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EFFECT OF 3′,4′-DIHYDROXY-2-METHYL-PROPRIOPHENONE (U-0521) ON CATECHOL-*O*-METHYLTRANSFERASE ACTIVITY AND ON DOPA ACCUMULATION IN RAT RED BLOOD CELLS AND CORPUS STRIATUM

AVINOAM RECHES,* DE-HUA JIANG and STANLEY FAHN

Department of Neurology, College of Physicians & Surgeons, Columbia University, New York, NY 10032, U.S.A.

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Abstract—The effects of the catechol-*O*-methyltransferase (COMT) inhibitor 3′,4′-dihydroxy-2-methyl-propriophenone (U-0521) were studied in red blood cells (RBC) and corpus striatum in the rat. *In vitro* U-0521 inhibited RBC COMT activity in a dose-dependent manner with an IC_{50} of 6×10^{-6} M. *In vivo* maximum inhibition (90%) of enzyme activity in RBC was obtained with 250 mg/kg with a peak effect at 5 min and enzyme recovery within 90 min. In U-0521-pretreated rats L-3,4-dihydroxyphenylalanine (L-DOPA) accumulation in RBC and corpus striatum, after injection of L-DOPA, was significantly higher than in nonpretreated rats. The use of COMT inhibitor along with L-DOPA may be of benefit in the treatment of Parkinson's disease.

One of the major problems in the chronic treatment of patients with Parkinson's disease is the rapid fluctuations in their motor ability throughout the day. These fluctuations are related to plasma levels of L-3,4-dihydroxyphenylalamine (L-DOPA) [1, 2], and it seems that improved availability of L-DOPA should be able to reduce their severity.

L-DOPA given to Parkinsonian patients is metabolized, in part, to its *O*-methyl derivative 3-*O*-methyldopa (OMD) by catechol-*O*-methyltransferase (COMT). Plasma and cerebrospinal fluid (CSF) levels of OMD were found to be greater than those of L-DOPA in L-DOPA-treated patients [3–5]. The administration of OMD to patients receiving L-DOPA for Parkinsonism led to a decreased response to therapy [6, 7] in direct proportion to OMD levels obtained in plasma [8]. Moreover, we were recently able to demonstrate that OMD interferes with striatal uptake and utilization of L-DOPA [9, 10].

It is therefore suggested that inhibition of *O*-methylation in Parkinsonian patients treated with L-DOPA might prevent any side effects which are possibly related to the methylated derivatives. The COMT inhibitor, 3′,4′-dihydroxy-2-methyl-propriophenone (U-0521), is a potentially useful drug in Parkinsonian patients. This drug has been administered previously to a limited number of humans (G. A. Johnson, personal communication).

In the experiments reported here, we have studied the *in vitro* and *in vivo* effects of U-0521 on COMT activity in rat RBC. We have also measured the effect of the drug on DOPA accumulation in rat RBC and corpus striatum. Since U-0521 is *O*-methylated by COMT [11], we were able to separate *O*-[^{14}C]-methyl-U-0521 from 3-*O*-[^{14}C]-methyl-dopamine as described below. This separation enabled us to measure COMT activity in the presence of its inhibitor, U-0521.

MATERIALS AND METHODS

L-DOPA was obtained from Calbiochem (La Jolla, CA), chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO), the Fisher Scientific Co. (Springfield, NJ), and Bioanalytical Systems (West Lafayette, IN). Adenosyl-L-methionine, *S*-[methyl-^{14}C]([^{14}C]-SAM), 57.6 mCi/mmole, was obtained from the New England Nuclear Corp. (Boston, MA). U-0521 was donated by the Upjohn Co., Kalamazoo, MI.

Male Sprague–Dawley rats weighing 180–250 g were fasted for 12 hr prior to all experiments. Injections were given intraperitoneally (i.p.) as a suspension in 0.1% methyl cellulose. Rats were decapitated, and blood was collected with heparin. Brains were rapidly removed, and corpora striati were dissected on an ice-cold glass plate and immediately frozen on dry ice.

