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# Age-related changes in benzylamine oxidase activity in rat tissues

HUNG CAO DANH, MARGHERITA STROLIN BENEDETTI*, PHILIPPE DOSTERT† AND ARLETTE MOUSSET
*Centre de Recherche Delalande – 10 rue des Carrières, 92500 Rueil-Malmaison, France*

Brain, liver, heart, lung, kidney and duodenum benzylamine oxidase (BZAO) activities were measured from young and old rats. Protein content was found to decrease in liver (−17%), kidney (−20%) and duodenum (−17%) but remained unchanged in brain, heart and lung of old rats compared with that of young rats. A significant decrease (−41%) of BZAO activity was found in lung whereas a significant increase of enzyme activity was found in brain (+49%) and kidney (+25%) and no change was found in heart and duodenum of old rats. BZAO was not detected in either young or old rat liver. Kinetic analysis for lung BZAO activity of old rats showed that $V_{max}$ was decreased but $K_m$ was unchanged in comparison with that of young rats. Since, as we have shown previously, MAO-A and -B activity in lung of old rats was also found to be decreased, the decrease of lung BZAO activity with increasing age merits further investigation, lung playing an important role in removing amines from the circulation.

Monoamine oxidase (MAO, EC 1.4.3.4) is a mitochondrial enzyme which exists in at least two forms termed MAO-A and MAO-B. These two forms are differentiated by their substrate-specificities (Tipton et al 1983; Fowler et al 1981) and their inhibitor sensitivities (Johnston 1968; Knoll & Magyar 1972). In addition to the MAO activities, many tissues of the rat, particularly those of the cardiovascular system, contain an amine oxidase. This amine oxidase prefers benzylamine (BZ) as substrate and it is resistant to inhibition by clorgyline, selegiline ((−)-deprenyl) and pargyline at concentrations which inhibit completely MAO-A and MAO-B (Coquil et al 1973; Lyles & Callingham 1975; Dial & Clarke 1977; Clarke et al 1982). Because of resistance to inhibition by clorgyline, this enzyme has also been called 'clorgyline-resistant amine oxidase' (CRAO) (Lyles & Callingham 1975; 1982a, b) while the name 'benzylamine oxidase' (BZAO) has been used by others (Lewinsohn et al 1978). BZAO is inhibited by certain carbonyl reagents such as semicarbazide, which at similar concentrations, has no effect on MAO activities (Coquil et al 1973; Lyles & Callingham 1975; Dial & Clarke 1977). This inhibitory property has led to the term 'semicarbazide-sensitive amine oxidase' (SSAO) also being used for this enzyme. In animal tissues, there is some evidence to suggest that BZAO is a membrane-bound enzyme mainly 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 (Callingham 1983). In a previous study on rat arterial tissue, BZAO activity was found mainly in the supernatant (Coquil et al 1973).

Age-related changes in MAO activity have been reported in tissues of animals (Shih 1979; Strolin Benedetti & Keane 1980; Lowe et al 1975; Della Corte & Callingham 1977; Cao Danh et al 1983) and man (Gottfries et al 1975; Robinson et al 1972; Oreland & Fowler 1979). Up to date, to our knowledge no data exist concerning BZAO activity in animal tissues with increasing age. The aim of the present study was therefore to compare the BZAO activity in several tissues from young adult (3 months) and old (23–26 months) rats.

## MATERIALS AND METHODS

[7-$^{14}$C]Benzylamine hydrochloride was obtained from the Radiochemical Centre, Amersham, UK; 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; the other reagents were standard laboratory reagents of analytical grade.

Male Wistar rats (Iffa Credo) aged 23–26 months (600–850 g) were compared with matched animals of 3 months (170–180 g). Animals were decapitated. Brains, livers, hearts, lungs, kidneys and duodenums

* Correspondence.
† Present address: Laboratoires Fournier, Centre de Recherche, 50 rue de Dijon, Daix 21121 Fontaine-lès-Dijon, France.

(10 cm after the pylorus) were immediately removed, rinsed in saline (0·9% NaCl w/v), and frozen in liquid nitrogen. All tissues were then stored at −20 °C until used.

