(-)DEPRENYL INDUCES ACTIVITIES OF BOTH SUPEROXIDE DISMUTASE (SOD) AND CATALASE BUT NOT OF GLUTATHIONE PEROXIDASE (GSH PX) IN THE STRIATUM OF MALE RATS

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SUMMARY

Daily s.c. injection of (-)deprenyl (2.0 mg/kg/day) for three weeks in young male Fischer-344 rats (6 months old) caused a three fold increase in SOD activities (U/mg) in the striatum compared with the value in saline-injected control rats (CuZn-SOD 8.56 $\pm$3.56 vs. 3.00 $\pm$ 2.00; Mn-SOD 0.913 $\pm$ 0.300 vs. 0.336 $\pm$ 0.145, mean $\pm$ SD). Furthermore, the activity of catalase (U/mg) (14.4 $\pm$3.21 vs. 8.50 $\pm$ 1.98) (but not of GSH Px) was also significantly increased by deprenyl treatment. Subsequent studies also confirmed that the drug is effective in young and old female

rats as well. However, the optimal dose was 10 fold lower in young female rats than in male rats. Furthermore aging reduced the sex difference in optimal drug dosage. The results confirm the original finding by Knoll on SOD activity but also provide evidence that catalase activity is significantly enhanced by this drug which is at variance with the work of Knoll. The radical scavenging effect of SOD is effective only when it is accompanied by activities of catalase and/or GSH Px, since SOD generates hydrogen peroxide which is more toxic than oxygen radicals. For this reason the present findings more logically support the original contention of Knoll that increased SOD activity may be beneficial in preventing possible tissue damage caused by free radicals during the aging process. Furthermore, future studies on deprenyl must be carefully performed, taking sex and age differences into account regarding the optimal dose of the drug.

Key words: *deprenyl, free radicals, superoxide dismutase, catalase, glutathione peroxidase, rat.*

INTRODUCTION

For many years, a number of attempts have been made to prolong the life span of animals by means of administration of various pharmaceuticals and nutrients. So far, however, there has been no definite success except for the well-known effect of dietary restriction in a variety of animals. Some years ago chronic administration of (-)deprenyl a MAO B inhibitor has been reported to prolong the life span of patients with Parkinson's disease[1].

More recently, Knoll[2,3] reported a dramatic prolongation of life span in rats by s.c. administration of deprenyl. A subsequent study from another group confirmed this effect[4], although the result of the latter study was not as robust as that initially reported by Knoll.

Knoll reported that the unique effect of deprenyl may be linked to the ability of this drug to enhance the activity of superoxide dismutase (SOD) in the striatum[2]. He also showed a moderate increase in activities of catalase and glutathione peroxidase but these increases were not statistically significant. Since technical details of enzyme measure-

ments were not available in his original report, we investigated in detail the ability of deprenyl to increase activities of these antioxidant enzymes in the brain.

MATERIALS AND METHODS

Chemicals

Hydrogen peroxide, xanthine, hydroxyammonium, sulfanilic acid, a-naphthylamine, potassium cyanide, reduced glutathione (GSH) and sodium azide were obtained from Wako Pure Chemicals Ltd. (Tokyo). Xanthine oxidase, GSH reductase and SOD were from Sigma. NADPH was purchased from Oriental Yeast Co. Ltd. (Tokyo).

Animals and treatment

Six-month-old Fisher-344 (F-344) rats of the male sex were used for this study. They were randomly divided into two groups. (-) Deprenyl was a generous gift of Fujimoto Pharmaceutical Co. Ltd. (Osaka, Japan). The preparation was dissolved in saline (4 mg/ml). Control rats received a daily s.c. administration of isovolumetric saline solution for 3 weeks. Treated rats received a daily s.c. administration of (-)deprenyl (2 mg/kg/day) during the same period of time. Twenty four hours after the last injection, animals were decapitated and the brains immediately excised. Striatal tissues were separated on an ice-cold surface. Tissues were homogenized in 1 ml of distilled cold water. The homogenates were sonicated for 15 sec in a Sonifier B-12 (Branson Sonic). The homogenates were then centrifuged for 2 min in an Eppendorf centrifuge (10000 x g). An aliquot of the supernatant was used immediately for the determination of catalase activity, and the remaining supernatant was stored at -20°C until the determination of SOD and GSH Px activities. This was performed within 24 to 48 hours after the preparation of samples.

