Upregulation of Antioxidant Enzyme Activities by Deprenyl

Implications for Life Span Extension[a]

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INTRODUCTION

As the role of oxidants has been increasingly discussed in terms of mechanisms of aging, the role of antioxidants in terms of antiaging strategies has become more and more intensely discussed in recent years. However, the exact role in the body of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH Px) in antiaging mechanisms remains unresolved.

An age-dependent decline in enzyme activities of SOD and CAT, especially in the liver, has been reported[1-3] and was suggested to be causally related to the life span of individual animals. If such a decline occurs consistently with aging, it will further accelerate tissue damage caused by oxidants, leading to a cascade of tissue damage ending most likely in cell death. Thus, if these enzyme activities are upregulated, it could have a beneficial effect on the natural aging process. However, when

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we look at past literature carefully, an age-dependent decline in antioxidant enzyme activities is not consistently seen and many discrepancies can be found in terms of alterations of antioxidant enzyme activities with aging, depending on the animal's species, sex, and tissues, and on the methodologies of different studies. Most of the past reports are concerned with changes in these enzyme activities in the liver, while information in the brain, especially in different regions of the brain, is more limited. The present study thus attempted first to determine whether antioxidant enzyme activities consistently decline with aging in several different regions of the brain in rodents.

Second, we examined how an upregulation of these enzyme activities, which is known to occur as a result of treatment with the monoamine oxidase B inhibitor, deprenyl, is regulated by several different factors such as sex, strain, and species of animals as well as dose and duration of deprenyl treatment, all of which turned out to be potent regulators for the modulation of antioxidant enzyme activities. Since, deprenyl treatment was also shown to significantly prolong the remaining life expectancies of old rats, the results will be discussed in terms of the role of antioxidant enzyme activities in the aging of animals.

MATERIALS AND METHODS

Unless otherwise stated, animals used in the present study are all from the specific-pathogen free (SPF) aging farm of the Tokyo Metropolitan Institute of Gerontology. Survivals and pathologies in their later lives have been reported elsewhere.[4]

Deprenyl Administration

For short-term treatment for 3 wks, we mostly used s.c. continuous infusion of deprenyl solution by means of s.c. implanted osmotic minipumps for practical convenience. For long-term treatment, ranging from 3 to 20 months, s.c. injection of deprenyl solution (mostly 3 times a week) was employed. For the comparison of dose effects of the drug between short-term and long-term treatments, doses were recalculated as a weekly dose.

Tissue Preparations

The details of tissue assays for enzyme activities have been reported elsewhere.[5,6] In brief, animals were sacrificed by decapitation. The brain and liver were quickly removed. Several different brain regions such as striatum, substantia nigra (S. nigra), hippocampus, cerebral cortex and cerebellum were dissected on an ice cold table.

Enzyme Activity Measurements

Activities of enzymes such as SOD, CAT and GSH Px were determined by the methods previously described.[5] In brief, SOD activities were determined by the

method described by Elstner and Heupel.[7] Mn-SOD activities were defined as the fraction which can be inhibited by the addition of KCN at a concentration of 0.5 mM. CAT activities were determined immediately after the preparation of tissue samples by the method described by Beers and Sizer.[8] GSH Px activities were determined by the method reported by Paglia and Valentine.[9] The use of two substrates permitted the measurement of two isozyme activities; these were a selenium-dependent GSH Px (Se-GSH-Px) which reacts with a variety of hydroperoxides as a substrate, 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. Protein concentration was determined by the method of Lowry *et al.*[10]

Life Span Study

Our initial study using male Fischer-344 (F-344) rats showed that chronic treatment with deprenyl (0.5 mg/kg/day, 3 times a week) can significantly increase the remaining life span of animals after 24 months of age when the treatment was started at the age of 18 months.[11] We next examined the life span extension effect of the drug using male and female rats and male mice using different doses; the preliminary results of these studies will also be discussed.

RESULTS

Comparisons of SOD enzyme activities in several brain regions between young (7-month-old) and old (28-30-month-old) F-344 rats are illustrated for male (FIG. 1) and female (FIG. 2) rats.[12] In male rats, total SOD activities tended to be higher in old rats than in young rats, especially in certain brain regions such as hippocampus, striatum and S. nigra. This effect was mostly due to about 3-5-fold increases in Mn-SOD activities in these regions in old animals. In contrast, changes in Cu, Zn-SOD activities in old male rat brains were not striking. In contrast with the remarkable increase in Mn-SOD activities in certain male rat brain regions, in female rat brains SOD activities (both Cu, Zn- and Mn-) remained essentially unchanged with age (FIG. 2).

Similarly, CAT and GSH Px activities in old animal brains remained essentially unchanged in comparison to young animals for both male and female rats.[12]

FIGURE 3 compares enzyme activities in control young male rats and in rats s.c. infused with deprenyl solution continuously for 3 weeks. Deprenyl treatment definitely increased both Cu, Zn-SOD and Mn-SOD activities as well as CAT activities in striatum and S. nigra but not in hippocampus or cerebellum. Interestingly, in some cerebral cortical regions SOD (and CAT) activities also tended to be higher in deprenyl-treated rats. We have confirmed that this trend is commonly seen in young and old rats of both sexes.[6,12-14] However, remarkable differences were observed in terms of an optimal dose of deprenyl for increasing these enzyme activities. While in young male rats, an optimal dose was about 2.0 mg/kg per day for 3 weeks, in young female rats, it was one-tenth of that dose (0.2 mg/kg per day).[6] Furthermore, aging caused a decrease in the optimal dose to one-fourth of the dose in young male

