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A Dietary Haloperidol Regimen for Inducing Dopamine Receptor Supersensitivity in Rats[1]

JOSEPH M. FREY,†[2] WILLIAM W. MORGAN,* MAHARAJ K. TICKU† AND RONALD D. HUFFMAN†[3]

*Departments of Pharmacology† and Cellular and Structural Biology**
The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7764

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FREY, J. M., W. W. MORGAN, M. K. TICKU AND R. D. HUFFMAN. *A dietary haloperidol regimen for inducing dopamine receptor supersensitivity in rats.* PHARMACOL BIOCHEM BEHAV 26(4) 661–669, 1987.—The induction of dopaminergic supersensitivity in rats by the administration of haloperidol in their diet for 30 days (CHAL) in three increasing concentrations (7–15 mg/kg/day) was compared to that induced by single daily subcutaneous injections (SCHAL, 0.7 mg/kg) on the basis of biochemical (radioimmunoassay of serum haloperidol levels, ^{3}H-spiroperidol binding) or behavioral (apomorphine stereotypy, spontaneous locomotor activity) parameters. The two modes of administration produced equivalent blood levels of haloperidol by day 30. At 48 hours post treatment: (a) spontaneous locomotor activity and stereotyped behavior were significantly increased in both groups of haloperidol-treated rats, (b) stereotyped behavior was significantly greater in CHAL- vs. SCHAL-treated rats at 8 days post treatment and (c) specific ^{3}H-spiroperidol binding was increased 64% and 236% within the striatum and GP, respectively, of CHAL-treated vs. control rats. Scatchard analysis of ^{3}H-spiroperidol binding isotherms revealed a significant increase in the B_{max} of high affinity binding sites [K_D~55 pM] within the striatum of both CHAL- and SCHAL-treated rats at 48 hours post treatment. A second, lower affinity site was resolved within the SCHAL-treated group which was not detected within striatal homogenates of CHAL-treated or control rats.

Chronic haloperidol Dopaminergic supersensitivity ^{3}H-Spiroperidol binding Basal ganglia Behavioral responses

CONSIDERABLE attention has been focused on the study of the various neurotransmitter systems within the basal ganglia (BG) in an effort to understand the cause(s) of the motor disturbances produced by neuroleptic drugs after prolonged administration to humans. A number of different experimental procedures have been employed to induce chronic blockade of dopamine receptors in rats to assess the effects of chronic haloperidol treatment on dopaminergic systems within the forebrain. The most popular modes of chronic haloperidol administration have been parenteral injections, either subcutaneous or intraperitoneal, and direct oral injections of the drug, either once or twice daily, for periods of a few days up to several months (for references, see [37]). Alternatively, other investigators have administered haloperidol in the diet [23] or in the drinking water [17,19]. For periods of administration lasting up to 18 months or longer, subcutaneous silicone (or silastic) implants containing haloperidol base have been utilized [29,44]. Almost all of the above methods of parenteral and enteral administration of haloperidol are associated with some degree of behavioral depression and catalepsy [2, 4, 9, 33]. In addition, a number of other problems are associated with the enteral and/or parenteral routes of administration including fluctuating concentrations of the drug in the blood and possible complications arising from local tissue damage resulting from multiple injections.

In a previous study, we examined the effects of chronic haloperidol administration on GABA receptor binding within the substantia nigra (SN) and striatum of rats [23]. In selecting a method for the long-term administration of haloperidol for that study, we sought a regimen which was simple to administer and offered a continuous mode of administration but which mimimized behavioral depression and catalepsy. We therefore chose to administer haloperidol via the diet for a period of 30 days. Several investigators have demonstrated the usefulness of this mode of haloperidol administration for

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[2]Present address: Department of Pharmacology, Northwestern University Medical School, 303 East Chicago Ave., Chicago, IL 60611. Medical School, 303 East Chicago Ave., Chicago, IL 60611.

[3]Requests for reprints should be addressed to Ronald D. Huffman, Ph.D., Department of Pharmacology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7764.

inducing dopaminergic supersensitivity in mice [20, 34, 46]. We have recently been conducting electrophysiological experiments to test the effects of chronic haloperidol administration on the sensitivity of globus pallidus (GP) and SN neurons to microiontophoretically-applied GABA in rats that had received haloperidol in their diet for 30 days [13,14]. Although we had previously demonstrated that this feeding regimen produced a significant increase in the number of GABA binding sites within the SN [23], we were concerned about the lack of adequate characterization of the effects of this feeding model on the dopaminergic system of the BG and by the fact that the large doses of haloperidol (7–15 mg/kg) that were being consumed by our rats seemed to be exerting little effect on their behavior. Since Gale [15,16] had employed the subcutaneous (SC) administration of haloperidol (0.7 mg/kg, once daily) to study the effects of chronic haloperidol administration on GABA binding within the SN, we decided to compare dopaminergic supersensitivity induced by this mode of haloperidol administration with that produced by our dietary mode of administration on the basis of biochemical (radioimmunoassay of serum haloperidol levels, ^{3}H-spiroperidol binding) and behavioral (apomorphine-induced stereotypy, spontaneous locomotor activity) parameters.

