Pergamon

Biochemical Pharmacology, Vol. 47, No. 8, pp. 1333–1338, 1994.

0006-2952/94 $6.00 + 0.00

0006-2952(93)E0096-P

THE EFFECT OF A LONG TERM (6 MONTHS) TREATMENT WITH (−)DEPRENYL ON ANTIOXIDANT ENZYME ACTIVITIES IN SELECTIVE BRAIN REGIONS IN OLD FEMALE FISCHER 344 RATS

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(*Received* 2 *July* 1993; *accepted* 30 *November* 1993)

Abstract—The effect of long term treatment with (−)deprenyl (s.c. injection three times a week for 6 months) on superoxide dismutase (SOD) and catalase (CAT) in selective brain regions was examined in old (22 months) female Fischer 344 rats. The three doses of deprenyl used (0.1, 0.25 and 0.5 mg/kg/day) increased the activities of both enzymes in substantia nigra, striatum and cerebral cortices essentially in a dose dependent manner. However, for CAT activities in cerebral cortices, the smallest dose of 0.1 mg/kg/day was most effective, while the highest dose (0.5 mg/kg/day) had no effect. In contrast to these brain regions, there were no significant differences in enzyme activities between control and deprenyl-treated groups in the hippocampus and cerebellum. If the effect of deprenyl on the life span of female F-344 rats is causally related to its effect on antioxidant enzyme activities in selective brain regions as shown in this study, then a dose of 0.25 or 0.5 mg/kg/day appears to be most appropriate. Since this dose is much lower than the dose suggested by our previous short term (3 week) experiments, an even longer term experiment is necessary to determine the optimal dose of deprenyl to increase free radical scavenging and thus possibly extend lifespan.

Key words: superoxide dismutase; catalase; striatum; long term effect; deprenyl

(−)Deprenyl was developed as a monoamine oxidase B inhibitor more than 30 years ago [1]. The drug has been reported to retard the progression of Parkinson's disease [2–4] as well as to prevent Parkinson's disease-like symptoms induced by MPTP§ in experimental animals [5, 6]. These effects have previously been interpreted as being due to the inhibition of the production of toxic monoamines by preventing their oxidation [7]. Furthermore, at least one retrospective [2] study reported that patients treated with levodopa and deprenyl lived longer than those treated with levodopa alone. Until recently, these results have been interpreted as due to deprenyl's effect in retarding the progression of Parkinson's disease.

In 1988, Knoll reported that old male rats treated with subcutaneous (s.c.) injections of deprenyl lived significantly longer than saline injected controls [7]. Two subsequent studies on male F-344 rats [8, 9] showed that (−)deprenyl's effect on increasing the life span of old rats was real, although not so marked as originally reported by Knoll. The mechanism(s) whereby (−)deprenyl prolongs the life span of animals remains unknown, however, Knoll reported increased activities of SOD in the striatum of rat brain and suggested that this may be causally related to its effect on life span in the rat [7].

Our group has examined in detail the effect of deprenyl on SOD and CAT in F-344 rats and found that the drug significantly increases activities of both types of SOD (Cu Zn-SOD and Mn-SOD) and CAT but not of glutathione peroxidase (GSH Px) [10]. The optimal dose for this effect is, however, one tenth lower in young female rats than in young male rats, and the optimal dose for young male rats actually decreased these activities in young female rats [11]. In old male rats, the optimal dose was one fourth lower than in young males [12], while in old females, the optimal dose was five times larger than in young females [11]. The effect of deprenyl is not specific to the striatum but is also seen in several regions such as substantia nigra (s. nigra) and cerebral cortices, but not in the hippocampus, cerebellum or liver [12, 13].

Until recently, our studies and those of Knoll [7] have exclusively employed deprenyl treatment for 3 weeks. On the other hand, in all three life span studies in rats including our own [7–9], rats were treated with s.c. injections, three times a week for 1–2 years. Furthermore, one recent study in our laboratory suggested that SOD and CAT activities tended to decrease as the treatment by deprenyl was continued, up to 4 weeks [14], suggesting that the optimal dose to increase these antioxidant enzymes may vary depending on the duration of the treatment.

