Pharmacology Biochemistry & Behavior, Vol. 19, pp. 199–204, 1983. © Ankho International Inc. Printed in the U.S.A.

The Effects of Chronic Haloperidol Administration on GABA Receptor Binding

RONALD D. HUFFMAN AND MAHARAJ K. TICKU

The University of Texas Health Science Center at San Antonio
Department of Pharmacology, 7703 Floyd Curl Drive, San Antonio, TX 78284

Received 8 November 1982

HUFFMAN, R. D. AND M. K. TICKU. *The effects of chronic haloperidol administration on GABA receptor binding.* PHARMACOL BIOCHEM BEHAV **19**(2) 199–204, 1983.—The effect of chronic administration of haloperidol for 30 days and its subsequent withdrawal for 0, 4 and 8 days on the binding of [^{3}H]-GABA and [^{3}H]-muscimol to GABA binding sites in rat brain membranes obtained from six discrete regions of the brain and spinal cord was investigated. The chronic administration of haloperidol resulted in a marked increase in the number of GABA binding sites within the substantia nigra, but did not affect GABA binding sites in other regions of rat brain or spinal cord. The increase in GABA binding sites in the substantia nigra was evident for at least 4 days following termination of haloperidol treatment; however, 8 days following withdrawal from haloperidol, when dopaminergic supersensitivity had become maximally expressed, the number of GABA binding sites in the substantia nigra had declined and was the same as that observed in the control rats. These observations suggest that blockade of dopamine receptors in the striatum by haloperidol results in a compensatory decrease in the activity of the GABAergic strionigral system that leads to an increase in the number of GABA binding sites within the substantia nigra. This increase in the number of GABA binding sites is gradually reversed when dopamine is again allowed to interact with its receptors.

Haloperidol GABA receptor binding Apomorphine-induced stereotypies Dopaminergic supersensitivity Substantia nigra Muscimol

IT is now well established that the chronic administration of neuroleptics such as haloperidol or chlorpromazine results in the development of dopaminergic supersensitivity [3, 14, 23]. Recent studies suggest that these neuroleptics may also be able to influence the responsiveness of other neurotransmitter-mediated synaptic responses; in particular, those mediated by acetylcholine, GABA, met-enkephalin and substance P [9, 13, 21, 22]. These effects on non-dopaminergic neurotransmitters are considered to be secondary to the blockade of dopamine receptors effected by the neuroleptics. In the basal ganglia, these neuroleptics are able to interact with dopamine receptors located postsynaptically on striatal intrinsic and projection neurons [29]. Many of these striatal projection neurons are GABAergic neurons that form a feedback loop that functions to modulate the activity of dopaminergic neurons located in the zona compacta of the substantia nigra (see review by Dray [5]). Thus, by blocking dopamine receptors located on these strionigral GABAergic neurons, the neuroleptics can indirectly alter the responsiveness of this GABA-mediated synaptic system.

Several lines of evidence suggest that dopamine agonists and antagonists are able to influence GABA-mediated synaptic transmission in the CNS. Acute administration of dopamine agonists, for example, has been reported to increase the turnover rate of GABA in the substantia nigra; while the acute administration of neuroleptics has been reported to increase the turnover rate of GABA in the globus pallidus and nucleus accumbens [21,22]. Chronic administration of neuroleptics has been reported to decrease the turnover rate of GABA in the substantia nigra and globus pallidus and increase it in the striatum and nucleus accumbens [21,22]. The acute or chronic administration of neuroleptics has been reported to decrease the content of GABA in the striatum and substantia nigra [15,24].

In a recent study on the effects of neuroleptics on GABA receptor binding in the basal ganglia, Gale [8] reported that the acute administration of haloperidol or chlorpromazine had no effect on GABA receptor binding in the substantia nigra; whereas the chronic administration of these neuroleptics resulted in a significant increase in GABA receptor binding in the substantia nigra, but not in the striatum. In the cerebellum, both acute and chronic haloperidol treatments were found to decrease GABA receptor binding in this brain area [17,32]. These studies, however, have not looked at the effects on GABA receptor binding that occurs during blockade of the dopaminergic receptors by the neuroleptics and the subsequent development and then remission of dopaminergic supersensitivity that occurs following withdrawal from chronic neuroleptic treatment. In the present study, we have investigated the effect of chronic haloperidol administration (30 days) and its subsequent withdrawal for 4 and 8 days on the binding of two GABA receptor radioligands in 6 discrete brain regions of the CNS.

