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Dopaminergic Alterations in Cotreatments Attenuating Haloperidol-Induced Hypersensitivity

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CARVEY, P. M., L. C. KAO, T. J. ZHANG, R. L. AMDUR, D. H. LIN, R. SINGH AND H. L. KLAWANS. *Dopaminergic alterations in cotreatments attenuating haloperidol-induced hypersensitivity.* PHARMACOL BIOCHEM BEHAV **35**(2) 291–300, 1990.—Chronic treatment of the laboratory rat with haloperidol results in an increased stereotypic behavioral response to subsequent dopamine agonist challenge. This behavioral hypersensitivity (BH) is thought to reflect an increase in DA receptor number following chronic pharmacologic denervation. Using a cotreatment strategy, we demonstrate here that a variety of agents can attenuate or prevent the development of BH when administered chronically with haloperidol. Cotreatment with lithium and amantadine prevented the changes in DA biochemistry as well as the proliferation of DA receptors normally associated with chronic haloperidol treatment. Cotreatment with thioridazine or scopolamine did alter the changes in DA biochemistry normally associated with haloperidol treatment, but failed to attenuate the DA receptor proliferation. Taken together, these data suggest that mechanisms in addition to DA biochemical and receptor changes participate in the development and subsequent expression of BH. DA receptor proliferation must, therefore, be considered permissive to the development of BH.

Dopamine Haloperidol Hypersensitivity Metabolism Lithium Amantadine Scopolamine Thioridazine Receptor Cotreatment Chronic Stereotypic behavior

CHRONIC treatment of the laboratory rat with dopamine (DA) receptor antagonist drugs, like haloperidol, results in a hypersensitive stereotypical behavioral response to subsequent challenge with the direct acting, mixed DA agonist, apomorphine (35,57). This phenomenon is commonly referred to as behavioral hypersensitivity (BH). The relative degree of BH is dependent upon the dose of chronic DA antagonist employed (38), as well as the duration of that treatment (39). In the absence of continued DA antagonist treatment, the state of BH is slowly reversible (62). Once BH has developed, acute antimuscarinic pretreatment accentuates the hypersensitive state (9). BH is commonly thought to reflect the up-regulation of DA receptors on striatal neurons resulting from chronic pharmacologic denervation of the striatum by DA antagonists (6,34).

Chronic treatment of humans with neuroleptic drugs, which are also DA antagonists, results in a choreiform movement disorder referred to as tardive dyskinesia (TD). The incidence of TD resulting from chronic neuroleptic therapy is estimated between 12 and 15% (58). In many cases, The development of TD is dose and duration of treatment dependent, and slowly subsides following neuroleptic withdrawal, although in some cases it can be permanent. Acute treatment with antimuscarinic agents can exacerbate TD. It has been hypothesized that TD similarly reflects striatal DA receptor site up-regulation resulting in a hypersensitive dopaminergic state (34). Since BH and TD show many similar pharmacologic characteristics, BH may be utilized to examine some of the underlying pathophysiologic processes responsible for the development of TD.

Chronic treatment with most neuroleptic agents reliably produces BH as well as D-2 receptor site proliferation (15). The coadministration of DA agonists, like levodopa (19) or amantadine (1), with neuroleptic agents has been shown to prevent the development of BH as well as D-2 receptor up-regulation. Coadministration of lithium or the neuropeptide l-poly-l-leucyl-glycinamide with haloperidol produces similar results (12,47). The prevention of D-2 receptor site proliferation, while at the same time preventing BH, has reinforced the belief that D-2 receptor up-regulation is solely responsible for the hypersensitive state. However, we have previously demonstrated that the development of BH is also dependent upon the "type" of chronic DA antagonist used. At administered doses, ostensibly equivalent in their DA antagonistic action, the subsequent degree of BH produced by a variety of DA antagonists was found to be inversely correlated to the DA antagonists' antimuscarinic potency (39).

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Similarly, the coadministration of antimuscarinic agents with haloperidol was shown to attenuate the eventual development of BH in a dose-dependent fashion without preventing the D-2 receptor up-regulation (7,8). This would suggest that D-2 receptor site proliferation is not the only factor responsible for the development of BH.

Neuroleptic agents with significant antimuscarinic activity are termed "atypical" since they are less associated with the development of acute extrapyramidal side effects (EPSE's) (44). There have also been scattered reports suggesting that atypical agents like thioridazine or that concurrent treatment with antimuscarinic agents can reduce the incidence of TD as well (22, 32, 43). Since it has been hypothesized that striatal function depends upon the relative activity of both the dopaminergic and cholinergic systems (52,56), one could assume that alterations in either system might influence the eventual expression of BH or TD. Neuroleptic agents have also been shown to significantly influence gabaergic (30,45) and adrenergic systems (46), as well as certain peptides (24,33). This would also intuitively suggest that mechanisms in addition to D-2 receptor site proliferation would participate in the development of BH. Yet, the focus of the hypersensitizing effects of chronic neuroleptic treatment in both animals, and in man, has centered around the proliferation of DA receptors.

In an effort to examine the potential relationship between DA receptor up-regulation and BH, we examined the effects of cotreatments with haloperidol and amantadine, lithium, the atypical neuroleptic agent, thioridazine, or the antimuscarinic agent scopolamine. The DA biochemistry and D-2 binding data presented here clearly demonstrate that the development of BH can be attenuated through mechanisms independent of the development of DA receptor site proliferation. This data, therefore, suggests that mechanisms in addition to D-2 receptor up-regulation participate in the development of TD.