METHOD

Chronic Haloperidol Administration

Dietary administration of haloperidol (CHAL). Male Sprague-Dawley rats, with initial weights of 140–200 g, received haloperidol in their feed for 30 days in three increasing concentrations: 0.01% for the first 12 days, 0.015% from day 13 to 24 and 0.02% for the remaining 6 days. On day 30, the haloperidol diet was terminated, and the rats were maintained on a drug-free diet of ground rat meal for two days. The haloperidol diet was prepared by mixing powdered haloperidol with ground rat meal (Teklab Rodent Meal) for 60 min in a Hobart mixer. The diet was then stored at 4°C in a light-protected container for a maximum of 5–7 days. The rats received fresh haloperidol diet at least every 3–5 days.

CHAL-control rats received drug-free rat meal throughout the entire 32 day feeding period and were matched by weight to the CHAL-treated group at the start of each experiment. CHAL-control rats were subsequently pair-fed to assure a weight gain comparable to that of the CHAL-treated rats. All rats were given access to water ad lib and were maintained in an environmentally controlled room on a 14:10 hr, light:dark cycle throughout the course of these experiments.

Subcutaneous haloperidol injections (SCHAL). Rats treated chronically with SC injections of haloperidol received a single 0.7 mg/kg injection (HALDOL injectable, McNeil Pharmaceuticals) at the same time of day for 30 consecutive days. On days 31 and 32, SCHAL-treated rats were injected with 0.9% NaCl. SCHAL-control rats received daily injections of 0.9% NaCl for the entire 32 day period. All rats received ground rat meal throughout the treatment period and were initially paired by weight. Animal weights ranged from 140 to 160 g at the start of experiments. All other environmental conditions were made as identical as possible to the CHAL-treated group of rats.

Determination of Serum Haloperidol Concentrations

Haloperidol (-butanone) and reduced haloperidol (-butanol) radioimmunoassays were performed by Dr. C. Davis (Texas Research Institute of Mental Sciences, Houston, TX) utilizing the method of Browning *et al.* [3]. The rats were sacrificed by decapitation and the blood was collected in a series of test tubes. Samples of rat serum (N=6) were collected 1 1/2 hr after SC-injections and 1 1/2 hr after termination of the dark cycle for the CHAL-treated group on days 6, 18, 30 and 32 of the treatment schedule. These samples were stored at −80°C and were shipped for assay packed in dry ice.

Behavioral Experiments

Apomorphine-induced stereotypy. Stereotyped behavior was assessed in both CHAL-treated and SCHAL-treated rats on the 30th day of treatment and again on days 2 and 8 following termination of the haloperidol treatments. The rats were tested individually in covered plastic cages (46×25.4×20.3 cm) in which a wire mesh had been placed on the bottom of each cage. The rats were allowed 30 min to acclimate to the cage before being given a 0.5 ml IP injection of 0.9% NaCl. Their behavior was then scored for 6 consecutive 5 min periods. After this 30 min period, apomorphine hydrochloride (Sigma Chemicals, St. Louis, MO) was administered IP at either 1.0 mg/kg on day 30 or 0.5 mg/kg on days 32 and 38; their behavior was then assessed for 12 consecutive 5 min periods. During each 5 min observation period, the rats were scored for the predominant behavior elicited during that period using the following modification of the scoring system developed by Ernst [8]: 0—the rats showed no stereotyped behaviors; 1—the rats walked around the cage, sniffing, licking, or pushing their nose into the wire mesh on the bottom of the cage; 2—the rats walked about the cage and occasionally bit or gnawed the wire or the sides of the cage, or they sat in one spot, without moving their hindlimbs, and intensely licked the wire or the side of the cage; 3—the rats restricted their activity to one small area (1/4 of the cage) and gnawed intensely on the wire or side of cage; 4—the rats remained in one spot for 5 min or longer without moving their hindlimbs, and intensely gnawed the wire or the side of the cage. The maximum possible stereotypy score for the 60 min period following the injection of apomorphine was 48.

Spontaneous locomotor activity. The rats were monitored during the dark cycle for changes in spontaneous locomotor activity during the course of the haloperidol treatments by using a series of Stoelting electronic activity monitors. All rats were tested individually in covered plastic cages (47×25.4×20.3 cm) containing bedding and were placed in this environment at least 4 hr prior to the start of each recording session. The rats were allowed free access to both food and water during the testing period. The cages were placed on individual Stoelting sensors 20 min prior to testing to allow warm-up of amplifiers and power supply as well as to acclimate the rats to a change in environment. Following this warm-up period, the lights were turned off and spontaneous locomotor activity was recorded for 5 consecutive 4 min periods. The average of the number of counts recorded during each of these 5 observation periods served as the control measure of spontaneous locomotor activity for each day of testing. Following this initial testing period, the lights were turned on and the rats were administered 0.5 ml SC injections of either 0.7 mg/kg haloperidol (SCHAL group) or 0.9% NaCl (CHAL group). The lights were again turned off and activity was monitored for an additional 8 hr (120 periods × 4 min). As a measure of the activity recorded during this 8

hr period, the total number of 4 min periods in which the activity was greater than or equal to 40% of the mean control activity recorded at the start of the recording session was tabulated. This method of quantification afforded the most consistent measure of locomotor activity recorded for the 32 day testing period. The monitoring of spontaneous locomotor activity as described above was performed on days 0, 2, 4, 6, 12, 18, 24 and 30. On days 31 and 32, all rats received 0.5 ml SC injections of 0.9% NaCl. Prior to the initiation of haloperidol treatment, spontaneous locomotor activity was monitored for 3–5 days using the procedure described above except that all rats received daily 0.5 ml injections of 0.9% NaCl. The activity recorded on the last day of this initial testing period was used as the baseline measure with which to compare the effects of the chronic haloperidol treatments or withdrawal from these treatments (day 0).

