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Behavioral and Neurochemical Effects Induced by Chronic L-DOPA Administration

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MOLINENGO, L., I. SCORDO, B. PASTORELLO, M. ORSETTI AND P. GHI. *Behavioral and neurochemical effects induced by chronic L-DOPA administration.* PHARMACOL BIOCHEM BEHAV **54**(4) 779–785, 1996.—L-DOPA, in combination with benserazide, in the ratio 4:1 (w/w), was administered orally to rats. In the staircase maze test a low dose of L-DOPA (3 mg/kg/day) reduced the increase in errors caused by 20 days interruption of daily training, while a higher dose (30 mg/kg/day) was ineffective. A decrease in levels of dopamine in the olfactory system and DOPAC in the striatum was seen at all tested doses of L-DOPA, while an increase in 5-HT levels was seen in the hippocampus and in the striatum. 5-HIAA levels did not change. Levels of ACh in the olfactory system were reduced at all doses of L-DOPA, while in the hippocampus this effect was seen only at the dose of 90 mg/kg/day. The density of muscarinic receptors was not altered. All tested doses of L-DOPA caused norepinephrine levels to fall in the hippocampus and increase in the striatum. The density of α_1-adrenoceptors was reduced only at the two lower doses of L-DOPA. A comparison of the neurochemical results with the behavioral modifications seen in the staircase maze test suggests that the catecolaminergic systems are implicated in the memory process.

L-Dopa plus benserazide Chronic administration Memory storage Staircase maze Dopaminergic system Serotoninergic system Cholinergic system Noradrenergic system

It has been proposed that certain drugs interfere with different steps of the memory process (acquisition, consolidation, storage, and retrieval), depending on when the drug is administered (16). Results obtained using the staircase maze, a test that makes it possible to evaluate drug action on storage, indicate that scopolamine inhibits the time-dependent decay of storage, resulting in an apparent improvement in memory (26) while scopolamine-induced inhibition of consolidation and retrieval (16) impairs an animal's performance in memory tests. Arecoline can have opposite effect on various steps of memory, resulting in improvement of memory by consolidation and retrieval (11–14) and in deterioration of memory by accelerating storage decay (27). If modification of memory is dependent on the step of the memory process on which arecoline acts, its therapeutic use in treatment of amnesia will be very limited. In agreement with these considerations, Christie et al. (8) concluded that arecoline is not a practical means of therapy for amnesia in Alzheimer type dementia.

The question may also be posed whether activation/inhibition of all the steps of memory is restricted to arecoline and scopolamine.

Evidence exists for the involvement of the dopaminergic systems in the memory process. Posttraining administration of dopamine receptor agonists (7,29,33,34) improves the performance of rats in memory tests, suggesting that activation of dopamine receptors improves memory consolidation. Intracerebral (1) or subcutaneous administration (31) of L-DOPA improves retrieval and Zis et al. (36) reported that the dopaminergic nigro-neostriatal projections serve a critical function in the acquisition of instrumental responses.

In the present study, we utilized the staircase maze test, which we have previously used to study modification of storage decay by scopolamine (26) and arecoline (27), to test if chronic L-DOPA administration, known to activate consolidation and retrieval, also activates spontaneous memory decay.

There is evidence for interactions between the dopaminergic and serotoninergic or cholinergic systems (10,19,20,28). Hence, behavioral effects of chronic L-DOPA administration may result from interference with other neurochemical systems. With the purpose of identifing biochemical-behavioral correlations, we, therefore, looked for any neurochemical modifications of the dopaminergic, noradrenergic, serotoninergic, and cholinergic systems induced by the same chronic L-DOPA administration used in the behavioral experiments.

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METHOD

Subjects

Male albino rats (Morini, Wistar derived strain) weight 150–200 g were used. They were housed five to a cage and fed ad lib with a standard rodent diet, except where otherwise stated, with free access to tap water.

Regions of the CNS Examined

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

The Staircase Maze

The staircase maze, described in previous publications (23,25,26), was used; it consisted of a 13-step staircase with a 17 cm long corridor in the vertical wall. Twenty-four rats were used. They were fasted from 1800 h to 1200 h 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 at the four reinforced steps. Once training was complete, a trial without reinforcement was performed.

In this trial (pretesting trial), a search for food on steps 3, 6, 9, and 12 was considered a correct response, while a search for food on any other steps was considered an incorrect response. The daily training was then interrupted for 20 days and a new trial, without reinforcement (testing trial) was performed. The overall experimental scheme was therefore: (a) 2 months of preliminary training, (b) pretesting trial, (c) no training/L-DOPA administration (15 days), (d) no training/no drug administration (5 days), (e) testing trial. Correct and incorrect responses were scored in both trials. As the staircase consisted of 13 steps in each trial, the rat had the possibility of making four correct (steps 3, 6, 9, and 12) and nine incorrect responses. The ratios (correct responses/total responses) $\times$ 100 were calculated from the experimental data for the pretesting and testing trials and the percent differences between these two indices used to evaluate the decay in performance following interruption of daily training.

