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Behavioral and Neurochemical Effects Induced by Subchronic *l*-Deprenyl Administration

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MOLINENGO, L. AND P. GHI. *Behavioral and neurochemical effects induced by subchronic* l-*deprenyl administration*. PHARMACOL BIOCHEM BEHAV **58**(3) 649–655, 1997.—(−)Deprenyl was administered orally to rats for 15 days. In the staircase maze, a reduction of incorrect responses was observed at 0.9 mg/kg/day; higher or lower doses (3.5 or 0.35 mg/kg/day) were ineffective. In the same range of doses, the subchronic administration of (−)deprenyl did not modify the levels of norepinephrine, 5-hydroxytryptamine, 5-hydroxyindolacetic acid or the density and affinity of alpha-noradrenergic receptors in the cortex, olfactory system, hippocampus and striatum. An increase of the dopamine and a reduction of dihydroxyphenylacetic acid levels was observed only at the highest tested doses, at which no behavioral modification was observed. Only at 1.0 mg/kg/day did (−)deprenyl increase the acetylcholine (ACh) levels in the olfactory system, hippocampus and striatum. This neurochemical effect may be correlated to the behavioral effect observed in the same range of doses. We propose that this increase of ACh levels is determined by an activation of dopaminergic systems, resulting from the increase in the levels of PE caused by the inhibition of monoamine oxidase B (MAO-B) by (−)deprenyl. © 1997 Elsevier Science Inc.

Deprenyl Memory retention Staircase maze Neurochemical effects

THE INCREASE in dopaminergic activity consequent to the inhibition of monoamine oxidase B (MAO-B) activity by (−)deprenyl is often considered to be the neurochemical mechanism of the improvement in performance on cognitive tasks caused by (−)deprenyl in aged rats (1) and in individuals affected by Alzheimer dementia (17,24,26).

Certainly (−)deprenyl inhibits MAO-B, but it also displays additional pharmacological actions in the brain, including a reuptake blocking effect of dopamine (DA) and an antagonistic action on DA receptors (13,14). Furthermore, (−)deprenyl undergoes a transformation to *l*-methamphetamine and *l*-amphetamine (6,11), and the inhibition of MAO-B can potentiate dopaminergic responsivity through the indirect mechanism of an elevation of endogenous 2-phenylethylamine (22,23).

The aim of the present research was to examine whether to find a correlation exists between the neurochemical and the behavioral effects caused by a chronic administration of (−)deprenyl.

The staircase maze test (19–21) was used to evaluate the behavioral modifications; in the same experimental conditions, we performed neurochemical measures to evaluate whether modifications in the dopaminergic, noradrenergic, serotonergic and cholinergic systems are correlated to the behavioral effects caused by (−)deprenyl.

METHODS

Chemicals

R(−)-deprenyl HCl (RBI Research Biochemicals International) was purchased from Amersham Italia Srl (Milano, Italy). ^{3}H-QNB (39 Ci/mM) and ^{3}H-prazosin (85 Ci/mM) were purchased from Amersham Italia Srl. Noradrenaline bitartrate salt, acetylcholine (ACh) chloride, 5-hydroxytryptamine (5-HT), DA hydrochloride, dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA) were all purchased from Sigma Chimica-Aldrich Srl (Milano, Italy).

The other reagents were of analytic grade.

Subjects and Pharmacological Treatment

Male albino rats (Morini, Wistar-derived strain), weighing 200–250 g at the beginning of the experiments, were used. They were housed 5 to a cage and fed ad libitum with a stan-

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dard rodent diet, except where otherwise stated, with free access to tap water.

(−)Deprenyl was administered dissolved in the water the rats drank at the following doses: 3 mg/l, 8 mg/l and 30 mg/l. The treatment with *l*-deprenyl lasted always 15 days. Based on the mean fluid consumed (30–35 ml/day/rat) and the weight of the rats (250–300 g), the doses of (−)deprenyl assumed by the rats were estimated. The real doses found for the different groups of rats are given in the following paragraphs.

The rats selected for the neurochemical studies were transported to the laboratory and handled daily for 20 days (5 days before and 15 days during (−)deprenyl administration). They were deprived of food in the same way and for the same length of time as the animals used in the behavior experiments and were killed 24 h after the final administration of (−)deprenyl.

Regions of the Central Nervous System (CNS) Examined

The following regions were selected: (a) frontal and parietal cortex (cortex); (b) olfactory bulb, plus the cortex piriformis and tuberculum olfactorium (olfactory system); (c) hippocampus; and (d) striatum.

