Uridine and Stimulant-Induced Motor Activity

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Abstract. **Chronic administration of uridine alters dopaminergic activity and related behavior. The present study investigated this effect using amphetamine and cocaine-induced activity and rotation in rats with unilateral dopaminergic lesions. Adult, female Sprague-Dawley rats with free access to food and water received daily intraperitoneal uridine (16 mg/kg) or an equal volume of saline. Activity was assessed for 10 min in a photocell chamber 30 min after intraperitoneal amphetamine or cocaine and 4 hr after the uridine or saline. Additional rats with unilateral dopaminergic lesions were treated comparably and assessed for stimulant-induced rotation. Uridine exerted no effect on body weight, activity, or rotation under baseline conditions. At higher doses, amphetamine and cocaine decreased activity and caused a dose-dependent increase in rotations. In the activity test, uridine-treated rats exhibited a significant increase in sensitivity to amphetamine but not to cocaine. In the rotation test, uridine-treated rats showed increased sensitivity to both stimulants. Finally, neurochemical analysis of a third set of comparably treated rats revealed that uridine blunted the amphetamine-induced increase in striatal dopamine. These observations are interpreted as indicating that chronic uridine modulates the stimulant-induced release of dopamine and, therefore, may be of therapeutic interest.** [P.S.E.B.M. 1993, Vol 204]

Although the primary role of the pyrimidine nucleoside, uridine, is in the synthesis of ribonucleic acid, it has been shown that its chronic administration alters both central dopaminergic activity and corresponding dopaminergic-mediated behaviors (1, 2). This modulation of the dopaminergic system is of importance because it has recently been proposed that uridine administration may be useful as a supplement to antipsychotic therapy (1). Purportedly, the coadministration of uridine would allow for a beneficial reduction in the dose of the antipsychotic agent.

Neurochemically, the chronic administration of uridine has been shown to decrease the number of striatal dopamine binding sites and to reduce haloperidol-stimulated dopamine release (1, 2). Behaviorally, the chronic administration of uridine has been shown to have no effect on general locomotor activity, but potentiated both the stereotypic behavior induced by the acute administration of apomorphine as well as the catalepsy induced by the acute administration of haloperidol (1).

Collectively, these observations are difficult to interpret. The reduction in the number of dopamine binding sites, reduced dopamine release, and potentiation of haloperidol-induced catalepsy are all indicative of a dopaminergic antagonist action by uridine. However, the potentiation of the apomorphine-induced stereotypy is indicative of dopaminergic agonist action. Furthermore, the reduction in haloperidol-induced dopamine release following chronic uridine was associated with an increase in extracellular 3,4-dihydroxyphenylacetic acid (DOPAC), a metabolite normally associated with increased oxidation of presynaptic dopamine.

Haloperidol-induced dopamine release is a depolarization-dependent event that may be affected by uridine at any of a number of stages (e.g., membrane ion permeability, exocytotic release, alterations in nigrostriatal feedback loops). The present study was designed to elucidate the effect of chronic uridine on dopamine release using amphetamine (a releaser of cytoplasmic dopamine) and cocaine (a dopamine reuptake blocker).

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Methods

Subjects. Adult, female Sprague-Dawley rats (Taconic Farms, Germantown, NY) were housed in individual cages in a temperature- and humidity-regulated colony room with free access to food and water. At the beginning of each experiment, mean ± SD weight was as follows: activity, 200 ± 20.4 g; rotation, 279 ± 20 g; and neurochemistry, 194 ± 16.6 g.

Activity. Ten rats received daily intraperitoneal injections of uridine (16 mg/kg dissolved in 0.9% saline at a concentration of 16 mg/ml; Sigma Chemical Co., St. Louis, MO), while 10 additional rats received an equal volume of saline. Daily injections were administered for 5 days before the start of activity testing and continued for the duration of the study, for a total of 25 days.

