3}H]noradrenaline uptake. Uptake assays were performed at 500 µM of noradrenaline in the presence of various inhibitors. All data represent the means ± SEM of four separate experiments.

human white adipocytes possess a noradrenaline transporter with the pharmacological properties consistent with those reported for the extraneuronal noradrenaline transporter. In addition, we demonstrated that, after uptake into adipocytes, noradrenaline is metabolized by MAOs. These data suggest that, in addition to their well-known metabolic functions, human adipocytes may also play a role in the degradation of peripheral catecholamines.

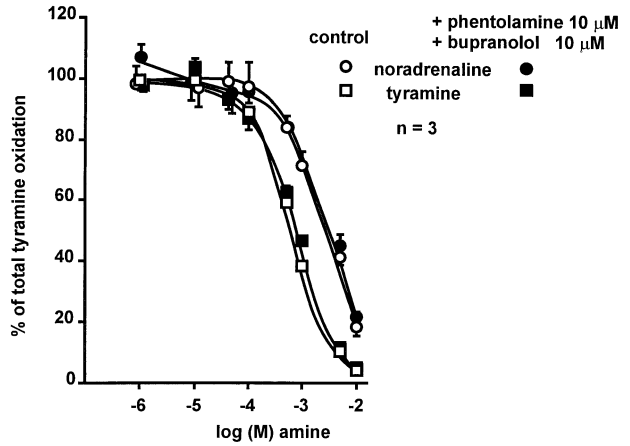

FIG. 5. Inhibition of tyramine oxidation in intact human adipocytes. Intact adipocytes were preincubated for 15 min with the indicated concentrations of tyramine (squares) or noradrenaline (circles). The preparations were then incubated for 30 min with 0.2 mM tyramine and oxidation products were measured as described in Materials and Methods. Tyramine oxidation was performed in the presence of intact cells equivalent to 27 ± 6 mg of cellular lipids. Results are expressed as percent of tyramine oxidation without competitor which was 1.4 ± 0.2 nmol/min/100 mg lipid in intact cells. Respective IC_{50} values for tyramine and noradrenaline were 704 ± 110 and 3038 ± 205 µM without (open symbols) or 741 ± 57 and 2930 ± 384 µM with phentolamine 10 µM plus bupranolol 10 µM (closed symbols). Mean ± SEM of 3 independent determinations.

Part of our results show that human white adipocytes contain high MAO activity, which is related to the expression of both MAO-A and MAO-B isoforms. The enzyme activity in human adipocytes was similar to that found in liver, one of the tissues expressing the highest MAO activity [11, 32]. Using complementary approaches, we were able to determine the relative expression of each MAO isoform. Inhibition studies of [^{14}C]tyramine oxidation by the subtype-selective inhibitors clorgyline and selegiline showed that MAO-A and MAO-B account for about 80 and 20% of the total enzyme activity, respectively. A similar proportion of the two isoenzymes was determined by Western blot using a polyclonal antiserum raised against homologous peptide of MAO-A and MAO-B [6, 27, 28]. These results clearly show that MAO-A is the predominant isoform expressed in human adipocytes.

Previous studies showed that, based on their regional localization, adipose tissue may differ in the expression of various proteins (e.g. adrenergic receptors and glucose transporters). In contrast, we found that the total amount of MAOs as well as the relative proportion of the MAO-A and MAO-B isoforms was similar in adipose tissues from abdominal and mammary deposits. These findings indicate that, in contrast to that observed for other proteins, the distribution of MAOs is conserved in two different fat depots.

Northern blot analyses showed that, as previously reported for different human tissues [33], the cDNA probe for

MAO-B identified a single 3-kb transcript. In contrast, the cDNA probe for MAO-A revealed two bands: one, which was slightly predominant, at 2 kb, and another at 5 kb. These two transcripts were previously identified in human placenta and small intestine. As observed in human adipocytes, each of these two tissues contains equivalent amounts of the 2- and 5-kb transcripts. In contrast, in a series of human tissues, including lung, heart, liver, and kidney, the abundance of the 2-kb transcript is much lower than that of the 5-kb transcript [33]. The significance of two transcripts for MAO-A in human adipocytes, placenta, and small intestine is unknown. In a previous report, Grimsby *et al.* suggested that the 2- and 5-kb transcripts may arise from different polyadenylation sites [33]. It is conceivable that the expression of the 2-kb transcript in adipocytes, placenta, and small intestine may be related to a regulation of the MAO pre-mRNA peculiar to these three tissues. Further studies are necessary to identify the products of each MAO-A transcript and determine their functional relevance.

The fact that human adipocytes contain a high amount of MAOs supplies additional evidence for the potential role of the adipose tissue in catecholamine metabolism. This possibility is further supported by the demonstration that adipocytes also contain a noradrenaline transporter. Indeed, transporters with kinetic and pharmacological properties similar to those that we have found in the adipocytes have been identified in peripheral organs (e.g. kidney and pulmonary vessels) involved in catecholamine clearance [34, 35]. This noradrenaline transport displays pharmacological properties different from those of the neuronal noradrenaline uptake 1 and has been defined as an extraneuronal noradrenaline transport (or uptake 2). According to the results previously obtained in other organs, high noradrenaline concentrations were required to activate the transporter. These concentrations are much higher than those inducing the maximal lipolytic and antilypolitic effects of noradrenaline. Thus, it seems unlikely that noradrenaline uptake and metabolism by MAOs plays a major role in the regulation of short-term noradrenaline effects on adipocytes. This is further confirmed by our results showing that adrenergic receptor-mediated regulation of lipolysis by noradrenaline is not modified after irreversible MAO inhibition (data not shown). In contrast, the kinetic characteristics of noradrenaline uptake support the role of adipocyte MAOs in «long-term» noradrenaline clearance. This is also supported by results obtained from the measurement of arteriovenous differences in adrenaline [16] or noradrenaline [17] concentrations after infusion across human adipose tissue. These results showed that both catecholamines were partially extracted from the plasma after the passage across adipose tissue. In one of these studies, the relative importance of muscle and adipose tissue, the two largest tissues in the body, in clearing infused noradrenaline from the blood was compared. The results showed that adipose tissue was about two-fold more efficient than muscle in removing noradrenaline.

The large mass of adipose tissue along with the high expression of MAOs suggests that this tissue may play an important role in the control of peripheral catecholamine concentrations. This may have a particular significance for the comprehension of the mechanisms leading to obesity and some related pathologies such as hypertension and non-insulin-dependent diabetes. In this context, it is important to note that twenty years ago, an increase in white adipose tissue MAO activity was reported in genetically obese mice [36], but the physiological relevance of this phenomenon and its extrapolation to human obesity remain to be clarified. Indeed, alterations in the activity of the autonomic nervous system are known to participate in and perhaps cause the development of obesity in rodents, although some controversies persist concerning human obesity [37].

At present, further studies are necessary to determine the role of MAO in adipose tissue as well as the functional significance of the prevalent expression of the MAO-A isoform. However, the high enzyme activity and large mass of the adipose tissue suggest that adipocyte MAOs may play a critical role in the control of regional and systemic effects of catecholamines. In addition, the high expression of MAO in adipocytes may have a pharmacological relevance. Indeed, MAO inhibitors are currently used in therapy. It is conceivable that, in addition to their central activity, these drugs may induce some peripheral effects through the interaction with MAO in adipocytes. This possibility should be taken into account to explain some unexpected therapeutical and/or side effects of MAO inhibitors.

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