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METABOLISM OF METHYLAMINE BY SEMICARBAZIDE-SENSITIVE AMINE OXIDASE IN WHITE AND BROWN ADIPOSE TISSUE OF THE RAT

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Abstract—The metabolism of [^{14}C]methylamine (MA) by amine oxidase activity in rat white and brown adipose tissue homogenates, and in mature adipocytes from these tissues has been studied. Oxidation of MA was completely inhibited by 0.1–1 mM semicarbazide, without being affected by the monoamine oxidase (MAO) inhibitor, pargyline (1 mM), indicating that MA is metabolized by semicarbazide-sensitive amine oxidase (SSAO) and not by MAO. The mean K_m for MA deamination in all of these sources was around 250–300 μM. SSAO activity towards MA was also demonstrated in white and brown pre-adipocytes, transformed to the adipose phenotype by treatment in culture for 7 days with lipogenic agents. These results are similar to previous findings that SSAO in vascular smooth muscle is able to metabolize aliphatic amines such as MA, and furthermore suggest that SSAO may play a role in adipose tissue function and/or maturation.

Many mammalian tissues contain an amine oxidase resistant to mitochondrial monoamine oxidase (MAO‡) inhibitors, particularly active towards benzylamine as a substrate, and inhibited by semicarbazide and related compounds which are believed to react with a carbonyl group in the enzyme cofactor. For this reason, the enzyme has conventionally been named semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6) [1]. The properties of this enzyme have been studied most extensively in vascular tissue, where it is associated predominantly with the smooth muscle cell plasma membrane [2, 3]. The physiological importance of SSAO is unclear, but its function is unlikely to be one associated solely with smooth muscle, since the enzyme has also been detected, for example, in rat chondrocytes [4]. Moreover, rat white (WAT) and brown (BAT) adipose tissues were found to be a good source of SSAO, and for this reason are another possible model for studying the biochemical and physiological properties of this enzyme [5, 6].

It is generally accepted that benzylamine is not a physiological amine and is therefore unlikely to be a naturally occurring endogenous substrate for the enzyme. SSAO in various rat tissue homogenates readily metabolizes certain physiologically active aromatic amines, including tyramine, β-phenylethylamine and tryptamine, suggesting that the enzyme could play a role in their turnover *in vivo* [7, 8]. In contrast, similar studies indicate that SSAO in human tissues has poor deaminating activity towards these amines, implying that the enzyme is less likely to play an important role in aromatic amine metabolism in all species [9, 10].

As a consequence, interest has arisen from recent reports that SSAO can metabolize some endogenously occurring aliphatic amines. For example, SSAO in homogenates of rat aorta and human umbilical artery shows high deaminating activity towards methylamine (MA), a product of pathways for the degradation of creatinine, sarcosine and adrenaline in mammalian tissues [11, 12]. Also, daily urinary excretion of MA was enhanced by treatment of rats with drugs (e.g. hydralazine, semicarbazide) capable of inhibiting SSAO, suggesting that SSAO or related enzymes may contribute to endogenous MA turnover [13]. Recently, MA was found to be a substrate for mouse, rabbit, pig and human WAT SSAO [14], and in order to investigate further if an ability to oxidize MA is a common characteristic of SSAO in WAT from various species, we have extended our observations here to a study of the enzyme in both WAT and BAT from the rat. In this respect, we have determined MA metabolism in tissue homogenates as well as in white and brown adipocytes at different stages of maturation.