Activity was assessed in a Plexiglas chamber (22 × 37 × 25 cm) divided across its length by three photocell beams. All rats were acclimated to the chamber for a total of four sessions of 10-min duration during the 2 weeks before drug testing, and for one additional session after daily uridine (or saline) injections began. Subsequently, rats were tested in the chamber only on days that amphetamine, cocaine, or vehicle were administered. Amphetamine (0.25, 0.5, 1.0, or 2.0 mg/kg; Sigma) or cocaine (1.0, 2.0, or 4.0 mg/kg; Merck & Co., Rahway, NJ) were administered intraperitoneally approximately 4 hr after uridine (or saline) injections and 30 min before activity testing. Drugs were administered in a volume of 1.0 ml/kg of saline in a randomized dosing order within drug type, with saline vehicle occasionally substituted for one of the test doses. In addition, all doses were repeated before the completion of the dose-response curve determination. At the completion of the study, rats were sacrificed 4 hr after the last uridine (or saline) injection, and the striatal region was dissected and assayed for dopamine, serotonin, and their major metabolites by high-performance liquid chromatography with electrochemical detection, as described in detail elsewhere (3).

Rotation. Sixteen rats received unilateral lesions of the nigrostriatal dopamine system. After surgical anesthesia with 50 mg/kg of Nembutal (Sigma), rats were placed in a stereotaxic instrument with incisor bars at +5 mm and a small scalp incision was made above bregma. A small burr hole was made above the right striatum (AP +0.2; ML −3.0) and a 10-μl syringe (no. 701; Hamilton Co., Reno, NV) was lowered into the striatum (DV −6.5). 6-Hydroxydopamine (Sigma) was dissolved in 0.1% ascorbate at a concentration of 1 μg/μl and 10 μg of the toxin were delivered over a 60-sec interval. Rats were allowed to recover for a minimum of 6 days after surgery.

Eight rats were then placed on a daily injection regimen of 16 mg/kg of uridine (prepared as above) and the remaining eight received an equal volume of the saline vehicle. Rotation was assessed 4 hr after the daily uridine (or saline) injection in a 30-cm^2 chamber with a clear plastic front by an observer blind to treatment condition. Rats were treated with amphetamine (0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg, ip) or cocaine (1.0, 2.0, 4.0, or 8.0 mg/kg, ip) 30 min before being placed in the chamber. Acclimation to the chamber, running schedule, and drug administration followed the protocol used for assessing activity. At the completion of the study (14 weeks), rats were sacrificed and assayed as described above.

Neurochemistry. Sixty-four additional rats were treated for 7 days with either 16 mg/kg of uridine or an equal volume of saline as described above. On the seventh day, 4 hr after the daily injection, rats were injected intraperitoneally with either saline or 2.0, 4.0, or 8.0 mg/kg of amphetamine and sacrificed 30 min later, and striatal regions were assayed as described above.

Protocols for the above studies were approved by the Rutgers University Institutional Review Board.

Results

Activity. In the uridine-treated rats, the chronic administration of uridine had no effect on body weight as compared with the saline-treated rats (229 ± 8.7 vs 235 ± 10.5 g, respectively). Likewise, the chronic administration of uridine did not alter the baseline levels of activity as compared with the saline-treated controls (148.6 ± 7.7 vs 165.1 ± 8.7 counts/10 min for the uridine- and saline-treated rats, respectively).

Both amphetamine ($F[4,60] = 4.6$; $P < 0.01$; Fig. 1) and cocaine ($F[3,45] = 52.4$; $P < 0.001$; Fig. 2) caused a significant reduction in activity levels during the 10-min test session. Fisher's PLSD post hoc analysis revealed that subjects in both the uridine- and saline-treated groups exhibited significantly fewer beam crossings after amphetamine 0.5 mg/kg and cocaine 4.0 mg/kg. In addition, the uridine-treated rats exhibited reduced activity levels after each dose of amphetamine as compared with the saline-treated controls and this difference reached statistical significance at the 1.0-mg/kg dose. Finally, the chronic treatment with uridine did not alter striatal concentrations of dopamine, serotonin, or their major metabolites (data not shown).

Rotation. Under baseline conditions, there were no significant differences in the number of rotations exhibited by the uridine- versus the saline-treated rats. Both amphetamine ($F[5,65] = 18.3$; $P < 0.001$; Fig. 3) and cocaine ($F[4,52] = 10.7$; $P < 0.001$; Fig. 4) caused dose-dependent increases in the number of rotations during the 10-min session. Fisher's PLSD post hoc analysis revealed that subjects in both the uridine- and saline-treated groups exhibited significantly more rotations (compared with baseline) after amphetamine 8.0 mg/