The Staircase Maze

The staircase maze, described in previous publications (19–21), was used; it consisted of a 13-step staircase with a 17-cm-long corridor in the vertical wall. Thirty rats were used. They were fasted from 6 p.m. to 12 a.m. and trained every morning to find food pellets (45 mg; Campden Instrument Ltd.) in the corridors corresponding to steps 3, 6, 9 and 12. After 2 months of preliminary training, all rats ran very quickly onto the staircase and only stopped for 1–2 s at the four reinforced steps. Once training was complete, a trial without reinforcement was performed.

In this trial (pretest trial), a search for food on steps 3, 6, 9 and 12 was considered to be a correct response, and a search for food on any other steps was considered to be an incorrect response. The daily training was then interrupted for 20 days and a new trial, without reinforcements, (test trial) was performed

Because the staircase consisted of 13 steps, in each trial, the rat had the possibility of making 4 correct (steps 3, 6, 9 and 12) and 9 incorrect responses. The ratio (correct responses: total responses) × 100 were calculated from the experimental data for the pretest and test trials, and the percentage of differences between these two indices was used to evaluate the decrease in performance following the interruption of daily training.

Determination of ACh Levels

Thirty-two rats were used and were killed by microwave irradiation of the head (2450 mHz, 1.5 kW, 3.0 s) 24 h after the final administration of (−)deprenyl. The skull was opened, and the brain was removed and frozen (−30°C). The four selected regions of the CNS were collected and weighed, the tissue was homogenized in a Polytron at 20,000 rpm for 3 s in 2.0 ml of boiling McIlvaine's citric disodium phosphate buffer (pH 4.0, 0.014 M), placed for 30 s in a boiling water bath, transferred to ice-cold water and diluted with an equal volume of frog and Ringer's solution, containing eserine hemisulfate (20 μg/ml and a double concentration of salt so that the final solution was isotonic) (1). The extracts were centrifuged (1,000 × *g* for 30 min) and the supernatant collected for bioassay of ACh on the frog rectus abdominis muscle.

The concentrations of ACh are given in micrograms per gram of fresh tissue.