MATERIALS AND METHODS

Materials. Male Sprague–Dawley (SD) rats (300–400 g) were obtained from the Departmental Breeding Colony, Biomedical Research Unit, University of Dundee. Pargyline hydrochloride, semicarbazide hydrochloride, bovine serum albumin, transferrin and triiodothyronine were obtained from the Sigma Chemical Co. (Poole, U.K.). Dulbecco's modified Eagles Medium (DMEM), nutrient mixture Ham's F12, streptomycin, penicillin and foetal calf

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‡ Abbreviations: SSAO, semicarbazide-sensitive amine oxidase; WAT, white adipose tissue; BAT, brown adipose tissue; MA, methylamine; MAO, monoamine oxidase; SD, Sprague–Dawley; DMEM, Dulbecco's modified Eagle's medium; FCS, foetal calf serum.

serum (FCS) were from Gibco (Paisley, U.K.). Collagenase (Type IA), calcium pantothenate, biotin and insulin were purchased from Boehringer Mannheim (Lewes, U.K. and Mannheim, Germany). BDH (Poole, U.K.) provided Amberlite resin CG-50 (type III, 400 mesh) and [^{14}C]methylamine hydrochloride (59 μCi/μmol) was purchased from Amersham International (Amersham, U.K.).

Adipose tissue homogenates. SD rats were killed by cervical dislocation and white epididymal adipose tissue (WAT) and brown interscapular adipose tissue (BAT) were dissected. Samples of WAT (0.5 g) and BAT (0.2 g) were homogenized in 5 and 2 mL, respectively, of 1 mM potassium phosphate buffer, pH 7.8. Homogenates were centrifuged for 10 min at 600 *g*, and the supernatants used as enzyme source in assays.

Preparation of mature adipocytes and pre-adipocytes. White and brown mature adipocytes were prepared by the method of Rodbell [15]. Briefly, adipose tissue from SD rats was minced with scissors into small pieces and incubated for 40 min at 37° in a centrifuge tube containing Krebs buffer (pH 7.4) of the following (mM) composition: NaCl (118), $NaHCO_3$ (25), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (0.6), glucose (11) and 2 mg/mL collagenase. Mature adipocytes were collected at the top of the tube after centrifugation (600 *g* for 30 sec) to separate them from stromal cells. The adipocytes were further washed by suspension and collection after centrifugation in fresh Krebs buffer. Adipocytes obtained from digestion of 1 g WAT and 200 mg BAT were then homogenized in 10 and 2 mL, respectively, of 1 mM potassium phosphate buffer, pH 7.8.

For pre-adipocyte preparation, samples of BAT and WAT were resected under sterile conditions and minced into small pieces. Stromal vascular cells were prepared as described previously [16, 17]. These were seeded in 1.5-mm multiwell culture dishes at a density of 10^5 cells/cm. They were grown in DMEM containing 100 U/mL of penicillin, 100 μg/mL streptomycin, 33 μM biotin, 17 μM pantothenate, 10% (v/v) heat-inactivated FCS (standard medium) and incubated at 37° in a humidified atmosphere of O_2 (95%) and CO_2 (5%). Stromal vascular cells from WAT reached confluence after 48–72 hr, whereas cells from BAT took around 92 hr. As soon as they reached confluence, cells were shifted to another medium, DMEM–F12 (1 : 1, v/v), containing transferrin 10 μg/mL, insulin 1 μM, triiodothyronine 0.2 nM and cultured for another 5–7 days. This time was previously established as that required for maximal cell transformation as assessed by the appearance of glycerol-3-phosphate dehydrogenase and SSAO activity [18]. They were then thoroughly washed with the standard medium without FCS before collecting them from the dish. Cells were scraped from the plates and pelleted by centrifugation for 5 min at 600 *g*. Pellets were then homogenized in 1 mL of 1 mM potassium phosphate buffer pH 7.8 for use in enzyme assays.

Assay of [^{14}C]*methylamine metabolism.* This was determined in tissue and cell homogenates by the radiochemical method for determining product formation developed by Lyles *et al.* [12], here using an assay incubation time of 60 min. In inhibitor studies, samples were preincubated with either pargyline (1 mM) or semicarbazide (1 mM) for 30 min at 37° before addition of substrate.

Protein assays. Protein content of homogenates was assayed by the method of Lowry *et al.* [19], using bovine serum albumin as standard.