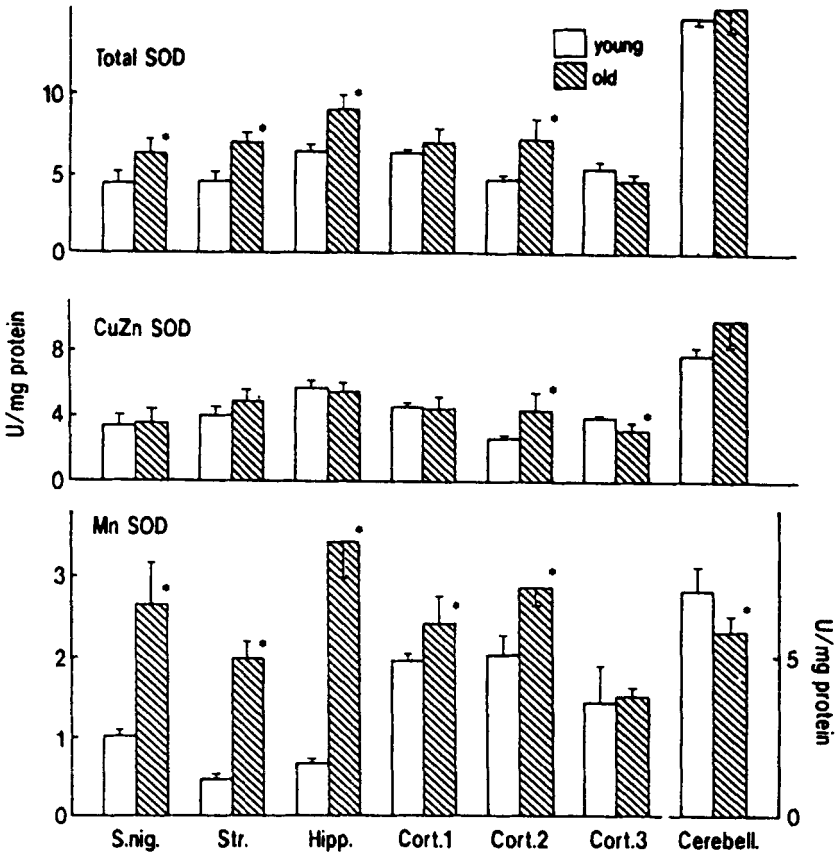


FIGURE 1. Superoxide dismutase enzyme activities in several brain regions in young (7-month-old) and old (28- to 30-month-old) male Fischer-344 rats. * Significantly different from corresponding values in young rats. S. nigra: substantia nigra; Str.: striatum; Hipp.: hippocampus; Cort. 1: frontal cortex; Cort. 2: parietotemporal cortex; Cort. 3: occipital cortex; Cerebell: cerebellum. (From Carrillo *et al.*[12] Reprinted by permission from *Mechanisms of Ageing and Development.*)

rats (0.5 mg/kg per day in old male rats),[14] while in old female rats the optimal dose increased to 5 times that in young female rats (1.0 mg/kg per day in old female rats)[6] (FIG. 4).

FIGURES 5 and 6 summarize changes in SOD (FIG. 5) and CAT (FIG. 6) activities in striatum of old female rats treated with deprenyl injections for the longer term of 6 months.[15] Interestingly, an optimal dose was found to be 0.5 mg/kg/day (3 times a week), and yet some decrease (rather than increase) for CAT activities was found with this dose. We therefore concluded that 0.25 mg/kg per day 3 times a week may be the most appropriate dose for a long-term treatment of 6 months in terms of maintaining antioxidant enzyme activities at significantly higher levels than control

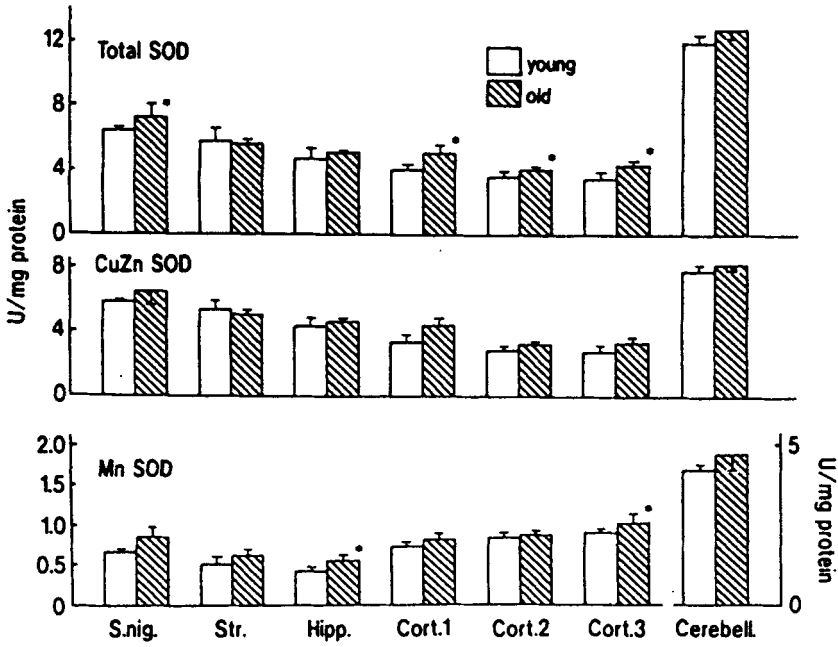


FIGURE 2. Superoxide dismutase enzyme activities in several brain regions in young (7-month-old) and old (28- to 30-month-old) female Fischer-344 rats. Abbreviations are the same as in FIG. 1. * Significantly different from corresponding values in young rats. (From Carrillo *et al.*[12] Reprinted by permission from *Mechanisms of Ageing and Development.*)

values.[15] We do not know what an optimal dose would be if we continued the treatment longer than 6 months.

DISCUSSION

Alteration of Antioxidant Enzyme Activities during Aging

No Evidence for Their Decrease

As discussed earlier, numerous studies have attributed deterioration of cellular functions during aging to free radical-induced cellular damage. An age-associated decline in antioxidant enzyme activities, if present, could facilitate such tissue damage. For example, Gershon and co-workers reported a more than 50% decline in SOD activities in the liver during aging and proposed that this decline is due to an alteration of the SOD enzyme molecule, with lowered enzyme activity but with unaltered antigenicity.[7,16] More recently, Richardson and his group reported a decline in CAT activity in the liver during aging.[2,3] They also showed a decline in mRNA levels for

