Neurochemical and Motor Effects of High Dose Haloperidol Treatment: Exacerbation by Tryptophan Supplementation[1] (43472)

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Abstract. **The neurochemical and motor effects of a high dose (25 mg/kg) of haloperidol were assessed in male Sprague-Dawley rats. In Experiment 1, this high dose of haloperidol caused dramatic increases in striatal dopaminergic and serotonergic turnover that only returned to control levels 100 hr after injection. In the second experiment, the same dose of haloperidol was administered twice over a 3-week interval in the presence or absence of a dietary tryptophan supplement added to the drinking water. Rats were assessed for disruption of locomotor behavior (using the rotorod) as well as the occurrence of spontaneous (dyskinetic-like) chewing and head twitching. It was observed that haloperidol impaired rotorod performance in a manner that paralleled the time course of the neurochemical changes in Experiment 1. In addition, the tryptophan (consumed at an average of 157 mg/kg/day) exacerbated the deficit in rotorod performance in haloperidol-treated rats after the first, but not after the second, haloperidol injection. Finally, the combination of haloperidol plus tryptophan was found to cause a long-lasting increase in spontaneous chewing movements that lasted 56 days after the first injection. These observations are interpreted in the context of tryptophan supplementation to antipsychotic therapy.** [P.S.E.B.M. 1992, Vol 200]

Although antipsychotic drugs have been widely prescribed for the treatment of schizophrenia since the 1950s (1), their beneficial effects are accompanied by involuntary movement disorders. These short-term extrapyramidal side effects can include akinesia, dystonia, and parkinsonism (2) and the later-appearing tardive dyskinesias, which manifest as tic-like movements, particularly of the oral-lingual-masticatory area (3).

To date, there are no adequate treatments for tardive dyskinesia. Attempted approaches include benzodiazepines, noradrenergic blocking agents, cholinergics, anticholinergics, antidepressants, and increased doses of the neuroleptic. However, these treatments may carry their own side effects and none have proved efficacious in a majority of patients (4).

Lack of an effective treatment for tardive dyskinesia is not surprising considering that its etiology is unclear. It has been well established that antipsychotic agents antagonize dopamine receptors (1) and that receptor supersensitivity develops with their prolonged administration (5). It has been proposed that this proliferation of dopamine receptors may result in the dyskinetic movements, but this conclusion has not lead to the development of treatments designed to lessen their occurrence.

In this regard, however, it has been speculated that the serotonin system may play a role in modulating the activity of dopaminergic neurons, and some preliminary investigations in our laboratory (6) as well as in the clinics (7) appear to support this hypothesis. The former animal studies showed that elevating brain serotonin through dietary tryptophan reduced haloperidol-induced head movements in rats. The clinical data indicate that an oral tryptophan supplement markedly reduced tardive dyskinesia in several patients who had been refractory to other treatments.

The nature of this serotonergic modulation of the dopamine system appears to be primarily one of inhi-

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bition (8–10). Lesions of serotonin cell bodies in the raphe nuclei augment apomorphine-induced locomotor behavior (11), whereas pretreatment with 5-hydroxytryptophan decreases apomorphine-induced circling in rats with unilateral lesions of the nigrostriatal pathway (12). In addition, it was found that L-tryptophan decreased the intensity of amphetamine-induced stereotypy (13). In contrast, the administration of dopaminergic antagonists has been shown to elevate both serotonin levels (6) and turnover (14). Collectively, these data indicate that compounds that enhance serotonergic function tend to diminish dopamine-mediated behaviors, whereas manipulations that diminish serotonin activity augment dopamine-mediated behaviors.

In the following experiments, the effects of a high-dose haloperidol treatment that had been shown to produce dyskinetic movements in previous studies (6, 15, 16) were examined. In the first experiment, we monitored the effects on dopamine and serotonin levels and turnover at a series of time points after 25 mg/kg of haloperidol. In the second experiment, we assessed rotorod performance and spontaneous motor behavior after the haloperidol with and without daily tryptophan supplementation.