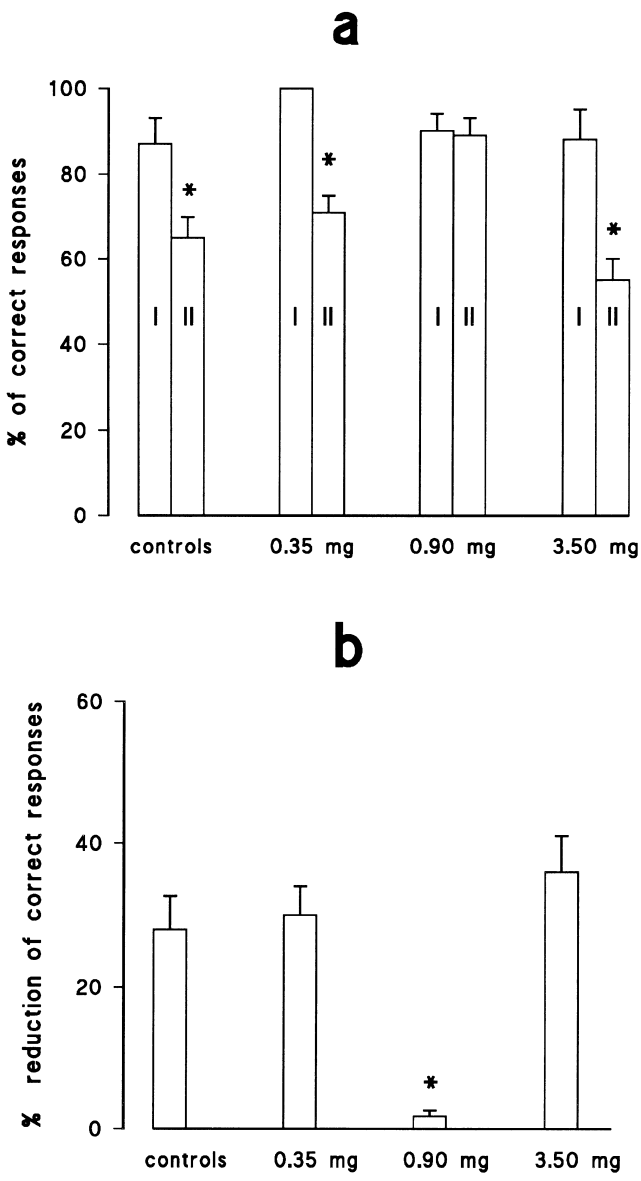

FIG. 1. a: Mean and standard error of the percentage of correct responses in the pretest (I) and test (II) trials (control group: $n = 9$; deprenyl group: $n = 7$). Between the two trials, daily training was interrupted for 20 days; in the first 15 days of this period, (−)deprenyl was administered. The differences between the results in the pretest (I) and test (II) trials are significant, $p < 0.05$, *t*-test for paired data. b: Mean and standard error of the percentage of reduction in the correct responses between the pretest and test trials shown in a. *Probability of a causal result of the difference from the control group (Dunnett's test for the comparison with a control) is less than 0.05.

Determination of Norepinephrine (NA), DA, DOPAC, 5-HT and 5-HIAA

Thirty rats were killed by decapitation, and the four selected brain regions were dissected. The tissue (80 mg in 300 μl) was dissolved by sonication in ice-cold 0.1 M perchloric acid containing 0.1% EDTA and 0.05% metabisulfite, and the samples were centrifuged at 50,000 × *g* for 30 min at 4°C. An aliquot of the supernatant for assaying the NA level was purified on acid-washed alumina, as described by Ehrenstrom and Johansson (5). The NA, DA, DOPAC, 5-HT and 5-HIAA concentrations in the samples were measured as described by Keller et al. (12) by using high-performance liquid chromatography with electrochemical detection.

The composition of the mobile phase was 8 ml of acetonitrile and 92 ml of a solution of 1.0 mM citric acid (pH 3.45), 0.1 mM Na_2HPO_4 0.1 mM EDTA and 1.0 mM heptanesulfonic acid. The flow rate was 0.8 ml/min, and the potential was +0.70 V. Peaks were automatically integrated by the data module and quantified with external standards. The instrumentation used were a μ Bondapak C18 column (Waters Associates, Italy), a pump (Waters 510), an electrochemical detector (Waters 460) and a data module (Waters 740).

Muscarinic Receptor Binding

^{3}H-QNB (39 Ci/mM, Amersham) binding to rat brain membranes was performed as described by Yamamura and Snyder (27).

Twenty rats were used. The animals were killed by decapitation, and the brains were quickly removed. The four selected brain regions were dissected out and homogenized in 10 ml of ice-cold 0.32 M sucrose solution by using a Potter-Elvehjem teflon–glass homogenizer. The homogenates were centrifuged at 1,000 × *g* for 20 min at 4°C, and the pellets were discarded. The protein concentration of the supernatant was determined as described by Lowry et al. (16) by using bovine serum albumin as the standard. Aliquots of supernatant (25–50 μl, 0.2 mg of protein) were incubated in triplicate, with increasing concentrations of ^{3}H-QNB (0.05— 2.0 nM) for 60 min at 25°C in NaK phosphate buffer, pH 7.4. Nonspecific binding was measured in the presence of 1 M atropine.

The reaction was stopped by adding 3 ml of ice-cold phosphate buffer, followed by filtration under reduced pressure on presoaked Whatman GF/B glass-fiber filters. After three washes of the filters with 5 ml of ice-cold buffer, the radioactivity was determined by placing the dried filters overnight in 5 ml of Beckman Ready-Gel scintillant, followed by liquid scintillation counting (Beckman LS-3801) at 40% efficiency. The B_{max} and K_d values were estimated by Scatchard analysis and are given in fentomoles per milligram of protein and picomoles, respectively.

α1-Adrenoceptor Binding

^{3}H-prazosin (85 Ci/mM; Amersham) binding to rat brain membranes was estimated by the method of Glossman and Hornung (9).

Twenty rats were killed by decapitation. The different selected brain regions were quickly removed and homogenized with a Potter-Elvehjem teflon–glass homogenizer in an ice-cold solution of 50 mM Tris-HCl, 1 mM EDTA, pH 7.4. The homogenates were centrifuged 48,000 × *g* for 15 min at 4°C, and the pellet was resuspended in ice-cold Tris-HCl buffer, pH 7.4. The protein concentration of the resuspended pellets was determined as described by Lowry et al. (16) by using bovine serum albumin as the standard.

