Biochemical Pharmacology, Vol. 28, pp. 1221–1225.

0006-2952/79/0401-1221 $02.00/0

EFFECT OF A CATECHOL-*O*-METHYL TRANSFERASE INHIBITOR, U-0521, WITH LEVODOPA ADMINISTRATION*

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(*Received* 12 *July* 1978; *accepted* 11 *September* 1978)

Abstract—The *in vivo* effect of 3,4-dihydroxy-2-methyl-propriophenone (U-0521) was studied in rats treated with L-DOPA, 250 mg/kg, intraperitoneally. In a time–course study, experimental animals received two doses of U-0521, 250 mg/kg, i.p., 30 min before L-DOPA and along with L-DOPA. Rats were decapitated at intervals of 30–120 min. U-0521-treated animals showed elevated levels of plasma and brain DOPA and of brain dopamine; they also had a reduction of 3-*O*-methyldopa (OMD) in plasma and brain, and of homovanillic acid (HVA) in brain, compared to rats treated with L-DOPA alone. A dose–response study of U-0521, given 30 min before L-DOPA, showed that there was effective inhibition of plasma and brain OMD accumulation at a dose of U-0521 of 100 mg/kg, i.p., or greater. Brain HVA accumulation was inhibited at a dose of 200 mg/kg, i.p., or greater. U-0521 can effectively block formation of *O*-methylated metabolites of L-DOPA and dopamine peripherally and centrally after high dosage L-DOPA administration.

Since the introduction of high dosage levodopa for the treatment of Parkinsonism by Cotzias *et al.* [1], this drug, with or without a peripheral DOPA decarboxylase inhibitor, remains the most effective agent to control the symptoms of this disorder. Adverse central nervous system reactions, such as dyskinesias and psychosis, have become a major dose-limiting factor. Other problems develop with chronic usage, including loss of efficacy, clinical fluctuations (on–off effect) and dementia [2–5], which are creating new considerations to delay the onset of levodopa therapy [6].

Plasma levels of levodopa and its metabolites have been correlated with clinical responses in patients with Parkinsonism [7, 8], and the levels of 3-*O*-methyldopa (OMD) were found to be greater than those of levodopa. Furthermore, the levels of OMD in cerebrospinal fluid were greater than those of DOPA in patients treated with levodopa [9]. Rivera–Calimlim *et al.* [10] and Feuerstein *et al.* [11] showed that high OMD levels in plasma correlated with DOPA-induced dyskinesias. In 1971, Ericsson [12] treated ten Parkinsonian patients taking levodopa, with a catechol-*O*-methyl transferase (COMT) inhibitor, *N*-butyl gallate. A lessening of abnormal involuntary movements and dystonia was reported. It was also found that a lower dosage of levodopa was required for therapeutic benefit, and that there was additional improvement in the control of rigidity, tremor, and bradykinesia. This study of Ericsson has never been confirmed due to cessation of clinical studies with *N*-butyl gallate. Most COMT inhibitors, including pyrogallol [13], are too toxic to be used in man [14]. The COMT inhibitor, 3′, 4′-dihydroxy-2-methyl-propriophenone (U-0521), is a more potent competitive inhibitor of COMT than pyrogallol, with a $K_i = 7.8 \times 10^{-6}$ M [15]. Prior to the introduction of levodopa, up to 6 g i.v. of U-0521 had been administered safely to humans, including pregnant women (G. A. Johnson, personal communication), and, therefore, could be a potentially safe and useful drug in levodopa-treated patients with Parkinsonism. Therefore, we have studied the time– and dose–response curves of U-0521 *in vivo* in rats treated with levodopa at high dosage.

METHODS

Male Sprague–Dawley rats, weighing 200–300 g, were fasted for 17 hr prior to all experiments. They were kept for at least 2 days on a day-light, night-dark cycle, and the experiments were conducted between 9:00 a.m. and 2:00 p.m. on the day following the fast. All injections were given intraperitoneally, and all drugs were given as a suspension in 1% methyl cellulose. L-DOPA was obtained from CalBiochem and U-0521 from the Upjohn Co., Kalamazoo, MI.

