Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 4, pp. 749–753, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00169-7

Uridine Reduces Rotation Induced by L-Dopa and Methamphetamine in 6-OHDA-Treated Rats

CAROL S. MYERS* HANS FISHER† AND GEORGE C. WAGNER*[1]

*Departments of *Psychology and †Nutritional Science,*
Rutgers University, New Brunswick, NJ 08903

Received 11 April 1994

MYERS, C. S., H. FISHER AND G. C. WAGNER. *Uridine reduces rotation induced by L-dopa and methamphetamine in 6-OHDA-treated rats.* PHARMACOL BIOCHEM BEHAV **52**(4) 749–753, 1995.—The pyrimidine nucleoside uridine may reduce side effects associated with antipsychotic medication by interacting with dopamine or GABA neurotransmission. Male Sprague–Dawley rats were used to investigate coadministration of uridine with agents that alter food intake (amphetamine, haloperidol, and chlordiazepoxide) and locomotor activity (methamphetamine and L-dopa). Results indicated that chronic uridine [32.0 mg/kg, intraperitoneally (IP)] alone did not alter milk intake or reduction of milk intake induced by amphetamine (dose range 0.5–2.0 mg/kg, IP) or haloperidol (0.125–1.0 mg/kg, IP), nor did it alter the biphasic response induced by chlordiazepoxide (5.0–40.0 mg/kg, IP). However, uridine-treated animals with unilateral striatal lesions exhibited no rotational behavior in the absence of drug challenge, but showed decreased rotation induced by the dopamine agonist, L-dopa (50.0–200.0 mg/kg, IP) compared with controls. In addition, uridine-treated rats exhibited reduced rotation after repeated injections of methamphetamine (4.0 mg/kg, IP) in contrast to increasingly greater rotation observed in control animals. These results are further evidence that chronic uridine may alter drug-induced dopaminergic activity without exerting effects itself.

Uridine L-dopa Methamphetamine 6-OHDA Rotation Psychosis

THE PYRIMIDINE nucleoside uridine freely crosses the blood–brain barrier, where it is phosphorylated and incorporated into RNA or is converted to uracil (11). Uridine has been shown to have a number of central effects. For example, intraventricular infusions of uridine administered to rats resulted in significant increases of slow-wave and paradoxical sleep episodes but did not affect duration of either stage, and these increases were apparent (though not significant) 24 h later (10). Therefore, uridine may have an auxiliary role in initiating and maintaining normal sleep patterns. It also has been suggested that pyrimidine nucleosides may be useful in reducing learning and memory deficits, as both uridine and cytidine facilitated acquisition of conditioned avoidance and enhanced retention of passive avoidance in young and aged rats (5,6).

Uridine has been shown to inhibit competitively GABA binding to both high-and low-affinity sites in the frontal cortex, hippocampus, and thalamus. In addition, the nucleoside protected rats against convulsions induced by the $GABA_A$ antagonist bicuculline, reduced cyclic GMP levels in a dose-dependent manner, and antagonized bicuculline-enhanced cyclic GMP levels in the cerebellum. It was suggested, therefore, that uridine may act as an endogenous GABA-receptor ligand (9).

Uridine's interaction with dopaminergic (DA) systems has been the focus of recent research to ascertain its possible use as adjunct treatment of psychosis. Neurochemical analysis of striatal DA levels in rodents found that chronic treatment with the direct DA antagonist haloperidol increased DA release following acute haloperidol challenge. However, chronic (but not acute) administration of uridine alone, as well as chronic coadministration of uridine with haloperidol reduced the DA overflow evoked by acute challenge with haloperidol (1). Other research found that chronic uridine administration also

[1] Requests for reprints should be addressed to G. C. Wagner, Department of Psychology, Busch Campus, Rutgers University, New Brunswick, NJ 08903.

reduced DA release evoked by the indirect agonist amphetamine (14).

Uridine's effect on DA-mediated behaviors also has been characterized in rodents. Chronic treatment with uridine by itself did not induce catalepsy (7), but potentiated catalepsy induced by acute challenge with haloperidol (1). In the latter study, chronic administration of haloperidol alone or with uridine decreased haloperidol-induced catalepsy. When administered alone, either acutely or chronically, uridine potentiated haloperidol-induced disruption of conditioned avoidance responding (13).

