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Effects of L-DOPA on Behavior and on Brain Amines in Mice Treated with 6-Hydroxydopamine[1]

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BARNES, L., F. CANN, A. G. KARCZMAR, G. KINDEL AND V. G. LONGO. *Effects of L-DOPA on behavior and on brain amines in mice treated with 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 1(1) 35–40, 1973.–Mice treated intracerebrally with 6-OH dopamine (6-OHDA) developed exaggerated behavioral response to L-DOPA; the response was evaluated by employing a rating scale of mouse behavior. Presence of a monamine oxidase inhibitor was not necessary for the occurrence of the maximal response to L-DOPA. The hypersensitivity to L-DOPA appeared as soon as 4 hr after 6-OHDA treatment and was still marked 30 days later. Norepinephrine (NE) and dopamine (DA) brain content was drastically reduced in 6-OHDA treated animals; administration of L-DOPA to these animals increased significantly the brain levels of these two amines. Acetycholine levels were not affected by 6-OHDA treatment throughout the 20 days of the testing period. α-methyl DOPA prevented the exaggerated L-DOPA response. These data suggest that L-DOPA hypersensitivity is due to NE and DA formed by L-DOPA, and to the decrease of their uptake by the nerve terminals destroyed by the 6-OHDA treatment.

L-DOPA 6-Hydroxydopamine Norepinephrine Dopamine α-Methyl DOPA α-Methyl-p-tyrosine Acetylcholine Catecholamines Catecholamines on behavior

WHEN injected into the brain or into the cerebrospinal fluid of experimental animals, 6-hydroxydopamine (6-OHDA) selectively destroys catecholaminergic nerve terminals in the central nervous system [14]. This specific neurotoxicity induces behavioral effects which include symptoms of depression ranging from diminished exploration to adipsia, aphagia and catatonia. Many authors related this phenomenon to the marked reduction in brain dopamine (DA) and norepinephrine (NE) content consistently found in 6-OHDA treated animals; yet, a few days after treatment, animals which still exhibit low levels of brain catecholamines are overtly indistinguishable from normal ones. Moreover, pharmacological maneuvers which prevented catecholamine depletion did not influence the behavioral depression due to intraventricular administration of 6-OHDA to rats [20]. To explain the recovery of the behavioral deficit in the absence of complete neurochemical recovery, Ungerstedt [24] proposed that the denervation increases the sensitivity of the receptors which can then function adequately even in the presence of low levels of catecholamines.

The present experiments were designed to test the sensitivity of central catecholaminergic receptors by administering L-DOPA at various times after intracerebral injection of 6-OHDA to mice, and by employing a specific behavioral test to evaluate the central effects of L-DOPA. Indeed, the sensitization of central catecholamine receptors should be reflected by an enhanced pharmacological response to the L-DOPA administration; several drugs were used to further analyze the L-DOPA response after 6-OHDA. We also attempted to relate the behavioral changes to brain levels of catecholamines. In addition, to test the specificity of the 6-OHDA effect, and also since an imbalance of the catecholamines may induce changes in acetycholine (ACh) levels [9,27] brain ACh was also measured.

MATERIALS AND METHOD

A total of 300 ICR adult male mice, weighing 20–30 g were used. They were housed 5–7 in a cage and given food and water ad lib. Intracerebral injections were made using a 3.0 mm long, 27 gauge needle attached to a microsyringe according to the method of Haley and McCormick [10].

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6-OHDA hydrobromide was dissolved in 1:1,000 aqueous solution of ascorbic acid immediately before use, resulting in a final concentration of 10 mg/ml, calculated in reference to the weight of the base. The volume injected was 10 μl (i.e., 100 μg of 6-OHDA); the same volume of ascorbic acid solution was injected intracerebrally to mice which served as controls. L-DOPA was dissolved in saline with sufficient 0.1 N HCl to produce a final 10 mg/ml solution at pH 3.5; pargyline was dissolved in water and was administered by stomach tube. Other drugs employed in this study were α-methyl DOPA and α-methyl-p-tyrosine methyl ester; they were dissolved in saline and administered intraperitoneally.