The rats were divided into three groups of eight.

Determination of the ACh Levels

Thirty-two rats were used. They were killed by microwave irradiation of the head (2450 mHz, 1.5 kW, 3.0 s) 24 h after the final administration of L-DOPA, the skull opened, and the brain 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 about 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, then transferred to ice-cold water, and diluted with an equal volume of frog-Ringer solution containing eserine hemisulfate (20 μg/ml) and a double concentration of salts so that the final solution was isotonic (4). The extracts were centrifuged (1000 $\times$ *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 μg/g of fresh tissue.

Determination of Norepinephrine (NA), Dopamine, Dihydroxyphenylacetic Acid (DOPAC), 5-Hydroxytryptamine (5-HT), and 5-Hydroxyindolacetic Acid (5-HIAA)

Twenty rats were killed by decapitation and the four selected brain areas dissected. The tissue (80 mg tissue in 300 μl) was disrupted by sonication in ice-cold 0.1 M perchloric acid, containing 0.1% EDTA and 0.05% sodium metabisulfite, and the samples centrifuged at 50,000 $\times$ *g* for 30 min at 4°C. An aliquot of the supernatant to be used to assay NA level was purified on acid-washed alumina, as described by Ehrenstrom and Johansson (9). The NA, dopamine, DOPAC, 5-HT, and 5-HIAA concentrations in the samples were measured as described by Keller et al. (18), using high-performance liquid chromatography (HPLC) with electrochemical detection.

The composition of the mobile phase was 92 ml of a solution containing citric acid 1.0 mM (pH 3.45), Na_2HPO_4 0.1 mM, EDTA 0.1 mM, heptansulfonic acid 1.0 mM (pH 3.45), and 8 ml acetonitrile. The flow rate was 0.8 ml/min and the potential +0.70 V. Peaks were automatically integrated by the data module and quantified using external standards. The concentrations are given in ng/g of fresh tissue. The instrumentation used was 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 (35). Twenty rats were used. The animals were killed by decapitation and the brains quickly removed. The four selected brain regions were dissected out and homogenized in 10 ml of ice-cold 0.32 M sucrose solution using a Potter-Elvehjem Teflon-glass homogenizer. The homogenates were centrifuged at 1000 $\times$ *g* for 20 min and the pellets discarded. The protein concentration of the supernatant was determined as described by Lowry et al. (21), using bovine serum albumin as 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 Na-K phosphate buffer, pH 7.4. Nonspecific binding was measured in the presence of 1 μM atropine.

The reaction was stopped by addition of 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, radioactivity was determined by placing the dried filters overnight in 5 ml of Beckman Ready-Gel scintillator, followed by liquid scintillation counting (Beckman LS-3801) at 40% efficiency. B_{max} and K_d values were estimated by Scatchard analysis and are given in fmol/mg protein and pM, respectively.

α_1-Adrenoceptor Binding

^{3}H-prazosin (85 Ci/mM, Amersham) binding to rat brain membranes was estimated by the method of Glossman and Hornung (15). 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 at 4°C for 15 min at 48,000 $\times$ *g* for 15 min and the pellet 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. (21) using bovine serum albumin as 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, 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 liquid of scintillation (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 μ M unlabelled prazosin. The values of B_{max} (fmol/mg protein) and K_d (nM) were estimated by Scatchard analysis

Pharmacological Treatment

A 4:1 (w/w) combination of L-DOPA and benserazide was used. In the following text, we will only indicate the dose of L-DOPA given.

In the behavioral tests, doses of 3 and 30 mg/kg/day of L-DOPA were used. Higher doses were avoided to avoid toxic peripheral effects that could interfere with the rat's performance. In the last 5 days of the no-training period, drug administration was discontinued to avoid any interference from peripheral effects of L-DOPA/benserazide on the rat's performance in the staircase maze. The doses selected for the neurochemical studies were 3, 30, and 90 mg/kg/day. The dose of 90 mg/kg/day was used to confirm, at a higher dosage, neurochemical changes seen at lower doses. The rats were transported to the laboratory and handled daily for 20 days (5 days before and 15 days during L-DOPA/benserazide administration). They were food deprived 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 drugs.