METHOD

Drugs and Drug Abbreviations Used

Apomorphine HCl (APO; Sigma) was prepared fresh in distilled water and delivered SC at 0.75 mg/kg. Haloperidol HCl (HAL; McNeil) was dissolved in acidified normal saline and delivered IP at 0.75 mg/kg. Thioridazine HCl (THIO, 10 mg/kg; Sandoz), amantadine HCl (AMAN, 50 mg/kg; Dupont), and scopolamine HBr (SCOP, 0.6 mg/kg; Sigma) were dissolved in normal saline and delivered IP. Lithium chloride (Li; Sigma) was formulated in Purina Rat Chow (Teklad) at 1.696 g/kg with cellulose reduced to 48.304 g/kg. Serum levels in rats run prior to the chronic treatment regimen revealed that this diet resulted in a range of lithium levels of 0.71 to 1.13 mEq/ml. When cotreatments were formulated with HAL (H+T, H+S, H+A), all drugs were administered in their full dose as described above, dissolved in the HAL vehicle, and delivered IP except for Li (H+Li) where the animals received HAL in addition to their Li diet. All injected drugs were delivered in a volume of 1 ml/kg. Control animals (CONT) received an equivalent volume of normal saline delivered IP.

Animals

A total of 396 male Sprague-Dawley rats (Sasco), initially weighing between 250 and 275 grams, were used in the study. The animals were adapted to the environmentally regulated animal facilities (lights-on 0600–1800) for at least 5 days prior to testing.

Stereotypic Behavior (SB) Assessment

Prior to any chronic treatment, all animals were tested for their behavioral response to APO. Each animal was weighed and placed in a standard perspex rat cage containing a wire grid floor. Each cage was enclosed in a sound-proofed cell equipped with its own lighting and ventilation system, and visible through a one-way mirror. The animals were allowed to adapt to this environment for at least 30 minutes prior to APO challenge. Eighteen animals could be evaluated at one time.

Ten minutes following APO injection, and at 5-minute intervals thereafter (through 65 minutes), each animal was scored for SB using a 6-point rating scale described as follows:

0—Animal resting quietly.

1—Animal grooming, rearing, sniffing, or exploring its environment.

2—Animal chewing the grid or licking the cage wall less than 50% of a 15-second observation interval, but having at least 2 chewing or licking episodes during that interval.

3—Animal chewing the grid or licking the cage wall greater than 50% of the interval but not continuously.

4—Animal chewing the grid or licking cage wall 100% of the interval.

5—Animal gnawing the grid 100% of the interval without locomotion.

The interval scores for each animal were then summed to yield the animal score (AS), which represented the primary behavioral dependent variable. Additional characteristics of the behavioral response were derived from the interval scores. Response duration was defined as the last observation interval in which a SB score of 2 was recorded. AS average (ASAVG) was computed by averaging the interval scores through the last interval in which a SB score of 2 was recorded. Peak response rate was defined as the average of the four consecutive interval scores (intervals 20, 25, 30, and 35 minutes) judged to represent the highest interval scores by examining the CONT animals behavioral response.

The AS's from all animals, tested at the same time of day, were then ranked in ascending order. Treatment groups were generated from these ranks such that prior to chronic treatment all treatment groups had the same group mean AS and standard deviation (overall AS mean was 27.74 ± 3.73; F-ratio between all groups was 0.322, $p = 0.967$).

Chronic Treatment Protocol

Since SB is time of day dependent (10) and multiple behavioral assessments were performed in a given day (between 0930 and 1430), animals pretested at a given time of day were subsequently tested at the same time of day. The behavioral assessment times for all treatment groups was also balanced across time of day. CONT animals were evaluated with every behavioral assessment. In order to examine all treatment groups, two separate studies were performed under identical protocols (October and January). Study 1 consisted of CONT, HAL, THIO, SCOP, H+T, and H+S treatment groups. Study 2 consisted of CONT, HAL, AMAN, Li, H+A, and H+Li treatment groups.

The animals were treated for 24 consecutive days. They were then withdrawn from treatment for 96 hours and again assessed for SB using the protocol described above, with the exception that the assessment duration was 90 minutes and Peak response rate was 30, 35, 40 and 45 minutes (Post Test I). The following day, a regimen of 24 consecutive treatments was again instituted followed by a 96-hour drug withdrawal period and SB assessment for 105 minutes with Peak response rate set at 35, 40, 45 and 50 minutes (Post Test II). All behavioral assessments and drug treatments were performed blind.

Tissue Harvest

Forty-eight hours following behavioral assessment (6 days

following the last treatment) the animals were sacrificed. All treatment groups were subsequently divided into 4 subgroups destined for different sacrifice protocols. These subgroups had been generated from the pretreatment AS's and, therefore, began treatment with equivalent group mean AS's. Treatment groups 1 and 2 were sacrificed by cervical dislocation for subsequent biochemical analysis. Treatment group 1, destined for biochemical analysis, was sacrificed exactly 45 minutes following a saline injection (OFF-APO), while treatment group 2 was sacrificed 45 minutes following an APO injection (ON-APO). This time was chosen from the Post Test II behavioral data (CONT animals) as representing the peak behavioral response. Exactly 90 seconds following cervical dislocation, the brain was immersed in liquid freon for 2 minutes and stored at −60°C for subsequent dissection. Treatment group 3, destined for DA receptor binding, was sacrificed as described above and then imbedded (Lipshaw Matrix) for subsequent sectioning. Treatment group 4 was sacrificed ON- or OFF-APO under light halothane anesthesia followed by exsanguination. The brains from these animals were perfused with normal saline for 5 minutes and subsequently examined for 2-deoxyglucose uptake (data not presented here).