Evaluation of the response of the mice to L-DOPA was carried out as described by Everett [6]. In brief, groups of four to six animals treated IP with L-DOPA, 100 mg/kg, were placed in large (40 cm dia.) round containers; during one hour after the injection the mice were evaluated every 10 min for the presence of piloerection, salivation and Straub tail phenomenon as well as for reactivity to external stimuli (evidenced by jumping, squeaking, running) and aggressive and stereotypic behavior. The score of +1 was assigned to animals showing slight salivation, slight increase in motor activities, tail in either an extended or vertical position (Straub tail Grade 1–2), and piloerection; +2 score mice exhibited distinct motor activity and salivation, slight hyperactivity, tail extended horizontally in rostral direction (Straub tail Grade 3), and exophthalmos; +3 score was characterized additionally by marked irritability, hyperreactivity, jumping, squeaking, running and stereotypic behavior (for details, cf. Everett, [6]). A global score of +1, +2, or +3 was assigned to each animal by two independent observers, who were unaware of the treatment; without L-DOPA treatment none of the above symptoms were present and the baseline score was essentially 0. The percentage of the animals in each group exhibiting a given score was calculated as the mean of the percentages obtained by the two observers (cf. Table 1).

Measurements of the whole brain levels of dopamine (DA), norepinephrine (NE) and acetylcholine (ACh) were carried out. The amine content was assessed in 6-OHDA and vehicle treated mice as well as after the L-DOPA challenge. Mice were killed by decapitation; the entire brain, with exception of the cerebellum and olfactory lobes, was weighed and frozen in liquid nitrogen. Assays were carried out on single brains; groups of at least four animals were used for each determination. The data obtained were averaged, and the standard error calculated. The method of Carlsson and Waldeck [5] as modified by Glisson *et al.* [9] was used for the determination of DA and NE. After homogenization in perchloric acid, the purification and separation of NE and DA were carried out using ion exchange column chromatography. The two amines were then determined fluorometrically in an Aminco-Bowman spectrophotofluorometer. This method may present certain difficulties in experiments involving L-DOPA administration as the NE and DA readings may be affected by the presence of unmetabolized L-DOPA. Indeed, in this method [5] ferricyanide and iodine oxidation are employed to obtain the NE and DA readings, respectively, and L-DOPA oxidized by idoine and ferricyanide exhibits fluorescence spectra similar although not identical to those of DA and NE, respectively. However, it was established at present that upon addition of L-DOPA to DA and NE recovery standards the readings did not show values higher than expected for either NE and DA. While this could be due to elution of DOPA in the first 3 ml of the acid eluate [9], we established at present that any of bound DOPA was eluted following the 40 ml water wash of the column prior to actual elution with HCl.

Bioassay of the levels of ACh was carried out on the guinea pig ileum by the methods of Turner [23], and Blaber and Cuthbert [2]. Two brain samples were employed for each experimental series, duplicate readings were carried out per each sample. Acid extraction was used; thus, the values obtained reflect the brain concentrations of total (including labile and bound) ACh. Specific antagonists were used to correct for the presence of histamine, serotonin and substance P. As the assay is based on two regression lines, obtained from the sample (unknown) and from the standard ACh solutions, each value was tested for parallelism of the regression and for the 95% confidence limits. The *t*-test was carried out and *p* values calculated to establish the significance of the difference between any two values.

TABLE 1

RESPONSE TO L-DOPA CHALLENGE IN 6-OHDA TREATED MICE

Time After 6-OHDA Treatment	Group	% of Population Showing the Response* +1	+2	+3
4Hr	Vehicle	40	60	0
	6-OHDA	22	44	33
8 Hr	Vehicle	50	40	10
	6-OHDA	7	7	86
16 Hr	Vehicle	29	57	14
	6-OHDA	7	7	86
24 Hr	Vehicle	40	40	20
	6-OHDA	0	29	71
2 Days	Vehicle	70	30	0
	6-OHDA	0	0	100
10 Days	Vehicle	100	0	0
	6-OHDA	0	10	90
20 Days	Vehicle	80	20	0
	6-OHDA	6	35	59
30 Days	Vehicle	100	0	0
	6-OHDA	0	25	75

*The percentages represent averages of two independent evaluations of the +1 to +3 L-DOPA response carried out by two observers (cf. Method).