Experiment 1

Animals. Experiment 1 employed 60 5-month-old male Sprague-Dawley rats (Charles River, Wilmington, MA). Animals were housed individually with free access to food and water in metal suspension cages in a temperature- and humidity-regulated colony room with a 12:12-hr light:dark cycle.

Procedure. Fifty of the rats were administered a single dose of 25 mg/kg of haloperidol intramuscularly and sacrificed in groups of 10 at 1, 3, 10, 30, and 100 hr after injection. Two animals per time period were administered a vehicle injection.

Neurochemistry. At the time of sacrifice, the brains were removed and the striatal region was quickly dissected (17) and stored in liquid nitrogen until assayed by high-performance liquid chromatography (HPLC) with electrochemical detection for dopamine, serotonin, and their metabolites dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid (5-HIAA). The HPLC unit used was a Waters system with a LC-4B electrochemical detector and Digital Professional 350 integrator/computer. The mobile phase was sodium phosphate and citrate buffer containing 15% HPLC-grade methanol, and the flow rate was 0.8 ml/min.

Experiment 2

Experiment 2 was conducted to investigate the effects of high-dose (25 mg/kg) haloperidol intramuscularly and daily tryptophan on locomotor performance and to assess whether any tolerance to haloperidol's effects on locomotor behavior developed after a second injection.

Subjects. Sixty male Sprague-Dawley rats (Charles River) were used. They were 60 days old at the start of testing, and housed as described for Experiment 1.

Procedure. The animals were divided equally into four groups: (i) vehicle with daily tryptophan (VEH/TP); (ii) vehicle alone (VEH); (iii) haloperidol (25 mg/kg) with daily tryptophan (HAL/TP); and (iv) haloperidol (25 mg/kg) alone (HAL). The animals were administered two intramuscular injections of haloperidol 3 weeks apart. Tryptophan methyl ester hydrochloride (2 mg/ml; Sigma Chemical Co., St. Louis, MO) and saccharin (1.5 mg/ml) were dissolved in the drinking water. The spouts of the water bottles were affixed to the front and center of the home cage. The solution was changed and consumption was measured every 3 days. There was no evidence of fluid spillage (confounding consumption measurement) or gnawing on the sipper tube in any of the groups.

Rotorod. Locomotor performance was assessed for all rats on a rotorod. The cloth-covered rotorod had a 15-cm circumference and a speed of 12 revolutions/min. Prior to any treatment, rats were trained in a single session until they attained 120 sec on the rod. Performance was measured at 10, 30, 100, 172, and 244 hr after each haloperidol/vehicle injection.

Behavioral Observations. Animals were assessed by an observer blind to treatment condition and scored for (1 = presence, 0 = absence) chewing or head twitching. The home cage was removed and placed on a cart for 10 min before the observation period of 2 min (see Ref. 15 for details).

Neurochemistry. Animals were sacrificed on Day 70 after the first injection and on Day 49 after the second injection. Striatal regions were removed and assayed by HPLC with electrochemical detection following the same procedure as described for Experiment 1.

Statistics. One-way and repeated-measures analyses of variance were used to determine group differences. Post hoc differences between individual groups were assessed by Fisher PLSD tests for the neurochemical and rotorod data. The behavioral studies were assessed using one-way chi-square tests.

Results

Experiment 1. *Neurochemistry.* There were decreases in levels of dopamine and dramatic increases in its metabolites after acute intramuscular injection of haloperidol compared with control vehicle injection (Table I). Although dopamine ($F(5,53) = 4.7$; $P < 0.01$) was only significantly lower at the 3-hr time point, dihydroxyphenylacetic acid ($F(5,53) = 16$; $P < 0.001$) and homovanillic acid ($F(5,53) = 13.6$; $P < 0.001$) were significantly elevated at all of the time points analyzed,

Table I. Levels of Striatal Dopamine and Its Metabolites in Tissue at Various Times after Acute Injection of Haloperidol[a]