Biochemical Analysis

The brains from treatment groups 1 or 2 were mounted on a cryostat chuck and sectioned until the anterior portion of the striatum was identified (ant 9.41, König and Klippel atlas). Two 875 μm coronal sections (anterior striatum) were then rolled off. Two 875 μm coronal sections (posterior striatum) were also rolled off. Still frozen, these sections were placed on dry ice and the anterior and posterior striatal sections were dissected using a knife and fork technique. These tissue sections were then placed into 1 ml of an ice-cold solution of 0.4 N perchlorate, 0.05% EDTA, 0.01% bis-metabisulfite solution and homogenized immediately on a Kinematica GmbH Homogenizer (Switzerland) for 20 seconds at setting 6. The homogenates were then allowed to sit at room temperature for 20 minutes, vortexing occasionally. The samples were then spun down at 18,000 × g for 30 minutes. The supernatants were poured off and stored at −60° for subsequent analysis using HPLC. The pellets were resuspended in 3 N NaOH and protein content analyzed using a Bio-Rad protein assay kit.

The supernatants were separated on a C-18 partisil column (25 cm, 5 μm ODS; Alltech) using a 0.067/0.05 M citrate/phosphate buffer (pH 3.9) containing EDTA (0.1 mM), heptanesulfonic acid (Kodak, 0.75 mM) and 14% methanol (HPLC grade, Fisher). DA was oxidized at 0.15 V while homovanillic acid (HVA) was oxidized at 0.40 V using an ESA dual electrode electrochemical detector (Model 5100A). Samples were analyzed in duplicate against 6-point standard curves (as the free base) run daily. The samples were sequenced during these analytical runs so that 2 samples from all treatment groups would be run in a given day thereby controlling for a run sequence effect. The run sequences were inverted daily.

Dopamine Binding Analysis

Coronal sections, 18 μm in thickness, were prepared at −10°C using a microtome cryostat (IEC). Four sets of sections were taken through the striatum which corresponded with the 4 sections used in the biochemical studies. Sections were thaw-mounted onto subbed slides and kept frozen at −60°C. On the day of the assay, sections were thawed at 23°C for 1 hr and then washed twice using ice-cold Tris buffer (50 mM) containing 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 120 mM NaCl (pH 7.7). Incubation was performed for 90 minutes in the dark at 23°C in the presence of 40 nM ketanserin and 1 nM [^{3}H]-sipiperone (spec.act. 20 Ci/mM). Nonspecific binding was determined by incubating the adjacent sections with 1 μM unlabeled spiperone. The incubation was terminated by rinsing sections twice for 10 minutes in ice-cold 50 mM Tris-HCl (pH 7.7). Sections were then dipped in ice-cold distilled water for 3 sec, fan dried, and kept overnight in a desiccator.

Autoradiograms were prepared by placing the slide mounted tissue sections and tritium standards in light-proof X-ray cassettes (Kodak) along with tritium-sensitive film (LKB or Amersham) for 2 weeks at 4°C. The films were developed using Kodak D-19 developer and Kodak fixer and then air-dried. The optical density of the autoradiograms was quantified against the tritium standards following digital subtraction of the nonspecific section using a RAS 1000 system (Amersham). This system automatically converted the optical densities into femtomoles.

Statistical Analysis

A repeated measures multiway ANOVA using Tukey's multiple range post hoc test was utilized to determine statistical differences among the various treatment groups for the behavioral data. Pearson correlation analysis was performed on a number of the behavioral parameters. One-way ANOVA using the least significant difference multiple range post hoc test was utilized to determine statistical differences among the various treatment groups for the biochemical and DA binding data.

RESULTS

Animals

By and large, the animals tolerated the drug treatments well with the exception of Li, H+Li, THIO and the H+T treatments which resulted in a significantly decreased weight gain over the 2-month study period (see Table 1). One Li animal was sacrificed for humane reasons. Because of these weight gain differences among the treatment groups, the behavioral data was analyzed using weight as a covariate and the multiple range tests, examining differences among the groups, were adjusted for that covariate. An infection, apparently unrelated to treatment group, broke out in the colony during study 1. The animals were placed on tetracycline for a period of 10 days which effectively treated the infection. However, 11 animals were dropped from that study because of a lack of significant weight gain over that 10-day period. These 11 animals were equally distributed over the 6 treatment groups. The following data therefore depicts the results from the 384 animals which completed the study.

Stereotypic Behavior

Figure 1 depicts the group mean AS's for the various treatment groups during Post Test I (top) and Post Test II (bottom). Overall the F-ratio's for both behavioral tests were highly significant [$F = 34.884$, $p < 0.0001$; $F = 57.644$, $p < 0.0001$, respectively]. The overall F-ratio from the MANOVA was also statistically significant (26.57; $p < 0.0001$). This analysis likewise revealed a significant time effect ($F = 16.49$; $p < 0.0001$), suggesting that behavioral responsiveness, overall, increased during the 2-month period, probably as a result of weight gain. There was not, however, a significant treatment group/time effect suggesting that the treatment group effect did not significantly change over the course of the experiment. The covariate (weight), however, was not statistically significant ($F = 0.21$), suggesting that although significant weight differences were observed across the various treatment groups, these differences did not contribute significantly to the treatment effect. Indeed, the correlation analysis of weight

TABLE 1

THE EFFECT OF VARIOUS CHRONIC DRUG TREATMENTS ON STEREOTYPIC BEHAVIOR: POST TEST II

Treatment Group (n)	Weight	ASAVG	PEAK	Res. Dur.
CONT (101)	431.17	1.90	3.67	56.43
HAL (78)	413.62	2.69*	4.01*	79.16*
THIO (20)	386.85*	1.79	3.29	57.25
SCOP (21)	430.76	1.81	3.55	57.38
AMAN (30)	421.50	1.86	3.64	56.83
Li (29)	351.03*	1.69	3.26	53.50
H+T (22)	381.18*	2.11*	3.48	67.50*
H+S (23)	414.21	2.16*	3.45	67.82*
H+A (30)	408.03	2.00	3.68	61.83*
H+Li (30)	351.60*	1.95	3.64	58.16
Total (385)	406.42 ± 45.99	2.07 ± 0.43	3.65 ± 0.51	62.80 ± 12.0

* = $p<0.05$ relative to CONT.

and AS revealed a nonsignificant effect explaining only 3% of the observed variation. Despite this apparent lack of effect by animal weight, the behavioral data were still adjusted for the effect of this covariate in keeping with the overall experimental design.