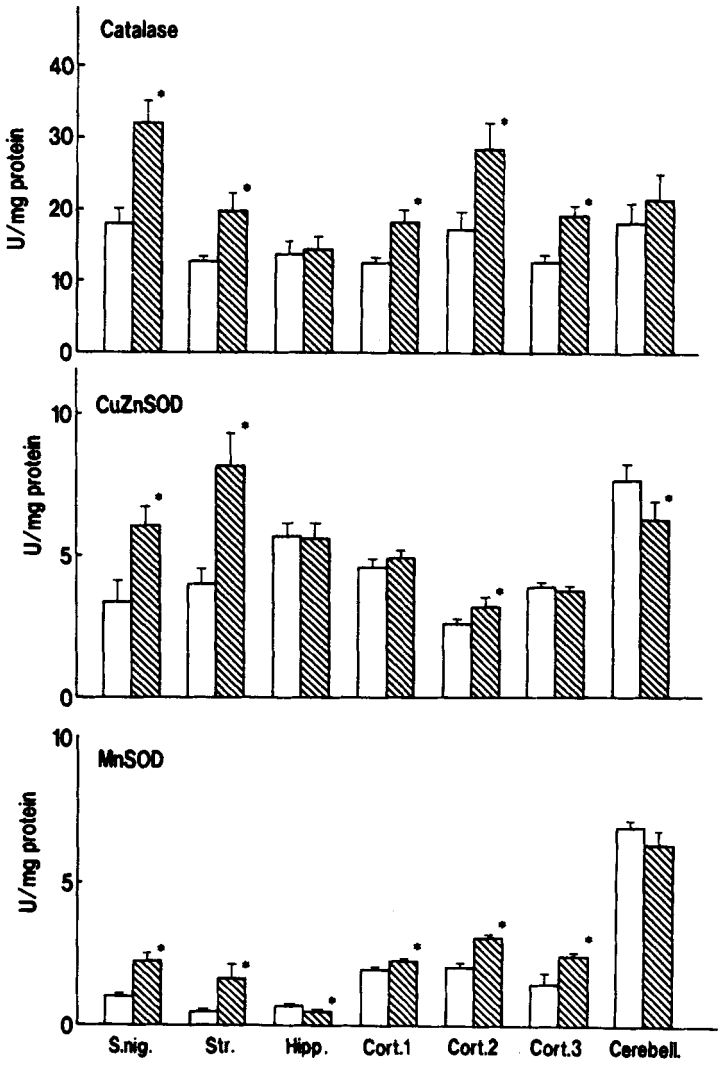


FIGURE 3. Catalase (*top panel*) and superoxide dismutase (*lower two panels*) enzyme activities in young control (*white columns*) and deprenyl-treated (*shaded columns*) young male rats. The dose of deprenyl is 2.0 mg/kg/day, s.c. continuous infusion for 3 wks. (From Carrillo *et al.*[13] Reprinted by permission from *Life Sciences.*)

CAT and SOD and attributed the age-dependent decline in CAT and SOD activities in the liver to the decrease in their mRA levels.[2,3]

As demonstrated previously by us[12] and others,[17,18] however, SOD activities in the liver barely decline with aging. In fact, Cu, Zn-SOD and Mn-SOD activities were slightly increased with age in male and female rats, respectively, in our hands.[12]

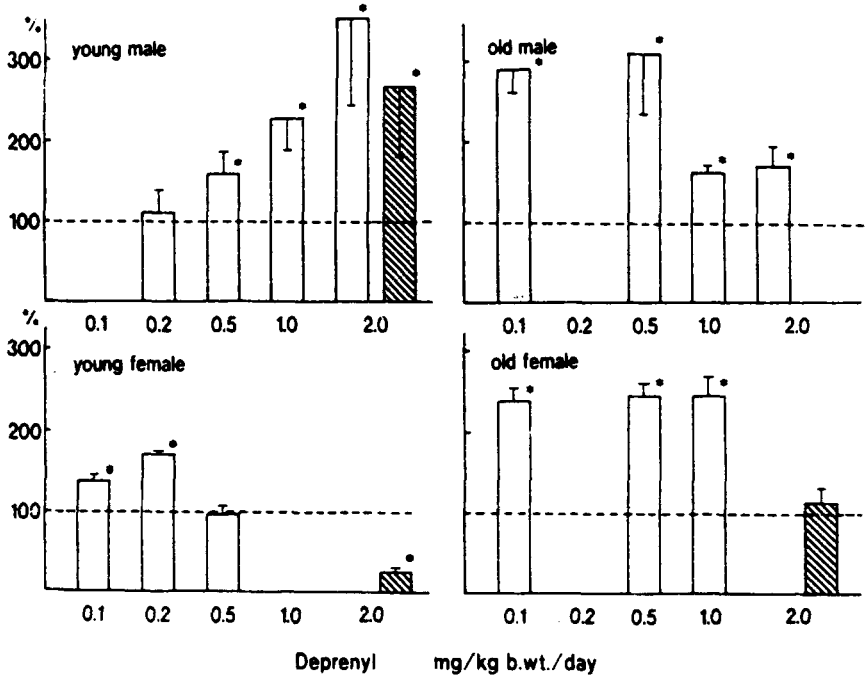


FIGURE 4. Relative enzyme activities of Mn-SOD in striata from young and old rats of both sexes treated with different doses of deprenyl. All values are expressed as percentages of respective control values. *White columns* indicate values in rats given 21 day s.c. infusion, and *shaded columns* represent values in rats given 21 day s.c. injection. * Significantly different from respective control values ($p < 0.05$). The number of rats studied in each group is 3-7 (mostly 4-5). (From Carrillo *et al.*[14] Reprinted by permission from *Life Sciences.*)

The discrepancy between the study of Gershon's group[7,16] and that of ours remains unresolved. However, many studies in the past reported unaltered (*e.g.*, Ref. 17) or even increased enzyme activities with age (*e.g.*, Ref. 18), although some others reported a decline. Regarding the CAT activity, our own study[12] has also shown a definite decline in enzyme activity in male rat livers as did studies by Richardson's group[2,3] who used exclusively male rats. However, we found that CAT activity showed a definite increase with age in female rat livers.[12] Similarly, Rikans *et al.*[17] also clearly showed that CAT activity decreased with age in male but increased in female rat livers. From these results, it is most likely that the age-associated decline in CAT activity in the liver is a phenomenon specific to male rats and cannot be generalized even to female rats.

As is shown clearly in brain regions examined in the present study, there is no evidence that these enzyme activities decline with age in the brain. Rather, in some brain regions such as hippocampus, striatum and S. nigra, Mn-SOD showed a 3-5-fold increase with age in male rats. A recent study by Williams *et al.*[19] showed a significant increase in Mn-SOD mRNA levels in the same brain regions with aging