	Control	1 hr	3 hr	10 hr	30 hr	100 hr
Dopamine	11.0 ± 0.73	9.2 ± 0.58	8.0 ± 0.57[b]	10.3 ± 0.85	10.7 ± 0.68	12.6 ± 0.88
Dihydroxyphenylacetic acid	0.62 ± 0.07	2.22 ± 0.16[b]	1.22 ± 0.12[b]	1.95 ± 0.21[b]	1.87 ± 0.15[b]	1.29 ± 0.11[b]
Homovanillic acid	0.46 ± 0.06	1.59 ± 0.13[b]	1.43 ± 0.10[b]	1.80 ± 0.19[b]	1.76 ± 0.17[b]	1.02 ± 0.13[b]
Dihydroxyphenylacetic acid/dopamine	0.06 ± 0.01	0.25 ± 0.02[b]	0.16 ± 0.02[b]	0.19 ± 0.01[b]	0.18 ± 0.01[b]	0.10 ± 0.01
Homovanillic acid/dopamine	0.04 ± 0.01	0.18 ± 0.02[b]	0.18 ± 0.02[b]	0.17 ± 0.02[b]	0.17 ± 0.02[b]	0.08 ± 0.02[b]

[a] Levels of striatal dopamine and its metabolites are expressed in μg/g ± SE of tissue at various times following an acute injection of 25 mg/kg of haloperidol. n = 10/group.
[b] $P < 0.05$ compared with control.

which indicates increased turnover. The ratios of dihydroxyphenylacetic acid to dopamine ($F(5,53) = 14.5$; $P < 0.001$) and homovanillic acid to dopamine ($F(5,53) = 14.8$; $P < 0.001$) were significantly higher than control at 1, 3, 10, and 30 hr, but returned to control levels by the 100-hr time point. These results reveal a marked increase in dopamine turnover that is only beginning to return to control levels 4 days after haloperidol injection.

Serotonin turnover was also increased after haloperidol injection ($F(5,53) = 2.4$; $P < 0.05$) (Table II). Serotonin levels were significantly decreased at 3 and 10 hr, and there was a general trend for 5-HIAA to increase, although this difference was not statistically significant. The ratios of 5-HIAA to 5-hydroxytryptophan ($F(5,53) = 10$; $P < 0.001$) were significantly elevated at 1, 3, 10, and 30 hr. Turnover also returned to control levels at 100 hr.

Experiment 2. The HAL/TP group consumed an average of 150 ± 5 mg/kg/day of tryptophan, while the VEH/TP group consumed an average of 164 ± 7 mg/kg/day of tryptophan over the 10 weeks of the study. There were no significant shifts in the amount of fluid consumed in the 10-week period in either of these groups. Furthermore, the tryptophan supplementation did not have any effect on body weight, although haloperidol-treated rats had depressed body weights compared with their vehicle-treated controls on Days 7 and 28.

Locomotor performance. Locomotor performance on the rotorod was dramatically impaired by haloperidol injections. After the first injection, both the HAL and HAL/TP groups had very short latencies to fall off the rotorod at the first two time points tested (Fig. 1). Both groups were significantly different from the two VEH groups at 10 ($F(3,56) = 32.7$; $P < 0.001$) and 30 ($F(3,56) = 48$; $P < 0.001$) hr. At 10 hr, the HAL group maintained an average of 32 ± 5 sec on the rotorod, while the HAL/TP maintained only 21 ± 2 sec, and this difference was significant. At 30 hr, the two HAL groups were not different from each other, but the HAL/TP group still had a shorter latency to fall, 31 ± 3 sec compared with the HAL group, which maintained 39 ± 9 sec. By 100 hr after the first injection, the HAL/TP group was performing significantly poorer than the other three groups ($F(3,56) = 4.5$; $P < 0.01$), whereas the HAL group was comparable to controls. These results indicate that after the first injection of haloperidol, daily tryptophan attenuated locomotor performance to a greater degree than haloperidol alone. At 172 and 244 hr, all groups were comparable to baseline.