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§ Abbreviations: SOD, superoxide dismutase; CAT, catalase; F-344, Fischer 344; s. nigra, substantia nigra; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Table 1. Body weights of rats at the start and the end of 6-month treatment with deprenyl

Deprenyl dose (mg/kg/day)*	Body weight (g) at the start (22 months)	at the end (28 months)
Control (saline)	233.8 ± 16.3 (4)†	252.5 ± 15.2 (4)
0.10	268.3 ± 13.1 (3)	285.0 ± 15.0 (2)
0.25	267.5 ± 26.6 (4)	261.3 ± 30.7 (4)
0.50	276.3 ± 31.9 (4)	270.0 ± 30.2 (4)

* Animals were s.c. injected three times a week for 6 months.
† Numbers in parentheses is the number of rats in each group.

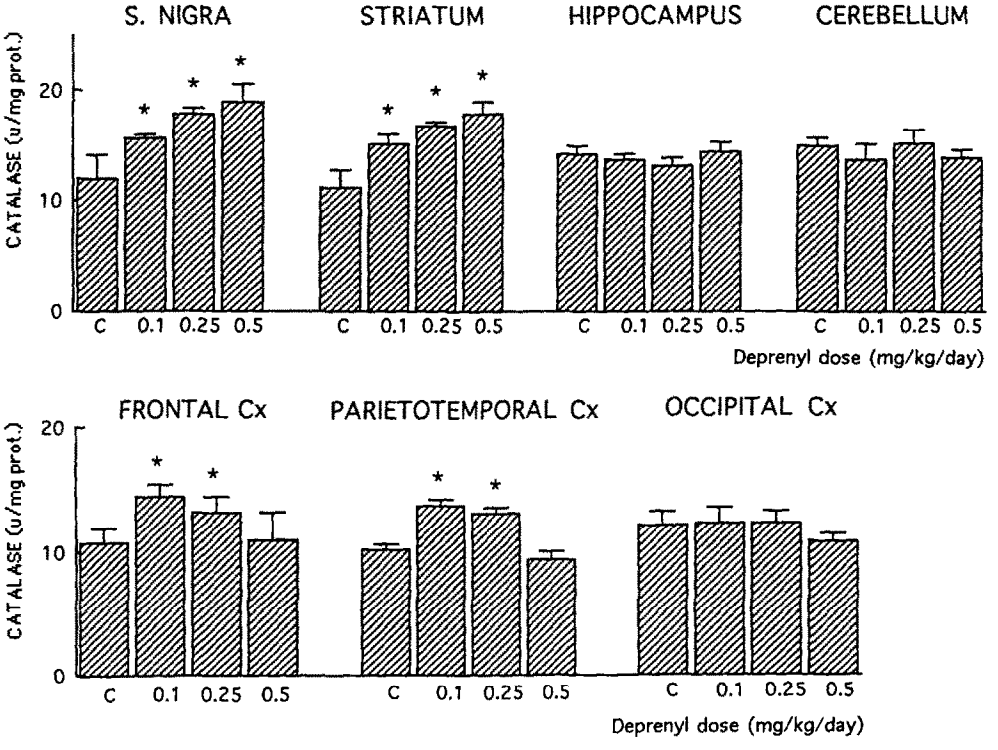

Fig. 1. Activities of catalase in seven brain regions from old female rats treated with different doses of deprenyl. Number of rats of each group is four except for the group given the dose of 0.1 mg/kg/day (N = 2). See Table 1. * Significantly different from corresponding control values ($P < 0.05$).

The present study, therefore, examined the effect of various doses of deprenyl on antioxidant enzyme activities in old female rats which were treated for 6 months.

MATERIALS AND METHODS

Specific pathogen free (SPF) female F-344 rats were purchased at the age of 4 weeks from Japan Charles River (Atsugi, Kanagawa). They were raised in the aging farm of the institute in SPF conditions until the start of deprenyl treatment.

