3-*O*-Methyldopa Blocks Dopa Metabolism in Rat Corpus Striatum

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3-*O*-Methyldopa (OMD) given to rats inhibits striatal uptake and utilization of L-dopa. Thus, the accumulation of L-dopa, dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid in OMD-pretreated rats after L-dopa injection is significantly lower compared with control rats. This effect of OMD is dose dependent. OMD inhibits L-dopa accumulation in the striatum after inhibition of aromatic amino acid decarboxylase activity with 3-hydroxybenzylhydrazine-HCL. This effect is not mediated through inhibition of firing in dopaminergic neurons, since the accumulation of dopamine in the striatum after γ-butyrolactone injection was also significantly reduced by OMD. It is suggested that OMD competes with L-dopa and tyrosine uptake into the brain. These findings are in line with clinical observations which indicate that high plasma levels of OMD in parkinsonian patients are associated with poor response to L-dopa. The data presented here indicate that use of catechol-*O*-methyltransferase inhibitors with L-dopa may be of value in the treatment of parkinsonian patients.

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Rapid fluctuations in the motor ability of parkinsonian patients is a major problem in chronic L-dopa therapy. Several clinical investigations have suggested that these fluctuations are associated in part with plasma levels of L-dopa [21, 22]. It is therefore reasonable to suggest that improved bioavailability of L-dopa may reduce the severity of these side effects. L-Dopa is metabolized in major part to 3-*O*-methyldopa (OMD) by catechol-*O*-methyltransferase [20]. Due to its prolonged half-life, OMD accumulates in plasma and cerebrospinal fluid of patients treated with L-dopa and reaches levels higher than those of the drug itself [8, 19]. Thus, for example, in 13 parkinsonian patients treated with L-dopa, the ratio of OMD to dopa in plasma ranged from 2.1:1 to 16:1 [15a]. High levels of OMD have been associated with dyskinesia induced by L-dopa [10] and with reduced response to therapy [18]. Moreover, when OMD was given to parkinsonian patients treated with L-dopa, it produced clinical deterioration in a dose-dependent manner [3, 5, 15]. In line with these observations, it was recently reported that parkinsonian patients with high catechol-*O*-methyltransferase activity in their red blood cells had a less favorable response to L-dopa [16]. In the experiments reported here, we studied the effect of OMD on L-dopa metabolism in rat corpus striatum.

Materials and Methods

Analytical-grade chemicals were purchased from Sigma Chemical Company (St. Louis, MO), Fisher Scientific Company (Springfield, NJ), and Bioanalytical Systems (West Lafayette, IN). L-Dopa was obtained from Calbiochem. γ-Butyrolactone, 3-hydroxybenzylhydrazine-HCl (NSD-1015), and L-4-hydroxy-3-methoxyphenylalanine (3-*O*-methyldopa, OMD) were purchased from Sigma. Iso-homovanillic acid (ISO-HVA) was kindly synthesized for us by Dr Sara Ginsburg of the Department of Neurology, Columbia University.

Male Sprague-Dawley rats weighing 180 to 250 gm were fasted for 12 hours before the experiments. All injections were given intraperitoneally. L-Dopa and OMD were given as a suspension in 0.1% methylcellulose. Rats were sacrificed by decapitation, brains were rapidly removed, and corpora striata were dissected on an ice-cold glass plate and immediately frozen on dry ice. Blood was collected with heparin, and plasma was separated by centrifugation.

