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Age-related changes in monoamine oxidase and semicarbazide-sensitive amine oxidase activities of rat aorta

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Aorta MAO-A and SSAO activities were measured on young (3 months) and old (23–26 months) rats. A significant decrease (30–40%) in SSAO activity was found with benzylamine as substrate and the decrease was due to a reduction in V_{max}. No significant changes in MAO-A activity were found in the aorta of old rats. β-PEA is oxidized mainly by SSAO in rat aorta. However, the significance of this is unclear since the physiological role of that enzyme remains unknown.

The heart and aorta of rat and man contain monoamine oxidases (MAO, EC 1.H.3.4) and other amine oxidases (Lyles & Callingham 1975; Lewinsohn et al 1978). Monoamine oxidase exists in two forms (MAO-A and MAO-B) which are differentiated by their specificity to substrates (Tipton et al 1983; Fowler et al 1981) and their sensitivity to inhibitors (Johnston 1968; Knoll & Magyar 1972). One of the other amine oxidases, has been called benzylamine oxidase (BZAO) (Lewinsohn et al 1978, 1980), prefers benzylamine as substrate; it is resistant to inhibition by clorgyline and selegiline ((−)-deprenyl) at concentrations which completely inhibit MAO-A and -B (Lyles & Callingham 1975, 1982a, b) and is sensitive to inhibition by semicarbazide. Therefore, this enzyme has also been called clorgyline-resistant amine oxidase (CRAO) but the preferred name is now semicarbazide-sensitive amine oxidase (SSAO) (Lyles & Callingham 1975; Dial & Clarke 1977; Coquil et al 1973).

Age-related changes in MAO and SSAO activities have been reported in tissues of animals (Lowe et al 1975; Della Corte & Callingham 1977; Fuentes et al 1977; Shih 1979; Strolin Benedetti & Keane 1980; Cao Danh et al 1984, 1985) and man (Robinson et al 1972; Gottfries et al 1975; Oreland & Fowler 1979). The aim of the present work was to compare MAO and SSAO activities in aorta of young and old rats.

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Materials and methods

5-Hydroxytryptamine-[side chain-2-^{14}C]creatinine sulphate (5-HT) and [7-^{14}C] benzylamine hydrochloride (BZ) were obtained from the Radiochemical Centre, Amersham, UK; β-phenethylamine-[ethyl-1-^{14}C] hydrochloride (β-PEA) was obtained from New England Nuclear, Boston, Mass., USA; clorgyline hydrochloride was synthesized in the Department of Organic Chemistry, Centre de Recherche Delalande, France; semicarbazide hydrochloride was obtained from E. Merck, Darmstadt, F.R. Germany. All other reagents were standard laboratory reagents of analytical grade whenever possible.

Male Wistar rats (Iffa Credo, L'Arbresle, France) aged 23–26 months (600–850 g) were compared with matched animals of 3 months (170–180 g). Animals were decapitated. Thoracic aortas were immediately removed, rinsed in saline(0·9% NaCl w/v), frozen in liquid nitrogen, then stored at −20 °C until used.

Aorta MAO and SSAO activity was measured by the micromethod of Clarke et al (1982) with some modifications as described by Guffroy & Strolin Benedetti (1984). In all cases, the final substrate concentrations in the assay tubes were 400 μM for 5-HT, 50 μM for β-PEA and 1 μM or 10 μM for BZ. The initial velocities were measured. MAO and SSAO activity was expressed as nmol (of deaminated substrate) mg^{-1} tissue (or mg protein)$^{-1}$ min^{-1}. Protein concentrations of the homogenates were determined by the method of Lowry et al (1951).

Oxidative deamination of each substrate by MAO-A, -B and/or SSAO in aorta was studied following the decrease of substrate oxidation as a function of in-

creasing concentrations of a selective MAO-A or SSAO inhibitor: clorgyline and semicarbazide, as described by others (Lyles & Callingham 1982a, b; Clarke et al 1982).