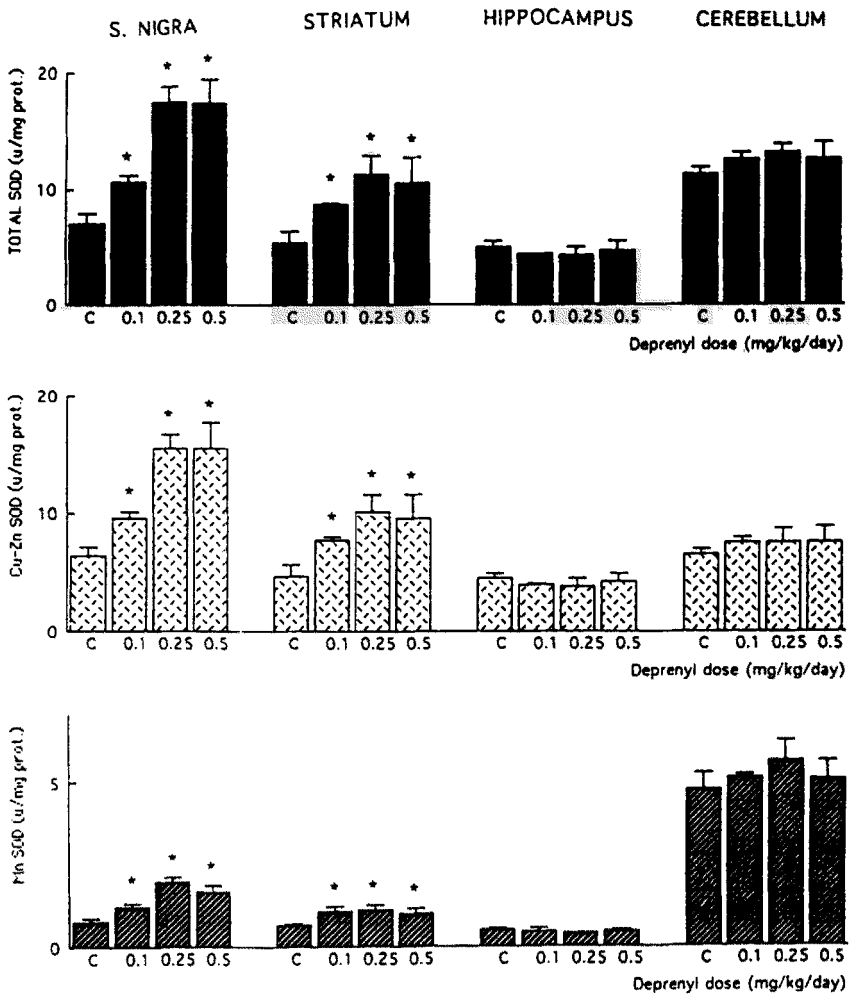
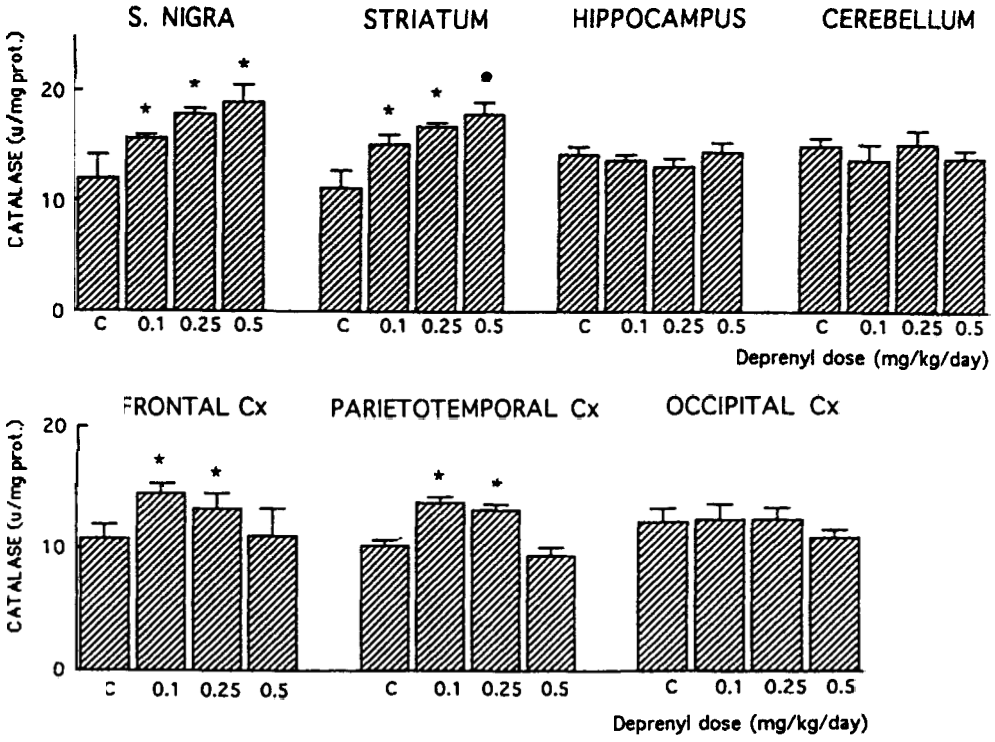


FIGURE 5. Superoxide dismutase activities in striatum of control and deprenyl-treated old female rats. Deprenyl was injected s.c. 3 times a week for 6 months. Doses indicated in the figure are doses per injection. * Significantly different from corresponding control values. (From Carrillo *et al.*[15] Reprinted by permission from *Biochemical Pharmacology.*)

in F-344 male rats as we have shown for Mn-SOD activities. They discussed these changes in relation to age-associated degenerative diseases such as Alzheimer's disease and Parkinson's disease, as in these disorders, these brain regions are specifically affected. However, our results in female rats showing almost identical enzyme levels between young and old rat brains cast doubt on such a simplification of the theory. At least, Williams and his group must confirm that Mn-SOD mRNA levels are also elevated during aging in female rat brains.



Catalase activities in different brain regions of control and deprenyl-treated old female rats. Deprenyl was injected s.c. 3 ti
. Doses indicated in the figure are doses per injection. * Significantly different from corresponding control values. (From Car
permission from *Biochemical Pharmacology.*)

Taken together, we believe that our results have provided enough evidence that antioxidant enzyme activities do not decline with aging in general. It is the opinion of the authors that any theory of aging should not be based on an assumption that antioxidant enzyme activities decline with aging.

Upregulation of SOD and CAT Activities

As is clear in our results, deprenyl definitely increases activities of SOD and CAT (but not of GSH Px) selectively in some brain regions. We found a definitive increase in activities of both species of SOD enzymes and that of CAT in striatum and S. nigra, while in hippocampus (and cerebellum) we have never seen an increase.[6,12-14]

Interestingly, in cerebral cortices, we demonstrated some (significant) increase in enzyme activities but it was not so striking as we had seen in striatum and S. nigra. In this regard, we recently found that deprenyl treatment can increase SOD and CAT activities in the same selective brain regions in mice[20] (FIG. 7) as we have shown here in rats. Further, SOD activities were also elevated by deprenyl in striatum but not in hippocampus of dog brains[21] (FIG. 8). Thus, this aspect of deprenyl's pharmacology appears to be reproduced in a variety of animal species, possibly including humans, if an appropriate dose is selected.

Although our results on deprenyl's pharmacology generally agree with those reported by Knoll, several uncertainties remain to be settled. 1. Knoll showed that the CAT activity increase was not statistically significant,[22] while our study showed a definite (and significant) increase in CAT activity by deprenyl.[5] 2. Knoll[22] showed an increase in GSH Px activities by deprenyl, although the increase did not attain a statistical significance. Our studies clearly revealed that deprenyl has no influence on GSH Px activities. 3. Knoll[23] showed data in a more recent study in another rat strain that he could not reproduce the increases in SOD activities with the same deprenyl dose as was used in the initial study in a Logan-Wistar cross of rat.[22] Based on our data presented here, as well as that reported previously by ourselves,[6,14] it is highly likely that Knoll's inability to reproduce their original data to increase SOD activities[23] was due to differences in the metabolism of deprenyl to inactive metabolites between different rat strains. Strain as well as sex differences in deprenyl's metabolism were clearly demonstrated in a previous study.[24] Furthermore, our recent findings that long-term treatment with deprenyl actually decreases the optimal dose of the drug by a factor of at least 5 in terms of upregulation of antioxidant enzymes (FIGS. 4-6), preclude any simple experiments on life span extension using an arbitrarily chosen dosage. Whether or not the upregulation of SOD and CAT by deprenyl may actually cause the life span extension reported for this drug will be discussed below.