Frozen corpora striata were weighed and sonicated (Kontes Micro-Ultrasonic cell disrupter) in approximately 20 vol of ice-cold 0.1 M perchloric acid containing 2 mM Na_2EDTA and 10 μl/ml of $NaHSO_3$ (0.1 M). After centrifugation (15,600 *g* for 5 minutes), 180 μl of supernatant was taken for analysis. Dopa and dopamine were isolated by the alumina extraction technique [14]. Dihydroxybenzylamine, 25 ng, was added as internal standard to quantitate recovery. Extracts were subjected to reverse-phase (C_{18}, ODS column, DuPont) high-performance liquid chromatography and eluted with 0.1 M monochloroacetate

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buffer (pH 3.05) containing 1 mM Na_2EDTA and 70 mg/L of sodium octyl-sulfate as an ion-pairing agent [6]. The flow rate was 1.0 ml/min at room temperature. The catechols were quantitated using an LC-4A glassy carbon electrode (Bioanalytical Systems) with an applied potential of 0.7 V. Typical retention times were 5.2 minutes for dopa, 7.27 minutes for dihydroxybenzylamine, and 11.6 minutes for dopamine. A Spectra-Physics graphic integrator was used for calculations [6]. The dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and HVA were determined in the perchloric extract by the diethylether extraction procedure of Hefti [12] and separated on the same chromatographic system. ISO-HVA, 10 ng, was added as an internal standard. Sodium acetate buffer, 50 mM, pH 5.0, containing 1 mM Na_2EDTA and 3.5% (v/v) propanol, served as a mobile phase. Flow rate was 1 ml/min at ambient temperature. Typical retention times were 4.0 minutes for DOPAC, 7.4 minutes for HVA, and 9.4 minutes for ISO-HVA. No ISO-HVA could be detected in homogenates of corpus striatum unless the compound was added to the assay. OMD and dopa assay in the plasma were determined fluorimetrically as previously described [9]. Results are expressed as nanograms per milligram of striatum, mean ± standard error of the mean, for 4 animals each. Statistical analysis was by two-tailed Student *t* test.

Results

EFFECT OF OMD ON DOPAMINE, DOPAC, AND HVA IN RAT STRIATUM. Rats were injected intraperitoneally with OMD, 400 $mg \cdot kg^{-1}$, and sacrificed at 15, 30, 60, 90, 120, and 240 minutes after the injection (N = 4 for each time point). In our experiment we were unable to detect any dopa formation from OMD during the time period studied. OMD also had no significant effect on dopamine, DOPAC, or HVA levels, although the dopamine level dropped from 11.03 ± 1.03 in untreated controls to 9.01 ± 0.83 one hour following OMD administration. Dopamine levels slowly returned toward basal levels at four hours (data not shown).

EFFECT OF OMD WITH TIME. Rats were injected intraperitoneally with OMD, 400 $mg \cdot kg^{-1}$, and two hours later with L-dopa, 100 $mg \cdot kg^{-1}$. Control rats were injected with L-dopa only. Animals were sacrificed at 0 to 120 minutes, and corpora striata were assayed for dopamine, DOPAC, and HVA. As shown in Figure 1, dopamine accumulation peaked 60 minutes after L-dopa injection. The dopamine level obtained at this time point was significantly lower in OMD-pretreated rats compared with L-dopa–injected controls: 9.6 ± 1.1 versus 17 ± 2.5 ($p < 0.02$). Moreover, in OMD-pretreated rats, dopamine levels obtained 60 minutes after L-dopa injection did not differ statistically from the level obtained in untreated controls. Similarly, OMD had an inhibitory effect on the accumulation of dopamine metabolites. As shown in Figure 2, DOPAC levels peaked 60 minutes after dopa injection, and the level

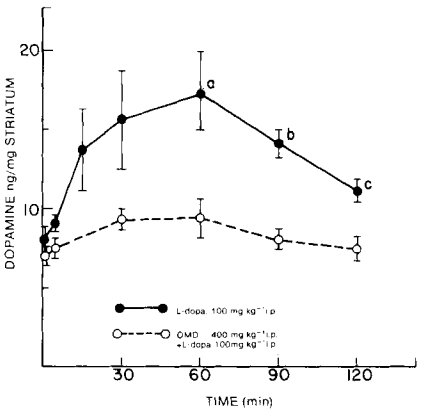


Fig 1. Effect of OMD on dopamine accumulation in rat striatum. Rats were injected intraperitoneally with OMD, 400 $mg \cdot kg^{-1}$, and two hours later with L-dopa, 100 $mg \cdot kg^{-1}$. Control rats were injected with L-dopa only. Dopamine in the striatum was determined as described in Methods. Circles represent the mean and vertical lines the SEM, ng/mg striatum, for 4 rats. a, b, *and* c: $p < 0.02$, $p < 0.005$, *and* $p < 0.025$, *respectively.*