After 24 treatments with the specified agents, HAL, H+T, and H+S AS's were statistically elevated relative to CONT AS's. After 48 treatments similar results were observed. However, 48 treatments with HAL led to a further increase in AS's whereas the H+T and H+S animals did not exhibit this progression. As a result, after 48 treatments, the HAL-treated animals exhibited a SB response which was statistically elevated relative to both the H+T and the H+S animals. This increased response by the HAL animals, relative to the H+T and H+S animals, appears to have been the result of the increase HAL animals exhibited in the PEAK response rate (Table 1), since after 48 treatments, only the HAL animals exhibited a statistically significant elevation in this behavioral parameter. Again, however, even though the H+T and H+S animals exhibited statistically significant elevations in ASAVG and response duration relative to CONT animals, both behavioral parameters in both groups were statistically reduced relative to the HAL-treated animals. Animals cotreated with H+A exhibited a similar statistical alteration in response duration, i.e., increased relative to CONT animals, although decreased relative to HAL animals.

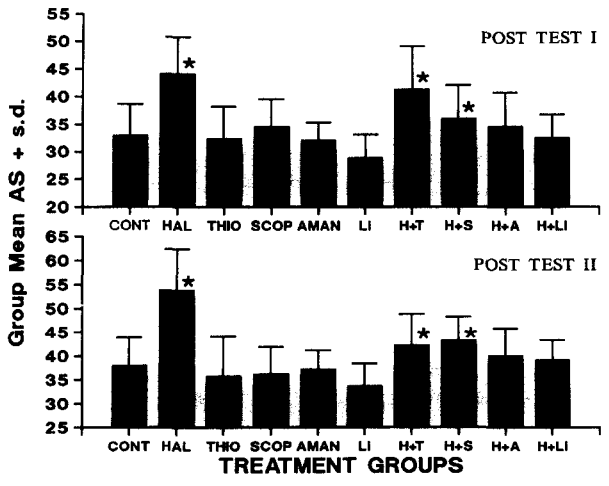

FIG. 1. Stereotypic behavioral response to apomorphine HCl (0.75 mg/kg) 96 hours following 24 treatments (Post Test I) or 48 treatments (Post Test II) with the agents specified. AS refers to the sum of the interval assessments of stereotypy recorded every 5 minutes through the completion of the response. (* = $p<0.05$ vs. normal saline- (CONT) treated animals.) See the Method section for abbreviations.

Striatal Biochemistry

Statistical analysis of anterior and posterior striatal protein content revealed no differences suggesting that the dissection technique employed segregated the structure into 2 physically equivalent regions. Figures 2 and 3 depict the alterations in anterior and posterior striatal DA, HVA and DA activity [(HVA)/(DA)] observed 6 days following 48 treatments. The data are expressed as percent of CONT in animals sacrificed 45 minutes following a saline injection (OFF-APO) or 45 minutes following an APO injection (ON-APO). One-way ANOVA's revealed overall statistically significant alterations in all parameters with the exception of the DA alterations in the posterior striatum ON-APO ($F=1.868$; $p=$ NS).

Control Animals

In CONT animals OFF-APO, DA content and HVA content were lower in the posterior (100.14 and 7.07 ng/mg protein, respectively) than in the anterior striatum (275.85 and 12.17 ng/mg protein, respectively). The administration of APO to CONT animals reduced DA content in the anterior striatum (225.75 ng/mg protein), while elevating it in the posterior striatum (160.85 ng/mg protein). HVA levels in these animals were reduced by APO challenge in both regions (5.41 anterior and 4.30 ng/mg protein posterior). DA activity in either region of the striatum in CONT animals was reduced by APO challenge (0.0243 anterior and 0.0272 posterior), although activity OFF-APO was higher in the posterior striatum (0.0713) than in the anterior striatum (0.0440).

Anterior Striatum

HAL treatment had its most pronounced effects in the anterior

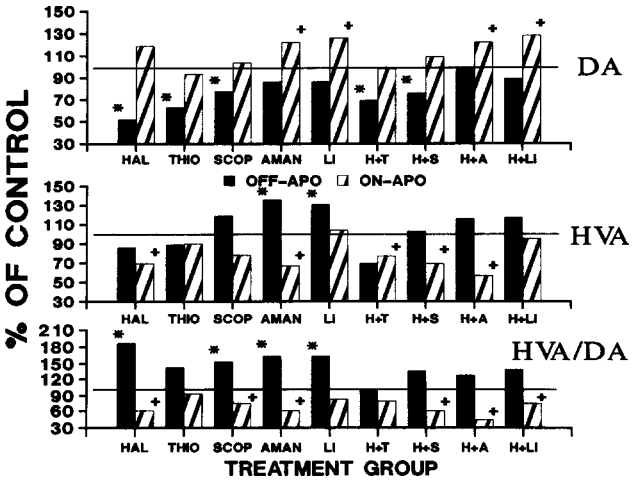

FIG. 2. Anterior striatal DA biochemistry in animals treated for 48 days with the specified agents and then withdrawn for 6 days. The values were derived from animals sacrificed 45 minutes following a normal saline injection (OFF-APO) or 45 minutes following an apomorphine HCl injection (ON-APO). Values are expressed as percent of CONT (see text for actual CONT values). (* and + = $p<0.05$ vs. normal saline-treated animals.)