RESULTS

Mice treated with 100 μg of 6-OHDA intracerebrally first exhibited excitation, running motion and, sometimes,

convulsions. This behavior which amounted essentially to increased psychomotor activity, was qualitatively different from that observed after L-DOPA challenge. Within a period of 2–3 hr these acute effects subsided and the animals became depressed and lethargic; mortality rates were approximately 30% at 24 hr [19]. Approximately 80% of the surviving animals recovered within 2 days of the treatment; a week later all the treated animals were indistinguishable, on the basis of gross visual examination, from those treated with the vehicle alone.

Behavioral Effects of L-DOPA in 6-OHDA Treated Mice

Three different mice populations were exposed to L-DOPA, 100 mg/kg, challenge, 2, 10 and 20 days after the intracerebral injections of 6-OHDA; in each case 4 groups of mice, 4 mice per group, were used. Each L-DOPA challenge was carried out 8 hr following oral administration of pargyline, 40 mg/kg; identical experiments were carried out with controls. Administration of L-DOPA to controls induced piloerection, exophthalmos, Grade 1 Straub tail phenomenon (cf. Method), and slightly increased the animal's reactivity to stimuli. The scoring of the control groups was usually +1 and only in 10% of animals it reached +2 score. In the case of 6-OHDA treated animals the DOPA challenge caused characteristic, abnormal behavior described in detail by Everett and Weigand [7]. This was true irrespective of whether L-DOPA challenge was tested in 6-OHDA treated animals at the time of the 6-OHDA induced depression (2 days after treatment, cf. above) or after the recovery took place. In other words, in the depressed animals L-DOPA did not induce a return to normal behavior but caused instead the typical L-DOPA syndrome. The 6-OHDA treated animals exhibited, within five min after injection of L-DOPA, hyperreactivity, squeaking, piloerection, exophthalmos, intense (Grade 3, cf. Method) Straub phenomenon and spontaneous, prolonged jumping and running; a few of them displayed aggressive and/or stereotypic behavior. They may be best described as hyperreactive and hyperirritable. Potentiation of the response to L-DOPA was still present on the tenth and twentieth day after treatment. The scoring of the 6-OHDA treated groups was always +3.

In view of the marked potentiation obtained in these initial trials, further experiments were carried out without pargyline treatment. L-DOPA challenge was evaluated 2, 10 and 20 days after 6-OHDA or vehicle treatment; at each time, at least four groups of mice, 4 mice per group were employed in both the experimental or control series. At all times, the score recorded was of the same intensity as that observed in the previous experiments in which pargyline was used prior to L-DOPA, at least 75% of animals exhibiting the 3+ score. The control mice exhibited a mild reaction to L-DOPA which only in 25% of animals exceeded the +1 score at any time.

Since inhibition of MAO did not seem necessary to evidence the potentiation phenomenon, also the subsequent trials were carried out without pargyline. In these experiments, the development and duration of the potentiation of the L-DOPA response was investigated in more detail. 6-OHDA treated mice were challenged with L-DOPA given 4, 8 and 16 hr, and 1, 2, 10, 20 and 30 days after 6-OHDA or vehicle treatment. At each time, at least four groups of mice, four mice per group were employed in the experimental or control series. An increased response to L-DOPA as compared to that observed with the controls was already noticeable four hours after treatment; at this time, approximately 80% of mice exhibited 2+ and 3+ scores, while no control mice exhibited a score of more than 2+ (Table 1). This increased sensitivity to L-DOPA of 6-OHDA treated mice maximized 1–2 days after treatment; at these times, from 70–100% of mice responded at the +3 level. This exaggerated response was still intense after 10 and 20 days, diminishing slightly on the thirtieth day (Table 1).

Effects of Drugs on the Potentiation of the L-DOPA Response Induced in the 6-OHDA Treated Mice.