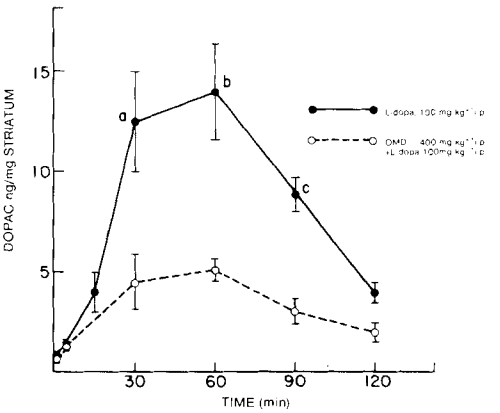


Fig 2. Effect of OMD on DOPAC accumulation in rat striatum. Rats were injected as described in Figure 1. DOPAC levels in the striatum were measured as described in Methods. Circles represent the mean and vertical lines the SEM, ng/mg striatum, for 4 rats. a, b, *and* c: $p < 0.05$, $p < 0.01$, *and* $p < 0.005$, *respectively.*

obtained in OMD-pretreated rats was significantly lower compared with L-dopa–treated controls: 5.1 ± 0.38 versus 14 ± 2.3 ($p < 0.01$). This significant decrease was maintained throughout the 30- to 90-minute period after L-dopa injection. In line with the previous observation, the peak accumulation of HVA was similarly lower in OMD-pretreated rats compared with L-dopa–injected controls: 5.4 ± 0.08 versus 11.5 ± 1.6 ($p < 0.02$) (Fig 3). This reduction was significant throughout the 30- to 120-minute period after L-dopa injection. Also, the peak level of HVA in OMD-treated rats was obtained at 90 minutes, a delay of 30 minutes compared with L-dopa–treated controls.

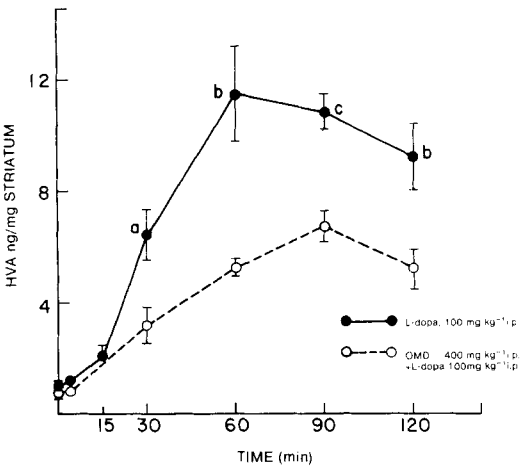
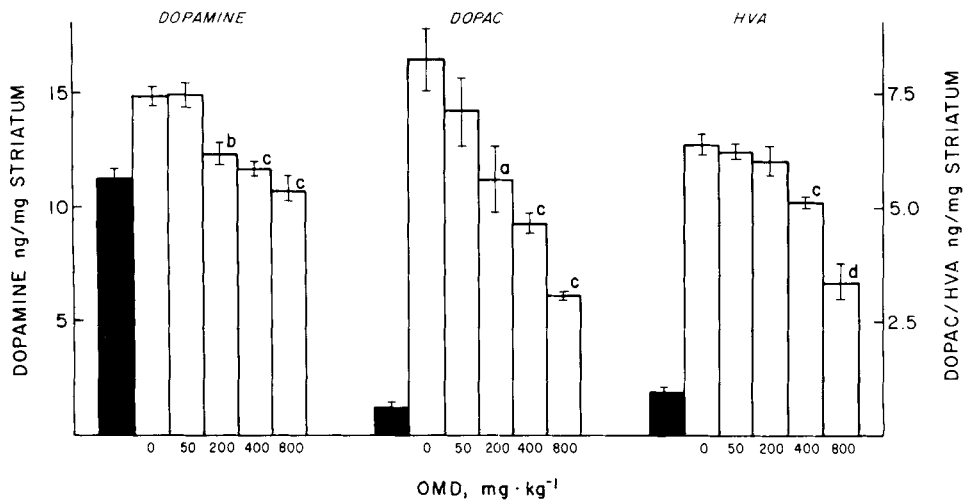


