# Effects of L-Dopa and L-3-Methoxytyrosine on D-Methamphetamine-Induced Motor Activity and Seizures Induced by Electroshock (37475)

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The finding that patients with Parkinson's disease had markedly reduced levels of dopamine in the corpus striatum (1) led to the successful use of L-dopa, the precursor that is converted to dopamine in the brain, as a treatment for Parkinsonism (2, 3). A major metabolite of L-dopa is its 3-O-methyl derivative, L-3-methoxytyrosine (L-3-MOT), which persists in blood and other tissues for a considerably longer period of time than L-dopa (4, 5). Subsequently, it was found that L-3-MOT was partially demethylated to L-dopa in rats (6), and man (7). These results led to the trial of L-3-MOT as a treatment for Parkinson's disease. It was reported that the administration of L-3-MOT for from 1 to 3 months led to improvement in the majority of patients (8, 9). These results suggested that the administration of L-3-MOT might lead to the formation of sufficient dopamine to produce the favorable clinical response. They also suggested that L-3-MOT might show some responses similar to L-dopa in laboratory test models.

In order to test this hypothesis we have examined L-3-MOT in 2 tests used in our laboratory to measure the ability to a compound to substitute for L-dopa.

*Methods.* L-Dopa was supplied by the Chemical Division of Parke, Davis and L-3-MOT was purchased from Monsanto Chemicals. The iproniazid was generously supplied by Roche Laboratories. Other drugs were purchased from commercial sources.

The effects of L-dopa and L-3-MOT on the tonic extensor seizures induced by electroshock were determined in male albino mice of the Webster strain weighing between 18 and 26 g. After the administration of drugs to the mice, tonic extensor seizures were produced using a Hans Technical Associates electroshock apparatus. Sixty cycle shocks of 250 msec duration and of controlled current strengths were delivered through small bulldog clips attached to the ears. At least four groups of 10 mice each (in which convulsions occurred in from 10 to 90% of the animals/group), were used to establish the seizure threshold. The current strengths required to produce seizures in 50% of the shocked mice were estimated from probit log-amperage regression lines obtained using Finney's method (10) of probit analysis and a computational computer program.

The mice were injected with the monamine oxidase inhibitor iproniazid, 3 hr before giving L-dopa or L-3-MOT. Treatment with iproniazid, which does not itself raise the seizure threshold, is required to show an elevation of the seizure threshold by L-dopa.

The effects of L-dopa and L-3-MOT on the increase in motor activity induced in rats by methamphetamine, as measured in jiggle cages (11), were determined with Sprague Dawley rats weighing between 180 and 200 g. Two hours prior to the test some groups of rats were given the tyrosine hydroxylase inhibitor, DL-α-methyltyrosine.

Brain levels of norepinephrine, dopamine, and dopa were determined using a modification of the method of Anton and Sayre (12). The catechols were adsorbed on 250 mg of alumina in 6 mm diameter columns and eluted with 0.2 *N* acetic acid. The levels of dopamine and dopa in the eluates were calculated from fluorescence measurements at pH 5.5 and 2.1 in separate samples of the eluates.

Duncan's multiple *t* test was used to assess the significance of differences in jiggle cage