Effect of U-0521 with time. Absolute control rats received no injections. DOPA controls were given L-DOPA, 250mg/kg. Experimental animals were pretreated with U-0521, 250 mg/kg, 30 min before receiving a combined injection of U-0521 and L-DOPA, each 250 mg/kg. Animals were decapitated at 30 min intervals between 0 and 120 min after L-DOPA. Blood and brain were collected.

Effect of dose of U-0521. Absolute and DOPA control rats received the same treatment as described above. Experimental rats were pretreated with U-0521 (50, 100, 150, 200 or 250 mg/kg) 30 min before receiving the injection of L-DOPA, 250 mg/kg. All animals were decapitated 90 min after receiving levodopa. Blood and brain were collected.

Analytical procedures. Brains were chilled on ice, homogenized in 3 vol. of ice-cold 0.4 M perchloric acid containing 0.025% ascorbic acid, and centrifuged at 15,000 rev/min for 20 min. The supernatant fractions were collected and the above procedure was repeated on the residue. The combined supernatant fractions were stored at −20°. Bloods were collected in

* Supported by the Parkinson's Disease Foundation and by The Samuel A. and Katharine B. Berger Foundation.
† Recipient of a student fellowship from the Parkinson's Disease Foundation.

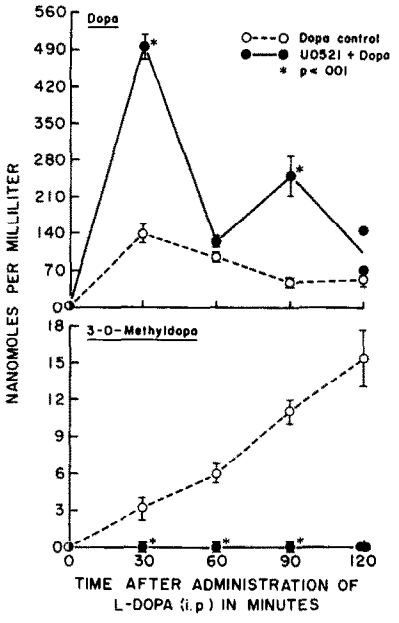

Fig. 1. Time course of plasma DOPA and OMD. Rats received either L-DOPA (250 mg/kg) alone or with U-0521 (500 mg/kg), as described in the text, and were killed at the indicated times. Each point represents the mean of at least four rats ±S.E.M., except for DOPA levels at 60 min (N = 3) and for the rats killed at 120 min. The DOPA controls have an N = 3, and only two of four animals treated with U-0521 survived; their results are plotted as individual points. Key: (*) $P < 0.001$.

heparinized tubes immediately after decapitation, chilled and centrifuged, and the plasma was pipetted off. Plasma proteins were precipitated in 4 vol. of ice-cold 0.4 M perchloric acid solution and centrifuged at 10,000rev/min for 15 min. Protein-free supernatant fractions were decanted and stored at −20°. All samples were chromatographed within 2 days of the experiment.

Column chromatography of the sample extracts was carried out on a strong cation resin (Bio Rad AG 50-X8) according to the method of Prasad and Fahn [16]. Homovanillic acid (HVA) remained in the combined effluent and subsequent water wash; these eluates underwent solvent extractions and were assayed by an automated fluorometric method [17]. DOPA and OMD were eluted together and assayed fluorometrically as described previously [17, 18]. After dopamine was eluted, it was measured by an automated fluorometric method [19] of Atack [20]. All eluates were assayed within 10 days of chromatography; they were stored at −20°, thawed and mixed thoroughly before assay.

Statistical analysis was carried out by the use of Student's *t*-test.