Life Span Prolongation by Deprenyl

Our previous study which showed a significant increase in the remaining life expectancy of rats treated with deprenyl[11] agrees with the previous two studies in male rats which both showed that deprenyl treatment caused a significant increase in life expectancy after 24 months of age.[22,25] However, quantitatively, there are large differences among the three studies. The initial study by Knoll[22] showed a greater

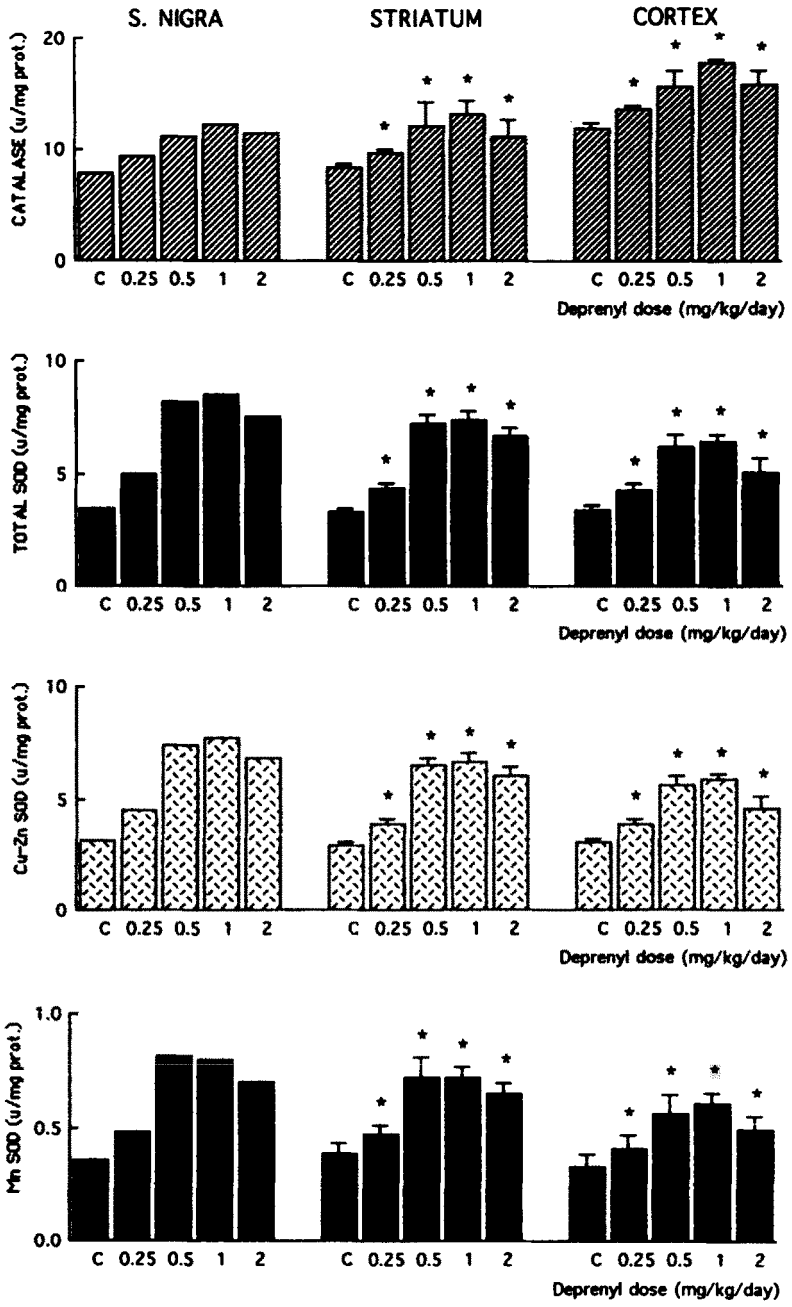
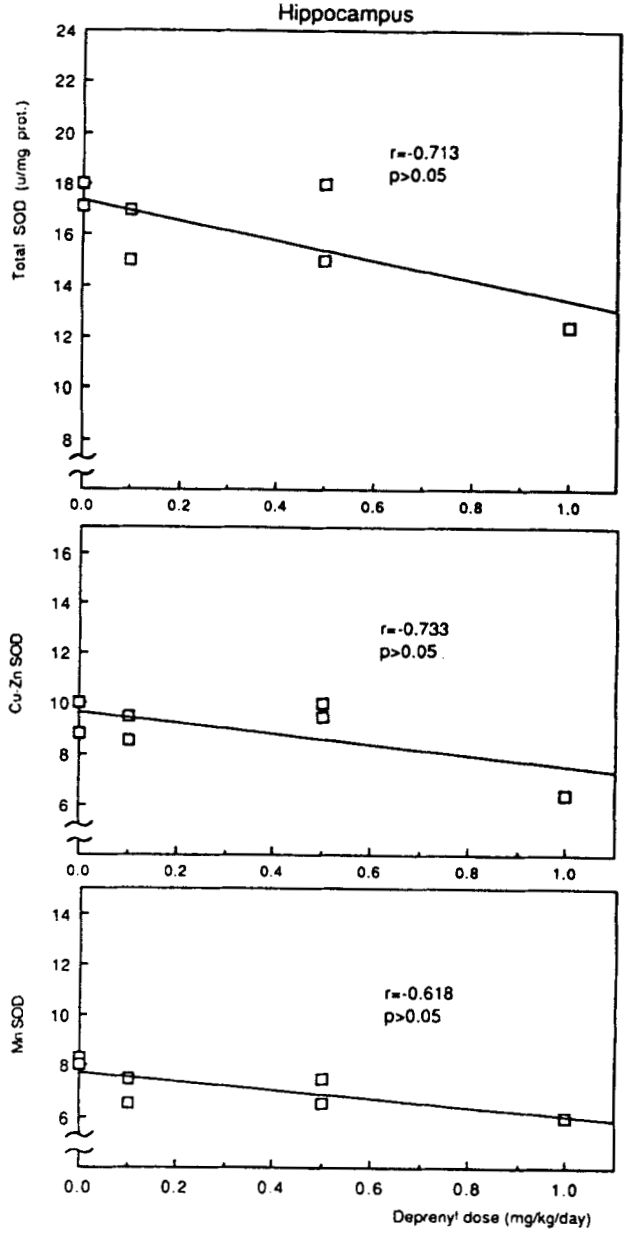


FIGURE 7. The effect of deprenyl on brain regions in old male C57BL/J6S1c mice. Deprenyl was continuously infused s.c. for 3 wks. * Significantly different from corresponding control values. (From Carrillo *et al.*[20] Reprinted by permission from *Life Sciences.*)