BZAO activity was assayed basically as described by Guffroy and Strolin Benedetti (1983), using BZ (1 μM) as substrate. Contribution of MAO-A, MAO-B and/or BZAO to the metabolism of 1 μM BZ in each tissue was defined from experiments where the decrease of oxidative deamination of BZ was measured as a function of increasing concentrations of the selective MAO-A inhibitor, clorgyline. In the case of lung, the decrease of oxidative deamination of 10 μM BZ was also measured as a function of increasing concentrations of clorgyline. Moreover, in the case of lung, as described by other authors (Lyles & Callingham 1982; Clarke et al 1982), the BZAO inhibitor, semicarbazide, was also used. Preincubation time with clorgyline was 20 min at 37 °C. The tissues were homogenized in phosphate buffer 0·1 M, pH 7·8, using an Ultra Turrax (1 g tissue/16–20 ml buffer).

Aliquots (0·1 ml) of tissue homogenates were taken for determination of BZAO activity in a final volume of 0·5 ml. The reaction was started by addition of 0·1 ml of [$^{14}$C]BZ. After incubation at 37 °C in normal air, the reaction was stopped by cooling the tubes on ice and acidifying with 0·2 ml of 4 M HCl. The deaminated products were extracted in 7 ml of toluene–ethylacetate (1 : 1, v/v). The tubes were then kept at −20 °C for 1 h to allow the aqueous layer to freeze. The organic layer was poured into a scintillation vial, and 10 ml of toluene containing PPO (0·4%, wt/vol) was then added. The samples were counted in a Beckman or Intertechnique scintillation counter and the values obtained were corrected for the efficiencies of extraction of the deaminated metabolites into the organic layer. Enzyme activity was expressed as nmol mg$^{-1}$ of protein or g$^{-1}$ fresh tissue min$^{-1}$.

Protein concentrations of the homogenates were determined by the method of Lowry et al (1951).

$K_m$ and $V_{max}$ of BZ oxidative deamination by BZAO in lung of young and old rats was determined by linear regression analysis using the Lineweaver-Burk plot representation with 7 substrate concentrations (0·5–10 μM), in presence of clorgyline (0·1 mM).

Statistical analyses were performed on the experimental data, using the Student's *t*-test when the hypothesis of equal variance was valid, as evaluated by the Fisher test, and the Wilcoxon non-parametric test when this hypothesis was rejected.

## RESULTS

### *Protein content in young and old rat tissues*

Since enzyme activity is frequently expressed as activity per mg of protein, the protein content of young and old rat tissues was analysed. A significant decrease of protein content was found in liver, kidney and duodenum of old rats but no difference was found in brain, heart and lung (Table 1).

Table 1. Protein content of tissue from young and old rats.

| Tissue | Young rats Mean ± s.e.m. | Old rats Mean ± s.e.m. | $\frac{\text{Old}}{\text{Young}} \times 100$ Mean ± s.e.r. |
|---|---|---|---|
| Brain | 130 ± 4 | 132 ± 3 | 102 ± 4 |
| Liver | 257 ± 4 | 214 ± 5 | 83 ± 2*** |
| Heart | 178 ± 4 | 170 ± 4 | 95 ± 3 |
| Lung | 154 ± 7 | 160 ± 7 | 104 ± 6 |
| Kidney | 196 ± 4 | 156 ± 4 | 80 ± 3*** |
| Duodenum | 107 ± 6 | 89 ± 5 | 82 ± 7* |

Protein content is expressed as mg g$^{-1}$ of tissue, n = 5–6. Student's *t*-test or Wilcoxon test: *$P < 0.05$, ***$P < 0.001$.

### *Inhibition of BZ deamination in tissues of young and old rats by clorgyline*

Oxidative deamination of BZ in tissues of young and old rats as a function of increasing concentrations of clorgyline is presented in Figs 1 and 2. The oxidative deamination of 1 μM BZ in tissues of young rats as a function of increasing concentrations ($10^{-9}$ to $10^{-3}$ M) of semicarbazide has been previously presented (Guffroy & Strolin Benedetti 1984). In brain and liver of both young and old rats, 1 μM BZ is metabolized practically only by MAO-B (Fig. 1). In heart, 1 μM BZ is a substrate of BZAO in young rats but of both BZAO and MAO-A in old rats. In kidney, 1 μm BZ is a substrate of both BZAO and MAO-B. Finally, in duodenum and lung of both young and old rats, 1 μM BZ is a substrate of BZAO but 10 μM BZ is deaminated in lung by both MAO-B and BZAO (Fig. 2).