Table 1. Metabolism of MA in adipose tissue

Homogenate source	Specific activity (nmol/min/mg protein)
WAT	3.12 ± 0.69
White mature adipocytes	1.57 ± 0.23
BAT	0.22 ± 0.03
Brown mature adipocytes	0.25 ± 0.08

Deaminating activity was determined by incubating tissue or cell homogenates with 200 μM [^{14}C]MA for 60 min.

Results are the means ± SE of values for four different samples in each category, obtained from triplicate assays on each sample.

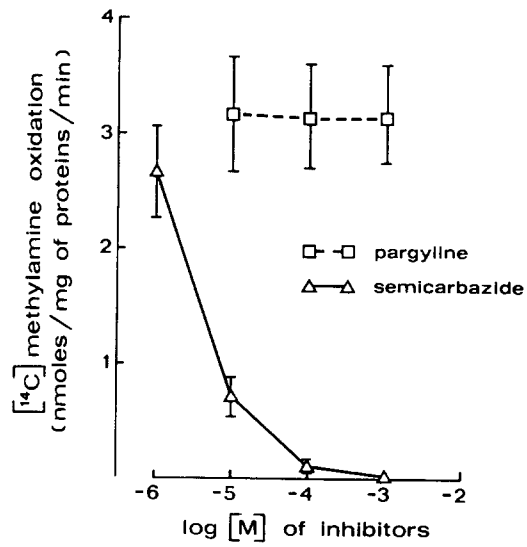

Fig. 1. Effect of pargyline and semicarbazide on [^{14}C]MA metabolism by rat WAT. Inhibitors were preincubated at the indicated concentrations for 30 min with homogenates, before the addition of 200 μM methylamine and further incubation for 60 min to determine remaining enzyme activity. Control samples were preincubated in the absence of inhibitors. Results are the means ± SE of four samples assayed in triplicate.

RESULTS

Table 1 shows that 200 μM MA is deaminated by homogenates prepared from samples of BAT and WAT from the rat, as well as by homogenates of mature adipocytes from these sources. Specific enzyme activity was about 15 times higher in homogenates prepared from WAT than from BAT.

Figures 1–4 show the effects of preincubating

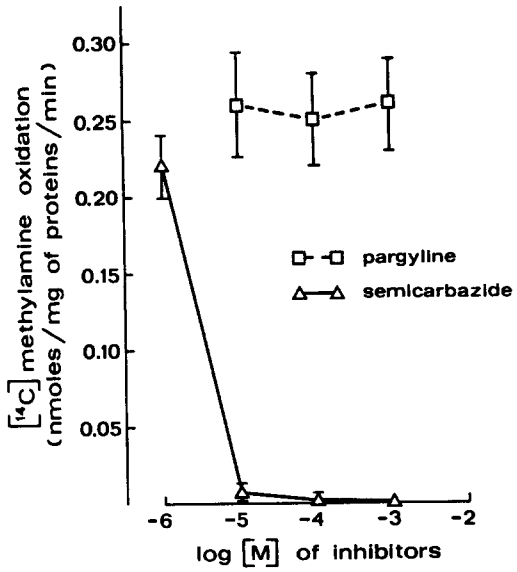

Fig. 2. Effect of pargyline and semicarbazide on [^{14}C]MA metabolism by rat BAT. Experimental details as in legend to Fig. 1.

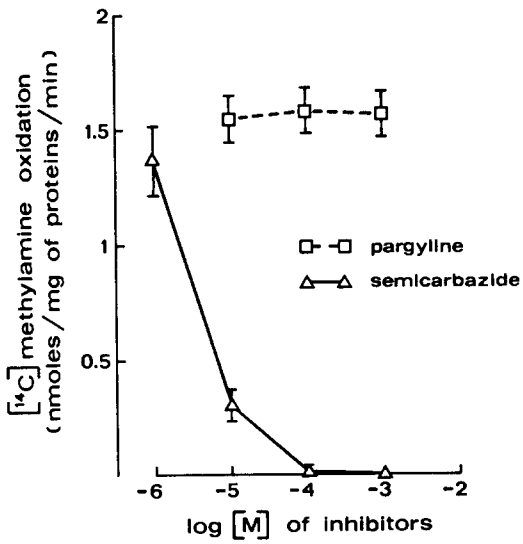

Fig. 3. Effect of pargyline and semicarbazide on [^{14}C]MA metabolism by rat white adipocytes. Experimental details as in legend to Fig. 1.