Hippocampus
Total SOD (u/mg prot.)
r=-0.713
p>0.05
Cu-Zn SOD
r=-0.733
p>0.05
Mn SOD
r=-0.618
p>0.05
Deprenyl dose (mg/kg/day)

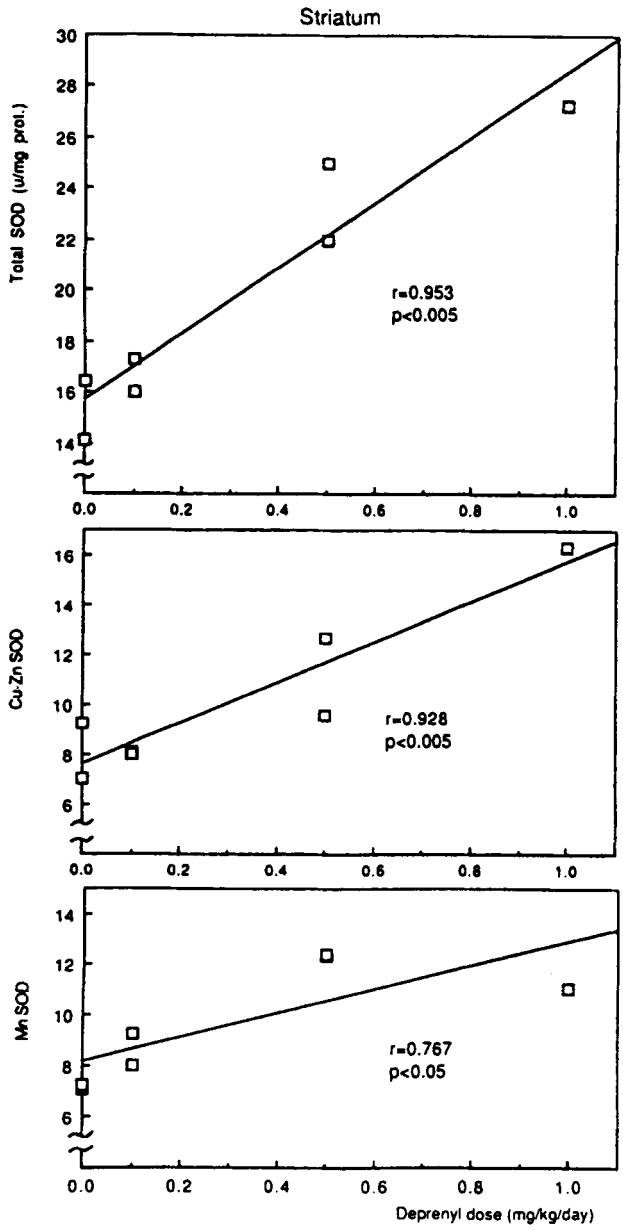


FIGURE 8. The effect of deprenyl administration on SOD enzyme activities in different brain regions in female beagle dogs. Deprenyl was administered orally by means of capsules for 3 consecutive weeks. (From Carrillo *et al.*[21] Reprinted by permission from *Life Sciences.*)

than 100% increase in life expectancy after 24 months, when the deprenyl treatment was started, while the second study from Canada on F-344 male rats showed only a marginal (16%) increase after the treatment was started at the age of 24 months[25] in male F-344 rats. A 34% increase in the life expectancy after 24 months observed in our study[11] is twofold higher than the value obtained previously by the Canadian study[24] but still far lower compared with results reported by Knoll.[22] Although we have no direct evidence that the upregulation of CAT and SOD by deprenyl caused an extension of average life expectancy in old male rats, a proper choice of the dosage in long-term experiments appears to be the key factor. While we have not done a long-term study for seeking an optimal dose for enzyme activities in old male rats, our old female rat studies have shown that the optimal dose will decrease by at least a factor of 5 (or 10) compared with the optimal dose in a relatively short-term study of 3 wks (FIG. 4 vs. FIGS. 5, 6). From the foregoing, it is possible that the optimal dosage for increasing SOD and CAT activities in a long-term study for old male rats may be 0.25 mg/kg/day (3 times a week) or smaller. Unfortunately, since these observations in old female rats came out much later than our second life span studies in male rats already in progress, we had unfortunately increased the dose up to 1.0 mg/kg/day (3 times a week) with the hope that this dose could be more effective in terms of life span extension.

Contrary to our expectation, deprenyl-treated animals lived shorter lives than control animals. After 13 months of treatment, we had sacrificed some animals and had found that there was no difference in SOD or in CAT activities between control and deprenyl-treated animals.[26] Since we already knew from short-term studies that excessive doses are less effective in increasing enzyme activities or that they may even reduce the activities,[6] we concluded that 1.0 mg/kg/day (3 times a week) could have been an excessive dose, such that enzyme activity upregulation and possibly also life span extension were either unaffected or even adversely affected. Our subsequent works in progress using 0.25 mg/kg/day (3 times a week) in both male and female rats appear to suggest that this dosage is closer to an optimal dosage, at least for life span extension.

Another recent study in male mice has shown that a long-term treatment (3 months) not only reduced the optimal dose, as we have observed in female rats, but reduced the effective dose range and, more importantly, the magnitude of increase in the SOD and CAT activities (Carrillo *et al.*, unpublished observations). From our study in C57BL male mice, the dose of 0.5 mg/kg/day (3 times a week for 3 months) was optimal for increasing enzyme activities, while the lower dose of 0.25 mg/kg/day was clearly less effective. Interestingly, in our preliminary studies using BDF_1 male mice, the 0.5 mg/kg/day dose increased the remaining life expectancy to some extent but the difference was not statistically significant (Kitani *et al.*, unpublished observations). Further, with a 0.25 mg/kg/day dose, the average life expectancy was almost identical for control and deprenyl-treated mice (TABLE 1). There are at least two published studies on the effect of deprenyl on the life span of mice which suggest that the effect was not so clear as we found in rats.[27,28] Considering all of this information together, it appears that a parallelism exists between the effects of deprenyl on enzyme activities and on life span in rodents.

In contrast with doses used for enzyme activities and life span studies, doses recently reported by other investigators for a direct radical scavenging effect or

TABLE 1. Summary of Life Span and Antioxidant Enzyme Activity Studies by Means of Long-Term Treatments with Deprenyl

Animals (Doses)	Life Span	Antioxidant Enzymes
Male F-344 (0.5 mg/kg, 3× a week)	+34% after 24 months (18 months ----)	not determined
Male F-344 (1.0 mg/kg, 3× a week)	no significant effect (18 months-31 months)	no significant effect (18 months-31 months)
Female F-344 (0.25 mg/kg, 3× a week)	significant effect (18 months -- in progress)	significant effect (22 months-28 months)
Male mice (0.5 mg/kg, 3× a week)	+10% (not significant) (18 months ----)	significant (26 months-29 months)
Male mice (0.25 mg/kg, 3× a week)	+2.5% (not significant) (18 months ----)	significant but much lower than values in mice given 0.5 mg/kg dose (26 months-29 months)

neuroprotective effect of the drug[29] are at least one magnitude lower. At the same time, these effects appear to affect damaged tissues very quickly, while deprenyl's effect on antioxidant enzyme activities requires at least 2-3 wks to be optimal.[30]

Although we have no direct evidence, the results discussed above are compatible with the original hypothesis raised by Knoll, that the life span extension by deprenyl is causally related to the upregulation of antioxidant enzyme activities.[22] A recent preliminary study showing that a significant life span extension was observed in old dogs treated by an oral administration of the drug[31] appears to be promising for the extrapolation of the data to humans. However, studies by ourselves and others in mice should make us all very cautious about simplifying any interpretation of the data or generalizing about the effects of the drug.