### *Inhibition of BZ deamination in lung of young and old rats by semicarbazide*

In lung of both young and old rats, 1 μM BZ is metabolized practically only by BZAO (Fig. 2).

### *Change in BZAO activity with age in rat tissues*

BZAO activity was determined in the absence (Table 2) or presence (Table 3) of clorgyline (0·1 mM) and expressed as nmol g$^{-1}$ of tissue or mg$^{-1}$ of protein min$^{-1}$. In the absence of clorgyline, when enzyme activity was expressed as mg$^{-1}$ of protein, a

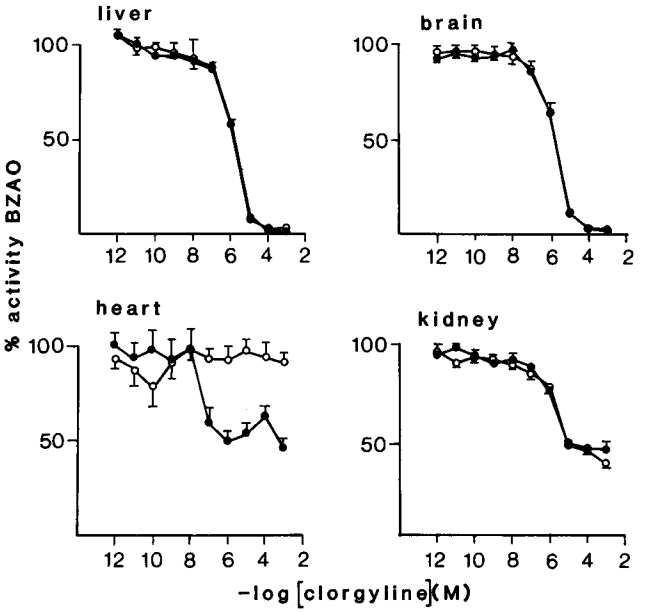


FIG. 1. Oxidative deamination of BZ (1 μM) in liver, brain, heart and kidney of young (○) and old (●) rats as a function of increasing concentrations of a selective MAO-A inhibitor, clorgyline. Each point represents the mean ± s.e.r. (determinations in triplicate).

significant decrease of BZAO activity was found in lung, whereas a significant increase of enzyme activity was found in brain and heart and no change was found in liver, kidney and duodenum of old rats (Table 2). In the presence of clorgyline, a significant decrease of BZAO activity was found in lung whereas a significant increase of enzyme activity was found in brain and kidney and no change was found in heart and in duodenum of old rats. BZAO activity in liver in presence of clorgyline was not detected (Table 3).

*Kinetic analysis of BZAO in the presence of clorgyline (0·1 mM) in lung of young and old rats*

In young rats, the $K_m$ and $V_{max}$ values (mean ± s.d.) of BZ oxidative deamination by lung BZAO are 3·79 ± 0·68 μM and 0·54 ± 0·06 nmol $mg^{-1}$ protein $min^{-1}$. The corresponding values (mean ± s.d.) in old rats are 4·20 ± 0·44 μM and 0·28 ± 0·04 nmol $mg^{-1}$ protein $min^{-1}$ (Fig. 3). Statistical analysis shows that the $V_{max}$ of BZAO in lung homogenates of old rats is significantly lower than that of young rats (−49%, $P < 0·01$), whereas the $K_m$ values do not differ significantly (Student's *t*-test).