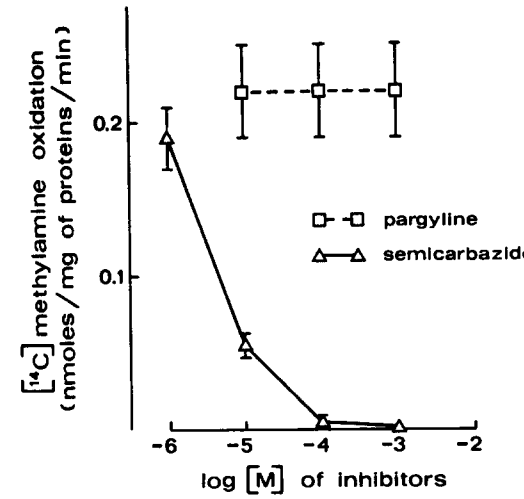

Fig. 4. Effect of pargyline and semicarbazide on [^{14}C]MA metabolism by rat brown adipocytes. Experimental details as in legend to Fig. 1.

Table 2. Effects of pargyline and semicarbazide on MA oxidation by pre-adipocytes transformed in culture

Enzyme source	Specific activity (nmol/min/mg protein)
White pre-adipocytes	
No inhibitor	0.06 ± 0.03
+ Pargyline	0.05 ± 0.01
+ Semicarbazide	0
Brown pre-adipocytes	
No inhibitor	0.50 ± 0.11
+ Pargyline	0.50 ± 0.17
+ Semicarbazide	0

Homogenate samples were preincubated for 30 min in the absence or presence of either 1 mM pargyline or 0.1 mM semicarbazide before adding 200 μM [^{14}C]-methylamine and incubation for 60 min to determine remaining enzyme activity.

Results are means ± SE of values for four to six preparations from different cultures, each preparation being assayed in triplicate.

homogenates with various concentrations of either pargyline or semicarbazide. It can be seen that at concentrations of up to 1 mM, the MAO inhibitor pargyline had no significant inhibitory effect upon MA metabolism either in homogenates of BAT and WAT, or in homogenates of brown and white mature adipocytes, whereas 1 mM semicarbazide produced complete inhibition.

Table 2 shows that brown pre-adipocytes, after being grown in culture in the presence of a lipogenic transforming cocktail, exhibited greater deaminating activity than white pre-adipocytes. The use of a single concentration (10^{-4} M) of semicarbazide produced complete inhibition of metabolism of 200 μM MA in homogenates of brown and white pre-adipocytes, whereas 1 mM pargyline produced no significant inhibitory effect. These results indicate that the deamination of MA in all of these sources could be attributed to SSAO and not MAO activity.

Apparent kinetic constants for MA metabolism were measured in WAT, BAT and in the

Table 3. Kinetic constants for MA metabolism by adipose tissue

	K_m (μM)	V_{max} (nmol/min/mg protein)
WAT	282 ± 20	7.50 ± 2.60
White adipocytes	252 ± 22	2.98 ± 0.55
BAT	289 ± 43	0.53 ± 0.04
Brown adipocytes	258 ± 36	1.11 ± 0.33

[^{14}C]MA metabolism was determined over 60-min incubation in triplicate assays on homogenates of the samples above, at final substrate concentrations of 50, 100, 250, 500 μM and 1 mM. Apparent kinetic constants were determined by linear regression from double reciprocal plots of product formation vs substrate concentration.