Chronic uridine pretreatment has also been studied on behavioral responses induced by DA agonists. For example, stereotypy evoked by acute challenge with apomorphine was enhanced by either uridine or haloperidol pretreatment, and further potentiated (though not significantly) by concurrent administration of uridine with haloperidol (1). Chronic uridine had no effect by itself on locomotor activity (7,14) but decreased activity induced by amphetamine while having no effect on cocaine-induced activity (14). Finally, uridine's effect was ascertained on rotational behavior in rats with unilateral striatal dopaminergic lesions. Chronic uridine alone did not elicit rotation, but potentiated rotation after acute challenge with both amphetamine and cocaine. However, this increase was elicited only by much higher doses of both psychomotor stimulants than those which altered activity levels (14).

The present studies were designed to further investigate uridine's interaction with GABAergic and dopaminergic agents. Experiment 1 assessed chronic uridine administration on milk intake after acute challenge with amphetamine, haloperidol, and chlordiazepoxide (a benzodiazepine receptor agonist that enhances GABA transmission). Amphetamine, a DA releaser, and the DA antagonist haloperidol are known to reduce milk consumption (8), whereas chlordiazepoxide has been shown to increase milk intake in rats (2).

The second experiment assessed uridine's effect on rotation induced by methamphetamine and L-dopa in 6-hydroxydopamine (6-OHDA)-lesioned rats. Unilateral striatal microinjections of 6-OHDA destroy DA terminals so that subsequent administration of DA agonists produce a robust rotational response (16). L-dopa (a DA precursor and the major therapeutic agent administered to parkinsonian patients) elicits contralateral rotation in 6-OHDA-lesioned rats, whereas methamphetamine (a DA releaser) elicits ipsilateral rotation (3,12,16).

METHOD

Experiment 1

Subjects. Twenty adult male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 530 g (±41.2) were individually housed in suspended stainless-steel cages in a temperature and humidity-controlled colony room with a 12 L : 12 D cycle (lights on at 0800 h). Animals had free access to water throughout the study and were provided a solution of one part sweetened condensed milk (Eagle Brand, Bordens, Columbus, OH) to two parts water, 20 min daily, followed by two pellets of laboratory chow.

Bottles containing the milk solution were weighed before and after consumption of the milk to determine intake. After establishing baseline milk intake for 1 mo, animals were divided into two groups matched for consumption. Ten of the animals began receiving chronic injections of uridine (32.0 mg/kg, IP, in a volume of 1.0 ml/kg) and 10 received an equal volume of 0.9% saline, 45 min before milk consumption. This dose of uridine was previously found to be sufficient to potentiate haloperidol-induced disruption of conditioned avoidance responding (13). Starting on the 7th day of chronic injections, dose–response curves were determined for amphetamine (0.0, 0.5, 1.0, and 2.0 mg/kg, IP), chlordiazepoxide (0.0, 5.0, 10.0, 20.0, and 40.0 mg/kg, IP), and haloperidol (0.0, 0.125, 0.25, 0.5, and 1.0 mg/kg, IP). Drug doses were administered twice in random order (for both dose and drug) and never <48 h apart. Daily uridine or saline injections continued over the course of this experiment, for approximately 4 mo.

Amphetamine and chlordiazepoxide were dissolved in 0.9% saline and administered in a volume of 1.0 ml/kg 15 and 30 min, respectively, before the milk solution. Haloperidol was dissolved in 0.2 N acetic acid with several drops of glacial acetic acid added to dissolve the drug and NaOH to neutralize the solution. This stock solution was diluted with saline such that the final injection volume was 1.0 ml/kg, delivered 90 min before testing.

Experiment 2

Subjects. Forty adult male Sprague-Dawley rats (Taconic Farms) weighing 275 g (±26) were housed as in Experiment 1. Animals were given free access to water and food throughout the study, except that food was withheld 14 h before surgery.

Procedure. All animals received unilateral lesions of the striatum. One hour before surgery, animals were pretreated with pargyline (25.0 mg/kg, IP, dissolved in 0.9% saline at a concentration of 25.0 mg/ml). Then, 20 min before surgery, animals were anesthetized with 50.0 mg/kg of sodium pentobarbital injected IP in a volume of 1.0 mg/ml. Animals were placed in a stereotaxic instrument with incisor bars at +5 mm. They received 11 μg of 6-OHDA hydrobromide dissolved in 10 μl of 0.9% NaCl with 0.1% ascorbic acid, delivered in unilateral infusion into the right striatum over a 60-s interval. Coordinates were AP +2.0, lateral −3.0, and skull −6.5 to bregma, according to the atlas of Paxinos and Watson (15). Behavior testing was initiated 15–17 days after surgery.