## DISCUSSION

Substrate oxidation by MAO-A and/or MAO-B varies from tissue to tissue and from species to species, although it should be necessary to discriminate when the oxidation of a substrate in a given tissue is due to the presence of undetectable amounts of one of the two enzyme forms or when the other form is capable of oxidizing the substrate in that particular tissue (Fowler & Callingham 1978; Garrick & Murphy 1982; Strolin Benedetti et al 1983). Recently, substrate specificity of BZAO in several tissues has also been reported (Clarke et al 1982; Barrand & Callingham 1982; Andree & Clarke 1981,

Table 2. Oxidative deamination of benzylamine by whole tissue homogenates of young and old rats without clorgyline.

| Tissue | Young rats Mean ± s.e.m. | Old rats Mean ± s.e.m. | $\frac{\text{Old}}{\text{Young}} \times 100$ Mean ± s.e.r. |
|---|---|---|---|
| Brain | 2·81 ± 0·07 (0·0216 ± 0·0006) | 3·28 ± 0·04 (0·0249 ± 0·0005) | 117 ± 3*** (115 ± 4)** |
| Liver | 21·77 ± 0·55 (0·085 ± 0·002) | 19·52 ± 1·30 (0·091 ± 0·005) | 90 ± 6 (107 ± 6) |
| Heart | 3·05 ± 0·21 (0·017 ± 0·001) | 4·18 ± 0·24 (0·025 ± 0·001) | 137 ± 12** (147 ± 12)** |
| Lung | 22·65 ± 2·0 (0·148 ± 0·012) | 14·50 ± 1·0 (0·090 ± 0·005) | 64 ± 7** (61 ± 6)** |
| Kidney | 1·01 ± 0·05 (0·0052 ± 0·0002) | 0·82 ± 0·09 (0·0053 ± 0·0005) | 81 ± 10 (102 ± 11) |
| Duodenum | 22·50 ± 0·47 (0·214 ± 0·017) | 20·96 ± 1·04 (0·243 ± 0·025) | 93 ± 5 (114 ± 15) |

Final concentration: benzylamine, 1 μM.
Benzylamine oxidase activity is expressed as nmol $g^{-1}$ of tissue (or $mg^{-1}$ of protein) $min^{-1}$, n = 5–6.
Student's *t*-test or Wilcoxon test: $^{**}P < 0·01$, $^{***}P < 0·001$.

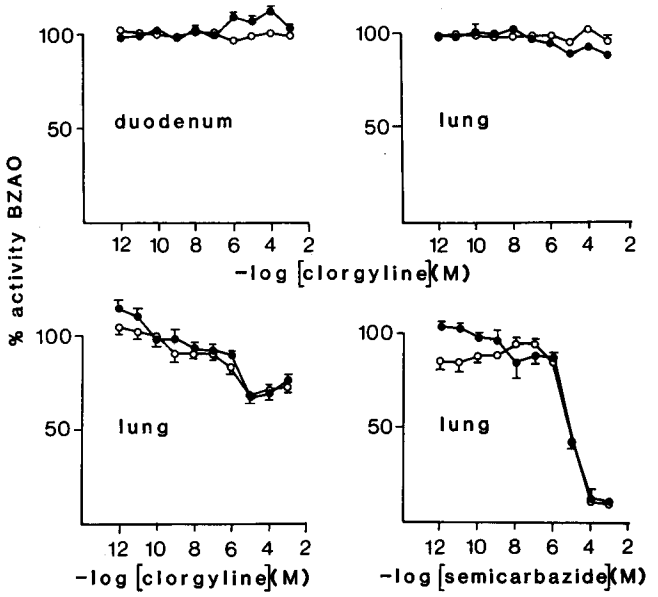


FIG. 2. Oxidative deamination of BZ (1 μM) in duodenum and lung (10 μM lower left) of young (○) and old (●) rats as a function of increasing concentration of a selective MAO-A (clorgyline) or SSAO (semicarbazide) inhibitor. Each point represents the mean ± s.e.r. (determinations in triplicate).

Table 3. Oxidative deamination of benzylamine by whole tissue homogenates of young and old rats with clorgyline.

| Tissue | Young rats Mean ± s.e.m. | Old rats Mean ± s.e.m. | $\frac{\text{Old}}{\text{Young}} \times 100$ Mean ± s.e.r. |
|---|---|---|---|
| Brain | 0·095 ± 0·006 (0·00073 ± 0·00003) | 0·143 ± 0·012 (0·00109 ± 0·00010) | 151 ± 16** (149 ± 15)** |
| Liver | ND | ND | ND |
| Heart | 3·01 ± 0·23 (0·017 ± 0·001) | 3·26 ± 0·29 (0·019 ± 0·001) | 108 ± 13 (112 ± 11) |
| Lung | 24·84 ± 1·58 (0·162 ± 0·012) | 15·19 ± 1·02 (0·095 ± 0·005) | 61 ± 6*** (59 ± 5)*** |
| Kidney | 0·47 ± 0·04 (0·0024 ± 0·0002) | 0·47 ± 0·02 (0·0030 ± 0·0001) | 100 ± 10 (125 ± 12)* |
| Duodenum | 24·53 ± 1·11 (0·234 ± 0·022) | 21·41 ± 0·87 (0·247 ± 0·022) | 87 ± 5* (106 ± 14) |