RESULTS

Behavioral responses. All rats treated with L-DOPA exhibited increased locomotor activity, piloerection, exophthalmos and other signs of sympathetic stimulation. Those treated with U-0521 showed striking reduction in motor activity within 10 min of injection, including prostration, lying on one side, rapid breathing and loss of responsiveness to handling and other stimuli. The degree of lethargy was related to the dosage of U-0521 employed. Administration of L-DOPA markedly attenuated all lethargic symptoms induced by U-0521, but did not abolish them. Little piloerection was apparent in animals receiving both inhibitor and L-DOPA. In the time–course experiment, in which the rats received a total of 500 mg/kg of the inhibitor in two injections of 250 mg/kg, five of twenty animals died after receiving the second injection. In the dose–response experiment in which a single dose of the inhibitor was administered, two of six animals receiving

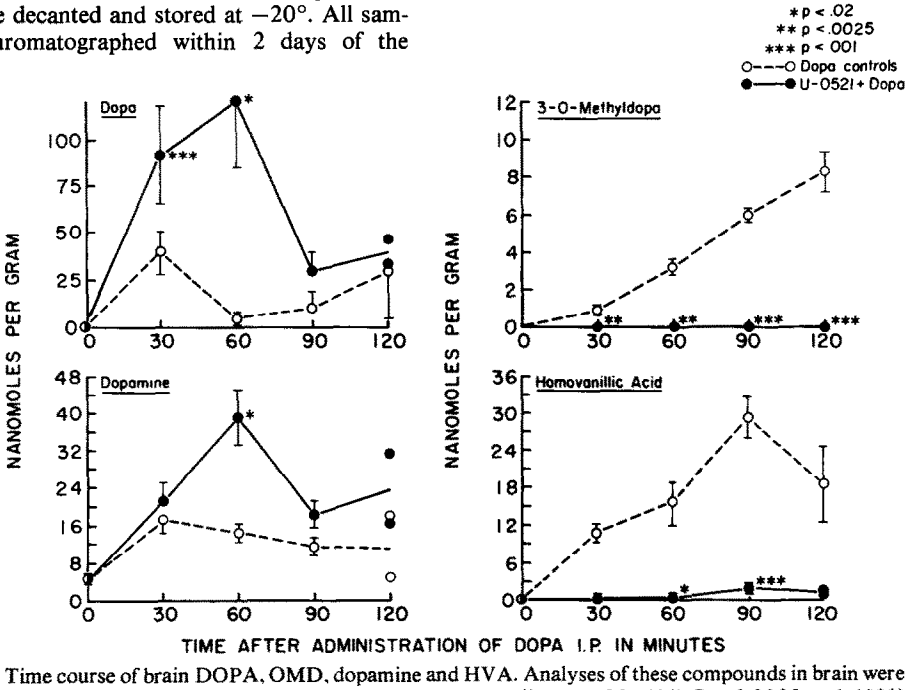

Fig. 2. Time course of brain DOPA, OMD, dopamine and HVA. Analyses of these compounds in brain were carried out in the same animals described in Fig. 1. Key: (*) $P < 0.02$; (**) $P < 0.0025$ and (***) $P < 0.001$.

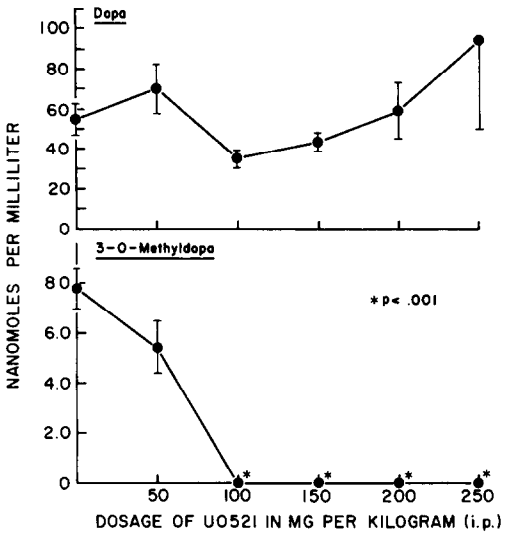

Fig. 3. Dose–response curves of plasma levels of DOPA and OMD to U-0521. Rats were pretreated with U-0521 at the indicated doses 30 min prior to administration of DOPA (250 mg/kg), as described in the text. Animals were killed 90 min after DOPA. Each point represents the mean ± S.E.M. of four animals. Key: (*) $P < 0.001$.

250 mg/kg died within 30 min, before receiving L-DOPA. Deaths did not occur with lower doses of U-0521.