Statistical analyses were made using Student's *t*-test (Armitage 1973) when the hypothesis of equal variance was valid as evaluated by the Fisher test, and the Wilcoxon non-parametric test (Hollander & Wolfe 1973) when this hypothesis was rejected.

Results

Protein content. The protein concentration did not change significantly in the aorta of old rats (0·12 ± 0·004 mg mg^{-1} tissue, mean ± s.e.m.) compared with young rats (0·14 ± 0·02 mg mg^{-1} tissue).

Inhibition of 5-HT, β-PEA and BZ oxidative deamination in the aorta of young and old rats. This was studied as a function of increasing concentrations of clorgyline (up to 10^{-3} M). 400 μM 5-HT is metabolized virtually only by MAO-A; the contribution, if any, of MAO to 50 μM β-PEA, is small; this is an indirect confirmation of our previous results in Wistar-Kyoto rats, i.e. the enzyme mainly responsible for β-PEA deamination in rat aorta is SSAO (Guffroy & Strolin Benedetti 1984) (Fig. 1). The inhibition of BZ oxidative deamination by semicarbazide (up to 10^{-3} M) was also carried out. BZ (1 and 10 μM) is metabolized only by SSAO in the aorta of both young and old rats (Fig. 1).

Change in SSAO activity with age in rat aorta. SSAO activity was determined with BZ in the absence or presence of clorgyline. A significant decrease of SSAO activity was found in the aorta of old rats (Table 1). Results obtained with β-PEA (Table 2) should also be viewed as representative of changes of SSAO activity with age: with this substrate, a decrease of SSAO activity, although not significant, is observed when data are expressed as nmol (mg protein)$^{-1}$ min^{-1}.

MAO-A activity in aorta from young and old rats. As shown in Table 2, no significant difference of MAO-A activity was found in the aorta of old rats compared with young rats. Concerning MAO-B, it is impossible to draw any conclusion from the results of this work, as none of the substrates used show any evidence for metabolism by MAO-B alone in rat aorta.

Kinetic analysis of SSAO in aorta of young and old rats., In young rats, the K_m and V_{max} values (mean ± s.e.m.) of BZ (0·5–10 μM) oxidative deamination by aorta SSAO are 2·6 ± 0·12 μM and 1·20 ± 0·09 nmol (mg protein)$^{-1}$ min^{-1}. The corresponding values in old rats are 2·4 ± 0·05 μM and 0·94 ± 0·03 nmol (mg protein)$^{-1}$ min^{-1}. Statistical analysis shows that the V_{max} of SSAO in aorta homogenates of old rats is significantly lower than that of young rats ($P < 0·05$, Student's *t*-test, n = 4), whereas the K_m values do not differ significantly (Fig. 2).