The selected doses of L-DOPA were mixed with a given quantity (about 0.5 g) of powdered and moistened purina chops. A bolus produced in this way was given to each rat after 15–18 h fasting. This procedure was used to avoid stress caused by stomach intubation.

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

The means and standard errors for the percentage of correct responses in the pretesting and testing trials for both control and L-DOPA treated rats are shown in Fig. 1A. The *t*-test for paired data shows a significant ($p < 0.05$) reduction in correct responses between pretesting and testing results for the control group and the group on 30 mg/kg/day of L-DOPA, but not the group receiving 3 mg/kg/day of L-DOPA. Figure 1B shows the percentage reduction in correct responses. Dunnett's test for comparison with a control indicates that the reduction seen with the dose of 3 mg/kg/day of L-DOPA is significant ($p < 0.05$).

Dopamine and DOPAC Levels

The levels of dopamine and its metabolite, DOPAC, are given in Figs. 2 and 3, respectively. Analysis of variance indicates differences in the level of dopamine in the various regions of the CNS, $F(3, 73) = 54.55, p < 0.001$, and that pharmacological treatment caused a significant change in dopamine levels, $F(3, 73) = 4.59, p < 0.01$. Dunnett's test shows ($p < 0.05$) that there is a significant reduction in dopamine level in the olfactory system at all tested doses of L-DOPA.

Significant differences of DOPAC levels, $F(3, 73) = 16.60$, $p < 0.001$, were seen between the various regions of the CNS. L-DOPA administration caused a significant change in DOPAC levels, $F(3, 73) = 5.36$, p 0.01–0.001. Dunnett's test indi-

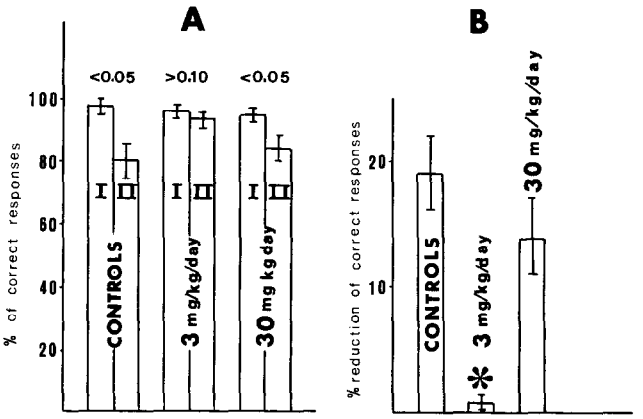

FIG. 1. (A) Mean and standard error of the percentage of correct responses in the pretesting (I) and testing (II) trials. Between the two trials, daily training was interrupted for 20 days; in the first 15 days of this period, L-DOPA + benserazide (dose ratio 4:1) were administered. The probability of a casual result (*t*-test for paired data) is given. (B) Mean and standard error of the percentage reduction in the correct responses between pretesting and testing trials shown in section A. An asterisk indicates that the probability of a casual result of the difference from the controls (Dunnett's test for the comparison with a control) is less than 0.05.

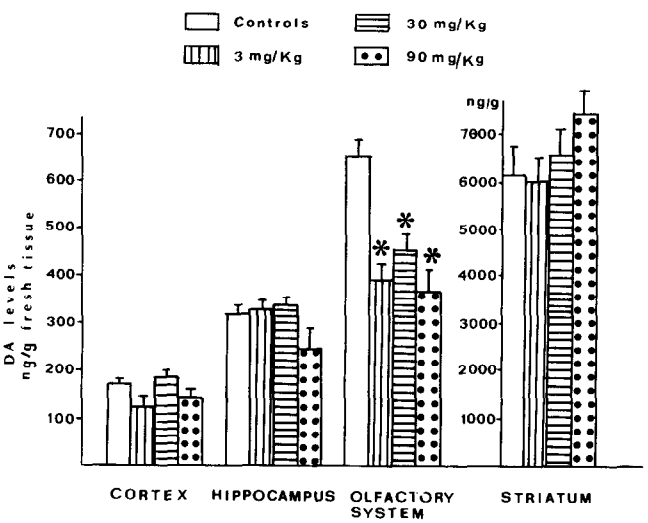

FIG. 2. Dopamine levels in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are the mean ± SEM for five rats per point. *The probability of a difference to controls (Dunnett's test) is < 0.05.