Effect of U-0521 with time. Maximal plasma levels of DOPA in rats treated with L-DOPA, alone or with U-0521, occurred 30 min after L-DOPA injections (Fig. 1). Inhibition of COMT resulted in more than a 3-fold increase in plasma DOPA concentration. In U-0521-treated rats, a second smaller increase in plasma DOPA occurred 90 min after L-DOPA administration.

After L-DOPA administration, the *O*-methylated derivative of DOPA, OMD, progressively accumulated in plasma (Fig. 1). In a separate experiment, we observed that OMD levels in plasma continued to increase for at least 4 hr after L-DOPA treatment (data not shown). Treatment with U-0521 completely prevented a measurable accumulation of OMD in plasma (Fig. 1).

In brain of DOPA control rats, DOPA rose to a peak value 30 min after L-DOPA administration (Fig. 2). With COMT inhibition, DOPA concentration was increased more than 2-fold at the 30 min time point, and continued to rise for an additional 30 min. Brain OMD levels rose slowly for the first 30 min in both DOPA control animals and then increased more rapidly and linearly (Fig. 2); levels were 18 nmoles/g 4 hr after L-DOPA administration (data not shown). The accumulation of brain OMD was blocked by U-0521 treatment (Fig. 2).

The dopamine concentration in brain was maximal at 30–60 min after L-DOPA treatment in both groups of rats, but inhibition of COMT increased the level of dopamine more than 2-fold at 60 min (Fig. 2). In DOPA controls, HVA accumulation in brain increased linearly for 90 min after administration of L-DOPA before beginning to decline. This accumulation was virtually abolished in rats treated with the COMT inhibitor.

U-0521 treatment did not have a significant effect on adrenal norepinephrine or dopamine levels, and no OMD accumulation was noted in that tissue (data not shown).

Effects of dose of U-0521. A time point of 90 min after L-DOPA treatment was selected to evaluate the effect of the dose of U-0521 on the accumulation of *O*-methylated derivatives of the catechols. Plasma DOPA levels at this time point were not affected by treatment with U-0521, 50–250 mg/kg (Fig. 3). However, plasma OMD accumulation was blocked completely at doses of U-0521 of 100 mg/kg or greater (Fig. 3). In brain, the accumulation of OMD or HVA was inhibited by U-0521 doses of 100 and 200 mg/kg, respectively (Fig. 4). DOPA and dopamine levels in brain were not

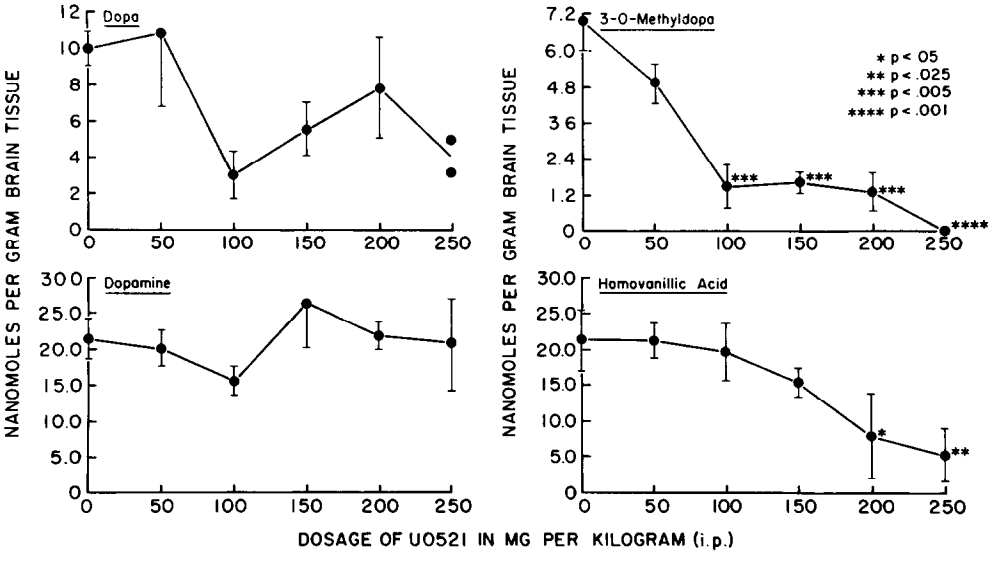

Fig. 4. Dose–response curves of brain concentrations of DOPA, OMD, dopamine and HVA to U-0521. Analyses of these compounds in brain were carried out in the same animals described in Fig. 3. Key (*) $P < 0.05$; (**) $P < 0.025$; (***) $P < 0.005$ and (****) $P < 0.001$.

