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# (−)-Deprenyl treatment restores serum insulin-like growth factor-I (IGF-I) levels in aged rats to young rat level

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**Abstract**

We studied the effects of treatment with (−)-deprenyl, a monoamine oxidase B inhibitor, on plasma levels of insulin-like growth factor-I (IGF-I) (as indicator of growth hormone (GH) secretion), levels of monoamines and their metabolites, and the activity and content of tyrosine hydroxylase – the rate-limiting enzyme in the biosynthesis of catecholamines – in the hypothalamus and hypophysis of old male rats. Male Wistar rats (22 months old) were treated with 2 mg deprenyl/kg body weight s.c. three times a week for 2 months. At the end of the treatment period, blood was collected for measurement of plasma IGF-I levels by radioimmunoassay (RIA). The concentrations of dopamine, serotonin (5-HT) and their main metabolites were determined by high performance liquid chromatography (HPLC) with electrochemical detection, and the tyrosine hydroxylase content in hypothalamus and hypophysis was determined by enzyme-linked immunoabsorbent assay (ELISA). (−)-Deprenyl treatment produced a pronounced increase in dopamine and 5-HT in both the hypothalamus and hypophysis ($P < 0.01$). The main dopaminergic metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), decreased in hypothalamus but not in hypophysis, and treatment had no effect on the concentration of 5-hydroxyindole-3-acetic acid (5-HIAA). The tyrosine hydroxylase activity and tyrosine hydroxylase content increased in hypothalamus and hypophysis ($P < 0.05$). In the hypophysis the increase in tyrosine hydroxylase activity was consistent with the increase in tyrosine hydroxylase amount. Moreover, (−)-deprenyl treatment restored the IGF-I plasma levels in old rats to a concentration similar to those found in young animals. Postulated anti-aging effects of (−)-deprenyl could hence be due to restoration of hypothalamic hormones such as GH.

*Keywords:* Aging; (−)-Deprenyl; IGF-I (insulin-like growth factor-I); Tyrosine hydroxylase; (Rat)

## 1. Introduction

(−)-Deprenyl, an irreversible selective inhibitor of monoamine oxidase B, has been reported to be a safe therapeutic agent for a variety of neurodegenerative diseases and also for aging. Multi-site studies of treatment of Parkinson's patients with (−)-deprenyl indicated that (−)-deprenyl treatment was capable of delaying the onset of disability (The Parkinson Study Group, 1993). (−)-Deprenyl has been reported to block nigrostriatal toxicity induced by some neurotoxic compounds used for animal models of Parkinson's disease: 6-hydroxydopamine in rats (Zsilla et al., 1986), MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) in mice (Tatton and Greenwood, 1991), and 1-methyl-4-phenylpyridinium ion ($MPP^+$) in rats (Vizuete et al., 1993). Furthermore, studies on the beneficial effects of (−)-deprenyl in Alzheimer's disease have been published (Heinonen et al., 1993). In rats, (−)-deprenyl may improve the functions of meso-limbo-cortical dopamine neurons, which are associated with cognitive processes (Brandeis et al., 1991). Many studies of the ability of (−)-deprenyl to protect against the aging process have also been reported upon. (−)-Deprenyl increases the lifespan of some animals (Milgram et al., 1990; Kitani et al., 1993) and slows down the age-related decline of performance in behavioral tests (Knoll, 1993). An increase in the survival of Parkinson's patients treated with (−)-deprenyl has been described (Birkmayer et al., 1985). (−)-Deprenyl treatment also prevents age-related pigment changes in the substantia nigra (Knoll et al., 1992). Recently, we have shown that (−)-deprenyl treatment pro-