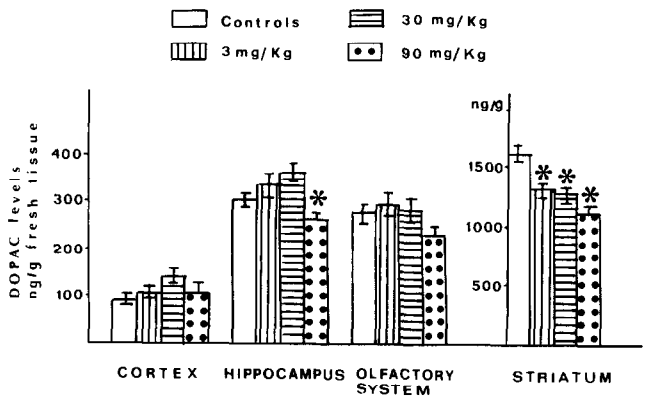

FIG. 3. DOPAC levels in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are the mean ± SEM for five rats per point. *The probability of a difference to controls (Dunnett's test) is < 0.05.

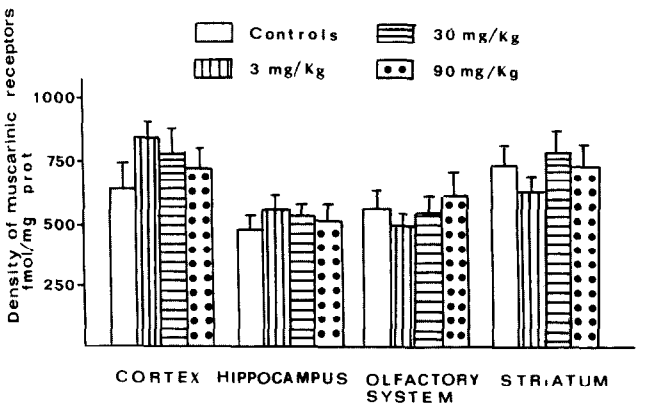

FIG. 5. Density of muscarinic receptors in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are mean ± SEM for five rats per point. *The probability of a difference to controls (Dunnett's test) is < 0.05.

cates ($p < 0.05$) that L-DOPA induced a significant reduction in DOPAC levels at all tested doses in the striatum and at 90 mg/kg/day in the hippocampus.

ACh Levels and Muscarinic Receptors Density

The ACh concentration (μg/g of fresh tissue) found in the CNS regions examined are given in Fig. 4. Two-way analysis of variance indicated that there was a significant difference between the regions, $F(3, 121) = 19.1$, $p < 0.001$. L-DOPA administration caused a significant modification of ACh levels, $F(3, 121) = 7.59$, $p < 0.001$. Dunnett's test for comparison with a control indicated ($p < 0.05$) that there was a significant decrease in levels of ACh in the olfactory system at all doses tested and in the hippocampus at the dose of 90 mg/kg/day.

The B_{max} (fmol/mg prot.) found in the different samples are given in Fig. 5. Two- way analysis of variance indicated a significant difference between regions, $F(3, 73) = 24.1$, $p < 0.001$. L-DOPA administration caused no significant change in the density of muscarinic receptors, $F(3, 73) = 1.91$, $p > 0.1$. No change in affinity constant (K_d) for the radioligand was seen, the maximal variation being from 105 ± 18 pM to 89 ± 13 pM.

5-HT and 5-HIAA Levels

The levels (ng/g of fresh tissue) of 5-HT and of its metabolite 5-HIAA are shown in Figs. 6 and 7, respectively. Analysis of variance indicated that there was a significant difference between the CNS regions, $F(3, 73) = 39.51$, $p < 0.001$. Pharmacological treatment produced a significant change, $F(3, 73) = 5.23$, p 0.01–0.001). Dunnett's test indicates that all tested doses of L-DOPA caused a significant increase in 5-HT levels in both the hippocampus and striatum. Pharmacological treatment had no significant effect on 5-HIAA levels, $F(3, 73) = 0.63$, $p > 0.2$.

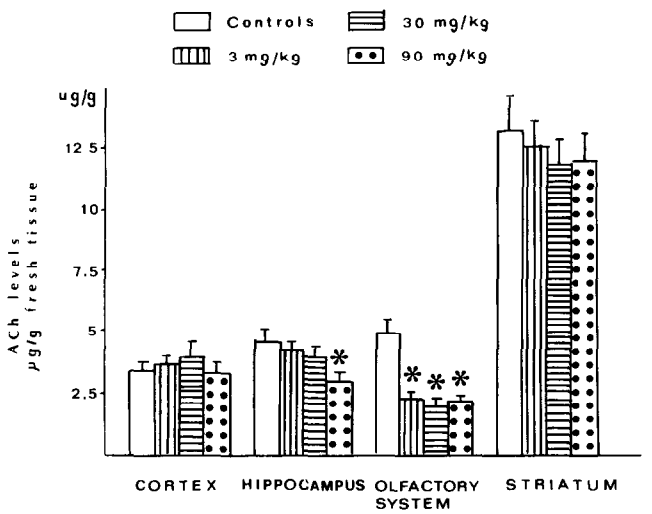

FIG. 4. Acetylcholine levels in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are mean ± SEM for eight rats per point. *The probability of a difference to controls (Dunnett's test) is < 0.05.