Tubes containing ^{3}H-prazosin (0.5— 2.5 nM) and an aliquot of resuspended tissue (1.5 mg of protein) were incubated in triplicate (final volume = 250 μl) for 15 min at 37°C, and then the samples were diluted with 3 ml of ice-cold Tris-HCl buffer and rapidly filtered on presoaked Whatman GF/B filters. After the filters were washed three times with 5 ml of ice-cold Tris-HCl buffer, they were placed in vials containing 5 ml of scintillant (Beckman Ready-Gel) and counted in a Beckman LS 3801 liquid scintillation counter (efficiency = 40%). Specific binding was defined as the excess over blanks containing 1 M unlabeled prazosin. The values of B_{max} (fmol/mg protein) and K_d (nM) were estimated by Scatchard analysis.

Statistical Methods

Data were analyzed by two-way analysis of variance followed by Student's *t*-test for grouped data or by Dunnett's test for comparison with a control.

RESULTS

Staircase Maze

Based on the mean fluid consumption (30–35 ml/day/rat) and the weight of the rats (350–400 g), the doses of (−)deprenyl assumed by the animals were estimated (0.35, 0.90, and 3.5 mg/kg/day).

The means and standard errors for the percentage of correct responses in the pretest and test trials for both control and deprenyl-treated rats are shown in Fig. 1A. The *t*-test for paired data shows a significant ($p < 0.05$) reduction in correct responses between pretest and test results for the control group and between groups receiving 0.35 and 3.5 mg/kg/day of (−)deprenyl but not in the group receiving 0.9 mg/kg/day of (−)deprenyl.

Figure 1B shows the actual percentage reduction in correct responses. Dunnett's test for comparison with a control indicates that the reduction seen with the dose of 0.9 mg/kg/day of deprenyl is significant ($p < 0.05$).

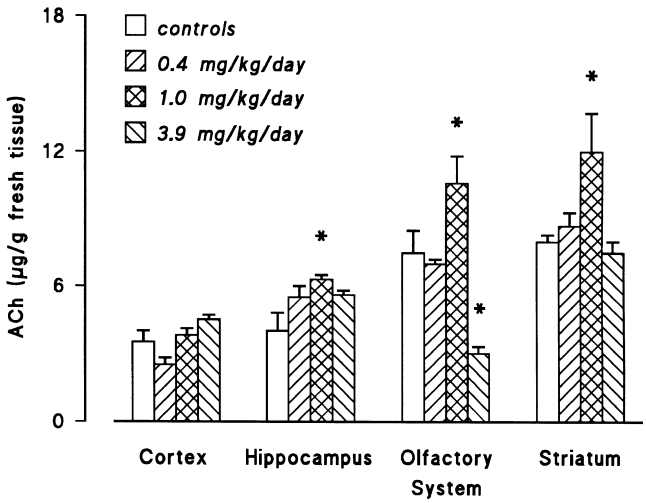

FIG. 2. Acetylcholine levels in regions of the CNS following chronic administration of (−)deprenyl. The values shown are mean ± SEM (n = 8/group). $p < 0.05$, difference from controls (Dunnett's test).

ACh Levels

The estimated doses of (−)deprenyl assumed by the rats were 0.4, 1.0, and 3.9 mg/kg/day.

The ACh concentrations (μg/g of fresh tissue) found in the CNS regions examined are shown in Fig. 2. Two-way analysis of variance indicated that there was a significant difference between the regions [$F(3, 121) = 10.32$, $p < 0.001$]. (−)Deprenyl administration caused a significant modification of ACh levels [$F(3, 121) = 33.18$, $p < 0.001$]. Dunnett's test for comparison with a control indicated that there was a significant ($p < 0.05$) increase in levels of ACh in the olfactory system, hippocampus and striatum at 1.0 mg/kg/day of deprenyl. The reduction in levels of ACh observed at 3.9 mg/kg/day in the olfactory system is also significant.

Affinity (K_d) and Density (B_{max}) of Muscarinic Receptors

The estimated doses of deprenyl assumed by the animals were 0.39, 0.98, and 3.4 mg/kg/day.

The B_{max} (fmol/mg protein) and the K_d (pmol/mg protein) found in the different samples are shown in Fig. 3. Two-way analysis of variance indicated a significant difference between regions of both B_{max} [$F(3, 73) = 9.24$, $p < 0.001$] and K_d [$F(3, 73) = 12.50$, $p < 0.001$]. Deprenyl administration caused no significant change in the density of muscarinic receptors, whereas pharmacological treatment caused a significant modification of K_d [$F(3, 73) = 2.86$, $p < 0.05$]. Dunnett's test for comparison with a control indicated a significant ($p < 0.05$) increase in K_d in the olfactory system at 3.4 mg/kg/day of (−)deprenyl.