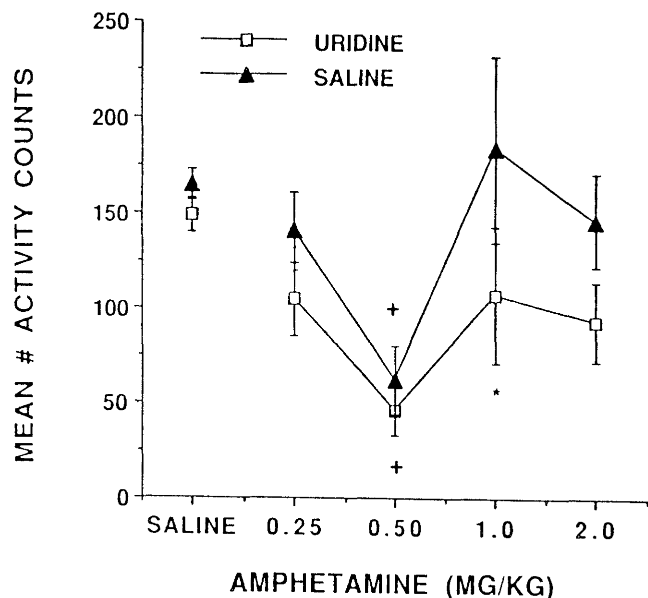

Figure 1. The effects of amphetamine on the mean number of activity counts during a 10-min session. Amphetamine was administered intraperitoneally 30 min before session to rats treated daily with uridine (16 mg/kg) or saline. *Significantly different from saline-treated rats; †Significantly different from saline baseline levels; $P < 0.05$.

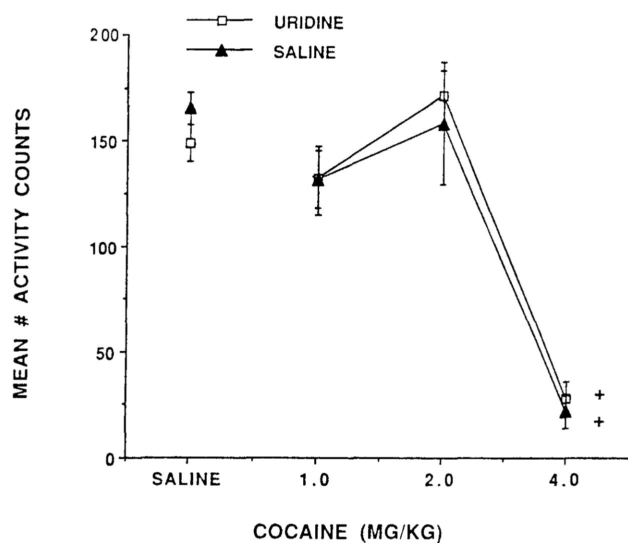

Figure 2. The effects of cocaine on the mean number of activity counts during a 10-min session. Cocaine was administered intraperitoneally 30 min before session to rats treated daily with uridine (16 mg/kg) or saline. †Significantly different from saline baseline levels; $P < 0.05$.

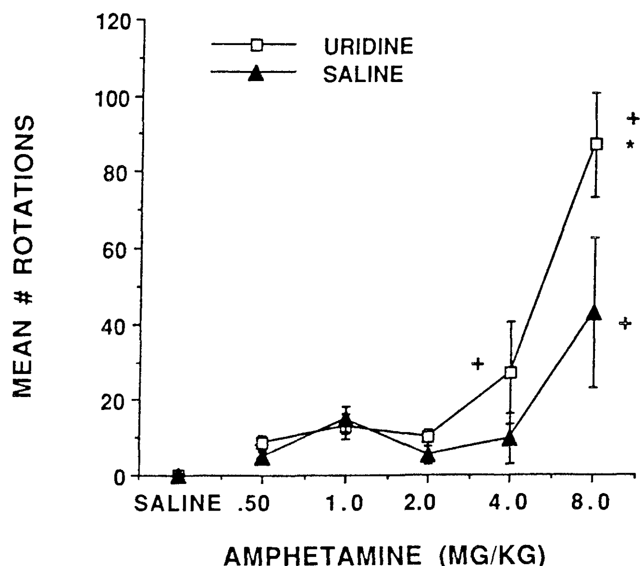

Figure 3. The effects of amphetamine on the mean number of rotations during a 10-min session. Amphetamine was administered intraperitoneally 30 min before session to rats treated daily with uridine (16 mg/kg) or saline. *Significantly different from saline-treated rats; †Significantly different from saline baseline levels; $P < 0.05$.