METHOD

Subjects

Male Sprague-Dawley rats, with an initial weight of 150–160 g, were employed in these studies. The animals were housed two per cage, with a 12-hr lighting regimen. The hal-

operidol used in this study was generously supplied courtesy of McNeil Laboratories, Inc. Apomorphine hydrochloride was purchased from Sigma Chemical Corporation. [^{3}H]-GABA (60 Ci/mmol) and [^{3}H]-muscimol (10.3 Ci/mmol) were purchased from New England Nuclear.

Chronic Haloperidol Administration

Powdered rat feed containing 0.01%, 0.015% or 0.02% haloperidol was prepared by adding haloperidol powder to powdered rat chow (Wayne LabBlox); the feed was then mixed for 2–3 hours in a Hobart mixer. The haloperidol-treated rats received haloperidol in their feed for 30 days as follows: 0.01% for 12 days (10.1±0.4 mg/kg), 0.015% for 12 days (15.2±0.7 mg/kg) and 0.02% for 6 days (11.6±0.6 mg/kg). The rats were not force-fed but were allowed to eat the haloperidol-treated feed ad lib. Control rats received the same powdered feed without haloperidol and were pair-fed to assure equal weight gain between the two groups of rats. The mean weights for the haloperidol-treated rats and control rats on day 30 were 257±6 g and 264±4 g, respectively, and were not significantly different.

Apomorphine-Induced Stereotypy

To assess the development of dopaminergic supersensitivity resulting from this haloperidol feeding regime, we employed a standard behavioral model based upon the determination of stereotypies that result following the administration of the dopamine agonist apomorphine [7]. For this part of the study, a second group of rats were fed haloperidol for 30 days and then were withdrawn from the haloperidol diet. Apomorphine-induced stereotypies were assessed on day 30 before withdrawal from haloperidol and again on 4–5, 8–9, 12–13 and 16–17 days following haloperidol withdrawal.

Apomorphine-induced stereotypies were measured according to a rating scale initially described by Ernst [7]. The animals were placed singly into cages which contained a wire mesh on the floor. Each rat was observed for apomorphine-induced stereotypies and the stereotype rating scores employed were as follows: 0—rats exhibited no stereotypies; 1—rats walked about the cage, sniffed and/or licked the wire mesh or sides of the cage; 2—rats walked about the cage and occasionally bit or gnawed the wire or cage sides, or sat in one spot and intensely licked the wire or side of the cage; 3—rats restricted their locomotion to one small area and gnawed intensely on the wire or side of the cage; 4—rats remained in one spot for 5 minutes or longer and intensely gnawed the wire or side of the cage.

The rats were observed for 10 consecutive five-minute periods, and each period was scored for the predominant behavior that occurred during each 5 min period. The total stereotypy score for the 50 min period was based upon scores obtained for every other 5 min period (total of 5 periods), starting with the initial 5 min period; thus, the maximum possible score was 20.

Treatment Groups for GABA Binding Study

For the assessment of the effect of chronic haloperidol administration and withdrawal on GABA receptor binding in discrete brain regions, 4 experimental groups of 8 rats/group were employed: treatment group 1 consisted of rats fed the control feed for 30 days, treatment groups 2, 3 and 4 consisted of rats fed haloperidol for 30 days and then sacrificed on day 30, 34 and 38, respectively. This sacrifice schedule resulted in the haloperidol rats being withdrawn from haloperidol for 0, 4 and 8 days, respectively. The rats employed in this binding study were not treated with apomorphine since it was felt that such treatment might alter GABA binding.

Tissue Preparation

Following chronic treatments, rats were decapitated, brain regions were rapidly dissected, rinsed and kept in iced 0.32 M sucrose. The tissue was prepared as described elsewhere [31]. Briefly, pooled discrete brain regions (striatum, substantia nigra, hippocampus, cerebellum, pons-medulla, spinal cord) were homogenized in 0.32 M sucrose and centrifuged at 580 × g for 10 min; supernatant was centrifuged at 140,000 × g for 45 min in a 60 Ti rotor to obtain the crude mitochondrial plus microsomal (P_2+P_3) fractions. The (P_2+P_3) fractions were osmotically shocked in iced distilled water, repelleted, osmotic shock treatment was repeated and the pellet resuspended in 0.05 M Tris citrate buffer (pH 7.1) and frozen at −20°C overnight. The tissue was then thawed, washed twice in 0.05 M Tris citrate buffer and frozen. On the day of the assay, the tissue was thawed at room temperature, pelleted, homogenized in 0.05 Tris citrate buffer, repelleted, washed twice in the buffer and finally resuspended in the same buffer at a protein concentration of 0.8–1.2 mg/ml. The freeze-thaw and excessive washing of the tissue was necessary to eliminate the uptake sites and endogenous inhibitors of GABA binding [11,30]. We have observed that two osmotic shock treatments, two freeze-thaw cycles and four buffer washes are adequate for removing endogenous inhibitors [30]. These procedures also eliminate the need for using Triton X-100 ([11] Ticku, unpublished observations). Protein was estimated according to the method of Lowry *et al.* [19]. All the tissue preparation was done at 0–4°C and in Na^+-free buffer.