COMT assay

COMT in lysed RBC-chelex resin mixture derived from whole blood was assayed as described by Raymond and Weinshilboum [12], with the following modifications. Dopamine (DA) at a saturating concentration of 1 mM was used as the substrate for the reaction instead of 3,4-dihydroxybenzoic acid (DBA). Blank samples for the assay included all reagents except DA. The reaction (150 μl final volume, 45 min at 37°) was stopped with perchloric acid

* Address all correspondence to: Dr. A. Reches, Department of Neurology, College of Physicians & Surgeons, Columbia University, 630 W. 168th St., New York, NY 10032, U.S.A.

(850 μl, 0.25 M final concentration), and the supernatant fluid after centrifugation (15,600 g × 2 min) was transferred to glass columns containing cation exchange resin (Bio-Rad AG-50W-X4, 200–400 mesh, H^+ form, 25 × 5 mm). Columns were first washed with 10 ml of 0.1 M NaCl–0.1 M sodium phosphate buffer, pH 6.5, and separation of *O*-[^{14}C]-methyl-U-0521 from 3-*O*-[^{14}C]-methyl-dopamine was performed by pooled collection of 1–4 ml and 5–10 ml, respectively, of eluent ethanol (25%)–HCl (1 N) mixture (Fig. 1). A 1-ml sample of the eluent from each fraction was added to counting vials that contained 10 ml of Aquasol-2 (New England Nuclear). Radioactivity was determined in a Packard liquid scintillation counter. Recoveries of 3-*O*-methyl-dopamine (81 ± 4.5% S.E.M., N = 10) were determined fluorometrically [13]. In a preliminary study, it was found that the addition of the monoamine inhibitor pargyline at a final concentration of 5×10^{-5} M had no effect on the separation procedure. The inhibitor was therefore omitted from other studies. A unit of enzyme activity represented the formation of 1 nmole of 3-*O*-methyl-dopamine per hr per 1 ml of packed RBC. With regard to the formation of *O*-methyl-U-0521, a unit of enzyme activity represented the formation of 1 nmole of methyl-labeled U-0521 per hr per 1 ml of packed RBC.

DOPA assay in RBC

Blood was collected with heparin, and RBC were separated (10 min × 4500 g) and washed once with ice-cold saline. Packed cells were weighed and 1 g of the packed cells was homogenized in 5 vol. of ice-cold perchloric acid (final concentration of 0.4 M). One milliliter of the supernatant fraction after centrifugation (10 min × 12,000 g) was taken for DOPA assay as previously described [14]. There was no cross interference with U-0521 either *in vitro* or *in vivo*.

Assay for DOPA in the striatum

Frozen 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 × 5 min) 180 μl of supernatant fluid was taken for analysis; DOPA was extracted using the alumina extraction technique [15]. Dihydroxybenzylamine (DHBA, 25 ng) was added as internal standard to quantitate recovery. For the separation and quantification of corpus striatum DOPA, the extracts were subjected to reverse phase (C_{18}, ODS column, DuPont) high performance liquid chromatography and eluted with 0.1 M monochloroacetate buffer (pH 3.05), containing 1 mM NA_2EDTA and 70 mg/l of sodium octylsulfate as an ion pairing agent [16]. 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 4.43 min for DOPA, 6.99 min for DHBA, 8.33 min for 3,4-dihydroxyphenylacetic acid (DOPAC), and 10.4 min for DA. A Spectra-Physics graphic integrator was used for calculations [16].

RESULTS

In vitro *effect of U-0521 on COMT activity in rat RBC*

As shown in Fig. 2, U-0521 inhibited COMT activity in a dose-dependent manner. Maximum inhibition (100%) was obtained at 10^{-4} M with an $IC_{50} = 6 \times 10^{-6}$ M.

In vivo *effect of U-0521 on COMT activity in rat RBC*

Time–response study. Rats were injected i.p. with U-0521 (200 mg/kg) and killed at 0–120 min after the injection. As shown in Fig. 3, maximum COMT inhibition (90%) was obtained at 5 min, with complete enzyme recovery at 90 min. The $T_{1/2}$ of *O*-[^{14}C]-methyl-U-0521 clearance in the blood was 30 min.