Fig 3. Effect of OMD on HVA accumulation in rat striatum. Rats were injected as described in Figure 1. HVA in the striatum was determined as described in Methods. Circles represent the mean and vertical lines the SEM, ng/mg striatum, for 4 rats. a, b, *and* c: $p < 0.05$, $p < 0.02$, *and* $p < 0.005$, *respectively.*

EFFECT OF OMD DOSE. Rats were injected with OMD at increasing concentrations (50 to 800 $mg \cdot kg^{-1}$). Two hours later they were injected with L-dopa, 100 $mg \cdot kg^{-1}$, and they were sacrificed 60 minutes after the second injection. Control rats were injected with L-dopa only. Dopamine, DOPAC, and HVA were assayed in corpora striata and compared with the levels obtained in untreated rats. Dopamine levels increased from 11.2 ± 0.5 to 14.8 ± 0.46 one hour after L-dopa injection. Pretreatment with OMD had an inhibitory effect on dopamine accumulation in a dose-dependent manner. As shown in Figure 4, at 200 $mg \cdot kg^{-1}$ of OMD the dopamine level was significantly lower than in dopa-injected controls: 12.3 ± 0.595 versus 14.8 ± 0.46 ($p < 0.02$). At 800 $mg \cdot kg^{-1}$ OMD completely abolished the increase in dopamine level following L-dopa injection. DOPAC levels rose from 0.629 ± 0.125 to 8.27 ± 0.748 following L-dopa injection. At 200 $mg \cdot kg^{-1}$, OMD attenuated this effect and the level obtained was significantly lower: 5.640 ± 0.776 ($p < 0.05$). Maximal inhibition of DOPAC accumulation (3.040 ± 0.07) was obtained at 800 $mg \cdot kg^{-1}$ of OMD, the highest dose tested (Fig 4). Similarly, OMD had an inhibitory, dose-dependent effect on HVA accumulation. HVA level increased from 0.982 ± 0.044 to 6.380 ± 0.232 one hour following L-dopa injection. A significant decrease was obtained at 400 $mg \cdot kg^{-1}$: 5.12 ± 0.131 ($p < 0.005$). This inhibitory effect was further enhanced at 800 $mg \cdot kg^{-1}$: 3.36 ± 0.425 ($p < 0.001$).

Fig 4. Effect of increasing concentrations of OMD on dopamine metabolism in rat striatum. Rats were injected with OMD at the indicated concentrations. Two hours later they were injected with L-*dopa, 100 mg* $\cdot kg^{-1}$, *and they were sacrificed one hour after* L-*dopa injection. Control rats were injected with* L-*dopa only. Solid bars represent untreated controls. Dopamine, DOPAC, and HVA were determined as described in Methods. Vertical lines represent the SEM, ng/mg striatum, for 4 rats.* a, b, c, *and* d: $p < 0.05$, $p < 0.02$, $p < 0.005$, *and* $p < 0.001$, *respectively, compared with* L-*dopa–injected controls.*