DA and DOPAC Levels

Based on the mean fluid consumption (33–38 ml/day/rat) and the weight of the rats (350–400), the doses of deprenyl assumed by the animals were estimated to be 0.4, 1.00, and 4.00 mg/kg/day.

The levels of DA and its metabolite DOPAC are shown in Fig. 4. Analysis of variance indicates differences in the level of dopamine in the different regions of the CNS [$F(3, 113) = 35.4$, $p < 0.001$], and the pharmacological treatment caused a

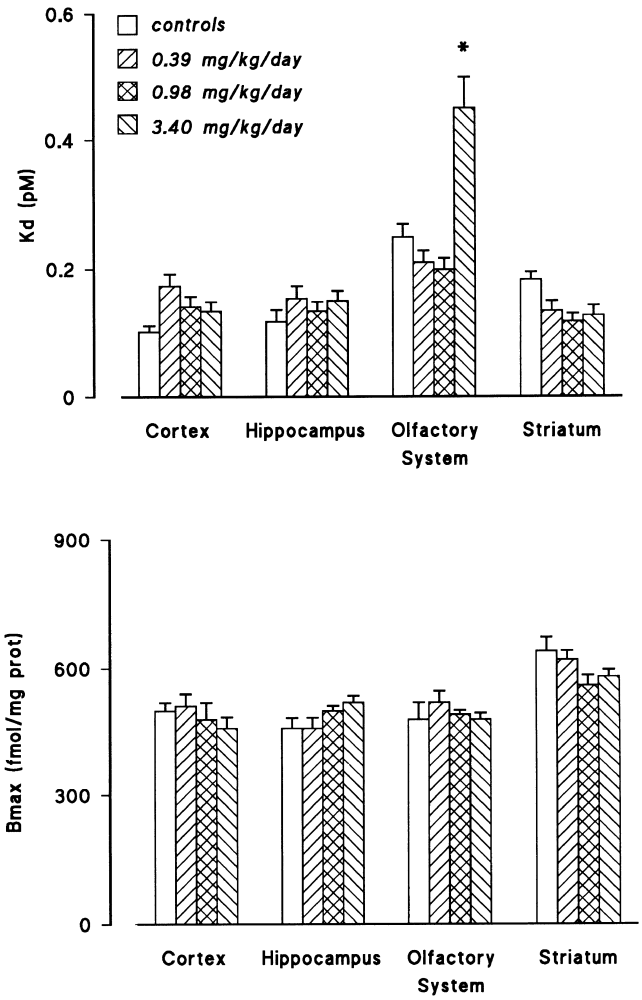

FIG. 3. Density (B_{max}) and affinity (K_d) of muscarinic receptors in regions of the CNS following chronic administration of (−)deprenyl. The values shown are mean ± SEM ($n = 5$/group). $p < 0.05$, difference from control group (Dunnett's test).