Final concentration: benzylamine, 1 μM; clorgyline, 0·1 mM.
Benzylamine oxidative activity is expressed as nmol $g^{-1}$ of tissue (or $mg^{-1}$ of protein) $min^{-1}$, n = 5–6.
Student's *t*-test or Wilcoxon test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
ND = not detected.

1982). Our results show that the oxidative deamination of 1 μM BZ is carried out practically only by BZAO in lung and duodenum of both young and old rats whereas it is deaminated by both MAO-B and BZAO in kidney from young and old rats. In liver of both young and old rats, 1 μM BZ is metabolized only by MAO-B. Finally, in brain, 1 μM BZ is deaminated mainly by MAO-B and probably slightly (<5%) by BZAO, although this requires further confirmation.

The present results are in agreement with those that other authors obtained in tissues of young rats (Lewinsohn et al 1978; Clarke et al 1982). The substrate specificity in heart tissue varies with age, as 1 μM BZ is deaminated only by BZAO in heart tissue from young rats but by both BZAO and MAO-A in heart tissue from old rats.

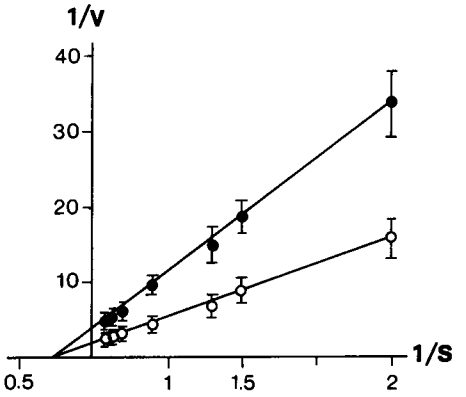


FIG. 3. Lineweaver-Burk plot of initial velocity (V) as a function of substrate concentration (S) for lung BZAO activity. Each point represents the mean ± s.d. of determinations from three lung homogenates of young (○) ($V_{max}$ = 0·54 ± 0·06 nmol $min^{-1}$ $mg^{-1}$ protein; $K_m$ = 3·79 ± 0·68 μM) and old (●) ($V_{max}$ = 0·28 ± 0·04 nmol $min^{-1}$ $mg^{-1}$ protein; $K_m$ = 4·20 ± 0·44 μM, n = 3) rats. Triplicate determinations of individual homogenates have been carried out.

The present results show that the protein content of rat tissues differs with age. Therefore, the age-related changes when BZAO activity is expressed as per mg of protein slightly differ from those observed when enzyme activity is expressed as per g of tissue. Our results indicate that BZAO activity decreases in lung whereas it increases in brain and kidney and it is unchanged in heart and duodenum of old rats. In a previous study, a significant increase of MAO-B was found in brain and liver and a significant increase of MAO-A in heart, liver and kidney. Both MAO-A and MAO-B activities decreased in lung of old rats (Cao Danh et al 1983). These results demonstrate that ageing exerts specific changes in MAO-A and MAO-B activities of the various peripheral organs of the rats. Since MAO activities are dependent on the microenvironment of the membrane, changes in the membrane composition, particularly of phospholipids, during ageing might lead to altered functional expression of MAO (Tipton et al 1973; Nohl & Krämer 1980; Huang & Faulkner 1980; Houslay & Marchmont 1980). Although the BZAO localization is not clearly elucidated, it is not excluded that similar phenomenon contributes to the changes of BZAO activity in old rat tissues.

Since lung plays an important role in removing amines from the circulation (Minchim et al 1982; Mais et al 1982; Olson et al 1983), the decrease of lung MAO-A, MAO-B and BZAO activity with increasing age needs further investigation.

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