Animals were divided into four groups. Control animals were injected s.c. with a physiological saline solution three times a week. Experimental groups were injected s.c. with deprenyl dissolved in saline solution. The doses selected were 0.1, 0.25, and 0.5 mg/kg/day three times a week. Treatment was begun at the age of 22 months and continued for 6 months. At the age of 28 months animals were killed by decapitation and brain regions such as s. nigra, striatum, hippocampus, three different cerebral cortical regions (frontal, parietotemporal and occipital) and cerebellum were dissected on an ice cold plate for enzyme measurements reported previously [10–14]. In brief, CAT activities were determined by the method of Beers and Sizer [15]. SOD activities were determined by the method using nitrite [16]. Mn-SOD activities were defined as the fraction which can be inhibited by the addition of

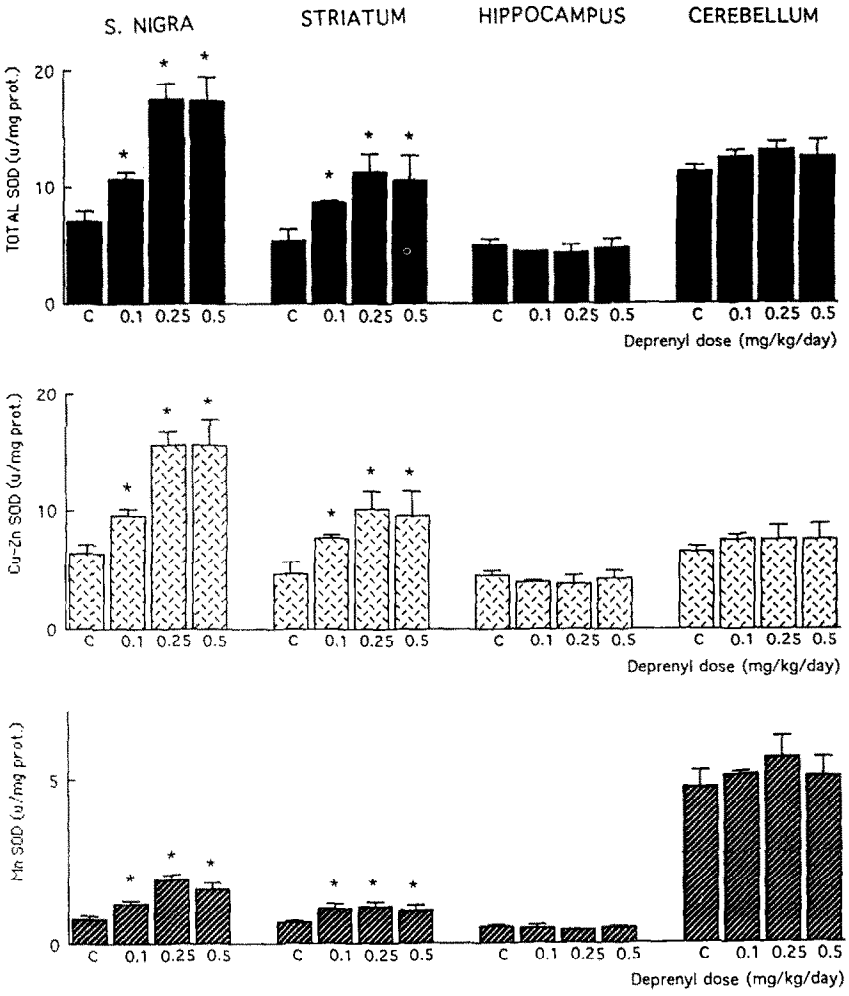

Fig. 2. Enzyme activities of total and of two species of SOD in s. nigra, striatum, hippocampus and cerebellum from old female rats treated with different doses of deprenyl. Number of rats of each group is the same as in Fig. 1. * Significantly different from corresponding control values ($P < 0.05$).

KCN at a concentration of 0.5 mM. CAT activities were determined immediately after the preparation of tissues. SOD activities were determined on the following day after 24 hr freezing which inactivates the CAT activity.

All values are expressed as means ± SD. Statistical analysis was done by means of one-way analysis of variance (ANOVA). When the difference was found to be significant with respect to deprenyl treatment, Scheffe's test was applied to compare any set of two different groups. P values lower than 0.05 were judged to be significant.