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tects the rat substantia nigra against oxidative damage (De la Cruz et al., 1996b). Much of this work has demonstrated the protective effect of (−)-deprenyl in the aging process, probably acting on the monoaminergic system. Catecholaminergic systems are involved in many functions involved in the aging process. The hypothalamic dopaminergic neurons have been reported to participate in the control of gonadotropin secretion (MacKenzie et al., 1988) and prolactin (Ben-Jonathan and Froehlich, 1985) and they also affect the secretion of thyrotropin (TSH), gonadotropin, adrenocorticotropin and growth hormone (GH) from the anterior pituitary (Weiner and Ganong, 1978; Tuomisto and Männistö, 1985). The alteration of these secretions has a significant role in determining the decline in many body functions with age (Meites, 1990). It is known that there is a decline in hypothalamic catecholamine activity in aged rats (Meites et al., 1987; Meites, 1988). The dopamine concentration was significantly lower in the median eminence, medial basal hypothalamus and preoptic area-anterior hypothalamus of aging rats (Simpkins, 1982). The mean concentration of dopamine in the median eminence of old rats was lower than that in young rats (Sarkar et al., 1982). A decrease of noradrenaline concentration, with some signs of neurodegeneration of serotonin (5-HT) fibers, in hypothalamus of aged rats has also been reported (Rodríguez-Gómez et al., 1995). All these results support the idea that the decrease of some important hormones such as GH and TSH, which are known to be reduced in aging (Meites et al., 1987; Meites, 1988), could be due to the decrease in catecholamines in hypothalamic areas. Administration of drugs that increase hypothalamic catecholamine activity has been shown to inhibit or reverse many effects of physical decline due to aging (Quadri et al., 1973). We now studied the effect of chronic treatment with (−)-deprenyl on aminergic systems in the hypothalamic areas and on the insulin-like growth factor-I (IGF-I) blood concentrations. Twenty-two-month-old rats were treated with deprenyl (2 mg/kg) for 2 months. We studied the levels of monoamines and their metabolites and the activity and content of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine and protein marker of dopaminergic neurons. All these measurements were carried out in the hypothalamus and hypophysis of treated and untreated aged animals.

## 2. Materials and methods

### 2.1. Animals, treatment and dissection

Male Wistar rats, 22 months old, were used for this study. They were divided into two groups: control animals ($n = 5$) and experimental animals ($n = 5$). Control animals were given s.c. injections of physiological saline solution three times a week for a period of 2 months. Experimental animals were injected s.c. three times a week for the same period with (−)-deprenyl (Research Biochemicals International, Natick, MA, USA; 2 mg/kg) dissolved in saline solution. We used 3-month-old male Wistar rats for the study of IGF-I plasma levels. They were divided into a control group ($n = 10$) and an experimental group ($n = 10$). Rats were given either s.c. injections of physiological saline solution or (−)-deprenyl (2 mg/kg) daily for 3 weeks. All rats were kept under controlled environmental conditions with food and tap water allowed ad libitum. The day following the last injection, the animals were decapitated between 10 and 11 a.m., and the brains were removed and placed on an ice-cold plate. The entire hypophysis (adenohypophysis and neurohypophysis) was removed from the skull. The diencephalon was separated from the telencephalon and the mesencephalon. The hypothalamus was dissected out from the diencephalon by cutting just under the ventral dorsal thalamus and from the optic chiasm to the mammillary region.

The aged animals used in these experiments were checked for, and found free of, brain tumors (Lamour et al., 1987). After dissection the brain parts were immediately frozen in liquid $N_2$ until assay. Blood samples were also collected. Heparinized plasma was separated by centrifugation at $2000 \times g$ and stored at $-80$°C until assay.

### 2.2. Measurement of neurotransmitters and their metabolites

Analyses were carried out using high performance liquid chromatography (HPLC) with electrochemical detection as described by Castaño et al. (1993).

The brain tissues were homogenized in 0.1 M perchloric acid containing 1 mM sodium bisulfite. Ultrasonic disintegration over ice, using a Labsonic 1510, was used for the purpose. Samples were centrifuged at $12\,000 \times g$ for 15 min at 4°C and the supernatant was then filtered through a 0.2 μm filter and used for HPLC.

The pellet was resuspended in carbonate-bicarbonate buffer pH 9.0. These samples were used to measure the content of proteins and tyrosine hydroxylase. Protein concentration of samples was determined as described by Lowry et al. (1951).