GABA Binding

The binding of [^{3}H]-GABA and [^{3}H]-muscimol to GABA receptor-like sites was assayed by a modification of the method of Enna and Snyder [6], by a centrifugation assay as described previously [31]. Binding assays were performed in triplicate by incubation of aliquots of P_2+P_3 homogenate (0.7–1.0 mg) with various concentrations of [^{3}H]-GABA (66 Ci/mmol) or 10 nM [^{3}H]-muscimol (10.3 Ci/mmol) with or without excess non-radioactive GABA (0.1 mM), for 5 min at 0°C in 1 ml volume in scintillation Biovials. For saturation binding isotherms, the concentration of [^{3}H]-GABA was varied for the low concentration points (0.1–4 nM); and for the high points (>10 nM), the concentration of non-radioactive GABA was varied, keeping [^{3}H]-GABA constant at 4 nM. Following incubation, the vials were centrifuged at 48,000 × g for 10 min in a JA 20.1 rotor. The supernatant was discarded and the pellets were rapidly rinsed, without disturbing the pellet, with iced buffer. Pellets were solubilized overnight with 0.3 ml Soluene-350 (Packard) and radioactivity was counted in 3 ml of Toluene containing 5 g/l of 2,5-diphenyloxazole. The counting efficiency, estimated by the external standard ([^{3}H]-toluene) method, was 40±1%.

The specific binding, obtained by subtracting from the total pelleted radioactivity the background, i.e., the amount not displaced by excess (0.1 mM) non-radioactive GABA, was converted into pmol/mg protein bound. Data were analyzed by Scatchard plots and the affinity constants (K_D) and binding capacities (B_{max}) were obtained by linear regression. Specific binding usually represented 76±5% of the total radioactivity in the pellet.

RESULTS

Behavioral Assessment of the Effect of Chronic Haloperidol Blockade of Dopaminergic Receptors

The effect of chronic haloperidol feeding on the responsiveness of the dopaminergic system was determined by assessing the development of stereotypies following the intraperitoneal administration of apomorphine to control and haloperidol treated rats. Two doses of apomorphine were employed in these studies: (1) 1 mg/kg of apomorphine was administered to control (N=14) and haloperidol-fed rats (N=16) prior to withdrawal from the haloperidol diet (0 days withdrawal), and (2) 0.5 mg/kg was administered to another group of control (N=25) and haloperidol-fed rats that had been withdrawn from the haloperidol-diet for 4–5 (N=20), 8–9 (N=22), 12–13 (N=18) and 16–18 (N=6) days.

Apomorphine-induced stereotypies were significantly decreased ($p<0.001$) in haloperidol treated rats as compared to control rats prior to their withdrawal from the haloperidol diet indicating that a good degree of dopamine receptor blockade was achieved by the chronic haloperidol feeding schedule (0 days withdrawal, Fig. 1). However, following four days of withdrawal from the haloperidol diet, the haloperidol treated rats exhibited a significant increase ($p<0.001$) in apomorphine-induced stereotypies that was evident for at least 13 days following withdrawal from the haloperidol diet. This enhancement of apomorphine-induced stereotypies peaked at 8–9 days and then began to subside. Thus the chronic haloperidol feeding schedule employed in this study led to the development of a significant degree of dopaminergic supersensitivity that persisted for more than 12 days. The haloperidol-treated rats did not exhibit any stereotypic behavior following haloperidol withdrawal without apomorphine administration.