The rotation chamber was made of wood with a Plexiglas front (24 × 24 × 90 cm). Animals were administered methamphetamine (4.0 mg/kg, IP, dissolved in 0.9% saline in a concentration of 4.0 mg/ml) 30 min presession, to select animals exhibiting rotational behavior. Those animals not meeting the criterion of at least 20 rotations/10 min were relesioned according to the methods detailed earlier. A total of 15 rats did not meet this criterion after the second surgery and were removed from the study. Five rats died, leaving a total of 20 rats used for behavior testing.

Animals then were divided into two groups matched for number of rotations. Ten rats received daily IP injections of uridine (32.0 mg/kg dissolved in 0.9% saline at a concentration of 32.0 mg/ml) and 10 animals received an equal volume of saline. These injections continued for the duration of the study, so that uridine was administered for 10.5 weeks. Following initiation of chronic uridine (or saline) treatment, rats were retested with 4.0 mg/kg methamphetamine on three subsequent occasions, approximately 3 weeks apart. Methamphetamine was administered 30 min after chronic uridine or saline injections, and rotations were counted 30 min after methamphetamine injections.

On the 8th day of chronic injections, challenge with L-dopa began. Animals were administered uridine or saline plus the peripheral dopa decarboxylase inhibitor, Ro4-4602 (benserazide hydrochloride; 25.0 mg/kg, dissolved in the saline or uridine solutions) 1 h before testing. Then, 20 min before

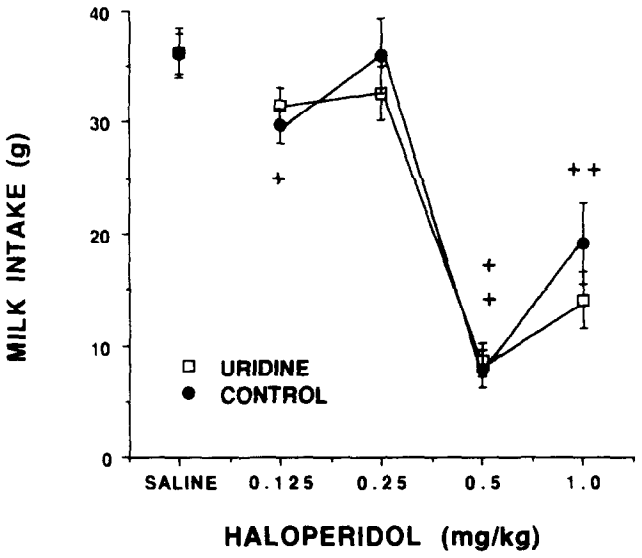

FIG. 1. Milk intake (g) as a function of haloperidol administration. Haloperidol was administered 90 min presession; sessions were 20 min. URIDINE animals received chronic uridine (32.0 mg/kg, IP; $n = 10$); CONTROL animals received chronic saline ($n = 10$). +, Significantly different from baseline, Fisher's PLSD, $p < 0.05$.

testing, they were injected with L-dopa (0.0, 50.0, 100.0, or 200.0 mg/kg, IP). On baseline days, animals received saline injections 20 min before testing and 40 min after the daily uridine or saline injection. L-dopa was administered in a randomized dosing order, with at least 24 h between test days. All doses were repeated before the completion of the dose-response curve.

At the completion of the study, rats were decapitated 4 h after the last uridine or saline injection, and the striatal region was dissected and assayed for DA, serotonin (5-HT), and their major metabolites by high-performance liquid chromatography (HPLC) with electrochemical detection as described elsewhere (4).

Statistical Analysis

In Experiment 1, milk intake was averaged across saline baseline days and Student's *t*-tests were used to determine between-group differences. Two-factor repeated measures analysis of variance (ANOVA) was performed to determine dose-response of amphetamine, haloperidol, and chlordiazepoxide, with dose as the within-subject factor and group (uridine vs. control) as the between-subject factor. The Fisher PLSD post hoc test was performed to determine significant differences within and between groups.