Dose–response study. Rats were injected with U-0521 (0–250 mg/kg) and killed 10 min after the injection. As shown in Fig. 4, COMT activity was inhibited by U-0521 in a dose-dependent manner; 50% inhibition of enzyme activity was obtained at 90 mg/kg and maximum inhibition of 90% was obtained at 250 mg/kg.

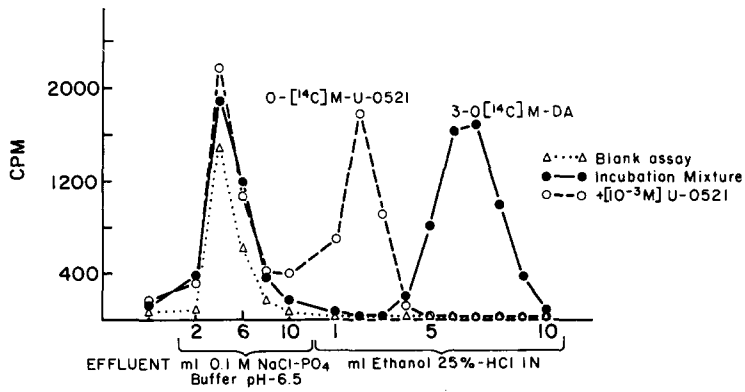

Fig. 1. Chromatographic separation of *O*-[^{14}C]-methyl-U-0521 from 3-*O*-[^{14}C]-methyl-dopamine. Separation of the *O*-methylated derivatives was performed as described in Materials and Methods. Key: (Δ . . . . . Δ) blank assay, i.e. in the absence of dopamine as the methyl acceptor; (●- - - -●) incubation mixture; and (○- - - -○) incubation mixture in the presence of 10^{-3} M U-0521.

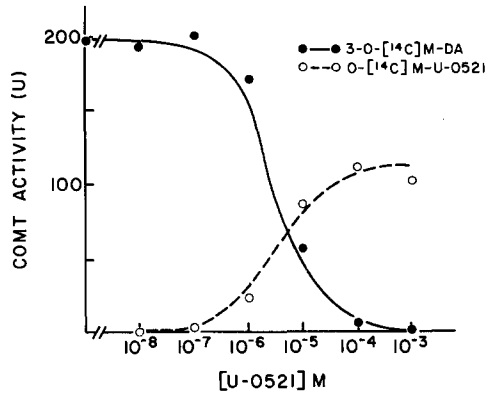

Fig. 2. *In vitro* effect of U-0521 on COMT activity in rat RBC. Rat RBC lysates were incubated in the presence of U-0521 at the indicated concentrations. COMT activity was determined as described in Materials and Methods. Results are given as the means of duplicate determinations.

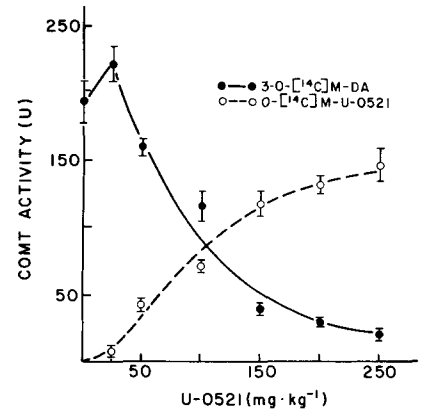

Fig. 4. *In vivo* dose–response of U-0521 on COMT activity in rat RBC. Rats were injected i.p. with U-0521, 0–250 mg/kg, and killed 10 min later. COMT assay was performed as described in Materials and Methods. Results are expressed as the means ± S.E.M. of N = 4.

In vivo *effect of U-0521 on DOPA accumulation in RBC and corpus striatum*

Rats were injected i.p. with U-0521 (150 mg/kg) at zero time, and then 10 min later with levodopa (100 mg/kg); they were killed 15 min after DOPA administration. Control rats were injected with DOPA only. As shown in Table 1, DOPA accumulation in RBC and corpus striatum was significantly higher in U-0521-pretreated rats.