EFFECT OF OMD ON DOPA ACCUMULATION. Rats were injected with OMD, 400 $mg \cdot kg^{-1}$, and two hours later with L-dopa, 100 $mg \cdot kg^{-1}$. Control rats were given L-dopa only. Rats were sacrificed 15 minutes after L-dopa injection. Two hours after the OMD injection the plasma OMD level was 137 ± 2.9 ng/ml, while 15 minutes after L-dopa injection the peak value of that drug in plasma was 9.8 ± 3.5 ng/ml. The ratio of OMD to dopa obtained in plasma was therefore close to the ratio found in humans treated with L-dopa. As shown in Figure 5A, the dopa level obtained in OMD-pretreated rats was significantly lower compared with L-dopa–treated controls: 0.890 ± 0.200 (N = 5) versus 1.58 ± 0.190



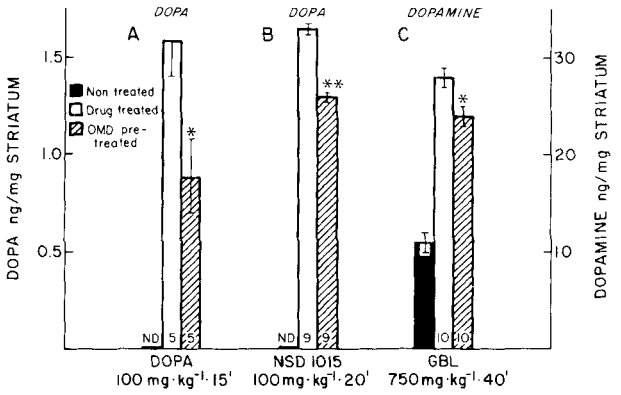


*Fig 5. Effect of OMD on (A) dopa accumulation, (B) NSD-1015–induced dopa accumulation, and (C) γ-butyrolactone (GBL)-induced dopamine accumulation in rat striatum. (A) Rats were injected intraperitoneally with OMD, 400 mg·kg⁻¹, and two hours later with L-dopa, 100 mg·kg⁻¹. Control rats received L-dopa injections only. Rats were sacrificed 15 minutes after L-dopa injection. L-dopa was assayed as described in Methods. (B) Rats were injected with OMD, 400 mg·kg⁻¹, and two hours later with NSD-1015, 100 mg·kg⁻¹. Control rats received saline injections instead of OMD. The accumulation of dopa was measured 20 minutes after NSD-1015 injection. (C) Rats were injected intraperitoneally with OMD, 400 mg·kg⁻¹, and two hours later with γ-butyrolactone, 750 mg·kg⁻¹. Control rats received saline injections instead of OMD. Dopamine accumulation was measured 40 minutes after injection of γ-butyrolactone. For all three experiments, values were compared between drug-treated and OMD-pretreated rats. * and **: $p < 0.05$ and $p < 0.025$, respectively. Solid bars represent untreated controls. ND = not detected.*

(N = 5) ($p < 0.05$). No dopa could be detected in the striatum of untreated controls.

EFFECT OF OMD ON DOPA ACCUMULATION FOLLOWING INHIBITION OF AROMATIC AMINO ACID DECARBOXYLASE ACTIVITY WITH NSD-1015. Rats were injected intraperitoneally with OMD, 400 mg·kg^{-1}, and two hours later with the dopa decarboxylase inhibitor NSD-1015, 100 mg·kg^{-1}. Control rats received saline injections instead of OMD. Animals were sacrificed 20 minutes after NSD-injection. As shown in Figure 5B, dopa accumulation was significantly lower in OMD-pretreated rats compared with saline-injected controls: 1.3 ± 0.04 (N = 9) versus 1.65 ± 0.117 (N = 9) ($p < 0.025$).