RESULTS

Table 1 summarizes the weights of animals at the start and the end of the deprenyl treatment. Only one of the 15 rats in the group given the dose of 0.1 mg/kg/day died during treatment.

Figure 1 summarizes CAT activities in seven different brain regions from rats treated with different doses of deprenyl. It is apparent that appropriate doses of deprenyl significantly increased CAT activities in striatum and s. nigra and to some extent in cerebral cortices. The dose of 0.5 mg/kg/day appeared to be most effective in striatum and s. nigra. However, unexpectedly, in frontal as well as in parietotemporal cortices, the smallest dose of 0.1 mg/kg was most effective and a dose dependent decrease occurred with the highest dose of 0.5 mg/kg being absolutely ineffective in increasing CAT activities in these regions. In the occipital cortex, CAT activities were almost the same for control and for the two deprenyl groups treated with the lowest two doses (0.1 and 0.25 mg/kg/day), while in the group given the highest dose (0.5 mg/kg/day), enzyme activities tended to be slightly lower, although this difference was not statistically significant. In contrast, in neither hippocampus nor in cerebellum, were there significant differences in the CAT activities between control and deprenyl treated animals.

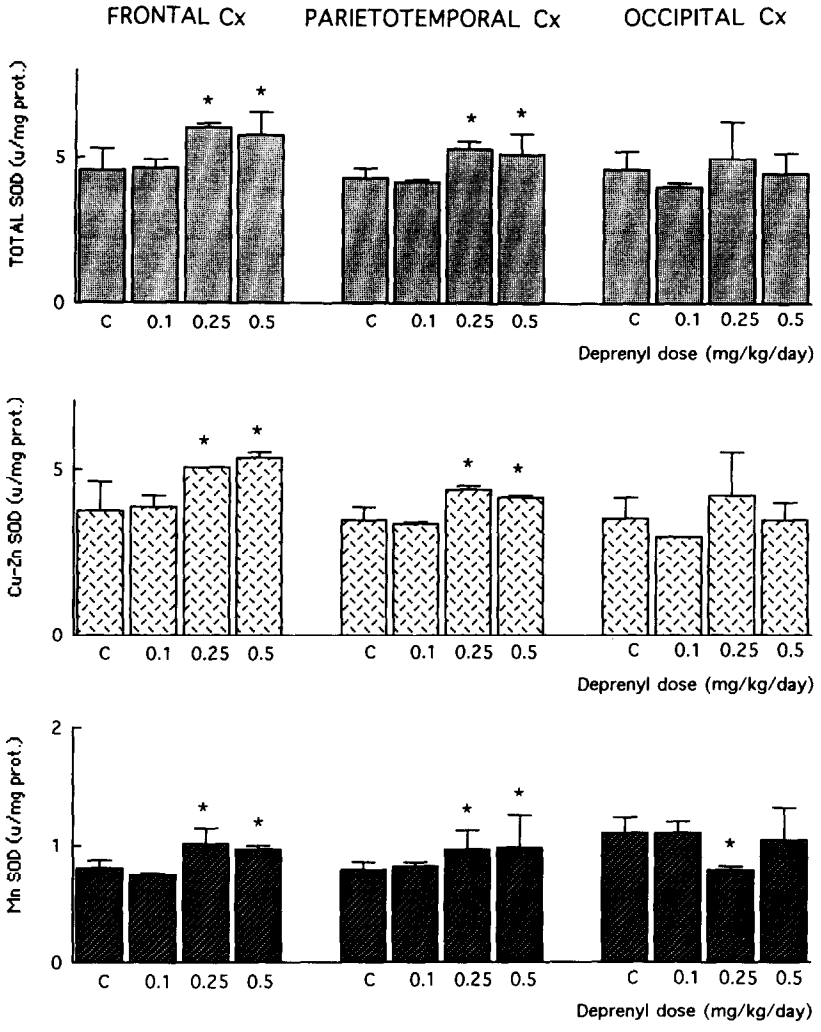

Fig. 3. Activities of total and of two species of SOD in cerebral cortices from old female rats treated with different doses of deprenyl. Number of rats of each group is the same as in Fig. 1. * Significantly different from corresponding control values ($P < 0.05$).