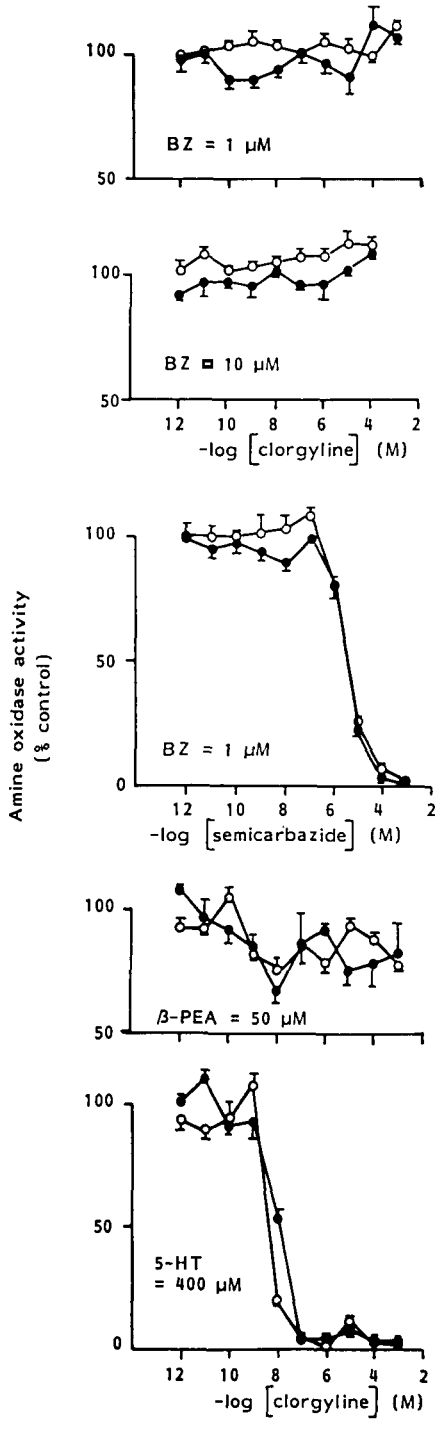

FIG. 1. Oxidative deamination of BZ, 5-HT and β-PEA in aorta of young (○) and old (●) rats as a function of increasing concentrations of a selective MAO-A inhibitor (clorgyline) or SSAO inhibitor (semicarbazide). Each point represents the mean ± s.e.r. (determinations in triplicate).

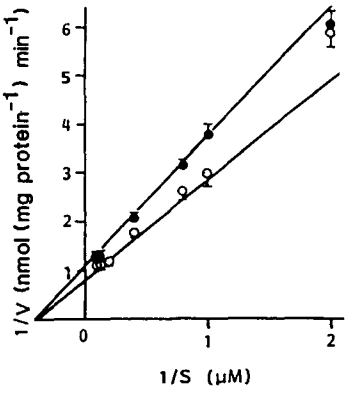

FIG. 2. Lineweaver-Burk plot of initial velocity (V) as a function of substrate concentration (S) for aorta SSAO activity. Each point represents the mean ± s.e.m. of determinations from four homogenates of young (○) and old (●) rats. Triplicate determinations of individual homogenates have been carried out.

Discussion

Substrate oxidation by MAO-A, MAO-B and/or SSAO varies from tissue to tissue and from species to species (Fowler & Callingham 1978: Garrick & Murphy 1982; Strolin Benedetti et al 1983: Clarke et al 1982: Barrand & Callingham 1982; Andree & Clarke 1981, 1982). The present work indicates that 1 and 10 μM BZ are metabolized virtually only by SSAO, that a major proportion of 50 μM-PEA metabolism is due to SSAO, and that 400 μM 5-HT is metabolized only by MAO-A in the aorta of both young and old rats. In the aorta of old rats a significant decrease of SSAO activity was found, due to a lower V_{max}, whereas no significant difference of MAO-A activity was observed. Age-related decreases of 5-HT and β-PEA oxidative deamination in rat aorta have been reported (Fuentes et al 1977), as well as age-related changes in MAO and SSAO activities in various tissues of rats (Cao Danh et al 1984, 1985). The findings of Fuentes et al (1977) agree with our results of a decrease of SSAO activity in the aorta of old rats. In contrast, data from those same authors on 5-HT oxidative deamination (1·2 mM) in the aorta of old rats do not correspond with our results (5-HT 400 μM).

In animal tissues, there is some evidence to suggest that SSAO is a membrane-bound enzyme localized in plasma membrane and microsomal membrane (Wibo et al 1980; Barrand & Callingham 1982; Clarke et al 1982). However, it has also been found in mitochondria of chick heart (Callinghm 1983) or in supernatant of rat arterial tissue (Coquil et al 1973). MAO activities are dependent on membrane composition (Tipton et al 1973; Nohl & Krämer 1980; Huang & Faulkner 1980; Houslay & Marchmont 1980) which may be altered with age. Although the SSAO subcellular localization is not clearly established, it is not excluded that membrane modifications contribute to the change of SSAO activity in aorta with age. Further study is merited in this respect, as age-related changes in amine metabolizing enzymes of aorta might have consequences for the function of amines in senescence.