DISCUSSION

In the experiments reported here, we have studied the *in vitro* and *in vivo* inhibitory effects of U-0521 on COMT activity in rat RBC. Our assay procedure (Fig. 1) enables us to measure COMT activity in the presence of this inhibitor.

In vitro, U-0521 inhibited COMT activity in a dose-dependent manner, with 50% inhibition obtained at 6×10^{-6} M (Fig. 2) in agreement with a previous report [11].

When given i.p. to rats, the *in vivo* inhibitory effect of U-0521 was very rapid, reaching maximum values at 5 min with enzyme recovery within 90 min (Fig. 3). This inhibitory effect was dose-dependent at 50–250 mg/kg with *in vivo* 50% inhibition of enzyme activity obtained at 90 mg/kg (Fig. 4). As reported earlier [17], U-0521 effectively inhibits the accumulation of OMD in the plasma of rats treated with L-DOPA. This effect is probably due to inhibition of COMT activity in tissues such as the liver and the kidneys which are rich with this enzyme. OMD which accumulates in the plasma of patients treated with L-DOPA [18] may induce dyskinesia [19] and poor response to therapy [20, 21]. This effect may be related to the fact that OMD inhibits brain uptake and utilization of L-DOPA [22–24]. Inhibition of COMT activity and the attenuation of OMD formation may thus enhance L-DOPA accumulation after administration of the drug. Indeed, in rats pretreated with U-0521, L-DOPA accumulation in RBC and corpus striatum after administration of the drug was significantly higher compared with control rats (Table 1).

In a similar study, we were able to show recently that U-0521 inhibits COMT activity in rat corpus striatum and accentuates L-DOPA metabolism in this brain region [25]. It is reasonable, therefore, to suggest that inhibition of COMT activity in Parkin-

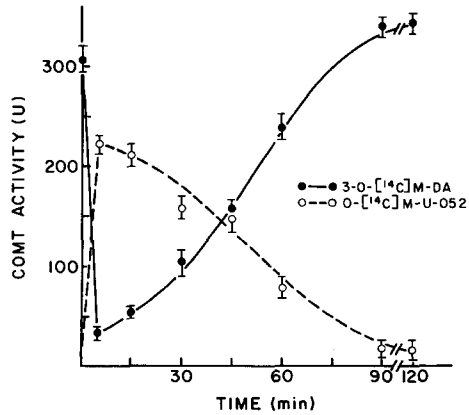

Fig. 3. *In vivo* effect of U-0521 on COMT activity in rat RBC. Rats were injected i.p. with U-0521 (200 mg/kg) and killed at 0–120 min. COMT activity was determined as described in Materials and Methods. Results are expressed as the means ± S.E.M. of N = 4.

Table 1. Effect of U-0521 on DOPA accumulation in rat RBC and corpus striatum*

Treatment	RBC	Corpus striatum
DOPA	1.91 ± 0.6 (5)	2.14 ± 0.27 (8)
U-0521 + DOPA	5.79 ± 0.4 (6)	6.31 ± 0.84 (8)
	$P < 0.05$	$P < 0.005$

* Rats were injected i.p. with U-0521 (150 mg/kg), then 10 min later with L-DOPA (100 mg/kg), and killed 15 min after DOPA administration. Other rats were injected with L-DOPA only. DOPA was measured as described in Materials and Methods. Results are expressed as the means ± S.E.M., ng/mg tissue; the number of determinations is given in parentheses. Statistics were obtained by the two-tailed Student's *t*-test.

sonian patients receiving L-DOPA could lead to substantial benefit. In a single clinical study [26] using *n*-butyl-gallate as the inhibitor, such an effect was indeed found. This study has never been confirmed due to cessation of clinical studies with this drug. The effect of U-0521 after oral administration in rats and in patients with Parkinson's disease is currently being studied.

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