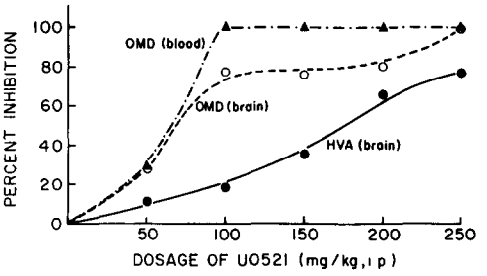

Fig. 5. Per cent inhibition of OMD and HVA accumulation as a function of dose of U-0521. Data are calculated from Figs. 3 and 4.

altered significantly by U-0521 at the 90 min time point for all doses of U-0521 (Fig. 4).

When the dose–response data for OMD and HVA in plasma and the brain were plotted as per cent inhibition relative to the DOPA control, the comparative effectiveness of U-0521 in preventing the accumulation of the methylated compounds was apparent (Fig. 5). A dose of 100 mg/kg completely inhibited OMD accumulation in plasma and inhibited the accumulation of OMD in brain by 80 per cent. At the same dose, HVA accumulation in brain was inhibited by only 20 per cent (Fig. 5).

DISCUSSION

The results indicate clearly that U-0521 can inhibit the accumulation of 3-*O*-methylated derivatives of catechols in plasma and brain when given at high dosage and after acute L-DOPA administration. As a result of this *in vivo* inhibition of COMT, striking enhancement and prolongation of DOPA and dopamine concentrations in the brain were achieved in animals treated with U-0521 (Fig. 2). Baldessarini and Chace [21] carried out a similar study using the COMT inhibitor, pyrogallol, and tracer doses of [^{3}H]-L-DOPA in rats. They found increases of [^{3}H]-DOPA and ^{3}H-catecholamines in brain 1 hr after [^{3}H]-DOPA administration, along with a reduction of [^{3}H]-OMD.

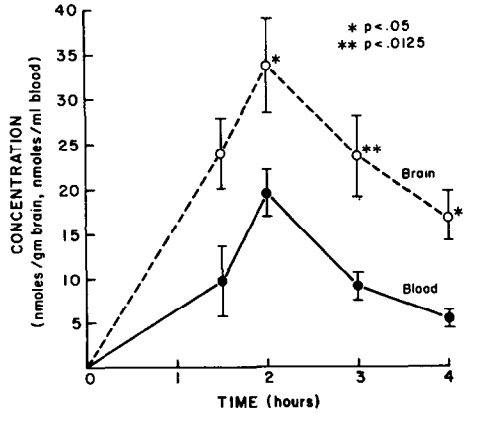

Fig. 6. HVA levels in brain and plasma after L-DOPA administration. Animals received L-DOPA, 250mg/kg, i.p., and were killed at the indicated times. Each point represents the mean ± S.E.M. of four animals. Key: (*) $P < 0.05$ and (**) $P < 0.0125$.

The data obtained from DOPA controls (Figs. 1 and 2, dashed lines) indicate that OMD continues to accumulate in plasma and brain for at least 4 hr after L-DOPA administration. The slow clearance of OMD, both centrally and peripherally, has been reported previously in animals by Bartholini and Pletscher [22], Kuruma *et al.* [23], and by Bartholini *et al.* [24], using low doses of [^{14}C]-DOPA and [^{14}C]-3-OMD. The biological half-life for OMD in blood, brain and heart was about 12–13hr [24]. The formation and concentration of [^{14}C]-OMD in plasma following acute [^{14}C]-DOPA administration to humans are enhanced when a peripheral DOPA decarboxylase inhibitor is administered concomittantly [25, 26]. The increase of plasma OMD with peripheral decarboxylase inhibition is not always seen in chronic levodopa-treated patients [8].