### 2.3. Tyrosine hydroxylase activity assay

Tyrosine hydroxylase activity was measured in vitro as described previously (Reinhard et al., 1986) with some modifications. Briefly, samples were homogenized (5 w/v) in 50 mM Tris buffer containing 0.2 mM dithiothreitol and 8% sucrose. An aliquot was further diluted 1:60 with lysis buffer (30 mM Tris containing 0.1% Triton X-100, pH adjusted to 6.5 with acetic acid) and incubated with 2.5 nmol of tyrosine-HCl (containing 0.4 μCi/nmol of L-[*ring*-3,5-$^3$H]tyrosine), 50 nmol of the cofactor, 6(*R*)-L-

erythro-5,6,7,8-tetrahydrobiopterin, 5000 units of catalase and 5 nM dithiothreitol in 100 mM potassium phosphate, pH 6.0. The released $^3H_2O$ was separated with an aqueous slurry of activated charcoal, and the radioactivity was determined by liquid scintillation counting.

### 2.4. Quantification of tyrosine hydroxylase enzyme

The concentration of tyrosine hydroxylase in hypothalamus and hypophysis was measured in an enzyme-linked immunoabsorbent assay (ELISA), using a monoclonal antibody (Boehringer-Mannheim). This was performed as previously described by De la Cruz et al. (1996a). The antigen concentrations were 250 μg protein/ml. Blanks without the first antibody and negative controls with liver homogenates (at the same concentration) were included in triplicate on each plate. Absorbance was measured at 405 nm with a plate reader (Titertek Multiskan II).

### 2.5. IGF-I plasma concentration analysis

The plasma was acidified with 0.5 M HCl and extracted on octadecasilyl-silica cartridges ($C_{18}$ Sep-Pak, Waters, Milford, MA, USA) to separate IGF-I from binding proteins. The columns were sequentially pretreated, beginning with 5 ml isopropyl alcohol followed by 5 ml methanol, and finishing with 10 ml 4% glacial acetic acid/HPLC $H_2O$ (all solutions were fully aspirated off under vacuum before the addition of samples). An aliquot of 200 μl of acidified plasma was added to the $C_{18}$ Sep-Pak column and incubated for 3 min before it flowed through the column slowly. The columns were washed with 10 ml 4% glacial acetic acid/HPLC $H_2O$. After washing, 2 ml of methanol was added to the column and the eluate was collected. The eluate was dried under nitrogen and reconstituted with 1.5 ml of phosphate buffer.

The IGF-I concentration in extracted plasma was measured in triplicate using a radioimmunoassay kit from Nichols Institute (San Juan Capistrano, CA, USA) as previously described (Underwood and Murphy, 1987). The standard curve was generated using DNA-recombinant IGF-I standards.

Table 2
Tyrosine hydroxylase activity in hypothalamus and hypophysis of aged rats after treatment with (−)-deprenyl

| | Controls | (−)-Deprenyl |
|---|---|---|
| Hypothalamus | 2.897 ± 0.562 | 4.027 ± 0.331 [a] |
| Hypophysis | 0.455 ± 0.145 | 0.745 ± 0.169 [a] |

Tyrosine hydroxylase activity in vitro is expressed as nmol/mg protein/h. The results were obtained as described in Section 2. All data are means ± S.D. from five determinations. Statistical significance: one-way analysis of variance followed by the Scheffé test: [a] $P < 0.05$, (−)-deprenyl compared with controls.

### 2.6. Statistical analysis

The results are expressed as the means ± S.D. The mean differences found were analyzed by one-way analysis of variance (ANOVA) followed by the parametric Scheffé test.

## 3. Results

### 3.1. Effects of (−)-deprenyl treatment on concentrations of neurotransmitters

#### 3.1.1. Hypothalamus

The (−)-deprenyl treatment produced changes in the monoamine content: there was a pronounced increase in dopamine (193%; $P = 0.0003$) and 5-HT (218%; $P = 0.0084$) content in treated animals as compared with controls. The main dopaminergic metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), showed a significant decrease (−58%; $P = 0.0146$). The 5-HT metabolite, 5-hydroxyindole-3-acetic acid (5-HIAA), did not show any statistically significant differences (Table 1).

#### 3.1.2. Hypophysis

In the hypophysis, (−)-deprenyl treatment produced a significant increase in dopamine content (206%; $P = 0.043$) but no significant change in its metabolite, DOPAC. 5-HT also was increased (132%; $P = 0.0014$), while 5-HIAA remained unchanged (Table 1).