Each value is the mean ± SE of four samples in each category.

corresponding mature adipocytes from these sources. Results were obtained from determinations of MA deamination at concentrations between 50 μM and 1 mM. Table 3 shows that the K_m values obtained (ranging from 252 to 289 μM) were quite close for the four types of sample (WAT, BAT, white and brown mature adipocytes) examined. The V_{max} values obtained also indicated, as already noted above, that WAT has a higher specific deaminating activity than BAT towards MA.

DISCUSSION

The presence of SSAO activity in adipose tissue has been established in several previous studies. Barrand and Callingham [20] reported that the enzyme found in homogenates of rat BAT deaminated not only benzylamine, but also tyramine and β-phenylethylamine. Solubilization and partial purification of SSAO from this source was later achieved, with evidence being presented that the enzyme is a glycoprotein with an estimated molecular mass (determined by irradiation inactivation techniques) of 183 kDa [21]. Furthermore, SSAO was subsequently shown in histochemical and subcellular fractionation studies to be associated with brown adipocytes, as a component of the plasma membrane [6], a subcellular localization previously described for SSAO in vascular smooth muscle [2].

More recently, SSAO activity towards benzylamine has been found in homogenates of WAT from several species (mouse, rabbit, pig and human) [14], as well as in rat mature white adipocytes [5]. The rat adipocyte enzyme was membrane-bound and capable of oxidizing not only benzylamine but also β-phenylethylamine and histamine, a characteristic more recently demonstrated to be shared by mouse, pig and rabbit WAT SSAO, although in contrast the human enzyme did not metabolize β-phenylethylamine and histamine [14]. The appearance of SSAO activity towards benzylamine in rat white and brown pre-adipocytes has previously been followed during maturation of committed cell type (pre-adipocytes) in culture, which under appropriate conditions can acquire the adipose phenotype. In this model, SSAO activity appears concurrently with lipid deposition, suggesting a possible relationship between SSAO and adipose differentiation [18, 22].

The present study has shown that MA oxidation in rat adipose tissues, in mature adipocytes and in transformed pre-adipocytes in culture is clearly due to SSAO without any participation of MAO since MA oxidation is not inhibited by 1 mM pargyline, an irreversible MAO inhibitor, whereas 0.1 mM semicarbazide completely abolishes this enzyme activity. In general, the specific activity for MA metabolism in homogenates was much higher in WAT than in BAT, whereas in contrast the transformed pre-adipocytes from BAT were more active than those from WAT. Perhaps these results indicate differences in the properties of transformed brown and white pre-adipocytes compared with the parent BAT and WAT, or in possible physiological functions of the SSAO activities within these different enzyme sources [22]. The K_m of around 250–300 μM for MA metabolism in homogenates of WAT, BAT and mature adipocytes was quite similar to values found for this amine with rat aorta SSAO [11, 12, 23]. However, in contrast to our earlier study using benzylamine as substrate [5], we did not find an enrichment of SSAO activity towards MA in white mature adipocytes compared with the parent WAT. The reason for this is not entirely clear since these earlier studies had indicated that the activity towards benzylamine was concentrated in adipocytes and that no other stromal cell contains this enzyme activity. Since the current study did not use benzylamine and MA in parallel on the same samples, it is impossible at this stage to be sure if these results indicate some differences in the distribution of the SSAO activity towards these two amines. However, as an extension of earlier findings that MA is a substrate for SSAO in WAT from various species (mouse, rabbit, pig, human) [14], it is evident from the current study that MA is also deaminated by SSAO in adipose tissue and constituent adipocytes from the rat.

Whether or not MA is an endogenous physiological substrate for SSAO in adipose tissue cannot be determined from the present study. However, there is now increasing evidence that SSAO from various sources can deaminate readily *in vitro* some biogenic and xenobiotic aliphatic amines with the production of potentially cytotoxic aldehyde metabolites such as formaldehyde (from MA), methylglyoxal (from aminoacetone) and acrolein (from allylamine) [11, 12, 14, 23–26]. As a result, further investigations of possible physiological or toxicological consequences of aliphatic amine breakdown upon cellular function in animal tissues would appear worthwhile.

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