α-Methyl DOPA. Eight groups of four animals were used in these experiments. They were treated with 6-OHDA, and received 200 mg/kg IP of α-methyl DOPA on the following day; on the next day, they were given an additional dose of 200 mg/kg of α-methyl DOPA, 4 hr prior to the L-DOPA challenge. α-Methyl DOPA deepened the behavioral depression induced by 6-OHDA; the animals crouched in a corner of the cage with their eyes closed, and reacted sluggishly to stimuli. The administration of L-DOPA resulted in a very slight stimulation; no jumping or running were noticed, and the response was evaluated as the score of less than 1. On the other hand, when only one dose of α-methyl DOPA (200 mg/kg) was given on the second day after the 6-OHDA treatment four hours before L-DOPA, the mice showed the usual +3 reaction.

α-Methyl-p-tyrosine (αMPT). Four groups of four animals each were used in these experiments. They were treated with 6-OHDA, and on the following day received 250 mg/kg IP of the methyl ester of αMPT. On the next day they were challenged with L-DOPA, and displayed the usual +3 reaction.

Brain Amine Content

All the biochemical experiments were carried out concomitantly with the behavioral tests. The data reflect the levels of catecholamines following 6-OHDA administration as well as after L-DOPA challenge in 6-OHDA treated mice; pargyline was not employed in these animals. The animals employed in the neurochemical tests were selected at random from the pertinent experimental mice groups.

Norepinephrine and dopamine. Brain levels of these two amines were measured 2, 10 and 20 days after 6-OHDA or control treatment. All all time intervals both amines were drastically reduced in 6-OHDA treated animals. The effect seemed maximal on the tenth day, and some recovery appeared to have occurred by the twentieth day (Table 2); however, even on the twentieth day after 6-OHDA treatment the amine levels in the treated animals were approximately 1/3 of the controls in the case of NE and 1/4 of the controls in that of DA. Table 2 summarizes all the data.

The levels of DA and NE were also measured in animals exposed to L-DOPA challenge 2 and 10 days after 6-OHDA treatment; the mice were sacrificed 30 min following the administration of L-DOPA, 100 mg/kg. At 2 days, L-DOPA increased significantly levels of DA and NE; the levels of both NE and DA increased approximately twofold, without however reaching the control levels (Table 3). Similar increases were recorded following DOPA 10 days after 6-OHDA treatment; in fact, the increments were significantly higher than those recorded on the second day.

TABLE 2

BRAIN CATECHOLAMINES AT VARIOUS TIMES AFTER INTRACEREBRAL ADMINISTRATION OF 6-OHDA TO MICE

		Vehicle	6-OHDA	% Control
2 Day	NE	0.42 ± 0.04 (7)	0.13 ± 0.02 (9)	32.2
	DA	1.10 ± 0.06 (7)	0.14 ± 0.02 (9)	13.0
10 Day	NE	0.28 ± 0.03 (9)	0.06 ± 0.01 (16)	20.6
	DA	0.91 ± 0.06 (9)	0.11 ± 0.02 (16)	12.2
20 Day	NE	0.42 ± 0.03 (12)	0.14 ± 0.01 (13)	32.5
	DA	0.94 ± 0.04 (12)	0.24 ± 0.02 (13)	25.9

Whole brain DA and NE content in μg/g wet weight. Results are expressed as the mean ± S.E. The figures in parenthesis indicate the number of animals used for each assay. The differences between the vehicle and 6-OHDA values were significant of the level of $p<0.0005$.

Neither on Day 2 nor on Day 10 did L-DOPA induce increases in NE or DA in the case of vehicle treated controls; in fact, a significant reduction in DA was recorded on the Day 10.

Acetylcholine. Brain levels of ACh were not significantly modified at either 2, 10 or 20 days after 6-OHDA treatment. The relevant results are shown in Table 4.

DISCUSSION

The present experiments demonstrated that mice treated intracerebrally with 6-OHDA develop, as soon as 4 hr after treatment, a supersensitivity to parenterally injected L-DOPA. This phenomenon was still present after 30 days. It should be stressed that at that time the 6-OHDA treated animals, which initially exhibited the well-known depression and lethargy [19], were overtly indistinguishable from the untreated mice.