Table 1
Levels of neurotransmitters and their metabolites in hypothalamus and hypophysis of aged rats after treatment with (−)-deprenyl

| | Hypothalamus | | Hypophysis | |
|---|---|---|---|---|
| | Controls | (−)-Deprenyl | Controls | (−)-Deprenyl |
| Dopamine | 235 ± 32 | 690 ± 120 [a] | 138 ± 52 | 423 ± 82 [a] |
| DOPAC | 63 ± 18 | 27 ± 10 [a] | 85 ± 35 | 68 ± 11 |
| DOPAC/dopamine | 0.268 | 0.039 | 0.61 | 0.16 |
| 5-HT | 444 ± 96 | 1415 ± 390 [a] | 75 ± 12 | 179 ± 12 [a] |
| 5-HIAA | 474 ± 132 | 266 ± 134 | 142 ± 82 | 123 ± 58 |
| 5-HIAA/5-HT | 1.067 | 0.187 | 1.894 | 0.687 |

The results are expressed as ng/g wet tissue. They are means ± S.D. from five determinations and were obtained as described in Section 2. Statistical significance: one-way analysis of variance followed by the Scheffé test: [a] $P < 0.05$, (−)-deprenyl compared with controls.

Table 3
Immunoassayable tyrosine hydroxylase content in hypothalamus and hypophysis of aged rats after treatment with (−)-deprenyl

| | Absorbance at 405 nm | |
|---|---|---|
| | Controls | (−)-Deprenyl |
| Hypothalamus | 0.069 ± 0.002 | 0.114 ± 0.006 [a] |
| Hypophysis | 0.034 ± 0.006 | 0.055 ± 0.007 [a] |

The results were obtained by measuring absorbance at 405 nm using a monoclonal antibody to tyrosine hydroxylase as described in Section 2. All data are means ± S.D. from five determinations. Statistical significance: one-way analysis of variance followed by the Scheffé test: [a] $P < 0.05$, (−)-deprenyl compared with controls.

### 3.2. Effect of (−)-deprenyl treatment on tyrosine hydroxylase activity

The results are shown in Table 2. Tyrosine hydroxylase activity increased significantly in the hypothalamus and hypophysis after treatment (39%; $P = 0.0288$ and 63%; $P = 0.02$, respectively).

### 3.3. Effect of (−)-deprenyl treatment on tyrosine hydroxylase content

(−)-Deprenyl treatment produced significant increases in tyrosine hydroxylase content in the hypothalamus (63%; $P = 0.000$) (Table 3). In the hypophysis this increase was consistent with the increase in tyrosine hydroxylase activity (61%; $P = 0.0035$).

### 3.4. Effect of (−)-deprenyl treatment on plasma IGF-I levels

GH secretion can be measured indirectly by measuring the plasma concentration of IGF-I, which is produced and released by the liver and other tissues in response to GH (Clemmons and Van Wyk, 1984). There is little diurnal variation in plasma IGF-I concentration, and its measurement is therefore a useful indicator of GH secretion. Plasma IGF-I levels were reduced in old rats (24 months) (−46%; $P = 0.000$) as compared with those in young rats (4 months). The young animals treated with (−)-deprenyl did not show any changes in the plasma IGF-I levels, but the old rats showed a significant increase (53%; $P = 0.000$), and the concentration reached was similar to that found in young animals. The results are shown in Table 4.

Table 4
Levels of IGF-I in serum of rats after treatment with (−)-deprenyl

| | Young | Aged |
|---|---|---|
| Controls | 953 ± 85 | 611 ± 75 [a] |
| (−)-Deprenyl | 886 ± 61 | 935 ± 78 [b] |

The results were obtained as described in Section 2. They are expressed as ng IGF-I/ml. Data are means ± S.D. Young animals ($n = 10$), aged animals ($n = 5$). Statistical significance: one-way analysis of variance followed by the Scheffé test: [a] $P < 0.05$ for comparison of aged and young control animals; [b] $P < 0.05$ for comparison of treated and control aged animals.