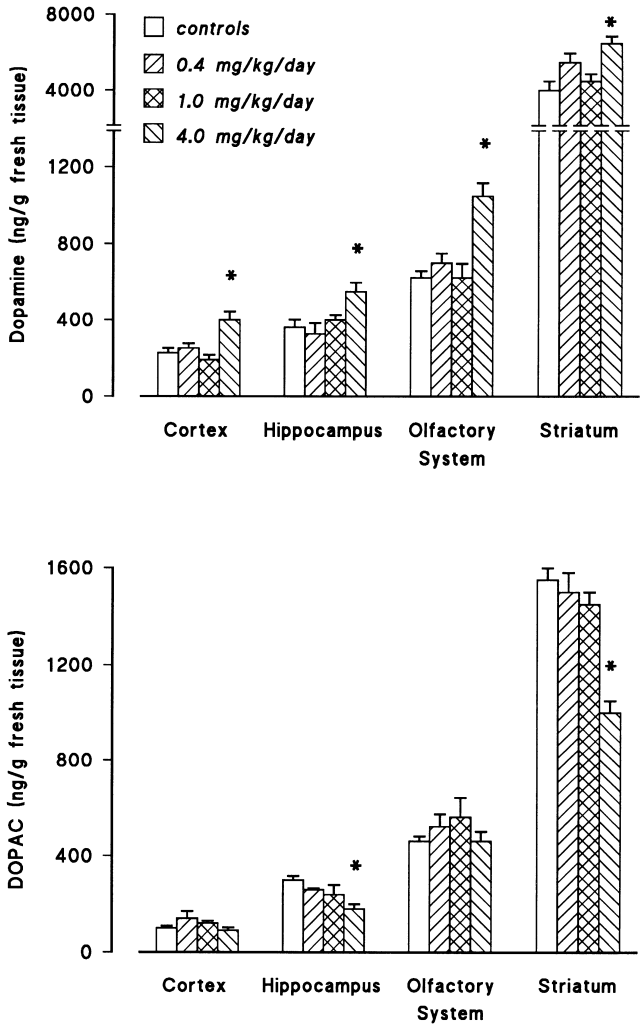

FIG. 4. Dopamine and DOPAC levels in regions of the CNS following chronic administration of (−)deprenyl. The values shown are the means ± SEM of 9 control and 7 deprenyl-treated rats. $p < 0.05$, difference from controls (Dunnett's test).

significant change in dopamine levels [$F(3, 113) = 6.06$, $p < 0.001$]. Dunnett's test shows ($p < 0.05$) a significant increase in DA levels in all the tested regions at 4.00 mg/kg/day of (−)deprenyl. In terms of DOPAC levels, significant differences [$F(3, 113) = 28.70$, $p < 0.001$] were seen between the different regions of the CNS. (−)Deprenyl administration caused a significant change in DOPAC levels [$F(3, 113) = 6.14$, $p < 0.001$]. Dunnett's test indicated that deprenyl induces a significant ($p < 0.05$) reduction in DOPAC levels at 4 mg/kg/day in the hippocampus and striatum.

5-HT and 5-HIAA Levels

The estimated doses of (−)deprenyl assumed by the animals were 0.40, 1.00, and 4.00 mg/kg/day.

The levels (ng/g of fresh tissue) of 5-HT and its metabolite 5-HIAA are shown in Fig. 5. The analysis of variance indicated a significant difference between the CNS regions in the levels of 5-HT [$F(3, 113) = 98.82$, $p < 0.001$] and 5-HIAA [$F(3, 113) = 51.30$, $p < 0.001$]. However, pharmacological treatment had no significant effect on 5-HT or 5-HIAA levels.

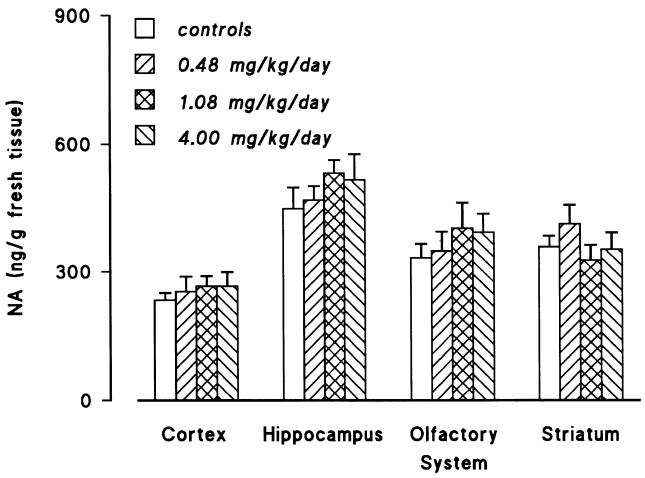

FIG. 6. Noradrenaline levels in regions of the CNS following chronic administration of (−)deprenyl. The values shown are mean ± SEM of 9 control and 7 deprenyl-treated rats.