Receptor Binding

Specific ^{3}H-spiroperidol binding to striatal, pallidal and nigral membranes. Binding assays were carried out on tissues pooled from 6 rats in each treatment group. On day 32, two days after the chronic treatments had been discontinued, the rats were anesthetized with chloral hydrate (600 mg/kg IP) and were sacrificed by decapitation. The brains were removed, blocked and frozen sections (600–800 μm) were cut through the areas of the striatum, GP and SN_R. Tissues from these three areas were dissected with the aid of a stereomicroscope, weighed and stored in polystyrene microbeakers at −80°C. The pooled tissues were thawed at room temperature and were homogenized in 9 volumes of 50 mM TRIS-HCl (pH 7.4) using a TRI-R Instruments, Model S63C homogenizer. The homogenate was centrifuged at 40,000 × g for 20 min at 4°C using a Beckman Model J_2-21 centrifuge. The resulting pellet was washed twice using the same buffer and then was frozen. The protein remaining in the sample following these two washes represented approximately 2% of the original tissue weight. Subsequently, the pellet was resuspended in the Tris buffer and ^{3}H-spiroperidol binding was performed by the method of Seeman *et al.* [45]. Protein content was determined by the method of Lowry *et al.* [31] and adjusted to a final concentration of 40–60 μg/ml. Triplicate aliquots (2 ml) of the washed membrane preparations were incubated in the presence of 0.02–0.8 nM concentrations of ^{3}H-spiroperidol for striatal membranes and 0.1 nM concentration for GP and SN_R membrane preparations. Two additional samples at each concentration of ^{3}H-spiroperidol were incubated in the presence of cold (+)-butaclamol (1 μM in 100 μM tartaric acid) to determine nonspecific binding. After incubation for two hr at room temperature, the reaction was terminated by filtration under vacuum through Whatman GF/B fiberglass filters which were washed twice with 5 ml ice cold TRIS-HCl buffer. The filters were placed immediately into liquid scintillation vials and 5 ml of scintillation fluid (2:1; Toluene: Triton X-100) was added. After cooling at 4°C overnight, the radioactivity was quantified by beta counting at 38–40% efficiency. Specific binding was determined by subtracting the total filtered radioactivity from background (the amount of radioactivity not displaced by 0.1 μM (+)-butaclamol) for each concentration of ligand and was subsequently converted to fmol/mg protein bound. Specific binding represented 72±3% of the total radioactivity in the filter at the 0.1 nM ^{3}H-spiroperidol concentration.

^{3}H-Spiroperidol (23.4 Ci/mmol) was purchased from New England Nuclear (Boston, MA) and (+)-butaclamol was supplied by Ayerst Research Laboratories (Montreal, Canada).

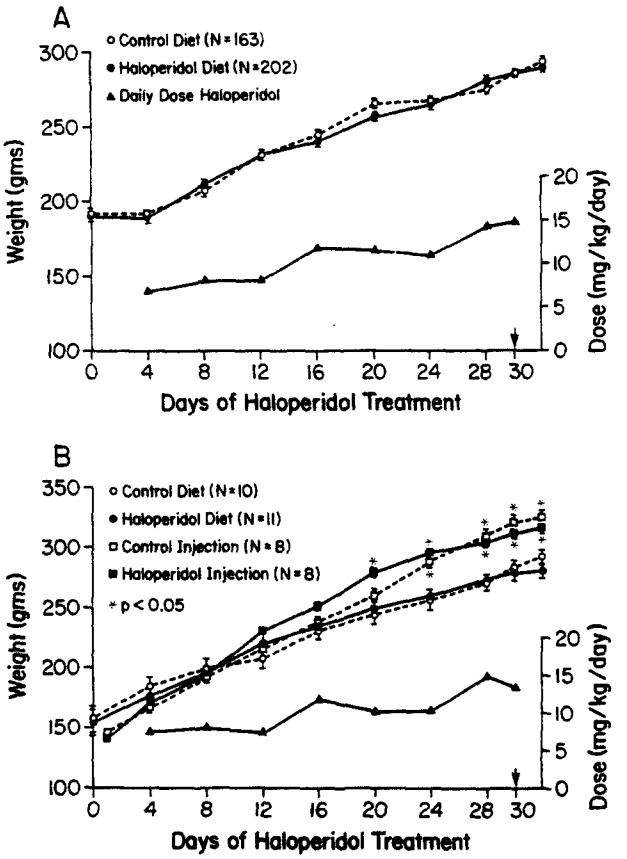

FIG. 1. (A) Growth curves for CHAL-control (Control Diet) and CHAL-treated (Haloperidol Diet) rats determined over the course of the 30-day treatment period. (B) Growth curves for CHAL- and SCHAL-treated (Subcutaneous Injections) rats tested for apomorphine-induced stereotyped behaviors. CHAL-control rats were pair-fed to assure a weight gain comparable to that of CHAL-treated rats. SCHAL-treated rats received daily injections of 0.7 mg/kg of haloperidol or saline (Control Injections) and were not pair-fed. The abscissa represents the 30 days of haloperidol treatment plus days 31 and 32 following termination of treatment (arrow). The ordinate on the left represents the average weight in grams; while the ordinate on the right represents the average calculated dose of haloperidol (mg/kg/day) consumed during the 30-day feeding regimen for the CHAL-treated rats. Each value represents the mean value±SEM for the number of animals indicated in the legend. The results were analyzed by one-way repeated measures of ANOVA with post-hoc pairwise comparison performed by the Bonferroni method [32]. Differences in weight between CHAL-treated and SCHAL-treated rats were statistically significant ($p<0.05$) for the last 10 days of drug treatment.