striatum, reducing DA content and increasing DA activity OFF-APO (Figs. 2 and 3). Interestingly, these effects were reversed by APO challenge. Both THIO and SCOP alone, as well as in combination with HAL, similarly produced a reduction in anterior striatal DA content OFF-APO, although the effect was not as pronounced. In contrast, cotreatment with AMAN and Li totally attenuated this effect. All four cotreatments attenuated the alterations in anterior striatum DA activity normally resulting when HAL was administered alone and sacrificed OFF-APO. In the anterior striatum ON-APO, cotreatments with SCOP, AMAN and

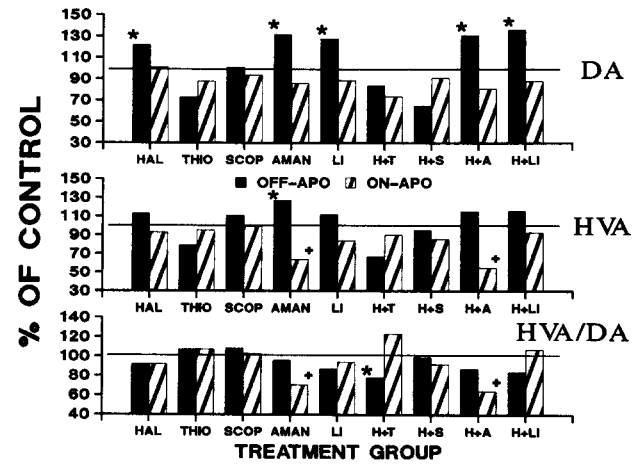

FIG. 3. Posterior striatal DA biochemistry in animals treated for 48 days with the specified agents and then withdrawn for 6 days. The values were derived from animals sacrificed 45 minutes following a normal saline injection (OFF-APO) or 45 minutes following an apomorphine HCl injection (ON-APO). Values are expressed as percent of CONT (see text for actual CONT values). (* and + = $p<0.05$ vs. normal saline-treated animals.)

Li failed to attenuate the reduction in DA activity normally produced by HAL when administered alone. Interestingly, THIO alone, or in combination with HAL, did not result in significant reductions in DA activity relative to CONT animals either during the withdrawal phase (OFF-APO) or in response to an APO challenge (ON-APO).

Posterior Striatum

THIO- and HAL-treated animals sacrificed OFF-APO exhibited opposite effects on DA content in the posterior striatum, i.e., HAL increased while THIO decreased DA. THIO, coadministered with HAL, similarly decreased posterior striatal DA. AMAN and Li, alone and in combination with HAL, produced increases in posterior DA similar to that seen in HAL-treated animals sacrificed OFF-APO. Few changes were observed in the posterior striatum in response to APO challenge, although AMAN alone and in combination with HAL produced a significant reduction in DA activity.

Whole Striatum

Table 2 displays the effects of various drug treatments on whole striatum DA biochemistry. HAL, THIO, H+T, and H+S treatments were all observed to statistically reduce DA content OFF-APO ($F=4.98$, $p<0.0001$), whereas no treatment significantly influenced DA content ON-APO ($F=1.385$; $p=$NS). Chronic treatment with AMAN or Li significantly elevated HVA levels OFF-APO ($F=6.756$; $p<0.0001$), whereas no other pairwise comparisons were statistically evident. All drugs, when administered alone, significantly elevated DA activity OFF-APO relative to CONT ($F=3.402$; $p<0.001$). In contrast, all 4 cotreatments attenuated the elevating effect that HAL, as well as the other single treatments had, on DA activity. AMAN, H+S and H+A treatments significantly reduced striatal HVA in response to APO ($F=3.481$; $p<0.005$). The attenuating effect that APO challenge had on striatal DA activity ($F=4.831$; $p<0.0001$) was statistically enhanced by HAL and AMAN treatments, as well as by H+S and H+A cotreatments.

Striatal DA-2 Binding

Overall, 4 animals' binding results were dropped from the analysis. Prior to decoding the groups it was determined that one HAL, one Li and two THIO animals had optical densities which were more than 3 optical density standard deviations from their respective group means. Figure 4, therefore, depicts the results of the quantitative autoradiographic analysis of spiroperone binding in both the anterior and posterior striatum of only 48 animals.

The overall one-way ANOVA in the anterior striatum was statistically significant ($F=3.3036$; $p<0.005$). Pair-wise comparisons revealed that HAL- and H+T-treated animals exhibited a statistically elevated increase in spiroperone binding in the anterior striatum relative to CONT animals. No other pair-wise comparisons were statistically evident, although H+S and H+A cotreatments tended to exhibit increased spiroperone binding. The overall one-way ANOVA in the posterior striatum was likewise statistically significant ($F=2.1015$; $p<0.05$). HAL- and H+S-treated animals exhibited significant increases in D-2 binding. Interestingly, Li, which did not alter anterior striatal D-2 binding, had the most pronounced effect of any drug treatment on posterior D-2 receptor density.

Table 3 presents the mean binding density (in femtomoles) for the various treatment groups in the whole striatum. The overall one-way ANOVA was statistically significant ($F=2.801$;

TABLE 2

THE EFFECT OF VARIOUS CHRONIC DRUG TREATMENTS ON STRIATAL DA BIOCHEMISTRY IN RESPONSE TO SALINE OR APOMORPHINE CHALLENGE

	OFF-APO			ON-APO		
	DA	HVA	HVA/DA	DA	HVA	HVA/DA
Group (n)	(ng/mg prot.)			(ng/mg prot.)		
CONT (16)	189.53	9.66	0.0510	194.41	4.84	0.0252
HAL (15)	132.62*	9.23	0.0731*	220.52	3.85	0.0180*
THIO (12)	125.09*	8.25	0.0659*	179.26	4.49	0.0250
SCOP (12)	157.63	11.14	0.0705*	196.07	4.22	0.0217
AMAN (16)	188.55	12.90*	0.0702*	206.81	3.20*	0.0159*
Li (15)	185.46	12.02*	0.0673*	216.16	4.63	0.0214
H+T (13)	141.89*	6.73	0.0479	168.03	4.01	0.0242
H+S (14)	154.26*	9.66	0.0630	195.39	3.69*	0.0188*
H+A (16)	199.84	11.14	0.0578	204.12	2.72*	0.0134*
H+Li (15)	191.40	11.12	0.0598	214.09	4.56	0.0216
Total (144)	167.73	10.24	0.0626	200.40	4.04	0.0205
s.d.	41.34	2.52	0.0143	36.14	1.18	0.0061

Values were determined by summing the chromatographic values for DA or HVA and then dividing by the sum of the anterior and posterior protein content. (* = $p<0.05$ relative to CONT.)