## 4. Discussion

(−)-Deprenyl treatment of aged rats produced a significant increase in the biogenic amines (dopamine and 5-HT) in the hypophysis and hypothalamus along with a decrease in the ratios, DOPAC/dopamine and 5-HIAA/5-HT. These results are consistent with other (−)-deprenyl effects reported for other structures (Heinonen et al., 1993; De la Cruz et al., 1996b). The results suggest that, at the concentration used by us (2 mg/kg), (−)-deprenyl blocks the activity of both monoamine oxidase B and monoamine oxidase A, since at the higher concentration of 0.5 mg/kg it produces non-selective inhibition of both forms of monoamine oxidase (Knoll, 1978). The inhibition of monoamine oxidase A by (−)-deprenyl is confirmed by the increase of 5-HT (Oguchi et al., 1985).

(−)-Deprenyl treatment produced an increase in tyrosine hydroxylase activity in hypothalamus and hypophysis, along with an increase in tyrosine hydroxylase amount. These results demonstrate that (−)-deprenyl treatment produced an increase in biogenic amine content and the induction of tyrosine hydroxylase in aged rats, in agreement with another report for a different structure of the central nervous system in aged rats (De la Cruz et al., 1996b) and in young rats (Rodriguez-Gómez et al. (in press)). The tyrosine hydroxylase induction produced by (−)-deprenyl treatment could be a result of the dopamine release-enhancing effect (Knoll, 1983, 1987; Oguchi et al., 1985; Zsilla et al., 1986; Lamensdorf et al., 1996) with, as consequence, depletion of the dopamine pool.

(−)-Deprenyl treatment increased the IGF-I concentrations significantly so that they reached the levels in young rats, but it had no effect on the IGF-I concentration in young animals. As there was no change in the food intake during (−)-deprenyl treatment, our results suggest an increase in GH levels in aged rats treated with (−)-deprenyl. The circulating levels of IGF-I in plasma are GH-dependent (Florini et al., 1985; Marcus et al., 1990; Rudman et al., 1990; DeMellow and Baxter, 1988; Clemmons et al., 1989). Plasma IGF-I level decreases with age (Florini et al., 1981; Donahue et al., 1990; Breese et al., 1991), probably as a secondary effect of the decline in the pulsatile GH secretion that occurs with aging (Sonntag et al., 1980; Prinz et al., 1983). Our suggestion is supported by the report of Takahashi et al. (1987) that treatment with exogenous GH increases plasma IGF-I concentration in aged rats to levels as high as those in young animals, but has no effect on plasma IGF-I concentration in young rats. The increase of IGF-I caused by (−)-deprenyl could be produced by the increase in dopamine concentrations, so that dopamine increases the secretion of GH (Muller, 1987). The increase in catecholamines is involved in other

(−)-deprenyl effects. Thyagarajan et al. (1995) and Mohan Kumar et al. (1994) reported that (−)-deprenyl re-initiates estrous cycles, reduces serum prolactin, and decreases the incidence of mammary and pituitary tumors in old acyclic rats. This relationship could extend to other effects such as the increase in lifespan (Knoll, 1988; Ivy et al., 1989). Certain other drugs that elevate hypothalamic dopamine activity, e.g., chronic administration of 3,4-dihydroxyphenylalanine (L-DOPA) (Cotzias et al., 1974) and lergotrile mesylate (a dopamine receptor agonist) (Clemens et al., 1979), reduced the death rate and increased survival. This effect could also be related with the increase in GH. The increase in life expectancy of mice following chronic treatment with a low dose of GH has been reported (Khansari and Gustad, 1991). GH and IGF-I secretion is reduced with age and some of the physical alterations associated with this reduction can be reversed by the administration of recombinant GH (O'Neill, 1992). GHRH given as twice daily s.c. injections to healthy older men with reduced IGF-I levels produced GH secretory dynamics and IGF-I levels similar to those in young men (Corpas et al., 1992). The transient induction of GH release by levodopa in healthy volunteers was potentiated by (−)-deprenyl (Koulu and Lammintausta, 1983). However, direct induction of GH secretion by (−)-deprenyl has not been reported (Koulu and Lammintausta, 1981). These experiments were carried out in healthy young persons and with low concentrations of (−)-deprenyl (Koulu et al., 1989). Taken together, the results suggest that the postulated anti-aging effects of (−)-deprenyl could be due to restoration of hypothalamic hormones such as GH.

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