Figures 2 and 3 summarize SOD activity with different doses of deprenyl in seven different brain regions. Again the doses of 0.5 and 0.25 mg/kg were significantly effective in increasing SOD activities in s. nigra and striatum. The smallest dose of 0.1 mg/kg was also effective in striatum and s. nigra but its effect was much smaller than the two higher doses (0.25, 0.5 mg/kg). In the cortical regions, only the two higher doses increased activities in frontal and parietotemporal cortices, but not in occipital cortex. Deprenyl treatment did not increase SOD activities in either hippocampus or cerebellum (Fig. 1).

DISCUSSION

The results of the present study showed that chronic administration of (−)deprenyl significantly increases activities of CAT as well as of SOD in selective brain regions of old female rats. The brain regions in which enzyme activities were increased essentially corroborate our previous observations in young and old rats of both sexes treated with continuous infusion of deprenyl for a short term of 3 weeks [12–14]. It is also clear from the present results that the increase in CAT and SOD activities is dose dependent. In our previous study [11], doses from 0.5 to 1.0 mg/kg/day for 21 successive days in old female rats were almost equally effective in increasing CAT and SOD activities in striatum, while 0.25 mg/kg/day was less effective in old female rats. Since in the present study we injected deprenyl only three times a week, the dose of 0.5 mg/kg/day is actually lower than a continuous infusion of 0.25 mg/kg/day, the weekly dose used in our previous study [11]. The optimal dose of deprenyl in old female rats for increasing antioxidant enzyme activities found in our previous short term study (1.0 mg/kg/day by continuous infusion) is higher than 2.0 mg/kg/day (three times a week) as a weekly dose, which may be too high to optimally increase

enzyme activities in a study for 6 months. The results of the present study compared with our previous results [11] thus suggest that the optimal dose obtained with short term (up to 3 weeks) treatment of deprenyl cannot be regarded as appropriate in the longer term. Indeed, longer treatment appears to reduce the optimal dose needed to increase these antioxidant enzyme activities. The results of the present study indicate that if the effect of deprenyl on life prolongation of animals is causally related to its effect in enhancing CAT and SOD activities in certain brain regions, an optimal dose in increasing these enzyme activities must be examined in old animals treated on a long term basis. This is especially important, since an overdose of deprenyl not only becomes less effective in increasing activities but even reduces activities to the level below those before the deprenyl treatment [11].

The results of our present study thus suggest that much smaller doses of 0.25 or 0.5 mg/kg/day (three times a week) may be more appropriate for a longer term study than the dose found in our previous short term study [12]. For most enzyme activities examined in the present study, 0.5 mg/kg/day was slightly more effective than 0.25 mg/kg/day. However, the decrease in CAT activities in cerebral cortex with 0.5 mg/kg/day compared with lower doses raises some concern about this dose. It appears that if the treatment is continued for a longer term, CAT activities in other regions, as well as SOD activities tend to decrease with this dose. While we are unable to draw a final conclusion, 0.25 mg/kg/day appears to be more appropriate than the higher dose of 0.5 mg/kg/day, if the treatment is continued for longer than 6 months. A life span study in Fischer 344 female rats is in progress, utilizing a dose of 0.25 mg/kg/day.

It remains unclear whether deprenyl's ability to increase CAT and SOD activities is at least a partial cause for the life prolonging effect of this drug, as was demonstrated in three previous studies [7–9]. Furthermore the mechanism(s) whereby deprenyl increases SOD and CAT activities remains unknown. There are indications that deprenyl induces neurotrophic factor(s) [16, 17]. Interestingly, recent studies suggest that some neurotrophic factors can increase SOD and CAT activities [18–20]. Whether deprenyl increases antioxidant enzyme activities by means of trophic factors remains unknown. It has been reported that some trophic factors have selectivity to certain brain regions [21, 22]. The selectivity of deprenyl's effect could have an explanation if deprenyl induces some trophic factor(s) having a selectivity to catecholaminergic neurons.

Acknowledgement—The skilful secretarial assistance of Mrs T. Ohara is gratefully appreciated.

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