Statistical Analysis

Quantitative comparisons were assessed statistically using the Mann-Whitney U-test (apomorphine-induced stereotyped behavior), Student's *t*-test and Pearson product-moment coefficient of correlation (serum haloperidol concentrations and animal weights), one-way repeated measures ANOVA with post-hoc pairwise multiple comparisons performed by the Bonferroni method

TABLE 1

DOSE VS. SERUM CONCENTRATIONS OF HALOPERIDOL AND REDUCED HALOPERIDOL DETERMINED BY RADIOIMMUNOASSAY

Mode of Administration	Days on Haloperidol 6	18	30	−2
		Diet		
CHAL Dose (mg/kg/day)	8.5 ± 0.7	11.0 ± 0.5	13.6 ± 0.7	0
Haloperidol Concentration (pmol/ml)	58.3 ± 18.0	68.9 ± 27.5	107.1 ± 6.6	4.1 ± 0.7
Reduced Haloperidol Concentration (pmol/ml)	9.0 ± 1.2	15.3 ± 9.2	9.0 ± 3.1	1.5 ± 0.7
		Subcutaneous Injection		
SCHAL Dose (mg/kg)	0.7	0.7	0.7	0
Haloperidol Concentration (pmol/ml)	101.0 ± 26.2	106.3 ± 9.0	105.0 ± 8.4	2.0 ± 1.0
Reduced Haloperidol Concentration (pmol/ml)	13.7 ± 3.3	8.4 ± 2.0	6.3 ± 3.3	N.D. (not determinable)

Each value represents the mean ± SEM. Serum haloperidol and reduced haloperidol concentrations were determined from 6 animals on days 6, 18, 30 and 2 days after cessation of haloperidol treatments. Samples were obtained 1½ hours after SC-injections or after the termination of the dark-cycle for the CHAL-treated group (DIET). The average dose of haloperidol administered to the CHAL-treated rats (N=6) on days 6, 18 and 30 was calculated by the following equation:

$$\text{CHAL Dose (mg/kg/day)} = \frac{\text{average daily diet consumed (g)} \times \text{HAL concentration (mg/g diet)}}{\text{animal weight (kg)}}$$

(locomotor activity and animal weights) [32] and one-way ANOVA and the Dunnett test for comparisons between control and several experimental groups (receptor binding experiments) [25]. All quantitative measurements represent the mean±standard error of the mean (SEM).

RESULTS

Effects of Chronic Haloperidol Treatment on Animal Weight

The growth rate of CHAL-treated (N=202) and pair-fed CHAL-control (N=163) rats, and the average calculated doses of haloperidol (mg/kg/day) consumed during the 30 day feeding regimen are illustrated in Fig. 1A for a relatively large number of experimental groups (N=18). There was no difference in the weight gained between the CHAL-control and CHAL-treated rats during the course of these experiments (CHAL-treated 93±3 g; CHAL-control: 94±3 g; $t(363)=0.11$, N.S.). The SCHAL-treated and SCHAL-control rats, however, gained significantly more weight over the course of these treatments (Fig. 1B) than did their CHAL-treated counterparts (SCHAL-treated: 175±4.5 g; SCHAL-control: 172±5.4 g; CHAL-treated: 124±8.5 g; CHAL-control: 126±8.8 g; $t(14)$ CHAL/SCHAL=4.21, $p<0.001$). This difference in weight became evident by day 20 and progressively increased until, by the end of the treatment period, the two groups of rats differed in weight by more than 30 g. Significant differences in body weight were observed for the last 10 days of the haloperidol treatments (Fig. 1B).

Serum Haloperidol Concentrations

Serum concentrations of haloperidol and reduced haloperidol were determined for CHAL-treated and SCHAL-treated rats on days 6, 18, 30 and at two days after termination of the haloperidol treatments. These values are presented in Table 1 along with the injected or calculated dietary dose of haloperidol administered on each of the days that blood samples were obtained. The serum concentration of haloperidol in the CHAL-treated rats increased in a dose-dependent manner throughout the 30 day treatment period and was found to be significantly correlated with the calculated dose of the drug, $r(4)=0.95$, $p<0.01$. This dose-dependent relationship was not observed for the serum concentration of reduced haloperidol, which increased in relation to dose until day 18 and then it decreased to the day 6 value. Daily injections of haloperidol (0.7 mg/kg, SC) produced equivalent serum haloperidol concentrations on days 6, 18 and 30 of the treatment schedule (Table 1). These concentrations were nearly twice that achieved by the