EFFECT OF OMD ON DOPAMINE ACCUMULATION FOLLOWING INHIBITION OF DOPAMINERGIC NEURONAL ACTIVITY WITH γ-BUTYROLACTONE. Rats were injected intraperitoneally with OMD, 400 mg·kg^{-1}, and two hours later with γ-butyrolactone, 750 mg·kg^{-1}, which inhibits impulse flow in dopaminergic nigrostriatal neurons. Control rats were injected with saline instead of OMD. Rats were sacrificed 40 minutes after the γ-butyrolactone injection. As shown in Figure 5C, dopamine levels increased from 11.03 ± 1.03 to 28.2 ± 1.1 (N = 10) following treatment with γ-butyrolactone. In OMD-pretreated rats, this accumulation was inhibited and the dopamine level obtained was significantly reduced: 24.5 ± 1.1 (N = 10) ($p < 0.05$).

Discussion

These experiments did not detect any formation of dopa from OMD in rat striatum, in keeping with the results of Chalmers et al [4], who were unable to demonstrate in vitro demethylation activity in rat brain tissue. We cannot support earlier reports which suggested that OMD may undergo partial demethylation in rat brain and contribute to dopa levels [1, 2].

In OMD-pretreated rats the accumulation of dopamine, DOPAC, and HVA after L-dopa injection was significantly lower than in control rats. This inhibitory effect of OMD was dose dependent. At 200 mg·kg^{-1}, OMD significantly attenuated the increase in dopamine and DOPAC levels determined 60 minutes after injection of 100 mg·kg^{-1} of L-dopa. A similar inhibitory effect on HVA accumulation was obtained at 400 mg·kg^{-1} of OMD pretreatment. OMD also significantly inhibited the accumulation of L-dopa in rat striatum 15 minutes after L-dopa injection compared with L-dopa–injected controls.

This effect of OMD is probably mediated through competition with L-dopa uptake into the brain and is consistent with the findings of Rivera-Calimlim [17] that [^{14}C]OMD is absorbed better from gut into brain than [^{14}C]dopa. Similarly, when injected simultaneously with [^{14}C]dopa into the carotid artery of the rat, OMD inhibited dopa uptake into the brain [23].

In OMD-pretreated rats the accumulation of L-dopa in the striatum after inhibition of aromatic amino acid decarboxylase activity by NSD-1015 was significantly lower compared with control rats. This indicates that L-dopa synthesis is reduced. Since the neutral amino acids L-dopa and tyrosine both share the same transport system [11], it is possible that OMD also interferes with brain uptake of tyrosine, the precursor of L-dopa. Kehr [13], who demonstrated a similar inhibitory effect of OMD on dopa accumulation, suggested that this effect of OMD may be mediated through inhibition of the firing rate in dopaminergic neurons. To test this possibility, rats were injected with γ-butyrolactone, which completely inhibits neural firing in the nigrostriatal pathway [24]. In OMD-pretreated rats, the accumulation of dopamine after injection of γ-butyrolactone was significantly lower compared with γ-butyrolac-

tone-injected controls. Since the inhibitory effect of OMD in this experiment is independent of firing rate, we suggest that the decreased accumulation of dopamine is due to diminished availability of tyrosine. It is unlikely that OMD interacts with presynaptic receptors, thus modulating tyrosine hydroxylase activity. However, this question, as well as direct measurements of brain tyrosine levels in OMD-treated rats, is still open.

The results of the present experiments are consistent with clinical studies which found that OMD given simultaneously with levodopa to parkinsonian patients produced clinical deterioration [3, 5, 15]. Similarly, high levels of OMD in plasma have been associated with L-dopa-induced dyskinesia [10] and a poor response to therapy [18]. We recently demonstrated that inhibition of catechol-*O*-methyltransferase activity by U-0521 decreased OMD formation and promoted L-dopa uptake in striatal tissue of rats [15b, 15c]. These findings agree with those of Ericsson [7], who, in a small group of parkinsonian patients, found that the L-dopa effect was potentiated by use of the catechol-*O*-methyltransferase inhibitor *n*-butylgallate. The data presented here should promote a search for safe inhibitors of catechol-*O*-methyltransferase for future treatment of parkinsonian patients.

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