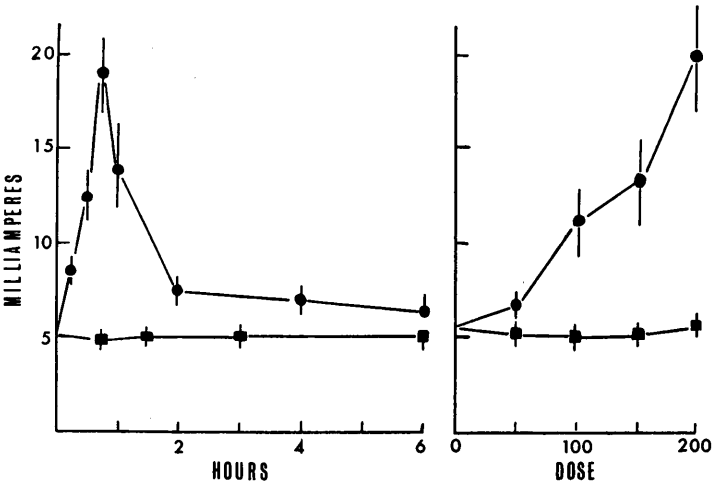


FIG. 1. Current strength (*m*A) required to produce extensor seizure in 50% of the mice. See text for method of calculation. Left diagram, at intervals after 200 mg/kg ip of L-3-MOT (■) or L-dopa (●). Right diagram, at 45 min after various doses of L-3-MOT or L-dopa. In both cases the mice were treated with 100 mg/kg ip of iproniazid 3 hr before testing.

activity and brain levels of catechols among the treatment groups (13).

All drugs were administered as aqueous solutions. When necessary, just sufficient HCl was added to bring the drugs into aqueous solution. Doses are expressed as active free base. Drug solutions contained sufficient drug to deliver the correct dose when 0.01 ml of the solution was given/g of body weight. Appropriate controls were carried along with each experiment, control animals being dosed with vehicle at pretreatment and/or treatment times.

*Results.* The time- and dose-response relationships for the effects of L-dopa and L-3-MOT on the threshold of electrically induced seizures are shown in Fig. 1. L-Dopa caused a dose dependent increase in the threshold. With 200 mg/kg of L-dopa this effect reached a maximum 45 min after the administration of L-dopa. At doses of up to 200 mg/kg L-3-MOT did not increase the extensor seizure threshold.

Combinations of 75 mg/kg of L-dopa given intraperitoneally, and L-3-MOT given intravenously at doses of from 25 to 200 mg/kg

TABLE I. Effect of Combinations of L-Dopa and L-3-MOT on the Tonic Extensor Seizures Induced in Mice by Electroshock.

| Dose of L-Dopa or L-3-MOT (mg/kg)[a] | Current strength (mA) required to produce seizures in 50% of the mice with 95% confidence limits; L-3-MOT given by: | |
|---|---|---|
| | Intraperitoneal injection | Intravenous injection |
| None | 5.53 (5.10– 6.01) | 5.79 ( 5.36– 6.18) |
| L-Dopa (75) | 9.50 (8.71–10.35) | 10.97 (10.12–11.80) |
| L-Dopa (75) + L-3-MOT ( 25) | 8.68 (8.00– 9.45) | 10.63 ( 9.76–11.49) |
| L-Dopa (75) + L-3-MOT ( 50) | 8.80 (8.00– 9.64) | 11.55 (10.74–12.46) |
| L-Dopa (75) + L-3-MOT (100) | 9.02 (8.33– 9.78) | 10.10 ( 9.36–10.83) |
| L-Dopa (75) + L-3-MOT (200) | 7.45 (6.88– 8.20) | 10.67 ( 9.81–11.43) |

[a] All mice were injected with 100 mg/kg of ipronazid 225 min before the test. L-Dopa was given by intraperitoneal injection 45 min before the test. L-3-MOT was given by either intraperitoneal or intravenous injection 45 min before the test.

TABLE II. Effect of L-Dopa and L-3-MOT on Mouse Brain Levels of Dopamine and Dopa.

| Treatment[a] | Brain levels ($\mu$g/g of whole brain)[b] Dopamine | Dopa |
|---|---|---|
| Vehicle | 1.01 ± 0.04 | —[c] |
| Iproniazid | 1.05 ± 0.04 | —[c] |
| Iproniazid + L-dopa | 9.15 ± 1.05 | 6.97 ± 1.07 |
| Iproniazid + L-3-MOT | 1.17 ± 0.12 | —[c] |
| Iproniazid + L-dopa + L-3-MOT | 5.67 ± 0.60[d] | 4.03 ± 0.56[d] |

[a] Iproniazid, 100 mg/kg; L-dopa, 200 mg; and L-3-MOT, 200 mg/kg were given by intraperitoneal injection on the same schedule described in Table I.