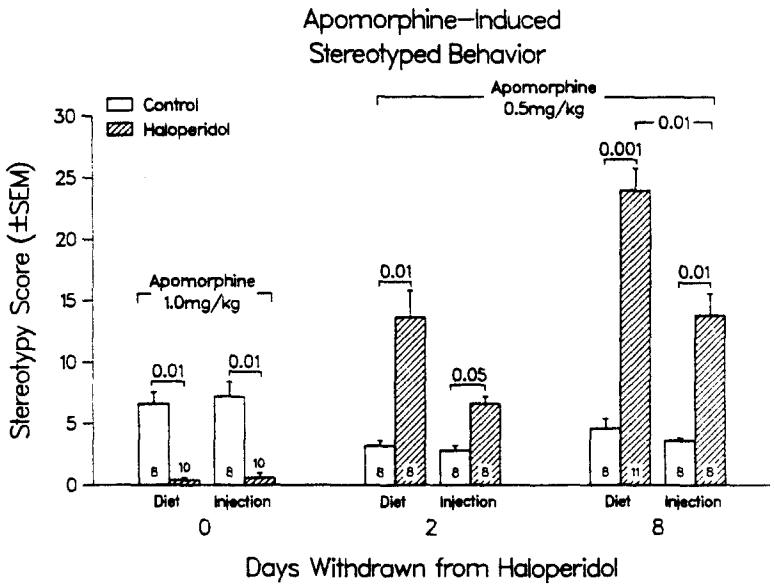

FIG. 2. Apomorphine-induced stereotyped behavior in control and haloperidol-treated rats. Stereotyped behavior was induced by the administration of apomorphine (IP) to rats on the last day of haloperidol treatment (day 0; 1.0 mg/kg) and again on 2 and 8 days (0.5 mg/kg) following termination of the haloperidol treatments. The results were compared by nonparametric statistical procedures using the Mann-Whitney U test.

haloperidol diet at 0.01% concentration on day 6 and 54% greater than the concentration produced by the diet at 0.015% concentration on day 18. By day 30, however, serum concentrations of haloperidol were not significantly different between the two haloperidol-treated groups of rats. Two days after cessation of haloperidol treatment (day 32), only a residual amount of haloperidol (less than 5 pmol/ml) could be detected in the serum of the CHAL- and SCHAL-treated rats. The serum concentration of haloperidol in CHAL-treated rats was greater than that recorded for the SCHAL-treated rats at this time, but the difference was not significant. The serum concentration of reduced haloperidol within the SCHAL-treated rats was greatest on day 6 and gradually declined to one-half this concentration by day 30. Although reduced haloperidol concentrations on day 30 were increased in the CHAL-treated rats relative to the SCHAL-treated rats, this difference was not significant. No detectable amount of reduced haloperidol was found in the serum of SCHAL-treated rats two days after cessation of the haloperidol treatment; however, small, but measurable concentrations were detected in the serum samples taken from the CHAL-treated rats.

Behavioral Experiments

Effects of the chronic haloperidol treatments on apomorphine-induced stereotyped behavior. Prior to termination of the haloperidol treatments on day 30, control and haloperidol-treated rats were administered apomorphine (1.0 mg/kg, IP) and were observed for stereotyped behavior. Both CHAL-and SCHAL-treated rats exhibited significantly lower stereotypy scores as compared to control rats (Fig. 2, day 0). These results indicate that the haloperidol treatments were producing a substantial blockade of DA receptors at this time. At 2 and 8 days after cessation of the haloperidol treatments, stereotyped behaviors were significantly increased in both CHAL- and SCHAL-treated rats in response to injections of 0.5 mg/kg of apomorphine (Fig. 2, days 2 and 8). Eight days after termination of the chronic haloperidol treatments (day 8), the CHAL-treated rats demonstrated a significantly greater sensitivity to apomorphine than did the SCHAL-treated rats. The CHAL-treated rats also had larger stereotypy scores as compared to SCHAL-treated rats two days after termination of the haloperidol treatment; however, this difference was not statistically significant.

Effects of the chronic haloperidol treatments on spontaneous locomotor activity. To determine the effects of the chronic haloperidol treatments on locomotor activity, spontaneous locomotor activity was monitored during the initial 20 min of the dark cycle throughout the 30 day treatment period. This time period, which preceded the period of active feeding by the CHAL-treated rats and the injection period for the SCHAL-treated rats, was chosen because the rats were generally more active at this time. When assessed during this period, spontaneous locomotor activity was not significantly altered throughout the course of the haloperidol treatments in either SCHAL- or CHAL-treated rats except for a reduction in activity on day two for the CHAL-treated rats (Fig. 3A). However, two days after termination of the haloperidol treatments, spontaneous locomotor activity was significantly increased within both groups (SCHAL: 152% of control, $p<0.05$; CHAL: 144% of control, $p<0.05$). Locomotor activity measured during the 8 hr period following the injection of haloperidol or during the most active period of feeding (dark cycle) (Fig. 3B) was significantly depressed in both groups of haloperidol-treated rats; however, the pattern of reduced locomotor activity recorded over the course of the 30 days of haloperidol treatment was distinctly different between the two groups. Daily injections of haloperidol produced a significant depression of spontane-