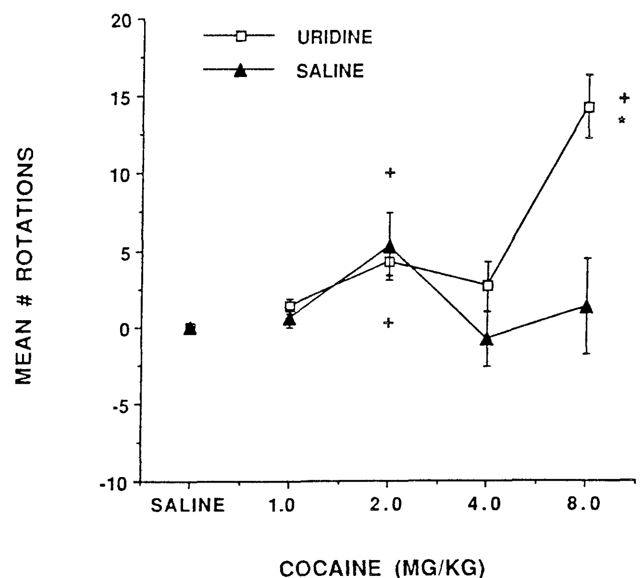

Figure 4. The effects of cocaine on the mean number of rotations during a 10-min session. Cocaine was administered intraperitoneally 30 min before session to rats treated daily with uridine (16 mg/kg) or saline. *Significantly different from saline-treated rats; †Significantly different from saline baseline levels; $P < 0.05$.

kg and cocaine 2.0 mg/kg. In addition, the uridine-treated rats exhibited significantly more rotations compared with baseline after amphetamine 4.0 mg/kg and cocaine 8.0 mg/kg. The uridine group rotated significantly more than saline-treated animals after amphetamine 8.0 mg/kg and cocaine 8.0 mg/kg. Finally, as above, chronic treatment with uridine did not alter striatal concentrations of dopamine, serotonin, or their major metabolites. However, the 6-hydroxydopamine treatment resulted in a significant ($t = 3.4$, $P < 0.01$) 76% depletion of dopamine on the lesioned side with no alteration in the concentration of serotonin (data not shown).

Neurochemistry. Amphetamine caused a significant ($F[7,62] = 3.6$; $P < 0.01$) increase in the concentration of striatal dopamine in both the uridine- and saline-treated rats (Table I). Fisher's PLSD post hoc analysis revealed that this difference reached statistical

Table I. Effects of Uridine and Amphetamine on Striatal Dopamine, Serotonin, and Metabolite Concentrations (n = 8/group)[a]

	Dopamine	DOPAC	HVA	Serotonin	5-HIAA
Saline					
Uridine	11.2 ± 1.7	2.2 ± 0.5	1.3 ± 0.3	0.5 ± 0.1	1.2 ± 0.1
Control	11.8 ± 1.0	1.9 ± 0.3	1.4 ± 0.1	1.9 ± 1.4	1.2 ± 0.2
Amphetamine 2.0					
Uridine	14.9 ± 1.6	0.9 ± 0.2[b]	1.1 ± 0.2	0.6 ± 0.1	1.2 ± 0.1
Control	19.0 ± 2.6[b]	1.1 ± 0.2[b]	1.2 ± 0.2	0.6 ± 0.1	1.2 ± 0.1
Amphetamine 4.0					
Uridine	17.4 ± 1.0[b]	0.7 ± 0.1[b]	1.3 ± 0.2	0.6 ± 0.1	1.3 ± 0.1
Control	16.8 ± 1.1[b]	0.8 ± 0.1[b]	1.2 ± 0.1	0.7 ± 0.1	1.6 ± 0.1[b]
Amphetamine 8.0					
Uridine	15.0 ± 0.9	0.9 ± 0.2[b]	1.5 ± 0.1	0.5 ± 0.1	1.2 ± 0.1
Control	13.1 ± 0.7	0.6 ± 0.1[b]	1.5 ± 0.1	0.6 ± 0.1	1.2 ± 0.1

[a] Values reported are in μg of compound/g of tissue ± SEM. Amphetamine (in mg/kg) was administered intraperitoneally 30 min before sacrifice to rats treated for 7 days with intraperitoneal uridine at 16 mg/kg/day (uridine) or an equal volume of saline (control). DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.
[b] Amphetamine effect significantly different from the saline treatment control; $P < 0.05$.