Dose-response data from Experiment 2 were analyzed by two-factor repeated measures ANOVA followed by the Fisher PLSD post hoc test. Factors were as in Experiment 1, except that the within-subject factor for methamphetamine data was time (four injections at 3-week intervals). Neurotransmitter and metabolite levels were analyzed by Student's *t*-tests.

RESULTS

Experiment 1

Both groups consumed nearly equal amounts of milk during baseline determination, showing that chronic administration of uridine by itself exerted no effect on milk intake (Student's *t*-test = 0.02; $p > 0.05$).

Haloperidol administration significantly reduced milk intake at all but one dose [0.25 mg/kg; $F(4, 72) = 73$; $p < 0.001$], and amphetamine caused a dose-dependent decrease in milk intake [$F(3, 54) = 119.7$; $p < 0.001$]. Although drug × treatment interactions were not significant, uridine blunted reduction of intake at the lowest dose of each drug (haloperidol 0.125 mg/kg; $t = 5.7$; $p < 0.05$; and amphetamine 0.5 mg/kg; $t = 5.32$; $p < 0.05$) (Figs. 1 and 2). The reduction in intake appeared to be consequent to nonspecific effects of each drug (i.e., stereotypy was apparent with amphetamine, and mild sedation with haloperidol).

Chlordiazepoxide significantly increased milk intake at 5.0 and 10.0 mg/kg, whereas the highest dose administered (40.0 mg/kg) resulted in significantly decreased intake [$F(4, 72) = 55.5$; $p < 0.001$]. There was no difference between groups at any dose (Fig. 3).

Experiment 2

Neither saline- nor uridine-treated animals rotated under baseline conditions. The administration of L-dopa exerted a dose-dependent increase in contralateral rotations in both uridine-treated and control animals compared with saline baseline [$F(3, 54) = 8.34$; $p < 0.01$]. Post hoc analysis showed this increase to be significant at 100.0 and 200.0 mg/kg for control animals but only at 200.0 mg/kg for the uridine-treated group ($t = 19.34$; $p < 0.05$). Although the mean number of rotations was lower at all doses of L-dopa for uridine-treated animals, between-groups analysis was not significant [$F(1, 18) = 0.34$; $p > 0.05$] (Fig. 4).

Administration of methamphetamine (4.0 mg/kg) produced opposite effects on the two groups, with control animals exhibiting increasingly higher numbers of ipsilateral rotations, whereas uridine-treated animals rotated less over time [$F(1, 18) = 0.34$; $p < 0.05$]. Post hoc analysis showed this

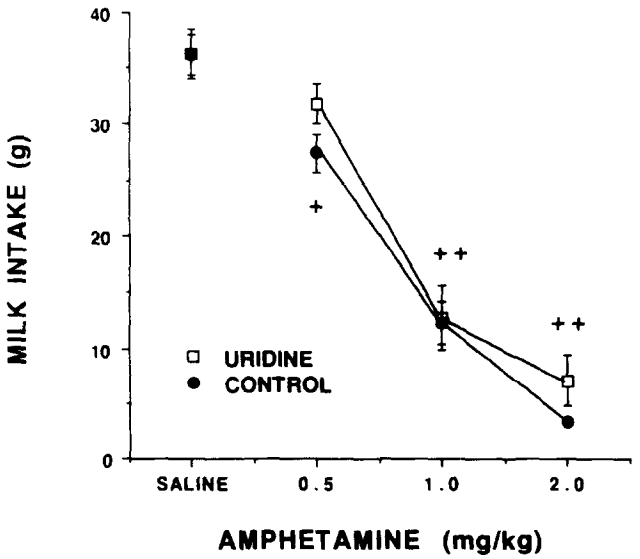

FIG. 2. Milk intake (g) as a function of amphetamine administration. Amphetamine was administered 15 min presession; sessions were 20 min. URIDINE animals received chronic uridine (32.0 mg/kg, IP; $n = 10$); CONTROL animals received chronic saline ($n = 10$). +, Significantly different from baseline, Fisher's PLSD, $p < 0.05$.