The 244-hr time point after the first injection was used for the baseline for performance after the second injection (Fig. 2). A repeated-measures analysis of variance revealed poorer performance at 10 hr after the second injection (as compared with the performance after the first injection), but this was due to both the vehicle and drug-treated groups' decline in performance and was not attributable to a sensitization to haloperidol. The differential attenuation between the HAL and HAL/TP groups was not maintained after the second injection, when the two groups performed at comparable levels. Dramatic differences between the vehicle- and drug-treated groups remained, however. At 10

Table II. Levels of Striatal Serotinin and Its Metabolites in Tissue at Various Times after an Acute Injection of Haloperidol

	Control	1 hr	3 hr	10 hr	30 hr	100 hr
5-Hydroxytryptamine	0.34 ± 0.03	0.26 ± 0.02	0.20 ± 0.01[b]	0.24 ± 0.03[b]	0.28 ± 0.03	0.26 ± 0.04
5-HIAA	0.29 ± 0.05	0.34 ± 0.03	0.31 ± 0.03	0.27 ± 0.04	0.30 ± 0.03	0.23 ± 0.04
5-HIAA/5-hydroxytryptamine	0.80 ± 0.09	1.36 ± 0.11[b]	1.55 ± 0.14[b]	1.14 ± 0.04[b]	1.07 ± 0.05[b]	0.80 ± 0.07

[a] Levels of striatal serotonin (5-hydroxytryptamine) and its metabolites are expressed in μg/g ± SE of tissue at various times following an acute injection of 25 mg/kg of haloperidol. n = 10/group.
[b] $P < 0.05$ compared with control.

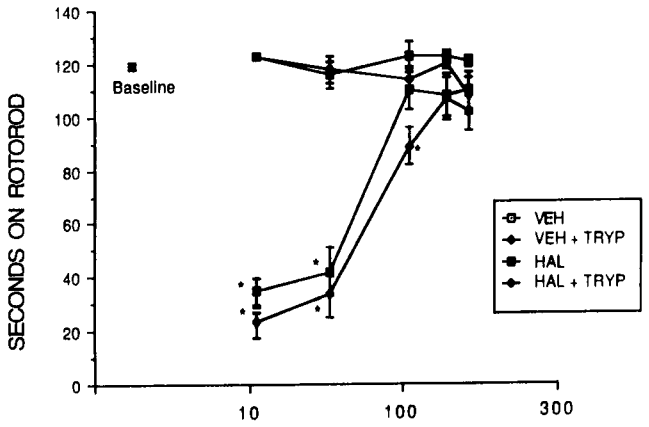

Figure 1. Locomotor performance in young (2-month-old) rats after first injection of VEH or HAL (25 mg/mg). The tryptophan (TRYP) groups received tryptophan in their drinking water. * Significantly different from baseline ($P < 0.01$), Fisher's PLSD.

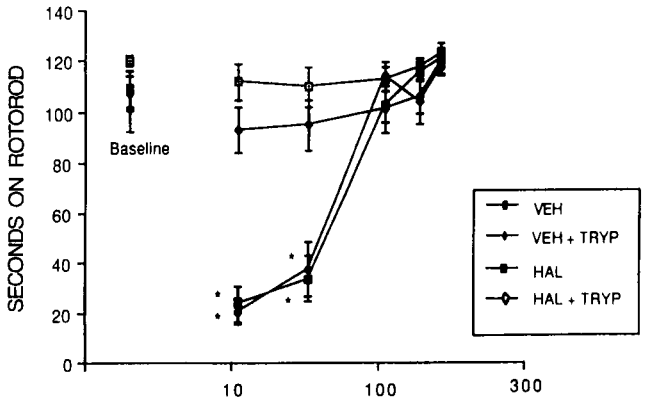

Figure 2. The 244-hr time point after the first injection was used as the baseline against which rotorod performance after the second injection was assessed. Performance was as impaired as after the first injection. * Significantly different from baseline ($P < 0.01$), Fisher's PLSD.

hr the performance of the HAL (22 ± 7 sec) and HAL/TP (18 ± 5 sec) groups was significantly lower than that of vehicle groups ($F(3,56) = 36$; $P < 0.001$). This difference remained at 30 hr ($F(3,56) = 14.5$; $P < 0.001$), when performance was still poor for HAL (31 ± 9 sec) and HAL/TP (35 ± 11 sec). However, by 100 hr after the second injection, there were no significant differences among the four groups ($F(3,56) = 0.87$; $P = 0.46$). Performance remained stabilized at 172 and 244 hr after the second injection.