significance after the 2.0-mg/kg as well as 4.0-mg/kg dose of amphetamine in the control group, but only after the 4.0-mg/kg dose in the uridine-treated group. Likewise, amphetamine caused a significant ($F[7,62] = 6.4$; $P < 0.001$) decrease in the concentration of DOPAC in both the uridine- and saline-treated rats. Post hoc analysis revealed that this difference reached statistical significance after each dose of amphetamine in both treatment groups. Amphetamine did not cause any statistically significant changes in the concentration of striatal homovanillic acid. Finally, amphetamine did not cause any statistically significant changes in the striatal concentration of serotonin or 5-hydroxyindoleacetic acid (5-HIAA), with the exception of the 4.0-mg/kg dose, which caused an increase in the concentration of 5-HIAA in the control subjects.

Discussion

On the basis of its putative dopaminergic antagonist action, it has been proposed that uridine may be an effective supplement in the treatment of the psychoses, allowing for a beneficial reduction in the dose of the antipsychotic agent. However, the mechanism through which uridine may exert its dopaminergic antagonist action remains elusive.

In the present study, chronic uridine was found to have no effect on body weight or baseline activity. However, after the acute administration of amphetamine, the uridine-treated rats were found to have significantly reduced activity levels as compared with the control-treated rats. This latter observation may be indicative of a potentiation of amphetamine-induced stereotypic behavior. "In place" stereotypic behavior in the photocell chamber would lead to a reduction in activity counts. Subjective observation of the rats after activity sessions was consistent with this notion.

In contrast, the chronic uridine treatment did not alter the activity levels of the rats after cocaine. This differential effect of uridine on amphetamine- versus cocaine-induced changes in activity is of interest because amphetamine is thought to release the newly synthesized pool of dopamine, whereas cocaine is thought to block the reuptake of depolarization-released (vesicular) dopamine (4–6). Thus, it appears that uridine may preferentially affect release of the newly formed as opposed to the storage pool of dopamine.

Ipsilateral circling behavior is evident in rats with unilateral lesions of the nigrostriatal dopamine system after the administration of indirect-acting agonists (7). Therefore, it is of interest that the chronic administration of uridine did not induce rotation under baseline conditions. However, the uridine treatment increased the sensitivity of the rats to both the amphetamine- and cocaine-induced increases in rotation. In comparing the results obtained in these two measures of stimulant-induced activity, it should be noted that higher doses were required to engender rotation than to alter activity. This may indicate that the proposed differential effect of uridine on newly synthesized versus storage pools of dopamine occurs only at the lower doses.

It has been reported previously that acute administration of amphetamine increases striatal dopamine concentrations (due to a release of end-product inhibition on tyrosine hydroxylase activity) and concomitantly decreases DOPAC concentrations (due to monoamine oxidase inhibition) (8). These observations were replicated in the present study in both the uridine- and control-treated rats. As was observed in the activity animals, the chronic administration of uridine did not alter striatal levels of dopamine, serotonin, or their metabolites under baseline conditions. However, the amphetamine-induced increase in dopamine concentrations was not apparent at the lowest dose in the uridine-treated rats, indicating a modulation of am-

phetamine's action on dopamine release by uridine in these rats. Finally, uridine also was found to blunt the amphetamine-induced increase in 5-HIAA at the intermediate dose. These effects may warrant further consideration with respect to the effect of uridine on the brain serotonin system.

It was demonstrated previously that chronic uridine treatment potentiated the extrapyramidal side effects of haloperidol as assessed by the catalepsy test (1). This observation was interpreted as evidence that cotreatment with uridine may effectively reduce the necessary maintenance dose of the antipsychotic agent. However, the mechanism through which uridine affects dopamine transmission has yet to be elucidated. The present findings indicate that treatment with uridine alone does not cause any detectable changes in activity or rotation nor does it affect striatal dopamine or serotonin concentrations. However, the otherwise cryptic effects of uridine were revealed after amphetamine and/or cocaine administration. While these results pertain more to the nigrostriatal as opposed to the mesolimbic dopamine system, they indicate that this compound warrants further consideration as a potential adjunctive therapy for a variety of psychiatric disorders.

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