Table 1. Oxidative deamination of BZ by aorta homogenates of young and old rats with and without clorgyline (0·1 mM). Final concentration: 1 μM BZ. Enzyme activity is expressed as nmol (mg tissue)$^{-1}$ or (mg protein)$^{-1}$ min^{-1}, n = 5–6. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, Student's *t*-test or Wilcoxon test.

Young rats	Old rats	$\frac{\text{Old}}{\text{Young}} \times 100$ Mean ± s.e.r.
Without clorgyline		
0·030 ± 0·003	0·018 ± 0·002	61 ± 8*
(0·221 ± 0·015)	(0·154 ± 0·014)	(70 ± 8)*
With clorgyline (0·1 mM)		
0·031 ± 0·002	0·018 ± 0·003	59 ± 10**
(0·232 ± 0·020)	(0·154 ± 0·021)	(66 ± 11)*

Table 2. Oxidative deamination of 5-HT and β-PEA by aorta homogenates of young and old rats. Enzyme activity is expressed as nmol (of deaminated substrate) (mg tissue)$^{-1}$ or (mg protein)$^{-1}$ min^{-1}, n = 6.

Substrate (μM)	Young rats (mean ± s.e.m.)	Old rats (mean ± s.e.m.)	$\frac{\text{Old}}{\text{Young}} \times 100$ (mean ± s.e.r.)
5-HT (400)	0·057 ± 0·004	0·073 ± 0·009	127 ± 17
	(0·56 ± 0·06)	(0·57 ± 0·06)	(102 ± 16)
β-PEA (50)	0·045 ± 0·004	0·047 ± 0·002	103 ± 11
	(0·45 ± 0·03)	(0·37 ± 0·03)	(83 ± 8)

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Age-related changes in the activities of the amine metabolizing enzymes of rat eye

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Eye MAO-A, MAO-B, semicarbazide-sensitive amine oxidase (SSAO) and aldehyde reductase (AR) activities were measured in young and old rats. When enzyme activity is expressed as nmol (mg protein)$^{-1}$ min^{-1}, a significant decrease (18–23%) of SSAO activity in the eye of old rats was found, whereas there was no significant difference in MAO-A and MAO-B activities. A significant increase of AR activity with D-xylose (67%), DL-glyceraldehyde (64%), D-glucuronate (43%) and D-glucose (21%) was found in the eye of old rats. These results suggest that changes in the activities of the amine metabolizing enzymes of rat eye with age might have consequences for their function in senescence; particularly, the increase of AR activity might be involved in cataract formation.

Monoamine oxidase (MAO, EC 1.4.3.4) is a mitochondrial enzyme which exists in at least two forms, MAO-A and MAO-B. These are differentiated by their substrate-specificities (Tipton et al 1983; Fowler et al 1981) and their inhibitor sensitivities (Johnston 1968; Knoll & Magyar 1972). Semicarbazide-sensitive amine oxidase (SSAO) is another enzyme with a widespread distribution in man and rat, particularly in aorta and lung (Lewinsohn et al 1978, 1980). This enzyme has also been called plasma or serum amine oxidase, benzylamine oxidase (BZAO) because it is able to catalyse the oxidation of benzylamine (Lewinsohn et al 1978, 1980), or clorgyline-resistant amine oxidase, as it is resistant to inhibition by clorgyline at in-vitro concentrations that are sufficient to inhibit MAO-A and MAO-B activities completely (Lyles & Callingham 1982).

The metabolism of catecholamines and 5-hydroxytryptamine (5-HT) in brain first involves their deamination to aldehydes, a reaction catalysed by MAO. These biogenic aldehydes are then either oxidized to acids by aldehyde dehydrogenase (ALDH, EC 1.2.1.3) or reduced to alcohols by NADPH-dependent aldehyde reductases (AR, EC 1.1.1.2) (Tipton et al 1977).

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