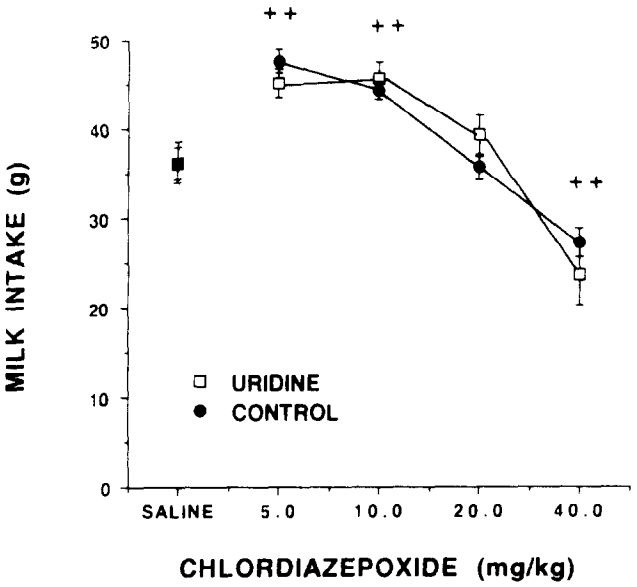

FIG. 3. Milk intake (g) as a function of chlordiazepoxide administration. Chlordiazepoxide was administered 30 min presession; sessions were 20 min. URIDINE animals received chronic uridine (32.0 mg/kg, IP; $n = 10$); CONTROL animals received chronic saline ($n = 10$). +, Significantly different from baseline, Fisher's PLSD, $p < 0.05$.

difference to be significant at the third and fourth administrations of methamphetamine (6 and 9 weeks after initiation of chronic uridine or saline; $t = 35.86$; $p < 0.05$) (Fig. 5).

HPLC analysis showed that 6-OHDA caused an 80% depletion of striatal DA in the lesioned side (2.15 μg/g tissue) compared to the nonlesioned side (11.12 μg/g tissue; Student's $t = 8.14$; $p < 0.01$). In addition, the toxin produced significant depletions in the DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) ($t = 7.2$; $p < 0.01$) and homovanillic acid (HVA) ($t = 7.8$; $p < 0.05$). 6-OHDA caused significant depletions of 5-HT ($t = 3.8$; $p < 0.01$) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) ($t = 3.6$, $p < 0.01$). Finally, t-tests performed between groups (control vs. uridine) showed that chronic treatment with uridine exerted no effect on these neurotransmitter and metabolite levels on either the lesioned or nonlesioned side of the striatum (data not shown).

DISCUSSION

Uridine has been proposed to serve as an important supplemental therapy in the treatment of the psychoses, potentially reducing side-effect liability associated with chronic haloperidol administration (1,13,14). The present studies were designed to determine whether uridine administered alone or in combination with various compounds would alter locomotor activity of rats without enhancing appetitive side effects.

In Experiment 1, milk intake was reduced after administration of amphetamine and all but one dose of haloperidol. Chronic uridine alone exerted no change in baseline milk consumption, but blunted amphetamine- and haloperidol-induced reduction in milk intake. However, this was marginal and only apparent at the lowest dose of each drug. Thus, although uridine previously has been shown to interact with dopaminergic systems that mediate locomotor activity (1,7,13,14), the effect on DA systems that regulate food intake is minimal.

At low doses, chlordiazepoxide increased milk intake, but decreased consumption at high doses. This biphasic effect was surprising given numerous reports of enhanced consumption at doses ranging from 2.0–50.0 mg/kg (2). Decreased con-

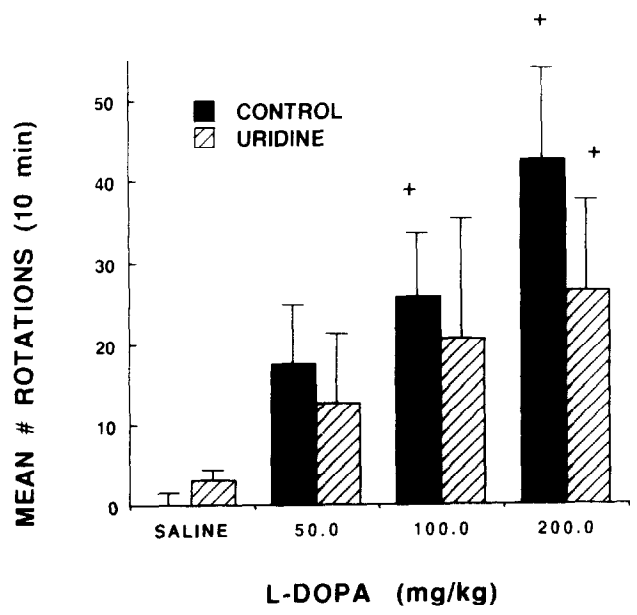

FIG. 4. The effect of L-dopa on mean number of rotations during 10 min sessions. L-dopa was administered together with Ro4-4602, 20 min presession to rats treated daily with URIDINE (32.0 mg/kg, IP; $n = 10$) or saline (CONTROL; $n = 10$). +, Significantly different from baseline, Fisher's PLSD, $p < 0.05$.