An increased locomotor response to L-DOPA was described by Uretsky and Schoenfeld [25] in rats treated intraventricularly three weeks before with 6-OHDA. In parallel biochemical studies, these authors demonstrated, in agreement with others [12,14], the decrease in NE and DA following the 6-OHDA treatment; this was confirmed at present. More particularly, they found that on the twenty-

TABLE 3

BRAIN NE AND DA LEVELS 30 MIN AFTER L-DOPA ADMINISTRATION TO 6-OHDA AND VEHICLE TREATED MICE

	2 Days after 6-OHDA Treatment			
	Vehicle	Vehicle + L-DOPA	Sig.	% Control
NE	0.37 ± 0.02 (8)	0.44 ± 0.02 (8)	$p<0.05$	119
DA	1.08 ± 0.08 (8)	1.11 ± 0.04 (8)	N.S.	103
	6-OHDA	6-OHDA + L-DOPA	Sig.	
NE	0.09 ± 0.01 (8)	0.19 ± 0.02 (8)	$p<0.005$	211
DA	0.19 ± 0.01 (8)	0.30 ± 0.03 (8)	$p<0.005$	158
	10 Days after 6-OHDA Treatment			
	Vehicle	Vehicle + L-DOPA	Sig.	
NE	0.44 ± 0.05 (6)	0.34 ± 0.03 (6)	N.S.	77
DA	0.99 ± 0.07 (6)	0.71 ± 0.06 (6)	$p<0.01$	72
	6-OHDA	6-OHDA + L-DOPA	Sig.	
NE	0.14 ± 0.01 (8)	0.33 ± 0.03 (7)	$p<0.0005$	236
DA	0.28 ± 0.03 (8)	0.81 ± 0.05 (7)	$p<0.0005$	289

Whole brain NE and DA content in μg/g wet weight two and ten days after intracerebral injection of 6-OHDA. The results are expressed as the mean ± S.E. The figures in parenthesis indicate the number of animals used for each assay. After L-DOPA, the brain content of NE and DA was markedly increased in 6-OHDA treated animals. Significance of the difference between various groups was calculated using the unpaired student t test, N.S. = not significant.

TABLE 4

BRAIN LEVELS OF ACETYLCHOLINE IN MICE AT VARIOUS TIMES AFTER INTRACEREBRAL ADMINISTRATION OF 6-OHDA

	Vehicle	6-OHDA
2 Day	1.16 ± 0.07 (8)	1.22 ± 0.05 (11)
10 Day	1.27 ± 0.14 (6)	1.27 ± 0.08 (9)
20 Day	1.30 ± 0.01 (2)	1.20 ± 0.05 (3)

Whole brain ACh content in μg/g wet weight; results are expressed as the mean ± S.E. The figures in parenthesis indicate the number of determinations for each experiment (2 brains pooled per determination). The brain concentration of ACh in normal untreated mice was 1.30 μg/g ± 0.10 (8 determinations).

first day after 6-OHDA L-DOPA induced smaller increase of DA in the 6-OHDA treated rats than in the controls. In our experiments, we confirmed the increase in DA after DOPA described by Uretsky and Schoenfeld [25,26] on the second and tenth days following 6-OHDA treatment (Table 3); our preliminary data [1] indicate that this situation prevailed also 20 days after 6-OHDA treatment, closely paralleling the data obtained in rats on the twenty-first day following 6-OHDA treatment [25]. Since in our hands DOPA did not increase DA and NE levels in the controls, the behavioral response to DOPA in 6-OHDA treated mice could have been due to increased levels of NE and DA, while Uretsky and Schoenfeld [25] who found an increase in catecholamines in their controls as well did not believe that DOPA hypersensitivity of 6-OHDA treated rats may be attributed to the catecholamine increment. The discrepancy between our data and these of Uretsky and Schoenfeld [25] may be due to the difference in the species employed by the two groups. Furthermore, Uretsky and Schoenfeld [25] measured DA and NE 75 min while in our case the catecholamines were measured 30 min after L-DOPA challenge. Finally Uretsky and Schoenfeld [25] employed, contrary to ourselves, a peripherally acting decarboxylase inhibitor.