$p<0.02$). The pair-wise comparisons revealed that HAL-, H+S- and H+T-treated animals were all statistically elevated relative to CONT animals.

DISCUSSION

The use of BH as an animal model of TD is subject to a number of criticisms. The absence of spontaneous movements associated with chronic treatment, the observation that most animals eventually develop BH, and the short duration of treatments used in rodents, are often criticised as major differences between the animal model and the disease state it is supposed to reflect. It is important to point out, however, that the facial musculature of the rodent is exceedingly less complex than that of man and one would, therefore, expect that the threshold to produce spontaneous movements in humans would be lower than in rodents. Although treatment with neuroleptic agents is associated with the development of peri-oral behaviors in rodents (61), it is important to recognize that this behavior is attenuated with antimuscarinic agents and is probably, therefore, more analogous to acute EPSE's than TD (49).

BH more appropriately reflects the form of TD observed after the withdrawal from neuroleptic agents (39). Most animals chronically treated do manifest the BH state, but it must also be remembered that BH is observed following a challenge with a DA agonist. Studies in human have suggested that patients at risk for the development of DA hypersensitivity behaviors can express abnormal movements or exhibit exacerbations in their abnormal

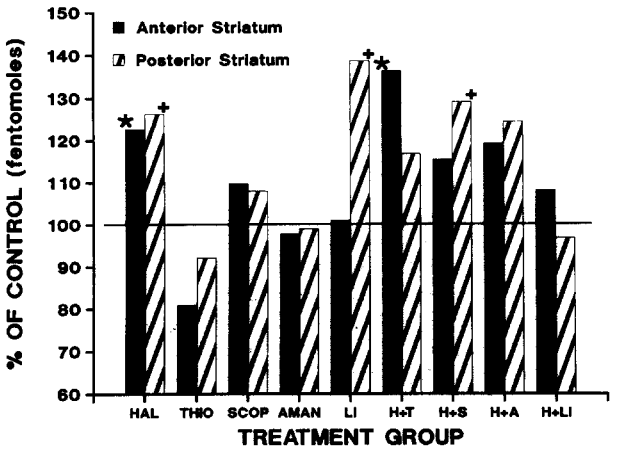

FIG. 4. The effect of 48 treatments with the agents specified on D-2 receptor density in the anterior and posterior striatum from animals sacrificed 6 days following their last treatment (see the Method section for description of anterior and posterior sections). Data, in femtomoles, is expressed relative to normal saline-treated controls. (* and + = $p<0.05$ vs. normal saline-treated controls.)

TABLE 3

THE EFFECT OF VARIOUS CHRONIC DRUG TREATMENTS ON STRIATAL D-2 BINDING DENSITY

Treatment Group (n)	Mean Binding Density ± s.d. (in femtomoles)
CONT (12)	225.00 ± 26.35
HAL (7)	266.90 ± 34.95*
THIO (2)	195.46 ± 65.27
SCOP (4)	244.95 ± 12.86
AMAN (4)	219.62 ± 33.52
Li (3)	264.45 ± 25.23
H+T (4)	287.37 ± 43.44*
H+S (4)	275.98 ± 38.42*
H+A (4)	262.96 ± 40.41
H+Li (4)	227.14 ± 11.64
Total (48)	248.83 ± 39.04

Values were derived by averaging the femtomoles density of the four striatal regions studied. (* = $p<0.05$ relative to CONT.)

movements in response to DA agonist challenge (23). In addition, the threshold for the expression of such behaviors can be altered by administering antimuscarinic drugs, as is often seen in TD [so-called masked probable TD; (36,54)]. Although the appropriate studies in humans have not been performed, one must consider what the incidence of choreiform movements would be in a patient population chronically treated with a neuroleptic, withdrawn, and then challenged with a DA agonist. Although the rodent model will probably never replace the nonhuman primate model of TD, the pharmacologic similarities between BH and TD should enable one to make statements about the possible pathophysiologic processes that contribute to TD from the use of rodent models.

Chronic Neuroleptic Treatment

Chronic HAL treatment resulted in the development of BH as has been reported by numerous investigators (13, 38, 33, 48). HAL-induced BH was associated with elevated D-2 receptor density and decreased striatal DA content during withdrawal from chronic treatment as has been previously reported (41, 42, 51). HAL-treated animals responding to the challenge dose of APO exhibited an elevation in DA and a reduction in HVA which, together, translated into a significant reduction in DA activity. The ratio of metabolite to its neurotransmitter is thought to reflect the state of release of the transmitter. These HAL-induced changes can be interpreted to reflect the alteration in D-2 receptor density in combination with the well established striato-nigral feedback loop. During withdrawal from HAL, the increase in D-2 receptor density would ostensibly signal increased DA tone with subsequent feedback diminution in DA synthesis. The administration of the direct acting DA agonist, APO, would result in more negative feedback than would be seen in CONT animals with a resultant decrease in DA activity.