The increased plasma concentrations of DOPA in U-0521-treated animals (Fig. 1) suggest that peripheral *O*-methylation may be a significant reaction of L-DOPA metabolism. The slow accumulation of OMD in plasma not only reflects its slow half-life [24], but also indicates that OMD continues to be formed from DOPA. Most tissues in the body, particularly pancreas and kidney, store DOPA after it is administered intraperitoneally [27–29]. It is likely that some of the stored DOPA continues to be metabolized with time to OMD. Some of the stored DOPA in tissues may also enter the circulation, which could explain the delayed small rise of plasma DOPA in U-0521-treated animals (Fig. 1).

The question of whether U-0521 is active centrally as well as peripherally must be addressed. Although OMD accumulation in brain is inhibited by U-0521 (Fig. 2), this phenomenon need not be due to central inhibition of COMT; it could be secondary to lack of entry of peripheral OMD into brain, for the accumulation of OMD in plasma is blocked completely by U-0521 (Fig. 1). Bartholini *et al.* [24] showed that OMD penetrates easily into brain from blood. On the other hand, the data on brain HVA (Fig. 2) would suggest that brain COMT is inhibited because HVA enters brain poorly from peripheral sources [30, 31]. However, if the plasma level of HVA is high enough, diffusion allows for accumulation of HVA in brain tissue [32]. In one study we compared simultaneous plasma and brain HVA concentrations following L-DOPA administration (250 mg/kg, i.p.) (Fig. 6). At all time points, the concentration of HVA in brain was higher than in plasma, suggesting that brain HVA is most likely synthesized directly in this tissue. Most of brain HVA probably comes from dopamine, since Bartholini *et al.* [24] showed that very little HVA in brain is generated from administered OMD. Moreover, the higher dose of U-0521 required to block accumulation of brain HVA compared to OMD (Fig. 5) possibly reflects that a higher dose of U-0521 is needed to be effective centrally compared to peripherally. The similar dose–response curves for plasma and brain OMD (Fig. 5) might indicate that most of brain OMD comes from peripheral sources.

COMT activity is present in most mammalian tissues, especially liver [14], and is almost evenly distributed in brain [33]. This widespread distribution probably accounts for the enhanced levels of plasma and brain DOPA after L-DOPA administration with U-0521 treatment (Figs. 1 and 2). These results indicate that high dosage exogenous levodopa normally may be

metabolized to a considerable extent by COMT, in addition to the well-known decarboxylation catalyzed by L-amino acid decarboxylase. Clinically, peripheral decarboxylase inhibitors reduce the dose of levodopa necessary to treat patients with Parkinsonism. COMT inhibitors might also reduce the required dose, as already indicated by Ericsson [12]. In addition, there is the possibility that COMT inhibition might reduce some of the unpleasant dose-limiting central adverse effects seen with levodopa therapy if these are related to *O*-methylated derivatives of DOPA or dopamine. These adverse effects are often accentuated rather than reduced with the use of a peripheral decarboxylase inhibitor in conjunction with levodopa. The potential of combining levodopa with both a COMT and a decarboxylase inhibitor should be considered seriously. With L-DOPA therapy there is also the possible formation of 4-*O*-methylated derivatives of both L-DOPA and dopamine [34], which could also contribute to adverse effects of therapy. COMT inhibition should block the synthesis of these 4-*O*-methylated compounds. Both the inhibition of formation of *O*-methylated derivatives and the enhancement of brain DOPA and dopamine concentrations by U-0521 implicate its potential usefulness in the treatment of Parkinsonism.

The question of toxicity of U-0521 needs to be addressed if the drug is to be tested in man. For the initial study on the effect of U-0521 as a function of time, we purposely selected a very high dose of U-0521 (500 mg/kg) in order to determine (1) that this compound does indeed have an inhibitory effect on COMT, and (2) the ideal time-point to select for the subsequent dose–response study. A dose of 500 mg/kg was lethal in 25 per cent of the rats. Doses of 200 mg/kg or less were not lethal. However, the toxicity of U-0521 in humans will have to be explored in more detail, particulary if it is to be administered in the presence of levodopa.

Acknowledgements—The authors thank Dr. Garland A. Johnson of Upjohn Co. for providing us with U-0521, and appreciate the technical help of Vernice Jackson and Carl Miller.

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