Enzyme assays

Catalase. Catalase activity was assayed by the method described by Deers and Sizer[5] in which the disappearance of peroxide is followed spectrophotometrically at 240 nm. The incubation mixture contained 0.05 M potassium phosphate, pH 7.0, 0.020 M hydrogen peroxide and a sample (0.05 ml) of the supernatant fluid, in a final volume of 3 ml. The decrease in absorbance was recorded at 240 nm for 2 minutes. The rate of decrease in absorbance per minute was calculated from the initial linear portion of the curve (45 seconds). The value of 0.0394 cm^2/mmol was used as the extinction coefficient of H_2O_2. One unit of catalase was defined as the amount of enzyme which decomposed one mmol of H_2O_2 per minute at 25°C and pH 7.0 under the specific conditions.

Superoxide dismutase (SOD). The activity of SOD was assayed by the method of Elstner and Henpel[6] which is based on the inhibition of nitrite formation from hydroxylammonium in the presence of 0_2.generators. Nitrite formation from hydroxylammonium chloride was determined under the following conditions. The incubation mixture (2 ml total volume) consisted of phosphate buffer pH 7.8 (65 mM, 1 ml), xanthine oxidase (40 mg prot, 0.3 ml), xanthine (1.5 mmol, 0.1 ml), and hydroxylammonium chloride (1 mmol, 0.1 ml). The reaction was started by the addition of xanthine oxidase and was conducted at 25°C in a water bath for 20 minutes. The determination of nitrite as a product of hydroxylammonium chloride was assessed in a 0.5 ml sample of the above reaction mixture with sulfanilic (0.01 mM) acid and L-naphthylamine (0.001 mM) (total volume, 1.5 ml). The optical density of the mixture was determined at 530 nm. Addition of SOD (or 0.015 ml of supernatant) to the incubation mixture yielded an inhibition of nitrite formation. A curve of activity units vs. percentage of inhibition was recorded with known amounts of purified SOD from Sigma Chem. Co., which contained 3600 units/mg protein as assayed by the method of McCord and Fridorich[7]. Approximately one-fifth of the activity unit yielded a 50% inhibition of hydroxylammonium chloride oxidation. The amount of SOD activity units of the samples was calculated using this curve.

Differentiation between the two different types of SOD (Cu Zn-SOD

and MN-SOD) was performed by the addition of potassium cyanide (5 x 10^{-4} M) at the incubation medium. On Zu-SOD was inhibited by potassium cyanide, while Mn-SOD was not affected by its presence.