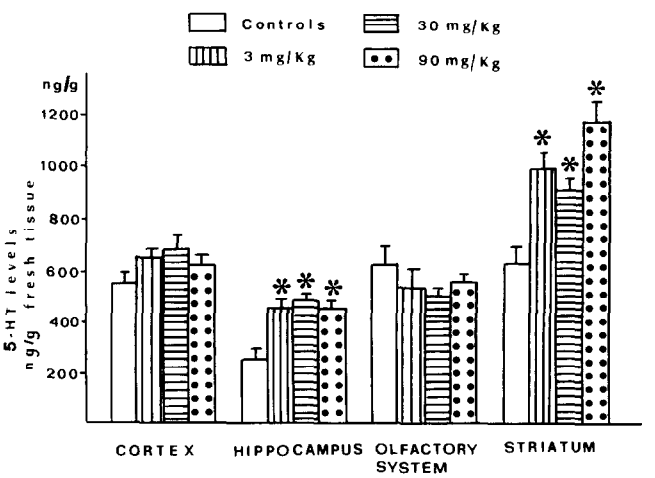

FIG. 6. 5-HT levels in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are mean ± SEM for five rats per point. *The probability of a difference to controls (Dunnett's test) is < 0.05.

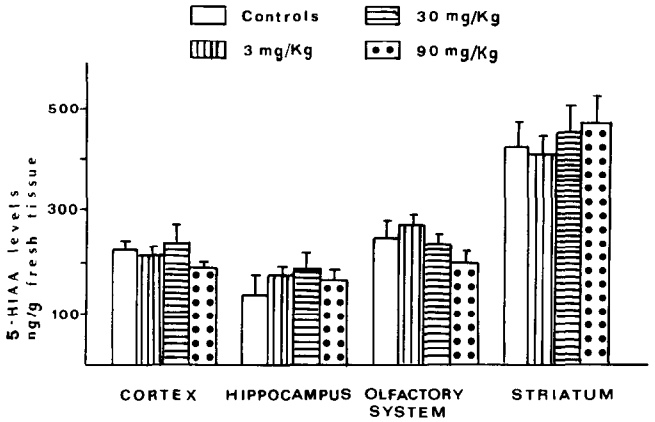

FIG. 7. 5-HIAA levels in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are mean ± SEM for five rats.

NA Levels and α_1-Adrenergic Receptors

The levels of NA (ng/g fresh tissue) and the B_{max} (fmol/mg prot) for the α_1-adrenergic receptors found in the various regions of the CNS are given in Figs. 8 an 9, respectively. Analysis of variance shows that there were significant differences in NA level between different regions of the CNS, $F(3, 73) = 11.18, p < 0.001$, and that there was no significant change in NA levels following L-DOPA administration. However, it should be noted that, in the hippocampus, there was a significant reduction in NA levels that compensated for the significant increase seen in the striatum.

The differences in α_1-adrenoceptors density (B_{max}) in the various regions of the CNS did not reach significance, $F(3, 73) = 2.52$, p 0.05–0.1. L-DOPA administration caused a significant change in B_{max}, $F(3, 73) = 16.63$, $p < 0.001$, while Dunnett's test indicated ($p < 0.05$) that the decrease in B_{max} seen at 3 and 30 mg/kg/day in the cortex, hippocampus, and olfactory system and in the striatum at 3 mg/kg/day were all significant. At 90 mg/kg/day of L-DOPA, there is no significant change in the density of α_1-adrenoceptors in the regions of the CNS examined.

No variation in affinity constant (K_d) for the radioligand was observed with the maximal variation being from 0.32 ± 0.06 nM to 0.27 ± 0.05 nM.

DISCUSSION

The results obtained in the staircase maze indicate that chronic administration of L-DOPA/benserazide for 15 days only caused a reduction in incorrect responses at the dose of 3 mg/kg/day L-DOPA/0.75 mg/kg/day benserazide; a higher dose of L-DOPA (30 mg/kg/day) was ineffective. In agreement with this observation Packard and White (29) reported that a D_2 agonist improved memory consolidation only over a limited range of doses. It may be noted that a lack of dose–response proportionality on memory was also noted with arecoline (27) and alpha-methylparatyrosine (23). Such an effect is difficult to explain and is certainly a drawback to the therapeutic use of drugs that improve memory processes. However, it still allows a correlation to be made between behavioral and neurochemical effects when the two functional changes are seen over the same range of doses.