Increased responsiveness of 6-OHDA treated animals was described also by other authors. Jalfre and Haefely [13] described increased sensitivity in so treated rats to the central effects of apomorphine and haloperidol. Ungerstedt [24] described an increased rotation response to L-DOPA in rats with unilateral degenerative lesions of the nigrostriatal system induced by 6-OHDA stereotaxically injected into the substantia nigra. He attributed his results to an increased sensitivity to DA of denervated striatal receptors while Jalfre and Haefely [13] hypothesized that drugs reach more easily the receptors when they become uncovered after the degeneration of the nerve terminals.

It is generally accepted that the central effects observed after L-DOPA are due to its metabolites, DA and, to a lesser extent, NE. It is possible to obtain a marked enhancement of the effects of L-DOPA by a variety of manipulations such as pretreatment with MAO inhibitors [7], with peripheral decarboxylase inhibitors [16], or with compounds which inhibit the catecholamines re-uptake by the nerve terminals such as the tricyclic antidepressants [6] and the antiparkinsonian drugs [8]. A possible, common explanation may be suggested on the basis of the present observations for the above instances of the L-DOPA potentiation as well as for the enhancement induced by 6-OHDA. Destruction of nerve endings due to 6-OHDA impairs uptake and removal of catecholamines from the synaptic cleft and diminishes decarboxylase activity [26]. Following the intracerebral injection of 6-OHDA, the histochemical changes of the central adrenergic terminals are noted within a few min, although the neuronal degeneration takes longer; [24]; at the periphery, the actual destruction of the nerve terminals may occur even faster [21]. Altogether, the diminution of the NE levels and the impairment of the NE uptake may occur even in the case of the brain as early as to coincide with the early time of the increased DOPA response reported at present. Upon L-DOPA administration, however, DA may still be formed by the catecholaminergic neurons which escaped destruction as well as by the noncatecholamine neurons which contain a decarboxylase enzyme such as the serotonergic neurons which are not affected by 6-OHDA; evidence that DA and NE are still formed in the brain of 6-OHDA treated animals is provided by the present experiments as well as by the data of Uretsky and Schoenfeld [25]. The transmitter thus formed can act longer on the receptor because of the inefficient re-uptake mechanisms. This hypothesis is consistent with our findings that α-methyl DOPA prevented almost completely the L-DOPA response; this could have been due to the formation of false transmitters [15], or to the inhibition of decarbaxylase and the concomitant block of the formation of catecholamines. A marked decarboxylase inhibition may be expected 24 hr following the administration of two doses of α-methyl DOPA [17], although it is not clear why the single administration of the drug was ineffective after four hours in blocking the DOPA response as at that time decarboxylase should be significantly inhibited [11]. Moreover, our hypothesis is consistent with the lack of the effect of α-MPT on the DOPA metabolism. It should be added that the impairment of the uptake was proposed as a major mechanism underlying the sympathetic supersensitivity phenomenon [22]. Furthermore, this line of reasoning also explains the return of the behavior to normal after 6-OHDA in the absence of complete restoration of the levels of DA and NE; this would be expected because of supersensitivity and of the capacity of the brain to form catecholaminergic mediators.

Admittedly, the assumption of the early destruction of the nerve terminal may be the weak point of our hypothesis, particularly as the hypersensitivity recorded by us occurred as early as 4 hr after 6-OHDA. Thus, the explanations offered by the previous authors [13,24] cannot be excluded at present; furthermore, 6-OHDA treatment may increase brain penetration of L-DOPA which is consistent with our demonstration of the increased levels of catecholamines following the administration of L-DOPA to 6-OHDA treated animals.

Finally, our data provide additional evidence as to the specificity of the action of 6-OHDA. At the periphery, it was shown that 6-OHDA destroys the adrenergic innervation of the iris leaving intact its cholinergic nerves [21]. Similar specificity seems to exist with regard to the central action of 6-OHDA, as in our hands as well as in those of Jacks *et al.* [12] the 6-OHDA treatment did not affect the brain levels of ACh.

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