In contrast, THIO treatment did not result in BH despite the fact that the dose administered (10 mg/kg) is approximately equivalent to the effect on the conditioned avoidance response as that produced by 0.75 mg/kg HAL (31). This would suggest that THIO is less likely to develop BH as has been reported previously (25) [although see (18)]. Longer treatments with THIO have been shown to produce a hypersensitive state although the characteristics of that hypersensitivity were not as pronounced or as long-lived as that produced by typical neuroleptics (14). This reinforces previous reports suggesting that chronic treatment with different neuroleptics do not possess the same risk for the development of BH (26). THIO treatment did not alter D-2 receptor density, although the caveat of the small sample size alluded to above must be considered. Despite this caveat, however, THIO treatment produced effects very similar to HAL treatment in terms of striatal DA biochemistry during the withdrawal phase (i.e., OFF-APO). This might suggest that alterations in DA biochemistry can occur in the absence of alterations in DA receptor number, possibly, as has been suggested, through alterations in DA autoreceptors (40). In contrast to the similarities observed during the withdrawal phase, the effects of THIO treatment following an APO challenge were very similar to those observed in the CONT animals. This suggests a "normalization" of changes in DA observed during withdrawal from THIO which could explain the normosensitive response to APO challenge. It would further suggest that a striatum chronically treated with THIO responds to an alteration in DA tone in a fashion different from a striatum chronically treated with HAL. Therefore, the typical neuroleptic HAL and the atypical neuroleptic THIO do not produce similar alterations in striatal DA biochemistry.

It is also interesting to note that the effects that HAL or THIO treatment had on striatal DA biochemistry were dependent upon the region of the striatum studied. Thus, the effects of both agents in the anterior striatum were very similar, whereas the effects of THIO in the posterior striatum were opposite to those of HAL in almost all of the biochemical parameters examined. This attests to the regional heterogeneity now thought to exist in terms of striatal anatomy, biochemistry, and possibly function. This would further suggest that the differential effects that neuroleptic agents have on DA in various regions of the striatum may participate in the development of BH. Thus, chronic typical and atypical neuroleptic treatment does not produce the same changes in striatal DA biochemistry further suggesting that the basis for atypical classification (i.e., production of acute EPSE's) extends to chronic treatment as well.

Chronic THIO and SCOP Cotreatments

The behavioral effects that the various cotreatments had on the development of BH are similar to those reported previously. Thus, THIO and SCOP when administered with HAL, have both previously been shown to attenuate the development of BH (9,37). Twenty-four and 48 treatments with H+T and H+S resulted in a SB response which was statistically elevated relative to CONT animals. Following 48 treatments, however, the SB response of both these cotreatment groups was also statistically reduced relative to HAL treated animals. This suggests that continued cotreatment interferred with the normal progression of BH over the second set of 24 treatments as was seen in the HAL animals. This "plateauing" effect has been reported in both patients and in other animal models on single neuroleptic therapy (59,61). The fact that both THIO or SCOP cotreatment induced a "plateau" effect earlier than HAL-only-treated animals may suggest that such cotreatment may reduce the relative proportion of patients at risk for developing TD during chronic therapy or, alternatively, that the mechanisms responsible for attenuating BH through this cotreatment strategy participate in the plateauing effect in humans.

The H+T and H+S treatment groups both exhibited an elevated D-2 binding density which, like HAL-treated animals, was statistically different from CONT animals. In both cases, the degree of elevation of D-2 binding density relative to CONT animals was greater than that seen in the HAL group, although these differences were not statistically different from the binding density of HAL-treated animals. Thus, cotreatment with both THIO or SCOP did not prevent the receptor proliferation normally induced by HAL treatment, despite the fact that the animals exhibited a SB response which was significantly reduced relative to HAL. Likewise, neither cotreatment prevented the reduction in striatal DA content normally produced by HAL treatment during the withdrawal from chronic treatment. The inability of SCOP to prevent the receptor proliferation and striatal DA reductions normally induced by HAL, while significantly attenuating BH, is similar to the results we reported previously using the antimuscarinic/DA reuptake inhibitor benztropine (7). Taken together, this data strongly suggests that D-2 receptor proliferation or striatal DA content are not the only factors contributing to the development of BH.

We originally hypothesized that chronic treatment with an atypical neuroleptic agent with significant antimuscarinic activity, such as THIO, would induce muscarinic receptor site proliferation. Chronic treatment with antimuscarinic agents or atypical neuroleptics has been shown to elevate muscarinic binding sites in the striatum (3, 21, 63) and the balance between DA and ACh activity, as it relates to striatal function and DA-mediated behaviors, is well established. This hypothesis assumed that D-2 up-regulation would also occur following treatment with atypical neuroleptics [not observed in the present study but reported by

other investigators (14,21)]. The tone of both the DA and the cholinergic system would be elevated following chronic treatment with an atypical neuroleptic, leaving striatal function in "balance" with regard to these two neurotransmitter systems. A normosensitive SB response would, therefore, be expected. Our previous reports utilizing 3 different antimuscarinic agents in combination with HAL demonstrated that the development of BH could be attenuated in a dose-dependent fashion in support of our hypothesis (8). However, it is important to note that the dose of THIO used in the present study is only one-tenth as effective in displacing QNB binding to muscarinic receptors as the protecting dose of SCOP employed in the present study (44,53). This might suggest that THIO and SCOP attenuated the development of BH through different mechanisms. Both THIO and SCOP administered alone, or in combination with HAL, tended to result in similar alterations in anterior and posterior striatal DA biochemistry, which for the most part, were similar to the effects of HAL. Thus, if THIO and SCOP do attenuate BH via different mechanisms, alterations in dopaminergic function cannot be employed to identify them.