Behavioral observations. Presence or absence of chewing between HAL/TP versus HAL, HAL versus VEH, and HAL/TP versus VEH/TP was compared by chi-square analysis. The HAL/TP group showed more chewing than the HAL group on Days 7 ($\chi^2 = 7.5$; $P < 0.01$), 21 ($\chi^2 = 6.1$; $P < 0.05$), 24 ($\chi^2 = 3.9$; $P < 0.05$), 42 ($\chi^2 = 6.0$; $P < 0.05$), and 56 ($\chi^2 = 5.4$; $P < 0.05$). There were no significant differences in chewing when the HAL alone group was compared with the VEH alone group, nor when the VEH/TP group was compared with the VEH alone group. The HAL/TP group displayed more chewing than the VEH/TP group on Days 14 ($\chi^2 = 6.0$; $P < 0.05$), 21 ($\chi^2 = 6.1$; $P < 0.05$), 24 ($\chi^2 = 6.1$; $P < 0.05$), 28 ($\chi^2 = 9.5$; $P < 0.01$), 49 ($\chi^2 = 3.9$; $P < 0.05$), and 56 ($\chi^2 = 10.9$; $P < 0.01$).

The only significant difference in head twitching was found on Day 24 when the HAL/TP group showed more than the VEH/TP group ($\chi^2 = 6.0$; $P < 0.05$).

Neurochemistry. The rats were sacrificed 49 days after the second haloperidol injection. There was no significant alterations in dopamine or its metabolites as a result of either the haloperidol injection or tryptophan supplementation. The tryptophan supplementation, however, caused a significant ($F(3,53) = 2.8$; $P < 0.05$) elevation (84%) in striatal serotonin in the VEH/TP group as compared with the VEH alone group, an effect that failed to reach statistical significance when comparing the HAL/TP group with the HAL alone group. In addition, the tryptophan supplementation caused a moderate but nonsignificant (31%) elevation in striatal 5-HIAA in the VEH/TP group as compared with the VEH alone group.

Discussion

The administration of a high dose of haloperidol was found to cause a long-lasting effect on both dopaminergic and serotonergic neurons that paralleled severe impairment of locomotor performance. The animals could not be evaluated on the rotorod at 1 or 3 hr after the injection because the sedative effect of haloperidol at those time points obviated testing. The increase in neurotransmitter turnover was still markedly elevated at the 10- and 30-hr time points, when there were correspondingly extreme deficits in locomotor performance. The neurochemical data show dopamine and serotonin turnover returning to control levels by 100 hr, a time when locomotor performance had also largely returned to baseline.

The tryptophan supplementation in Experiment 2 resulted in a significant elevation in striatal serotonin, along with a slight increase in 5-HIAA levels. Surprisingly, although the trend was still evident, the tryptophan-induced elevation in serotonin was blocked by the haloperidol. Nonetheless, the behavioral observations indicate that serotonergic activity exacerbates the effects of haloperidol on locomotor behavior. In addition, the daily tryptophan combined with haloperidol also had the effect of increasing chewing and head twitching over the levels of the other groups. It was reported previously that tryptophan supplementation caused a reduction in head movements (6), an observation at variance with the present results. However, variation in the topography of the behavior and amount of the daily tryptophan may account for these discrepant observations.

Serotonin antagonists such as ritanserin and seto-

perone have been shown to be effective in clinical trials in treating negative symptoms of schizophrenia and in producing a low incidence of extrapyramidal side effects (18). Janssen *et al.* (19) reported that risperidone, a combination of S2 and D2 antagonist, alleviated schizophrenic symptoms with no tendency toward manifestations of extrapyramidal side effects. These effects are consistent with the idea that serotonin antagonists act to disinhibit dopamine.

Because the serotonin system has an inhibitory influence on the dopamine system in the basal ganglia, it was predicted that a serotonin agonist such as tryptophan would exacerbate the early-appearing extrapyramidal side effects, but ameliorate the late-appearing tardive dyskinesia. The results do not support any differential effect of tryptophan at different times during treatment. Rather, tryptophan seems to have the general effect of exacerbating or unmasking dyskinetic behaviors after high-dose haloperidol treatment.

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