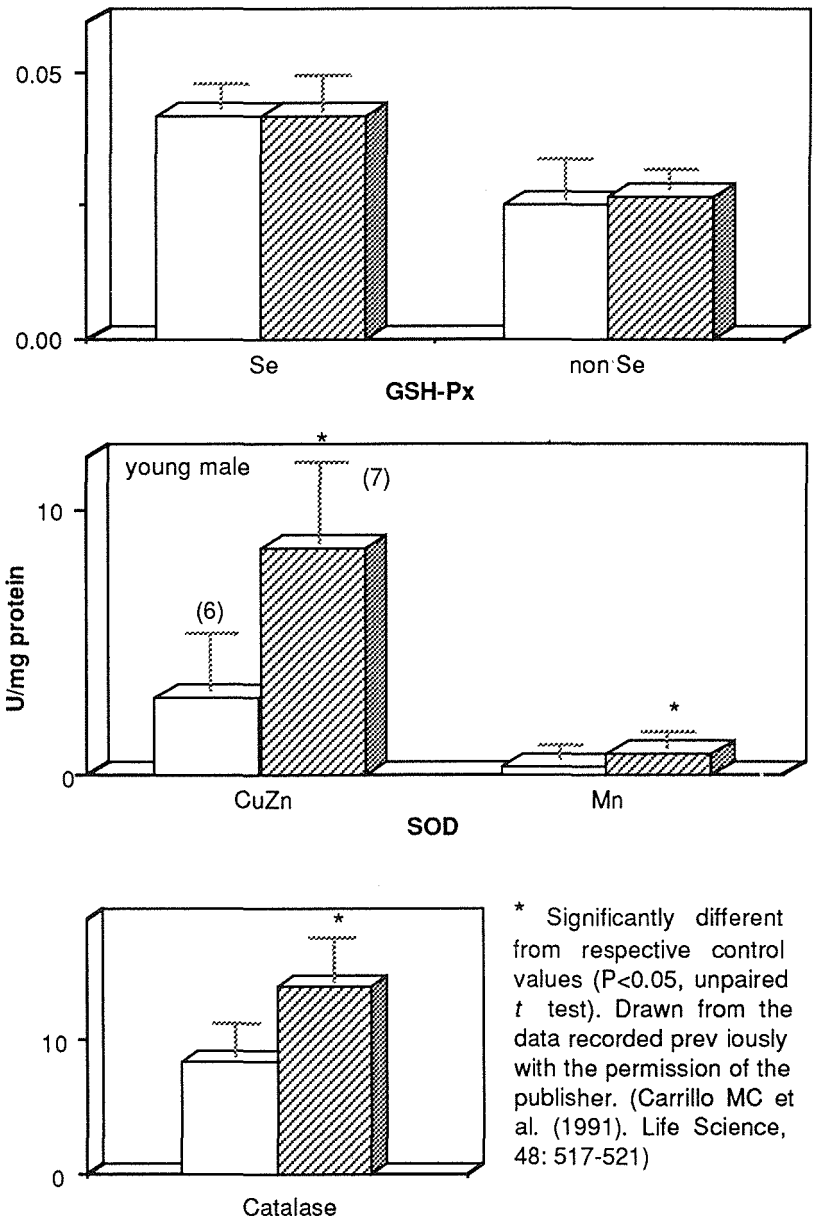
Glutathione peroxidase (GSH Px). The GSH Px activity was measured by a modification of the procedure described by Paglia and Valentine[8]. The standard assay mixture (3 ml) contained: 0.1 M buffer phosphate pH 7, 1 mM GSH, 0.2 mM NADPH, 1.4 IU GSH-reductase, 0.25 mM H_2O_2 or 1.2 mM cumene hydroperoxide, and a sample (0.04 ml) of supernatant fluid. Hydrogen peroxide was used as a substrate, and 1 mM sodium oxide was added to the reaction mixture in order to inhibit possible remnant catalase activity after the freezing of the samples. Blank values obtained without the addition of samples were subtracted from the assay values. The use of two substrates permitted the measurement of two isozymes: a selenium-dependent GSH Px (Se-GSH Px) which reacts with a wide variety of hydroperoxides including both hydrogen peroxide and organic hydroperoxides, and a nonselenium-dependent GSH Px (non Se-GSH Px), which does not use hydrogen peroxide as a substrate but reacts with organic hydroperoxides.

Statistical analysis. All values were expressed as mean $\pm$ SD. The difference between control and deprenyl-treated rats was analyzed by Student's *t* test for unpaired values. P values lower than 0.05 were judged to be significant.

RESULTS

There was no significant differences between control and deprenyl treated animals in body weight, striatal weight or protein concentration in striatum. Figure 1 summarises enzyme activities in striatal tissues from saline-treated (control) and deprenyl-treated rats. The SOD activity was three times greater in deprenyl-treated rats than in control rats. The difference was highly significant. A significant increase was observed in both Cu Zn-SOD and Mn-SOD activities. The catalase activity was also significantly higher (by 60%) in deprenyl-treated rats. In contrast, the GSH Px activity did not differ significantly between the two groups.

Fig. 1. Enzyme activities in the striatum of the brain of male F-344 rats.