Chiodo and Bunney (11), as well as others (64), have hypothesized that chronic cotreatment with an antimuscarinic agent and haloperidol prevents the development of striato-nigral feedback depolarization block in the substantia nigra. This hypothesis suggests that the occurrence of depolarization block in the substantia nigra, normally induced by HAL, and the resulting diminution of striatal DA release it would produce, would further enhance the pharmacologic denervation of the striatum and thereby enhance DA receptor site proliferation. However, in the present study, neither THIO nor SCOP cotreatment attenuated the DA receptor proliferation or the alterations in striatal DA content normally induced by HAL. This would suggest that preventing depolarization block with THIO or SCOP does not interfere with the pharmacologic denervation process, and, therefore, is not the mechanism responsible for the reduced ability of atypical neuroleptics to produce BH.

Finally, it could be argued that THIO, with its lower affinity for the D-2 receptor, may competitively inhibit the access of HAL to its receptor and thereby attenuate BH. However, THIO was administered at 13 times the administered dose of HAL in an effort to overcome these differences. In addition, H+T cotreatments resulted in a D-2 receptor density which was 9% elevated relative to HAL animals and also produced similar changes in anterior striatal DA content OFF-APO. This would suggest that THIO cotreatment did not interfere with HAL for access to the D-2 receptor. It could also be argued that the attenuating effect THIO cotreatment had on HAL-induced BH was related to cotreatment with a butyrophenone and phenothiazine type neuroleptic, potentially interacting with different states of the DA receptor or at different sites within the DA receptor complex. However, we have previously reported that cotreatment with HAL and another typical neuroleptic of the phenothiazine class, fluphenazine, not only failed to prevent BH, but actually significantly increased the degree of BH relative to animals treated with HAL or fluphenazine alone (37). Taken together, the data is most consistent with an hypothesis which suggests that mechanisms involving systems other than DA are responsible for the attenuating effect of THIO cotreatment.

Chronic AMAN and Li Cotreatments

Unlike THIO or SCOP cotreatment, both AMAN and Li prevented the development of BH after 24 as well as after 48 treatments. The only behavioral parameter affected by these treatments was a slight, although statistically significant, elevation in response duration in the H+A treatment group.

Allen and his co-workers (1) previously demonstrated that AMAN was able to prevent the development of BH as well as D-2 receptor proliferation. Chronic cotreatment with other indirect acting DA agonists, such as levodopa, similarly prevented BH as well as D-2 up-regulation (19). It is generally assumed that amantadine stimulates the release of DA (50,60), or inhibits the reuptake of DA (2,27). The increase of DA in the synaptic cleft these indirect acting DA agonists would produce would be expected to compete with HAL for access to the DA receptor, or alter the conformation of the receptor to reduce affinity for the antagonist, either of which would reduce the degree of pharmacologic denervation. Indeed, the present data demonstrates that AMAN did induce significant changes in striatal DA activity, suggesting increased DA activity during withdrawal and decreased DA activity in response to a APO challenge. Interestingly, H+A cotreatment was observed to "normalize" virtually all of the DA parameters, with the exception of posterior striatal DA, when examined OFF-APO. However, it is also important to note that D-2 binding density was elevated in both regions of the striatum although this difference was not statistically significant. This might suggest that doses larger than 50 mg/kg may be more effective at preventing the alterations in DA.

Li is thought to influence brain function, at least in part, by interfering with phosphoinoside metabolism (4). This effect might be expected to uncouple the production of important second messengers from receptor-transmitter interaction. This effect may likewise interfere with the mechanisms directly responsible for the induction of DA receptor proliferation thereby preventing the up-regulation of D-2 receptors as has been previously reported (47). The effects of subacute Li treatment on brain DA are conflicting. Various investigators have observed no changes in DA metabolism following Li treatment (5,26), while others have reported decreased DA synthesis and turnover (16,20). Our results are in agreement with Hesketh *et al.* (28) who reported increases in both HVA and the DA metabolite DOPAC. It is also important to note that these investigators examined the effects of lithium during withdrawal from Li treatment (24 hours), whereas the previous investigators examined the effects immediately following the last Li treatment. It is, therefore, interesting to note that Li produced the most dramatic increase in posterior striatal D-2 receptor density of any chronic treatment without influencing anterior striatal binding density. The selective increase in binding density in the posterior striatum was accompanied by alterations in DA biochemistry which were virtually identical to those observed following HAL treatment, while in the anterior striatum this correspondence did not occur. This might suggest that Li may have a preferential effect in the posterior region of the striatum. This region-specific effect may, in part, explain the numerous discrepancies reported on the effect of chronic Li treatment on DA metabolism as cited above. This region-specific effect might also explain the results of Staunton *et al.* (55) who demonstrated that cotreatment with H+Li for 3 weeks did prevent BH, but did not prevent the D-2 receptor proliferation. Despite this potential discrepancy, it appears that the mechanism responsible for D-2 receptor proliferation in the posterior striatum is different from that produced by HAL since the coadministration of these two agents totally normalized D-2 density in both the anterior as well as the posterior striatum.

Implications in Tardive Dyskinesia

DA receptor proliferation subsequent to chronic neuroleptic treatment has been one of the main features of BH which has broadly been accepted as contributing to the development of TD in humans. However, even this characteristic of the BH model has come under recent criticism when it was reported that DA receptor

site up-regulation was equivalent in chronically treated patients with or without TD (17). This might suggest that D-2 receptor site proliferation does not participate in the development of TD. However, the results from the present study would suggest that D-2 receptor proliferation does not necessarily correlate with BH. Since it appears that mechanisms in addition to alterations in striatal DA function participate in the expression of BH, it might be suggested that these additional mechanisms also participate in the expression of choreiform movements in man as well. Thus, it would be expected that not all patients with DA receptor site proliferation would express TD. It is, therefore, hypothesized that D-2 receptor proliferation is permissive to the development of BH and possibly TD. Thus, other factors, which must at least include striatal muscarinic tone, will determine whether or not the dopaminergic hypersensitivity induced by chronic neuroleptic treatment will manifest itself as a behavioral hypersensitivity state.

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