Further, our own data in rats and mice regarding upregulation of enzymes suggest that the selection of a proper dose and the timing of the start of deprenyl treatment may also be critical factors in prolonging the life span of animals including humans, if these two effects are really causally interrelated with each other. Thus, we need more time and work before we can confirm the practical usefulness of this drug in terms of human health and longevity. However, in order to advance our knowledge, as well as theories, regarding any apparent regulatory factors for the life span of animals, deprenyl appears to be a very useful tool. Clearly, much more research is needed to clarify the many uncertainties discussed above; however, these studies clearly appear to be worthy of continuation.

Physiological Implications of Upregulation of Antioxidant Enzyme Activities

Since antioxidant enzymes are all endogenously supplied machineries to defend organisms and organelles against oxidative stresses, it is generally thought that upreg-

ulations of these enzymes may be beneficial for the body. Accordingly, despite ample evidence that antioxidant enzyme activities do not generally decline with aging, it is still possible that an upregulation of these enzyme activities may counteract with age-associated alterations of cellular and organ functions.

Recent attempts to elevate Cu, Zn-SOD activities in transgenic rodents,[32-34] however, have given a general warning to us that a simple elevation of SOD activity may be hazardous rather than beneficial, since these transgenic animals demonstrated some similarities with human Down's syndrome, where SOD activities are known to be higher than in normal individuals. On the other hand, an elevation in Mn-SOD (but not Cu, Zn-SOD) mRNA levels caused by tumor neurosis factor (TNF)[35] has been shown to be effective in preventing oxidative stresses.[36,37]

Perhaps, the most exciting observation in terms of upregulation of antioxidant enzyme activities in relation to life span is the recent study of Orr and Sohal[38] that if proper modulations of both SOD and CAT genes are made, the life span can be significantly prolonged at least in *Drosophila.* If this observation is confirmed by a series of back-up studies in the future, the results would provide a very solid scientific basis for believing that it could happen in mammals. We have a long way to go in terms of gene modulation of life span in humans, both technically as well as ethically. However, as a number of individuals around the world are currently voluntarily taking low doses of deprenyl, a totally unplanned study to support or refute our hypothesis may already be underway.

SUMMARY

In order to elucidate the exact role of antioxidant enzyme activities such as superoxide dismutase (SOD) in the aging process of animals, we compared various enzyme activities in different brain regions and in the liver of young (6-8 mo) and old (28-30 mo) Fischer-344 (F-344) rats. While Mn-SOD activities were elevated 3-5-fold in specific brain regions such as hippocampus, striatum and substantia nigra in brains of old male rats compared with the young, in females both forms of SOD (Cu, Zn- and M*n*-) enzyme activities remained essentially unchanged with aging. Continued subcutaneous infusion of deprenyl for 3 weeks caused a 2-3-fold increase in activities of both Cu Zn- and Mn-SOD and a 50-60% increase in CAT activities in striatum and substantia nigra but not in hippocampus, cerebellum or the liver. Further, long-term treatment of old male rats with deprenyl caused a significant increase in the remaining life expectancy from 24 months of age by 34%.

In conclusion, activities of antioxidant enzymes in these regions examined do not show any uniform age-associated change, suggesting that changes in these enzyme activities do not have any specific role in the life span of rodents in general terms. In contrast, the results of our deprenyl study suggests the possibility that the protection of catecholaminergic neurons by an upregulation of SOD and CAT activities plays a significant role in the life span of animals.

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REERENCES

1. REISS, U. & D. GERSHON. 1976. Comparison of cytoplasmic superoxide dismutase in liver, heart and brain of aging rats and mice. Biochem. Biophys. Res. Commun. **73:** 255-261.
2. SEMSEI, I., G. RAO & A. RICHARDSON. 1989. Changes in the expression of superoxide dismutase and catalase as a function of age and dietary restriction. Biochem. Biophys. Res. Commun. **164:** 620-625.
3. RAO, G., E. XIA & A. RICHARDSON. 1990. Effect of age on the expression of antioxidant enzymes in male Fischer-344 rats. Mech. Ageing Dev. **53:** 49-60.
4. NOKUBO, M. 1985. Physical-chemical and biochemical differences in liver plasma membranes in aging F-344 rats. J. Gerontol. **40:** 409-414.
5. CARRILLO, M. C., S. KANAI, M. NOKUBO & K. KITANI. 1991. (-)Deprenyl induces activities of both superoxide dismutase and catalase but not of glutathione peroxidase in the striatum of young male rats. Life Sci. **48:** 517-521.
6. CARRILLO, M. C., S. KANAI, M. NOKUBO, G. O. IVY, Y. SATO & K. KITANI. 1992. (-)Deprenyl increases activities of superoxide dismutase and catalase in striatum but not in hippocampus: the sex and age-related differences in the optimal dose in the rat. Exp. Neurol. **116:** 286-294.
7. ELSTNER, E. F. & A. HEUPEL. 1976. Inhibition of nitrite formation from hydroxylammoniumchloride: a simple assay for superoxide dismutase. Anal. Biochem. **70:** 616-620.
8. BEERS, R. F. JR. & I. W. SIZER. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **195:** 133-140.
9. PAGLIA, D. E. & W. N. VALENTINE. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. **70:** 158-169.
10. LOWRY, O. H., M. J. ROSENBROUGH, A. L. FARR & R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265-275.
11. KITANI, K., S. KANAI, Y. SATO, M. OHTA, G. O. IVY & M. C. CARRILLO. 1993. Chronic treatment of (-)deprenyl prolongs the life span of male Fischer 344 rats: further evidence. Life Sci. **52:** 281-288.
12. CARRILLO, M. C., S. KANAI, Y. SATO & K. KITANI. 1992. Age-related changes in antioxidant enzyme activities are region and organ selective as well as sex in the rat. Mech. Ageing Dev. **65:** 187-198.
13. CARRILLO, M. C., K. KITANI, S. KANAI, Y. SATO & G. O. IVY. 1992. The ability of (-)deprenyl to increase superoxide dismutase activities in the rat is tissue and brain region selective. Life Sci. **50:** 1985-1992.
14. CARRILLO, M. C., S. KANAI, Y. SATO, M. NOKUBO, G. O. IVY & K. KITANI. 1993. The optimal dosage of (-)deprenyl for increasing superoxide dismutase activities in several brain regions decreases with age in male Fischer 344 rats. Life Sci. **52:** 1925-1934.
15. CARRILLO, M. C., K. KITANI, S. KANAI, Y. SATO, K. MIYASAKA & G. O. IVY. 1994. 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. Biochem. Pharmacol. **47:** 1333-1338.
16. REISS, U. & D. GERSHON. 1978. Mathionine sulfoxide reductase: a novel protective enzyme in liver and its potentially significant role in aging. *In* Liver and Aging—1978 K. Kitani, Ed. 55-61. Elsevier/North-Holland Biomedical Press. Amsterdam, New York, Oxford.
17. RIKANS, L. E., D. R. MOORE & C. D. SNOWDEN. 1991. Sex-dependent differences in the effects of aging on antioxidant defense mechanisms of rat liver. Biochim. Biophys. Acta **1074:** 195-200.