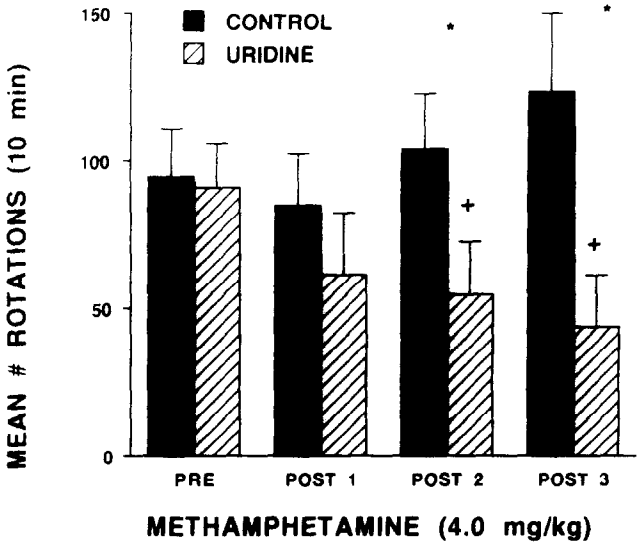

FIG. 5. The effect of methamphetamine (4.0 mg/kg, IP, 30 min presession) on mean number of rotations in four 10-min sessions. PRE = methamphetamine before chronic uridine or saline. POST 1, 2, and 3 = acute methamphetamine challenge, approximately 3 weeks apart over the course of 2 mo, to rats treated chronically with URIDINE ($n = 10$) or saline (CONTROL; $n = 10$). *Significant difference between groups; $p < 0.05$. +Significantly different from baseline (PRE); $p < 0.05$.

sumption in the present study appeared to be due to slight sedation at high doses. Nevertheless, the animals drank measurable quantities of milk at all doses, and chronic uridine treatment had no effect on chlordiazepoxide-induced alteration of milk intake. Although uridine has been reported to compete with GABA-receptor binding in some brain regions, and to antagonize bicuculline-induced seizure (9), the dose that blocked seizure was much higher than was used here (183.0 vs. 32.0 mg/kg, IP), making a comparison difficult. From our results it appears that the lower dose of uridine would not adversely affect food intake if used as supplemental therapy in the treatment of psychosis.

Chronic uridine treatment generated a shift to the right of the L-dopa dose-response curve compared with control animals. In addition, chronic administration of uridine elicited reduced rotation evoked by one dose of methamphetamine administered over time, in contrast to increased methamphetamine-induced rotation in control animals. These results suggest that uridine does not affect DA-mediated behaviors when administered by itself, but appears to alter DA transmission when combined with various dopaminergic agents.

It has been suggested that uridine's primary mechanism is reduction of presynaptic DA release (1). This notion is supported by results of Experiment 2, and by earlier reports that uridine decreased haloperidol-induced striatal DA release (1), and potentiated both catalepsy (1) and disruption of conditioned avoidance responding (13) induced by haloperidol. In addition, striatal DA release was blunted in uridine-treated animals after acute administration of 2.0, but not 4.0 or 8.0 mg/kg amphetamine (14).

Although these other studies are consistent with the interpretation that uridine blunts DA release, it should be noted that under other testing conditions a dopaminergic enhancement was reported (14). It was proposed that a secondary mechanism of action of uridine might be to increase DA receptor sensitivity, which could account for enhanced stereotypy induced by the direct-acting DA agonist apomorphine after chronic uridine (1), as well as behavioral changes elicited by acute haloperidol, amphetamine, and cocaine. It is possible that the increased dose of uridine used in Experiment 2 enhances the reduction of presynaptic DA release that was somewhat limited after chronic treatment with a lower dose of uridine (14), and this reduction may be sufficient to overcome secondary receptor sensitivity. In addition, long-term administration of uridine may further reduce DA release, as suggested by decreased rotation over time in uridine-treated animals evoked by acute methamphetamine challenge.