* Significantly different from respective control values ($P<0.05$, unpaired *t* test). Drawn from the data recorded prev iously with the permission of the publisher. (Carrillo MC et al. (1991). Life Science, 48: 517-521)

DISCUSSION

The results presented in Figure 1 clearly show that activities of both types of SOD (CuZn-SOD and Mn-SOD) significantly increased in striatum after the injection of deprenyl for 21 days. The results confirm the original report of Knoll[2] showing a significant increase of SOD activity in striatum with the dosage of 2 mg/kg/day for 21 days. In his work, however, he did not indicate which type of SOD was examined. According to his description, it appears that he measured the total SOD activity, although it is not clear. The present study has shown that both types of SOD activities are increased by deprenyl treatment. The magnitude of increase in male rats by deprenyl treatment was 10 fold in his study[2], while we observed only a 2 to 3 fold increase with the same dosage. In another paper, he reported that in rats of another strain (Wistar derived), he could find only a marginal increase (20%) of SOD activity in male rats and an insignificant increase in female rats[9]. In this context, we subsequently studied in detail, the dose-effect relationship in F-344 rats of different ages and sexes. We found that deprenyl treatment significantly increases SOD activities in striatum in all rat groups. However, the optimal dosage to increase the activity was 10 times lower in young female rats than in male rats (data not shown). Furthermore, aging reduced the sex difference in the optimal dosage, decreasing and increasing the optimal dosage with age in male and female rats respectively.

We believe that the large sex difference in the optimal dosage in young rats is due to a possible difference in the metabolism of the drug. It is well known that in most rats strains more than ten-fold sex differences exist for many enzyme activities in the hepatic microsomal cytochrome mono-oxygenase system (all activities are higher in male rat livers). If it is assumed that deprenyl is metabolised to non-effective metabolite(s) 10-fold more rapidly in young male than in female rat livers, the optimal dose for increasing enzyme activities in the striatum should be 10-fold greater in male than in female rats. Similarly, the increase of optimal dosage in old male rats can be explained by the drastic decrease in the mono-oxygenase function with age known to occur in male rats[10-13].

Since cytochrome mono-oxygenase activities in female rats do not change with age[10-13], the increase of the optimal dose of deprenyl in

female rats with age cannot be explained on the basis of changes in the metabolism of this drug. The most likely explanation for the increase of optimal dosage in old female rats appears to be due to the increase in monoamine oxidase activity known to occur in the brains of aged animals and humans[14-17]. If a greater amount of deprenyl is used in old rats by binding irreversibly to monamine oxidase B, a greater amount of the drug to affect SOD and catalase activities in the striatum would be needed in old rats.

The dosage used in the present study is the highest dose Knoll used[2,3]. Knoll, however, could not find a significant increase in catalase activity, although it tended to be higher in deprenyl-treated rats[2]. In contrast, the 60% increase in catalase activity induced by deprenyl treatment in the present study was statistically significant. In the study of Knoll[2], the activity of GSH Px was also higher in deprenyl-treated rats, although the difference did not gain statistical significance. In contrast, in the present study, GSH Px activities were almost identical in deprenyl-treated and control rats for Se dependent as well as Se independent enzyme activities. Since SOD generates hydrogen peroxide, which is biologically more toxic than oxygen radicals, a concomitant increase in catalase (or GSH Px) activity is essential if we expect a beneficial effect from the increase in SOD activity. The observation made in the present study, therefore, indicates that deprenyl treatment may be effective in preventing free radical induced tissue damage in the striatum, if such a damage increases during aging of animals. Although many studies have suggested the possibility of tissue damage caused by radicals during aging, evidence is still indirect and is largely based on the presence of TBA reactive substances. In future studies, direct measurements of superoxide radicals or the immediate products of lipid peroxidation are needed to validate such a thesis.

The causal relationship between the increase in these enzyme activities in the striatum and the life prolongation reported previously[2-4] remains unclarified. In our own study in progress, chronic treatment with deprenyl appears to increase the life span of male F-344 rats, however, our effect is not as robust as that originally reported by Knoll[2,3] but is close to the effect reported in the same rat strain (F-344) by Milgram et al[4].

It is also quite important to clarify how specific the effect of deprenyl is.

In our more recent study, we found that deprenyl increases scavenger activities in the striatum but not in hippocampus. Knoll also reported that the drug does not increase the activity in cerebellum[18]. Further work is necessary to clarify the selectivity of the effect of deprenyl in different regions of the brain as well as in other tissues.

In conclusion, deprenyl can increase SOD as well as catalase activity, but not activity of GSH Px in the striatum of male rats. The casual relationship between this effect and that of the life prolonging effect reported previously[2-4] must be examined in future studies.

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