[b] Average of values for 10 mice with standard error of the mean.

[c] Below the limit of detection.

[d] Significant difference from the value obtained in the group given iproniazid + L-dopa, Duncan's multiple $t$ test $p < 0.05$.

did not change the seizure threshold from that seen with L-dopa alone. When the L-3-MOT was given intraperitoneally, there was a partial inhibition of the increase in seizure threshold induced by L-dopa (Table I).

Table II shows the effects on brain levels of dopamine and dopa of intraperitoneal injections of 200 mg/kg of L-dopa and L-3-MOT, alone or in combination, in mice pretreated with ipronazid. L-Dopa but not L-3-MOT gave marked increases in the brain levels of both catechols. The combination of L-dopa and L-3-MOT gave significantly lower levels of dopamine and dopa than L-dopa alone.

The inhibitory action of L-3-MOT, on the elevation of the threshold for electrically induced seizures produced by L-dopa, was barely significant at the highest dose tested. Therefore, it was decided to explore the interaction of L-dopa and L-3-MOT in a second test system.

The effects of L-dopa given by subcutaneous injection, and L-3-MOT given by intraperitoneal injection, alone or in combination, on the motor activity induced in rats by D-methamphetamine, are shown in Table III. L-Dopa enhanced the motor stimulation induced by methamphetamine. This effect was more pronounced in those rats treated with $\alpha$-methyltyrosine. In these animals, L-3-MOT markedly inhibited the L-dopa-induced enhancement of motor activity in rats treated with methamphetamine.

Table IV gives the brain levels of dopa, dopamine, and norepinephrine in rats treated with $\alpha$-methyltyrosine and L-dopa, alone or with L-3-MOT.

*Discussion.* The elevation by L-dopa, of the threshold for electrically induced tonic extensor convulsions in mice treated with iproniazid, can be attributed to the conversion of L-dopa to dopamine and/or norepinephrine in the central nervous system. It has been shown that treatment with drugs that alter brain levels of the biogenic amines has a marked influence on the threshold for electrically induced seizures (14). The failure of

TABLE III. Effect of L-Dopa and L-3-MOT on Motor Activity Induced in Rats by D-Methamphetamine as Measured in Jiggle Cages.

| Treatment[a] | Jiggle cage counts in the 2 hr following the injection of D-methamphetamine[b] |
|---|---|
| D-Methamphetamine alone | 164.8 ± 18.5 |
| + L-dopa | 239.3 ± 85.4 |
| + L-3-MOT | 104.1 ± 16.3 |
| + L-3-MOT, L-dopa | 114.6 ± 18.7 |
| + DL-$\alpha$-methyltyrosine | 19.3 ± 7.5 |
| + DL-$\alpha$-methyltyrosine, L-dopa | 65.2 ± 10.9 |
| + DL-$\alpha$-methyltyrosine, L-3-MOT | 18.5 ± 4.1 |
| + DL-$\alpha$-methyltyrosine, L-3-MOT, L-dopa | 12.2 ± 2.1[c] |

[a] The route, dose, and time at which the compounds were given before the measurement of jiggle cage activity were: DL-$\alpha$-methyltyrosine, 32 mg/kg, subcutaneous, 120 min; L-3-MOT, 200 mg/kg, ip, 45 min; L-dopa, 128 mg/kg, subcutaneous, 30 min; D-methamphetamine, 2 mg/kg, subcutaneous, 0 time.

[b] Average of values for 6 rats with standard error of the mean. In the absence of D-methamphetamine neither L-dopa nor L-3-MOT had any effect on motor activity.

[c] Significant difference from the group given DL-$\alpha$-methyltyrosine, L-dopa, and D-methamphetamine, Duncan's multiple $t$ test $p < 0.05$.