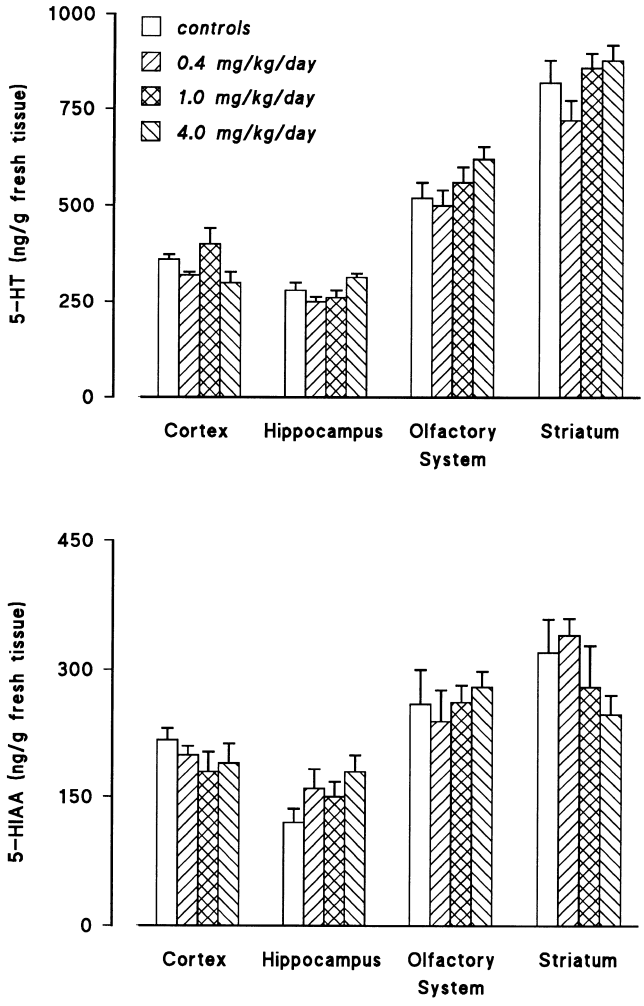

FIG. 5. The 5-HT and 5-HIAA levels in regions of the CNS following chronic administration of (−)deprenyl. The values shown are mean ± SEM of 9 control and 7 deprenyl-treated rats.

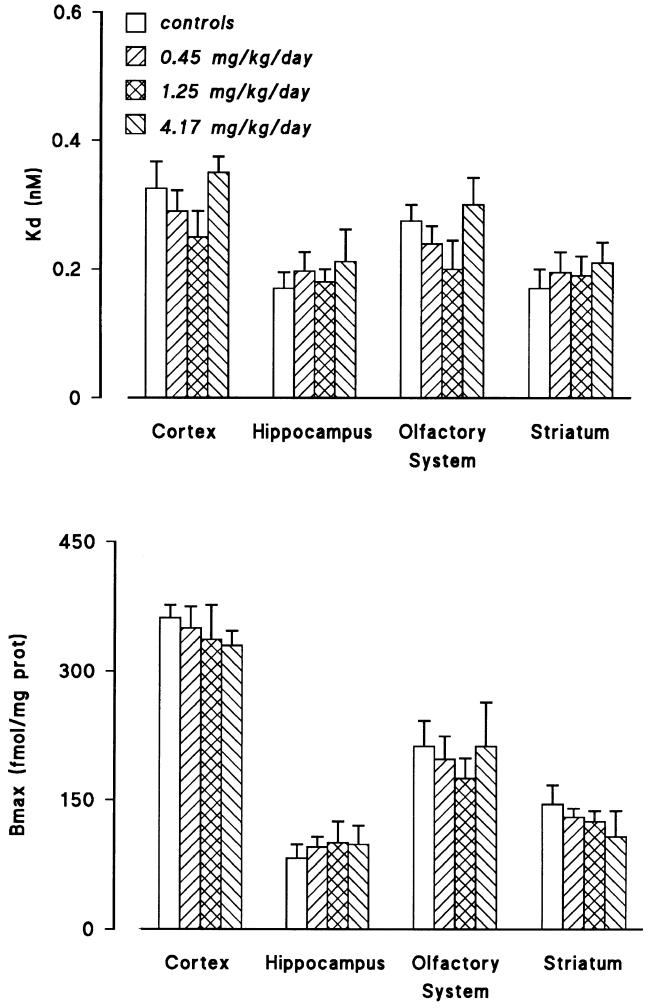

FIG. 7. Density (B_{max}) and affinity (K_d) of α-adrenoceptors in regions of the CNS following chronic administration of (−)deprenyl. The values shown are mean ± SEM ($n = 5$/group).

NA Levels, Density and Affinity of α-Adrenoceptors

The levels (ng/g of fresh tissue) of NA are shown in Fig. 6. The estimated doses of (−)deprenyl assumed by the rats were 0.48, 1.08 and 4.0 mg/kg/day. The analysis of variance indicated a significant difference between the CNS regions in the levels of NA [$F(3, 113) = 40.35$ $p < 0.001$], but pharmacological treatment had no significant effect on NA levels.

The B_{max} (fmol/mg protein) and K_d (nM) found in the different samples are shown in Fig. 7. The estimated doses of (−)deprenyl assumed by the rats were 0.45, 1.25 and 4.17 mg/kg/day. Two-way analysis of variance indicated a significant difference between regions of B_{max} [$F(3, 27) = 33$, $p < 0.001$] and K_d [$F(3, 27) = 5.87$, $p < 0.01$]; pharmacological treatment had no significant effect on either B_{max} or K_d.