In our experiments, L-DOPA administration was initiated after a long training period and an action of the drugs on memory acquisition or consolidation can be excluded. Hence, it can be proposed that chronic administration of L-DOPA, over a limited range of doses, blocked the decay in memory seen in the controls following interruption of daily training. It is also possible that the effects of chronic administration of L-DOPA persisted for 5 days after interruption of the daily administration and improved memory retrieval.

It was unavoidable that the behavioral and neurochemical experiments were performed on different sets of rats, because the animals in the neurochemical experiments were killed at the end of chronic L-DOPA administration before the final trial of memory retention. However, the rats used were equivalent in terms of strain, sex, and weight and underwent the same food deprivation and drug administration regimens. In addition, the rats used in the neurochemical experiments were transported to the laboratory and handled daily for 20 days to minimize differences between the sets of rats used in the behavioral and neurochemical experiments.

Chronic administration of L-DOPA (15 days) in combina-

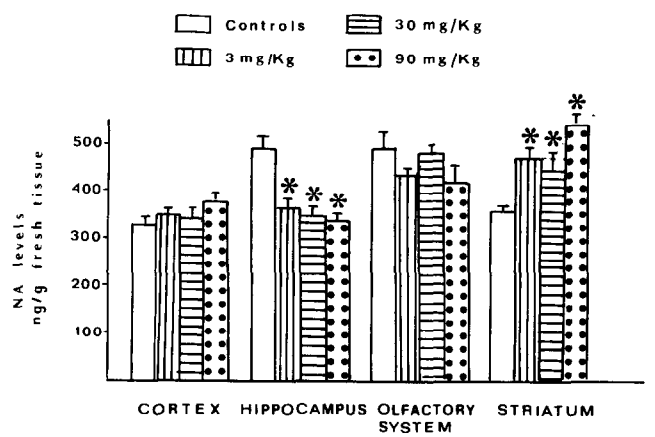

FIG. 8. Noradrenaline levels in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are mean ± SEM for five rats. *The probability of a difference to controls (Dunnett's test) is < 0.05.

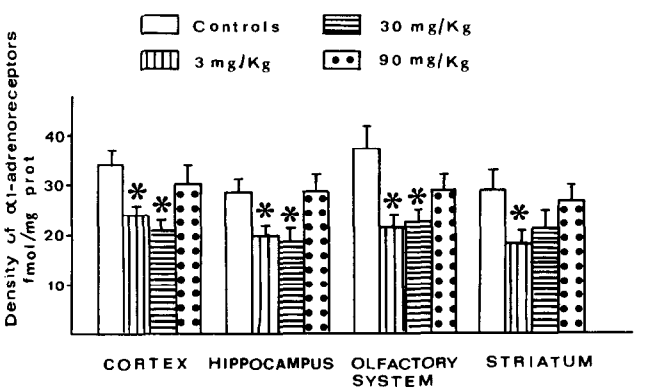

FIG. 9. Density of α_1-adrenoceptors in regions of the CNS following chronic administration of L-DOPA plus benserazide (4:1). The values shown are mean ± SEM for five rats per point. *The probability of a difference to controls (Dunnett's test) is < 0.05.

tion with benserazide reduced dopamine levels at all doses, but only in the olfactory system, while DOPAC levels were reduced at all tested doses in the striatum and at 90 mg/kg/day in the hippocampus.

These results are in agreement with other reports, which indicate that chronic administration of L-DOPA decreases the conversion of L-DOPA to dopamine (6,22), which may correlate with a downregulation of the dopaminergic systems (30).

Our results also indicate that, following chronic administration of L-DOPA, the levels of 5-HT in the hippocampus and striatum increase, while the levels of the metabolite 5-HIAA do not change. It should be noted that acute administration of L-DOPA reduces central stores of 5-HT (2,5). The increased 5-HT levels seen may, therefore, be interpreted as a further indication that chronic administration of L-DOPA causes downregulation of the dopaminergic systems.

The decrease in ACh levels seen in the olfactory system and the hippocampus may also correlate with downregulation of the dopaminergic systems. This would agree with the observation that dopamine receptor blockers (neuroleptic drugs) increase Ach release (3,33), which, in certain CNS sections, may result in a reduction in the ACh levels.

It should be noted that the neurochemical effects seen differed in the various regions of the CNS. This is particulary evident for NA levels, for which no change was seen in both the cortex and olfactory system whereas, at all doses tested, a decrease was seen in the hippocampus and an increase in the striatum. Differences between the various regions of the CNS in terms of neurochemical changes caused by disulfiram (25), alphamethyltyrosine (23), and choline (24) have been reported. These observations indicate that, when a drug modifies one neurochemical system, interference with many other different systems can occur to varying degrees and produce considerable differences in various regions of the CNS.