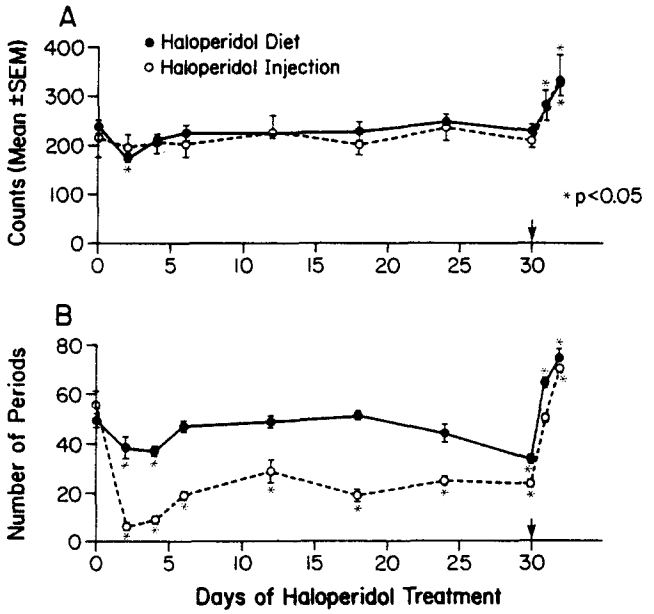

FIG. 3. Effects of CHAL and SCHAL treatments on spontaneous locomotor activity. Spontaneous locomotor activity was monitored during the dark cycle on days 0, 2, 4, 6, 12, 18, 24 and 30 of SCHAL treatment and for 2 days following termination of this treatment. CHAL-treated rats were given daily 0.5 ml injections of 0.9% NaCl while receiving haloperidol in their diet for 30 days. Two separate measures of activity were obtained during each period of testing: (A) The number of counts (COUNTS) for each of the first 5 consecutive 4 min periods of the dark cycle prior to each injection were averaged and plotted. (B) The total number of 4 min periods (NUMBER OF PERIODS) in which the activity was greater than or equal to 40% of the mean control count (A) was recorded for each animal during the 8 hr period of the dark cycle immediately after each injection. These values were averaged and plotted in (B). The results were analyzed by one-way repeated measures ANOVA and pairwise multiple comparisons by the Bonferroni method [32].

ous locomotor activity on each of the days of testing (Fig. 3B), and this motor depression was associated with a mild degree of catalepsy. The CHAL-treated rats, on the other hand, exhibited a significant decrease in spontaneous locomotor activity only on days 2 and 4 (Fig. 3B), but the activity returned to the control value by day 6 and remained at this value until day 24 when the activity again began to decline. It should be noted that on day 24, the haloperidol concentration of the diet was increased to its highest level (0.02%). Although spontaneous locomotor activity in these CHAL-treated rats was significantly reduced ($p<0.05$) on day 30, these rats never displayed catalepsy. Two days after termination of the haloperidol treatment (Fig. 3B), spontaneous locomotor activity was significantly increased in both groups of haloperidol-treated rats (NUMBER of PERIODS, SCHAL: 127% of control, $p<0.05$; CHAL: 152% of control, $p<0.05$).

Receptor Binding

Effects of the chronic haloperidol treatments on ^{3}H-spiroperidol binding within striatum, GP and SN_R. To determine whether this behavioral supersensitivity to dopamine was attributable to an increase in the number of dopamine binding sites (B_{max}) or to a change in affinity (K_D),

TABLE 2

EFFECT OF CHRONIC HALOPERIDOL TREATMENT ON THE BINDING CONSTANTS OF ^{3}H-SPIROPERIDOL IN THE STRIATUM

		Binding Site Affinity (pM)		Binding Site Density (fmol/mg protein)	
Treatment	N	K_{D1}	K_{D2}	B_{max1}	B_{max2}
Control	4	54 ± 8	—	319 ± 12	—
CHAL	4	51 ± 8	—	509 ± 26 60%†	—
SCHAL	3	70 ± 2	336 ± 74	444 ± 34 39%*	786 ± 139

Each value represents the mean ± SEM of the number of experiments (N) (each experiment performed in triplicate). For each treatment group, the striata from 6 rats were pooled and processed for tissue preparation as described in the text. With this procedure, the final protein content represented 2% of the original tissue weight; thus the B_{max1} for ^{3}H-spiroperidol binding in the control striatum was 6.4 ± 2.4 fmol/mg original tissue weight. The Scatchard data could be best fitted to a single binding site within the striatum of control and treated rats. Within the striatum of SCHAL-treated rats, two binding sites were determined. The results were analyzed by one-way ANOVA and the Dunnett Test for comparisons between control and several experimental groups [25].

*$p<0.05$, †$p<0.01$.

we examined striatal homogenates for ^{3}H-spiroperidol binding at 10 concentrations ranging from 0.02 to 0.8 nM, and analyzed the data by Scatchard plots drawn by the methods of least squares. No differences in ^{3}H-spiroperidol binding kinetics were detected between SCHAL-control and CHAL-control rats (SCHAL-control: K_D=58 pM, B_{max}=323 fmol/mg protein, N=1; CHAL-control: K_D=52±11 pM, B_{max}=318±17 fmol/mg protein, N=3); therefore, these control data were pooled and used for comparison with binding data obtained from both SCHAL-treated and CHAL-treated rats. Scatchard analysis of data obtained from ^{3}H-spiroperidol equilibrium-saturation binding to striatal membranes from control rats revealed a single high affinity binding site with an apparent dissociation constant (K_D) of 54±8 pM and a binding capacity (B_{max}) of 319±12 fmol/mg protein (Table 2). Two days following termination of the haloperidol treatment, ^{3}H-spiroperidol binding was significantly increased within the striatum of CHAL-treated rats, but the affinity (K_D) of this site for ^{3}H-spiroperidol was not altered.

Analysis of ^{3}H-spiroperidol binding data from the striatum of SCHAL-treated rats revealed two distinct populations of binding sites within the striatum (Table 2): a high affinity site with a K_{D1} similar to that determined for both CHAL-control and CHAL-treated rats (70±2 pM, CE(3,8)=14.3, N.S.), and a low affinity site with an apparent K_{D2} of 336±74 pM. The B_{max1} of the high affinity site was significantly increased (37%) by the SCHAL treatment. The B_{max2} of the low affinity site was estimated to be 786±139 fmol/mg protein. The K_D and B_{max} values of the SCHAL-treated group were obtained by linear regression of the binding data and resolved by Feldman analysis [10].