TABLE IV. Effect of L-Dopa and L-3-MOT on Rat Brain Levels of Dopa, Dopamine, and Norepinephrine.

| Treatment[a] | Brain levels ($\mu$g/g)[b] | | |
|---|---|---|---|
| | Dopa[c] | Dopamine[c] | Norepinephrine[c] |
| Vehicle | —[d] | 0.88 ± 0.02 | 0.40 ± 0.01 |
| DL-$\alpha$-methyltyrosine | —[d] | 0.55 ± 0.01 | 0.31 ± 0.01 |
| DL-$\alpha$-methyltyrosine, L-dopa | 1.31 ± 0.11 | 2.06 ± 0.25 | 0.60 ± 0.03 |
| DL-$\alpha$-methyltyrosine, L-dopa, L-3-MOT | 0.94 ± 0.07 | 1.59 ± 0.11 | 0.51 ± 0.02 |

[a] Route, dose and time of treatment as described in Table III.
[b] Average of values for 10 rats with standard error of the mean.
[c] Values of all combinations of pairs differ significantly, Duncan's multiple $t$ test $p < 0.05$.
[d] Below the limit of detection.

L-3-MOT to have a similar effect suggests that in this test system in mice, or in rats with the stimulation of motor activity as the test procedure, L-3-MOT is not converted to L-dopa, or the L-dopa metabolite responsible for its action, at a sufficient rate to have a measurable effect. This finding is in agreement with the report of Chalmers *et al.* (15) that in intact rats given L-3-*O*-$^{14}$C-methyldopa by intraperitoneal injection, less than 10% of the dose was demethylated in a 24 hr period following treatment. Furthermore in rats with a biliary fistula, no $^{14}C$-$CO_2$ could be detected in the expired air, suggesting that demethylation of L-3-MOT for form L-dopa had occurred primarily in the gut rather than in tissues.

It should be emphasized that the present finding that L-3-MOT failed to exert similar pharmacological actions to L-dopa or give measurable increases in brain catecholamine is not in conflict with previous reports that small doses of $^{14}$C-L-3-MOT give rise to detectable amounts of $^{14}$C-dopamine in rats and man (6, 7). The studies reported here were undertaken to determine whether or not the rate of conversion of L-3-MOT to L-dopa was sufficient to give a detectable effect in tests in which L-dopa shows pharmacological activity.

The partial inhibition by L-3-MOT, of the ability of L-dopa to raise the threshold for electrically induced seizures, and to increase brain levels of dopa and dopamine, could be due to inhibition of the absorption of L-dopa from the peritoneal cavity when both drugs were given intraperitoneally. This possibility is strengthened by the lack of effect of intravenous L-3-MOT on the increase in the threshold for seizures induced by L-dopa given intraperitoneally.

It seems improbable that L-3-MOT given by intraperitoneal injection would have interfered with the absorption of L-dopa given by subcutaneous injection. It is possible that L-3-MOT interferes with the transport of L-dopa into the brain. This would account for the lower levels of dopa, dopamine, and norepinephrine found in the brains of rats given both L-dopa and L-3-MOT compared to the levels found in rats given L-dopa alone.

The results of the experiments reported here, based on short-term exposure of rats and mice to the drugs, do not provide an explanation for the efficacy reported for L-3-MOT in the therapy of Parkinsonism in man. The reason for this discrepancy may be species differences or differences in the duration of treatment.

*Summary.* L-3-Methoxytyrosine did not substitute for L-dopa, in increasing the threshold for electrically induced extensor seizures in mice, or in restoring the ability of D-methamphetamine to stimulate motor activity in rats treated with DL-$\alpha$-methyltyrosine. At high doses L-3-methoxytyrosine inhibited the effect of L-dopa in both tests. L-3-Methoxytyrosine partially inhibited the increase in brain levels of dopa, dopamine, and norepinephrine produced by L-dopa. It is suggested that L-3-methoxytyrosine may in-

terfere with the uptake of L-dopa from the peritoneal cavity and from the blood into the brain.

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