It may also be noted that the neurochemical changes increased or persisted at increasing doses of L-DOPA, while the behavioral improvement in the memory test was lost at the highest dose. Our results, therefore, provide no evidence that the dopaminergic, serotoninergic, and cholinergic systems are implicated in the memory effect of L-DOPA that we studied.

In the striatum, the density (B_{max}) of α_1-receptors was only reduced at the low dose of L-DOPA, whereas in other regions it was reduced at the two lower doses, but was normal at the highest dose of L-DOPA. These results may suggest a correlation between neurochemical and behavioral effects. Actually, an analogous dose–effect proportionality was found for the behavioral modifications in the memory test. It may be noted that Johnson et al. (17) observed no reduction of receptor density when the adrenergic imput was modified. This suggests that the effect we observed may not be determined by L-DOPA induced modifications of adrenergic imput. We are unable to explain how chronic L-DOPA administration caused, over a limited range of doses, a reduction in density of α_1-noradrenergic receptors; nevertheless, our observations suggest that catecolaminergic systems are implicated in the mechanism of storage decay.

Finally, it should be noted that arecoline, scopolamine and choline (24,26,27) induce either an improvement or deterioration in rat performance in memory tests, depending on the phase (consolidation, storage or retrieval) of the memory process on which they act. In contrast, our results and those of other authors (1,7,29,33,34,36) indicate that the action of L-DOPA on the various phases of the memory process always results in an improvement in performance in memory tests.

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REFERENCES

1. Altman, H. J.; Quartermain, D. Facilitation of memory retrieval by centrally administered catecholamine stimulating agents. Behav. Brain Res. 7:51–63; 1983.
2. Bartholini, G.; Da Prada, M.; Pletscher, A. Decrease of cerebral 5-hydroxytryptamine by 3,4-dihydroxyphenylalanine after inhibition of extracerebral decarboxylase. J. Pharm. Pharmacol. 20:228–229; 1968.
3. Bartholini, G. Interaction of striatal dopaminergic, cholinergic and GABA-ergic neurons: Relation to extrapyramidal function. Trends Pharmacol. Sci. 1:138–140; 1989.
4. Beani, L.; Bianchi, C. The extraction of acetylcholine in small samples of cerebral tissue. J. Pharm. Pharmacol. 15:281–282; 1963.
5. Butcher, L.; Engel, J.; Fuxe, K. L-DOPA induced changes in central monoamine neurons after peripheral decarboxylase inhibition. J. Pharm. Pharmacol. 22:313–316; 1970.
6. Carey, R. J. Chronic L-DOPA treatment in the unilateral 6-OHDA rat: Evidence for behavioral sensitization and behavioral tolerance. Brain Res. 568:205–214; 1991.
7. Castellano, C.; Cestari, V.; Cabib, S.; Puglisi-Allegra, S. Posttraining dopamine receptor agonists and antagonists affect memory storage in mice irrespective of their selectivity for D_1 or D_2 receptors. Behav. Neural. Biol. 56:283–291; 1991.
8. Christie, J. E.; Shering, A.; Ferguson, J.; Glen, I. M. Physostigmine and arecoline: Effects of intravenous infusion in Alzheimer presenile dementia. Br. J. Psychiatry 138:46–50; 1981.
9. Ehrenstrom, F.; Johansson, P. A method for very rapid determination of catechols using ion-pairing reverse phase electrochemical detection: Effects of L-DOPA treatment on catechol content in various brain structures. Life Sci. 36:867–879; 1985.
10. Everett, G. M.; Borchherding, J. W. L-DOPA effect on concentration of dopamine, norepinephrine, and serotonin in brains of mice. Science 168:849–850; 1970.
11. Flood, J. F.; Landry, D. W.; Jarvik, M. E. Cholinergic receptor interactions and their effects on long term memory processing. Brain Res. 215:177–185; 1981.
12. Flood, J. F.; Smith, G. E.; Cherkin, A. Memory retention: Potentiation of cholinergic drug combination in mice. Neurobiol. Aging 4:37–43; 1983.
13. Flood, J. F.; Smith, G. E.; Cherkin, A. Memory retention test performance in mice: Improvement by chronic oral cholinergic drug treatment. Pharmacol. Biochem. Behav. 21:169–173; 1984.
14. Flood, J. F.; Smith, G. E.; Cherkin, A. Memory enhancement in mice: Role of drugs dose and training-testing interval. Pharmacol. Biochem. Behav. 29:635–639; 1988.
15. Glossmann, H.; Hornung, R. α adrenoceptors in rat brain: Sodium changes the affinity of agonists for prazosin sites. Eur. J. Pharmacol. 61:407–408; 1980.
16. Izquierdo, I. Mechanism of action of scopolamine as an amnestic. Trends Pharmacol. Sci. 10:175–177; 1989.
17. Johnson, R. D.; Iuvone, P. M.; Minneman, K. P. Regulation of alpha-1 receptor density and functional responsiveness in rat brain. J. Pharmacol. Exp. Ther. 242:842–849; 1987.
18. Keller, R.; Oke, A.; Mefford, I.; Adams, R. N. Liquid chromatographic analysis of catecholamines routine assay for regional brain mapping. Life Sci.19:995–1004; 1976.