To assess possible changes in DA receptors within other BG regions, specific binding of ^{3}H-spiroperidol (0.1 nM) was employed to assess the effect of CHAL treatment on DA binding within the striatum, GP and SN_R. Specific ^{3}H-spiroperidol binding (0.1 nM) was significantly increased

TABLE 3

EFFECT OF CHRONIC DIETARY HALOPERIDOL TREATMENT ON THE SPECIFIC BINDING OF ^{3}H-SPIROPERIDOL IN RAT BASAL GANGLIA

Region	N	Control	CHAL-Treated	% Change
Striatum	4	208 ± 10	343 ± 24*	64
GP	3	44 ± 2	147 ± 11†	236
SN_R	2	61 ± 10	80 ± 18	31

Each value indicates the mean specific ^{3}H-spiroperidol binding (0.1 nM) in fmoles/mg protein ±SEM. Each experiment was performed in triplicate. Details of the tissue preparation and assay techniques are described in the text.

*$p<0.01$, †$p<0.001$.

within the striatum (64%) and GP (236%) of CHAL-treated rats as compared to CHAL-control rats (Table 3). Although ^{3}H-spiroperidol binding was increased by 31% within the SN_R of CHAL-treated rats, this increase was not statistically significant (CHAL-control: 61±10; CHAL-treated: 80±18 fmol/mg protein; $t(2)=0.90$, N.S.).

DISCUSSION

In the present study we have employed several biochemical and behavioral measures to characterize the effects of chronic administration of haloperidol in the diet on the sensitivity of dopaminergic systems within the BG of rats. The ability of dietary haloperidol to produce dopaminergic supersensitivity within forebrain structures was also compared with that of the SC mode of administration which has been employed by numerous investigators to induce dopaminergic supersensitivity in rats (for references, see [37]). Our results demonstrate that two days after termination of CHAL treatment, DA binding was increased within the striatum and this increase was correlated with an enhancement in apomorphine-induced stereotyped behaviors and spontaneous locomotor activity. These changes were qualitatively similar to those observed in rats receiving daily SC injections of haloperidol, but were achieved without the behavioral depression and catalepsy that results from this parenteral route of administration. While CHAL treatment resulted in a significant increase in DA binding within the GP, there was no change in DA binding within the SN_R.

Although the 0.01% and 0.015% dietary concentrations of haloperidol resulted in lower serum concentrations of haloperidol (approximately 45% less) than did subcutaneously administered haloperidol, the serum concentrations of haloperidol were the same for both routes of administration when the concentration of haloperidol in the diet was increased to 0.02% on day 24. On day 30 of haloperidol treatment, serum concentrations in SCHAL- and CHAL-treated rats were comparable. The serum levels of haloperidol determined on day 30 for SCHAL-treated rats were similar to those reported in other studies in which serum levels have been measured 1–2 hr after acute IP injections of haloperidol [5,6]. The fact that the serum concentration of haloperidol was twice as high in CHAL-treated rats as compared to the SCHAL-treated rats 48 hr after termination of the haloperidol treatment suggests that a more constant daily serum concentration of haloperidol may have been achieved with the dietary regimen than with the once-daily SC injections of haloperidol.

Serum concentrations of reduced haloperidol were also measured since it has been suggested that this metabolite may possess neuroleptic activity similar to that of haloperidol [12]. More recent studies, however, question the ability of this metabolite to mimic the neuroleptic actions of haloperidol [26,28]. Nevertheless, reduced haloperidol is known to be rapidly reconverted to haloperidol in the liver [28]; therefore serum concentrations of reduced haloperidol are still of interest as an indicator of changes in haloperidol metabolism and of the total "potential" haloperidol concentrations in plasma. On day 30, however, the concentrations of reduced haloperidol were not significantly different between the SCHAL- and CHAL-treated rats, which indicates that by the end of the haloperidol treatment period, the total "potential" haloperidol concentrations did not differ as a result of the route of administration.

Although the two modes of haloperidol administration resulted in comparable blood levels of haloperidol by day 30, the degree of dopaminergic supersensitivity induced by the two modes of treatment was not identical. Scatchard analysis of ^{3}H-spiroperidol binding isotherms revealed a significant increase in the B_{max} of high affinity binding sites within the striatum of both SCHAL- and CHAL-treated rats following cessation of the haloperidol treatments. Although the B_{max} of this site was higher within the CHAL-treated rats as compared to the SCHAL-treated rats, this difference was not statistically significant. However, within the SCHAL-treated rats, a second, lower affinity site was resolved which was not detected within the striatum of CHAL-control or CHAL-treated animals. While the binding constants determined for the high affinity ^{3}H-spiroperidol binding site within the striatum of control and treated rats were quite similar to those that have been reported by other investigators [11,22], a second, lower affinity ^{3}H-spiroperidol site has not been previously reported. It remains to be determined if this lower affinity site represents the binding of ^{3}H-spiroperidol to a second (allosterically altered) population of striatal DA receptor sites or to a nondopaminergic binding site such as the serotonin (S_2) receptor sites located within the striatum [40,42]. In these experiments, S_2 antagonists such as ketanserin, cinanserin or methylsergide were not used to selectively displace ^{3}H-spiroperidol from S_2 binding sites [1, 38, 41]; therefore, it is possible that this low affinity binding site represents ^{3}H-spiroperidol binding to an S_2 site. It has been estimated that as much as 15–20% of the total ^{3}H-spiroperidol binding to striatal membranes may be to S_2 binding sites [24, 30, 39, 47]. Moreover, the binding affinity of ^{3}H-spiroperidol for S_2 sites within the rat brain is similar to that which we have found for this low affinity ^{3}H-spiroperidol site within the striatum of the SCHAL-treated rats [35]. Other modes of haloperidol administration have been shown to produce small (20%), but significant, elevations in striatal serotonin receptor binding when measured 48 hr after termination of haloperidol treatment [36]. However, it is not clear at this time why the lower affinity site was only detected in SCHAL-treated rats. One possible hypothesis is that the SC mode of administration was able to achieve a sufficiently high brain concentration of haloperidol over a relatively short period of time and was able to promote the saturation of D_2 sites and occupation of other (lower affinity) binding sites as well, thus leading to a proliferation of both high and low affinity binding sites.