DISCUSSION

The results obtained in the staircase maze indicated that subchronic administration of (−)deprenyl for 15 days only cause a reduction in incorrect responses at doses of 0.9 mg/kg/day; higher or lower doses (3.5 or 0.35 mg/Kg /day) were ineffective.

In agreement with our results, an improvement in spatial memory has been reported in rats following daily administration of (−)deprenyl for 5 days (4) or 4 months (8). A number of studies also have shown that chronic treatment with (−)deprenyl provides an improvement in episodic memory and learning in individuals with Alzheimer's disease (17,24,26). These improvements were observed at a dose of 10 mg/day but not at a dose of 40 mg/day. These observations in people are in agreement with the results we obtained in the rats tested in the staircase maze. The lack of a dose–response proportionality on memory is certainly a drawback to therapeutic use; however, it allows a correlation to be made between behavioral and neurochemical effects when the two functional changes are seen over the same range of doses.

(−)Deprenyl is a potent irreversible inhibitor for the oxidative deamination of dopamine (7) and seems to act by inhibiting DA catabolism in vivo (18).

The increase in the levels of DA and the reduction of its metabolite DOPAC observed after the subchronic administration of (−)deprenyl are in agreement with the observation that (−)deprenyl inhibits DA catabolism. However, these neurochemical effects are evident at much higher doses of (−)deprenyl (4 mg/kg/day) than the doses (0.9 mg/kg/day) that improve rat behavior in the staircase maze. These observations indicate that the inhibition of DA catabolism by (−)deprenyl is not correlated to the improvement of behavior observed in the staircase maze.

In the range of doses of (−)deprenyl we tested, there was no significant modification in the levels of NA or of the density (B_{max}) and affinity (K_d) of the alpha-noradrenergic receptors. Similarly, the levels of 5-HT and its metabolite 5-HIAA were not modified in the sections of the CNS we examined. These results are apparently in disagreement with the report of Knoll et al. (15) in which (−)deprenyl in a range of 0.05–0.25 mg/kg/day increased the output of NA from the locus coeruleous and reduced the output of 5-HT from the raphe. Differences in the regions of the CNS examined may explain the differences between the results. Moreover, our measurements were performed in regions of the CNS collected immediately after the killing of the rats, whereas Knoll et al. (15) evaluated the output of NA and 5-HT from regions of the CNS kept for a certain time in an artificial medium. However, independently of the interpretation, the discrepancy between our results and those of Knoll et al. (15) may be due to the neurochemical effects observed by these authors, which were evident at very low doses of (−)deprenyl (0.05–0.25 mg/kg/day). In the staircase maze, we observed no modification of behavior at 0.35 mg/kg/day. These observations indicate that a modification of the output of NA or 5-HT is not correlated to the modifications of the memory process, which is evident only at doses of (−)deprenyl that are higher than the doses that cause in vitro modifications of the output of the neuromediators.

Paterson et al. (22) reported that the administration of a dose of (−)deprenyl, which is selective for MAO-B inhibition, results in a potentiation of neuronal responses to DA agonists. The dose of (−)deprenyl did not alter the levels of DA or its metabolites but did result in an elevation of phenylethylamine (PE) levels, which activated DA receptors (2,3). There is evidence that an activation of dopamine receptors increased striatal ACh by about 40% and induced a 30% inhibition of ACh-evoked release from striatal slices (10). This finding was further supported by the observation that chlorpromazine, in consequence of the blockade of DA receptors, increased the release of striatal ACh. Apomorphine, which stimulates DA receptors, reversed the chlorpromazine-induced release of ACh (25). Thus, the increase of the levels of ACh we observed at doses of 1 mg/kn/day of (−)deprenyl, which did not modify the levels of DA or of its metabolite, may be determined by the activation of dopamine receptors due to the elevation of endogenous PE levels resulting from the inhibition of MAO-B. Our results indicate that the increase of the levels of ACh is no more evident at higher doses (3.9 mg/kg/day) of (−)deprenyl.

In the olfactory system, the levels of ACh and the affinity (increase of K_d) of muscarinic receptors were reduced. These observations suggest that doses of (−)deprenyl, which reduced the catabolism of DA, display collateral effects that also interfere with the cholinergic systems.

Our results indicate that in the same range of doses (−)deprenyl improves memory retention and increases the levels of ACh in the hippocampus, olfactory system and striatum. This neurochemical modification to the activation of dopaminergic systems consequent to the elevation of endogenous PE levels may be caused by the inhibition of MAO-B.

ACKNOWLEDGEMENTS

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