19. Levin, E. D.; McGurk, S. R.; Rose, J. E.; Butcher, L. L. Cholinergic-Dopaminergic interactions in cognitive performance. Behav. Neural. Biol. 54:271–299; 1990.
20. Levin, D. E.; Rose, J. E. The importance of D_1 and D_2 interactions with nicotinic and muscarinic systems for working memory. In: Levin, D. E.; Decker, M. W.; Butcher, L. L., eds. Neurotransmitter interactions and cognitive function. Boston: Birkhäuser; 1992: 144–158.
21. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
22. Melamed, E.; Globus, M.; Friedlender, E.; Rosenthal, J. Chronic L-DOPA administration decreases striatal accumulation of dopamine from exogenous L-DOPA in rats with intact nigrostriatal projections. Neurology 33:950–953; 1983
23. Molinengo, L.; Ghi, P.; Oggero, L.Orsetti, M. Behavioral and neurochemical modifications caused by chronic alpha-methylparatyrosine administration. Pharmacol. Biochem. Behav. 39:437–442; 1991.
24. Molinengo, L.; Orsetti, M.; Ghi, P.; Pastorello, B. Action of chronic choline administration on behavior and on cholinergic and noradrenergic systems. Pharmacol. Biochem. Behav. 44:587–594; 1993.
25. Molinengo, L.; Oggero, L.; Ghi, P.; Orsetti, M. Action of chronic disulfiram administration on memory decay and on central cholinergic and adrenergic system. Brain Res. 551:72–77; 1991.
26. Molinengo, L. The action of scopolamine on retrieval and memory storage in rats evaluated in the staircase maze. Behav. Neural. Biol. 59:18–24; 1993.
27. Molinengo, L.; Orsetti, M.; Pastorello, B.; Scordo, I.; Ghi, P. The action of arecoline on retrieval and storage evaluated in the staircase maze. Neurobiol. Learn. Memory 63:167–173; 1995.
28. Ng, K. Y.; Chase, T. N.; Colburn, R. W.; Kopin, I. J. L-DOPA-induced release of cerebral monoamines. Science 170:76–77; 1970.
29. Packard, M. G.; White, N. M. Memory facilitation produced by dopamine agonist: Role of receptor subtype and mnemonic requirements. Pharmacol. Biochem. Behav. 33:511–518; 1989.
30. Ponzio, F.; Cimino, M.; Achilli, G.; Lipartiti, M.; Perego, C.; Vantini, G.; Algeri, S. In vivo and in vitro evidence of dopaminergic system down regulation induced by chronic L-DOPA. Life Sci. 34:2107–2116; 1984.
31. Quartermain, D.; Judge, M. E.; Leo, P. Attenuation of forgetting by pharmacological stimulation of aminergic neurotransmitter systems. Pharmacol. Biochem. Behav. 30:77–81; 1988.
32. Stadler, H.; Lloyd, K. G.; Gadea-Ciria, M.; Bartholini, G. Enhanced striatal acetylcholine release by chlorpromazine and its reversal by apomorphine. Brain Res. 55:476–480; 1973.
33. White, N. M.; Viaud, M. Localized intracaudate dopamine D_2 receptor activation during the posttraining period improves memory for visual or olfactory conditioned responses in rats. Behav. Neural Biol. 55:255–269; 1991.
34. White, N. M.; Packard, M. G.; Seamans, J. Memory enhancement by posttraining peripheral administration of low doses of dopamine agonist: Possible autoreceptor effect. Behav. Neural Biol. 59:230–241; 1993.
35. Yamamura, H. L.; Snyder, S. H. Muscarinic cholinergic binding in rat brain. Proc. Natl. Acad. Sci. USA 71:1725–1729; 1974.
36. Zis, A. P.; Fibiger, H. C.; Phillips, A. G. Reversal by L-DOPA of impaired learning due to destruction of the dopaminergic nigroneostriatal projection. Science 185:960–962; 1974.