Apomorphine-induced stereotyped behaviors were sig-

nificantly reduced in the haloperidol-treated rats prior to termination of the treatments as compared to control rats denoting a substantial blockade of DA receptors at that time. However, 2 and 8 days after termination of the haloperidol treatments, stereotyped behaviors were significantly increased in both the CHAL- and SCHAL-treated rats in relation to the control rats. Stereotypy scores were higher within the CHAL-treated rats on both of these days; however, this difference was only statistically significant on day 8. Although these results could have been caused by differences in the experimental conditions experienced by the two treatment groups, such as the disparities in growth rate, degree of handling received by each group or conditioned responses developed to daily injections of haloperidol, it is more likely that the higher stereotypy scores for the CHAL-treated rats were due to the increase in the number (B_{max1}) of high affinity ^{3}H-spiroperidol binding sites within this group relative to the SCHAL-treated rats. If the low affinity binding site represents an S_2 binding site, then the lower apomorphine-induced stereotypy scores for the SCHAL-treated rats may have resulted from a serotonergic interaction with these striatal S_2 binding sites. The activation of the striatal serotonergic system has been shown to inhibit apomorphine-induced stereotyped behaviors in rodents [21].

Catalepsy and reduced locomotor activity are common behavioral effects associated with haloperidol administration [2, 4, 9, 27, 33]. Reduction in spontaneous locomotor activity has been reported in mice receiving chronic diets containing 0.005 and 0.01% haloperidol [34,46]. Except for an initial decrease during the first few days of treatment, little change in spontaneous locomotor activity was noted for the CHAL-treated rats until the dietary concentration was increased to its highest level on day 24. The SCHAL-treated rats, on the other hand, exhibited reduced spontaneous locomotor activity and mild catalepsy during the 8 hr period following each haloperidol injection throughout the 30 day treatment period. Tolerance did not develop to this behavioral effect of haloperidol. Two days after cessation of the haloperidol treatments, spontaneous locomotor activity was increased in both CHAL- and SCHAL-treated rats. Other investigators have reported an increase in spontaneous locomotor activity lasting up to 4 days after withdrawal from haloperidol [20, 34, 43, 46]. This increase in spontaneous locomotor activity following chronic haloperidol treatment has been attributed to the development of dopaminergic supersensitivity within the nucleus accumbens [7].

The results of the biochemical and behavioral test demonstrated that the effects of the CHAL and SCHAL modes of administration on forebrain DA systems were, in general, comparable. Since we have been using this dietary regimen to treat rats that are employed in electrophysiological experiments to assess the effects of chronic haloperidol administration on the responsiveness of neurons in the GP and SN_R to iontophoresed GABA, we were naturally interested in characterizing the effects of this mode of CHAL treatment on DA binding within the GP and SN_R and in comparing this binding data with that obtained from the striatum. The chronic administration of haloperidol via the diet caused a significant increase in dopamine (D_2) binding within the striatum and the GP. More interesting, however, was the finding that the increase in DA binding within the GP (>200%) was much greater than that observed for the striatum. In control animals, DA binding was low within the GP as compared to the striatum (~20%); however, after CHAL treatment, DA binding within the GP increased to over 40% of that detected within the striatum. Although DA binding was also increased within the SN_R of CHAL-treated rats (31%), it was not statistically significant and did not approach in magnitude the increase found within the striatum or GP. The lack of significant change in ^{3}H-spiroperidol binding within the SN_R may reflect an important difference in DA binding sites within the SN_R as compared to the striatum and GP [18]; however, it may also be a function of the larger variability associated with the binding measurements obtained within the SN_R. Additional binding experiments (only two were performed for the SN_R) and reduced tissue dissection variability relative to sampling the reticulata region of the SN might reduce this variability and facilitate the detection of smaller changes in ^{3}H-spiroperidol binding within the SN_R. The fact that the binding was markedly increased within the GP following chronic haloperidol administration suggests that the GP may play a significant role in the overall effects of chronic neuroleptic treatment.

In summary, the administration of haloperidol in the diet as described in this study constitutes a convenient and reliable method for inducing supersensitivity in DA receptor systems within the BG and has several advantages over parenteral and oral injection routes of administration. The diet is simple to administer, it provides a more continuous mode of drug administration over a 24 hr period than do parenteral or enteral injections, and as previously noted, it does not produce the pronounced behavioral depression and catalepsy in rats that is associated with the other methods of haloperidol administration. In addition, the animals are not stressed by daily or twice daily injections. Thus the effects of stress or prolonged behavioral depression, which could alter the activity of neurotransmitter systems within the BG, are eliminated.

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