Haloperidol is a widely prescribed medication effective at reducing psychosis. Its affinity, however, for D_2 receptors found in abundance in the striatum is thought to elicit serious motoric side effects. By reducing DA overflow subsequent to receptor blockade, coadministration of uridine with haloperidol may allow for a reduction in dose of the neuroleptic, thereby reducing side effects. Further studies with uridine are warranted to elucidate its mechanism of action, as well as the effective dose range and regimen of administration in conjunction with antipsychotic medication. In addition, uridine's effect on mesocortical and mesolimbic DA systems must be determined, because dysregulation of these systems is thought to underlie the thought disorder and psychotic behavior typical of schizophrenic patients.

ACKNOWLEDGEMENTS

This work was supported by state funds and a grant from Polifarma S.p.A. New Jersey Agricultural Experiment Station Publication no. 14503-2-94.

REFERENCES

1. Agnati, L. R.; Fuxe, K.; Ruggeri, M.; Merlo-Pich, E.; Benefenati, F.; Volterra, V.; Ungerstedt, U; Zini, I. Effects of chronic uridine on striatal dopamine release and dopamine related behaviours in the absence or presence of chronic treatment with haloperidol. Neurochem. Int. 15:107-113; 1989.
2. Cooper, S. J. Benzodiazepines as appetite-enhancing compounds. Appetite 1:7-19; 1980.
3. Creese, I.; Burt, D. R.; Snyder, S. H. Dopamine receptor binding enhancement accompanies lesion-induced behavioral supersensitivity. Science 197:596-598; 1977.
4. DeVito, M. J.; Wagner, G. C. Methamphetamine-induced neuronal damage: A possible role for free radicals. Neuropharmacology 28:1145-1150; 1989.
5. Drago, F.; Continella, G.; De Luca, G. Effects of pyrimidine nucleosides and n-acetyl-glutamine on avoidance behaviour of the rat. Acta Ther. 8:337-46; 1982.
6. Drago, F.; D'Agata, V.; Valerio, C.; Spadaro, F.; Raffaele, R.; Nardo, L.; Grassi, M.; Freni, V. Memory deficits of aged male rats can be improved by pyrimidine nucleosides and n-acetyl-glutamine. Clin. Neuropharmacol. 13:290-296; 1990.
7. Farabegoli, C.; Merlo-Pich, E.; Cimino, M.; Agnati, L. F.; Fuxe, K. Chronic uridine treatment reduces the level of [^{3}H] spiperone-labelled dopamine receptors and enhances their turnover rate in striatum of young rats: Relationship to dopamine-dependent behaviours. Acta Physiol. Scand. 132:209-216; 1988.
8. Foltin, R. W.; Woolverton, W. L.; Schuster, C. R. The effect of d-amphetamine and haloperidol alone and in combination on milk drinking in rats. Psychopharmacology 80:342-344; 1983.
9. Guarneri, P.; Guarneri, R.; LaBella, V.; Piccoli, F. Interaction between uridine and GABA-mediated inhibitory transmission: Studies in vivo and in vitro. Epilepsia 26:666-671; 1985.
10. Inoue, S.; Honda, K.; Komoda, Y.; Uchizono, K.; Ueno, R.; Hayaishi, O. Differential sleep-promoting effects of five sleep substances nocturnally infused in unrestrained rats. Proc. Natl. Acad. Sci. USA 81:6240-6244; 1984.
11. Mandel, P.; Wintzerith, M. Free nucleotides and nucleic acids during brain development. In: Kumar, S., ed. Biochemistry of brain. Oxford: Pergamon Press; 1980:241-282.
12. Melamed, E.; Hefti, F.; Bitton, V.; Globus, M. Suppression of *l*-dopa-induced circling in rats with nigral lesions by blockade of central dopa-decarboxylase: Implications for mechanism of action of *l*-dopa in parkinsonism. Neurology 34:1566-1570; 1984.
13. Myers, C. S.; Fisher, H.; Wagner, G. C. Uridine potentiates haloperidol's disruption of conditioned avoidance responding. Brain Res. 651:194-198; 1994.
14. Myers, C. S.; Napolitano, M.; Fisher, H.; Wagner, G. C. Uridine andstimulant-induced motor activity. Proc. Soc. Exp. Biol. Med. 204:49-53; 1993.
15. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
16. Ungerstedt, U.; Arbuthnott, G. W. Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. Brain Res. 24:485-493; 1970.