18. RIKANS, L. E., C. D. SNOWDEN & D. R. MOORE. 1992. Effect of aging on enzymatic antioxidant defenses in rat liver mitochondria. Gerontology **38:** 133-138.
19. WILLIAMS, L. R., D. B. CARTER, E. DUNN & J. R. CONNER. 1995. Indicators of oxidative stress in aged Fischer 344 rats: potential for neurotrophic treatment. *In* Alzheimer's and Parkinson's Diseases: Recent Advances. I. Hannin, A. Fischer & M. Yoshida, Eds. 641-646. Plenum Pub. Corp. New York.
20. CARRILLO, M. C., K. KITANI, S. KANAI, Y. SATO, K. MIYASAKA & G. O. IVY. 1994. (-)Deprenyl increases activities of superoxide dismutase and catalase in certain brain regions in old male mice. Life Sci. **54:** 975-981.
21. CARRILLO, M. C., N. W. MILGRAM, P. WU, G. O. IVY & K. KITANI. 1994. (-)Deprenyl increases activities of superoxide dismutase (SOD) in striatum of dog brain. Life Sci. **54:** 1483-1489.
22. KNOLL, J. 1988. The straital dopamine dependency of life span in male rats: longevity study with (-)deprenyl. Mech. Ageing Dev. **46:** 237-262.
23. KNOLL, J. 1990. Advances in Neurology. Vol. 53. Parkinson's Disease: Anatomy, Pathology and Therapy. 425-429. Raven Press. New York.
24. YOSHIDA, T., T. OGURO & Y. KUROIWA. 1987. Hepatic and extrahepatic metabolism of deprenyl, a selective monoamine oxidase (MAO) B inhibitor of amphetamines in rats: sex and strain differences. Xenobiotica **8:** 957-963.
25. MILGRAM, N. W., R. J. RACINE, P. NELLIS, A. MENDONCA & G. O. IVY. 1990. Maintenance of L-deprenyl prolongs life in aged male rats. Life Sci. **47:** 415-420.
26. KITANI, K., M. C. CARRILLO, S. KANAI, Y. SATO & G. O. IVY. 1993. Chronic treatment of (-)deprenyl abolishes the dose effect on the superoxide dismutase (SOD) and catalase (CAT) in striatum in the rat. The XVth Congress of the International Association of Gerontology. Abstract p. 164.
27. INGRAM, D. K., H. L. WIENER, M. E. CHACHICH, J. M. LONG, J. HENGEMIHLE & M. GUPTA. 1993. Chronic treatment of aged mice with L-deprenyl produces marked MAO-B inhibition but no beneficial effects on survival, motor performance, or nigral lipofuscin accumulation. Neurobiol Aging. **14:** 431-440.
28. PIANTANELLI, L., A. ZAIA, G. ROSSOLINI, C. VITIECHI, R. TESTA, A. BASSO & A. ANTOGNINI. 1994. Influence of L-deprenyl treatment on mouse survival kinetics. *In* Pharmacology of Aging Processes. Method of Assessment and Potential Interventions. I. Zs.-Nagy, D. Harman & K. Kitani, Eds. Ann. N. Y. Acad. Sci. Vol. 717: 72-78.
29. WU, R. M., C. C. CHIUEH, A. PERT & D. L. MURPHY. 1993. Apparent antioxidant effect of L-deprenyl on hydroxyl radical formation and nigral injury elicited by MPP^+ *in vivo*. Eur. J. Pharmacol. **243:** 241-247.
30. CARRILLO, M. C., S. KANAI, Y. SATO, G. O. IVY & K. KITANI. 1992. Sequential changes in activities of superoxide dismutase and catalase in brain regions and liver during (-)deprenyl infusion regions and liver during (-)deprenyl infusion in male rats. Biochem. Pharmacol. **44:** 2185-2189.
31. RUEHL, W. W., D. BICE, B. MUGGENBURG, D. D. BRUYETTE & D. R. STEVENS. 1994. *l*-Deprenyl and canine longevity: Evidence for an immune mechanism, and implications for human aging. 2nd Conference on Anti-Aging Medicine, Las Vegas. Abstract, p. 25-26.
32. ELROY-STEIN, O. & Y. GRONER. 1988. Impaired neurotransmitter uptake in PC12 cells overexpressing human Cu/Zn-superoxide dismutase—implication for gene dosage effects in Down syndrome. Cell **52:** 259-267.
33. AVRAHAM, K. B., M. SCHICKLER, D. SAPAZNIKOV, R. YARON & Y. GRONER. 1988. Down's syndrome: abnormal neuromuscular junction in tongue of transgenic mice with elevated levels of human Cu/Zn-superoxide dismutase. Cell **51:** 823-829.
34. EPSTEIN, C. J., K. B. AVRAHAM, M. COVETT, S. SMITH, O. ELORY-STEIN, G. ROTMAN, C. BRY & Y. GRONER. 1987. Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in Down syndrome. Proc. Natl. Acad. Sci. USA **84:** 8044-8048.

35. WONG, G. H. W. & D. V. GOEDDEL. 1988. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science **242:** 941-944.
36. EDDY, L. J., D. V. GOEDDEL & G. H. W. WONG. 1992. Tumor necrosis factor-α pretreatment is protective in a rat model of myocardial ischemia-reperfusion injury. Biochem. Biophys. Res. Commun. **184:** 1056-1059.
37. TSAN, M. F., J. E. WHITE & G. H. W. WONG. 1992. D-factor and growth hormone enhance tumor necrosis factor-induced increase of Mn superoxide dismutase mRNA and oxygen tolerance. Cytokine **4:** 101-105.
38. ORR, W. C. & R. S. SOHAL. 1994. Extension of life span by overexpression of superoxide dismutase and catalase in *Droshophila melanogaster.* Science **263:** 1128-1130.