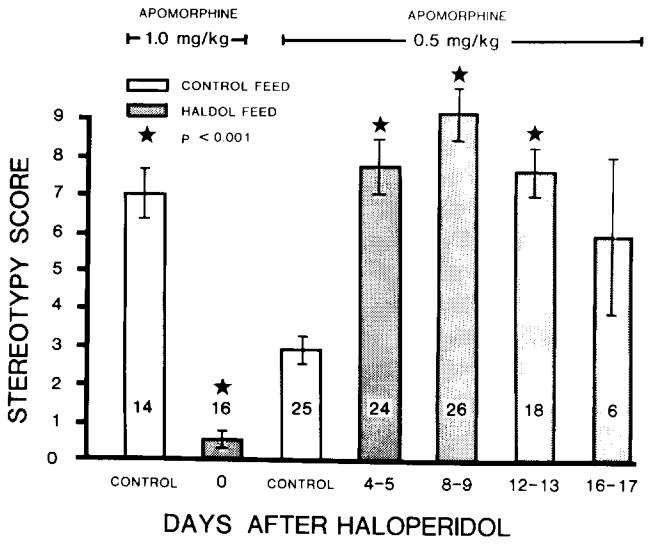

FIG. 1. Apomorphine-induced stereotypies in control and haloperidol treated rats. Chronic haloperidol treatment for 30 days (0 days after haloperidol) prevents the induction of stereotypies following the administration of apomorphine (1 mg/kg IP). Apomorphine-induced stereotypies (0.5 mg/kg IP) were significantly increased in rats that had been withdrawn from haloperidol treatment for 4–13 days. These results were analyzed by nonparametric statistical procedures including Kruskal-Wallace analysis of variance and Mann-Whitney U tests and significance was assessed at $p<0.001$.

Effects of Chronic Haloperidol Administration on GABA Receptor Binding

Binding studies have demonstrated that [^{3}H]-muscimol binds to the same synaptic receptors as does [^{3}H]-GABA and thus both can be considered to be ligands for synaptic GABA receptors [4]. Therefore, we have employed both ligands as probes aimed at characterizing the effects of chronic haloperidol administration on GABA receptors in the various brain regions. Table 1 illustrates the effect of chronic haloperidol treatment on the binding of [^{3}H]-muscimol in four brain regions and in spinal cord. Specific [^{3}H]-muscimol binding was significantly increased in the substantia nigra as a result of the chronic administration of haloperidol (0 day withdrawal) and remained elevated during the first 4 days following haloperidol withdrawal. Following 8 days of haloperidol withdrawal, however, muscimol binding had declined and was not significantly different from that observed in the control group. Under identical treatment conditions, the specific [^{3}H]-muscimol binding was not altered in the striatum, pons-medulla, spinal cord (Table 1) or hippocampus (not illustrated). Although muscimol binding was significantly elevated in the cerebellum, this increase was variable and did not appear to be correlated with changes in responsiveness of the dopaminergic system. Similar results were obtained in these same regions when equilibrium GABA binding was measured using 4 nM [^{3}H]-GABA (data not shown).

To further characterize this increase in GABA receptor binding following chronic haloperidol treatment, we determined [^{3}H]-GABA Scatchard plots for the substantia nigra. The substantia nigra from each group of rats were pooled for these binding studies using a range of GABA concentrations (0.25–504 nM). A ten-point Scatchard plot of [^{3}H]-GABA binding was measured for the substantia nigra of the control and three haloperidol treated groups of rats. The Scatchard data indicated that [^{3}H]-GABA was binding to a single high affinity binding site in the substantia nigra of the control group with an apparent K_D of 38 nM. Similar results have been reported by Gale [8]. Table 2 shows that chronic haloperidol treatment did not change the apparent K_D of GABA binding in substantia nigra in any of the treatment groups. However, the B_{max} of this high affinity GABA binding site was increased from a control value of 904 fmol/mg protein to 2527 fmol/mg protein at 0 day withdrawal and to 2862 fmol/mg protein at 4 day withdrawal. At 8 day withdrawal, the B_{max} returned to control values.

Similar [^{3}H]-GABA Scatchard plots were determined in the control and three haloperidol treated groups for the striatum, hippocampus, cerebellum and spinal cord. In contrast to substantia nigra, [^{3}H]-GABA bound to two classes of GABA binding sites in each of these regions. The K_D and B_{max} values for GABA binding in each of these regions in the control group were similar to the values described elsewhere [31]. No significant change in the number of GABA receptor sites (B_{max}) or affinity (K_D) of either GABA binding site was observed in any of these other brain regions following the chronic haloperidol treatment (data not shown).

DISCUSSION

The repeated administration of neuroleptics induces an increase in the number of dopamine receptors within the striatum and dynamic changes in other basal ganglia neurotransmitter systems, i.e., acetylcholine, GABA, substance P and enkephalins [9, 13, 21, 22]. Recent evidence strongly

TABLE 1

EFFECT OF CHRONIC HALOPERIDOL TREATMENT ON SPECIFIC [^{3}H]-MUSCIMOL BINDING

Region	Specific [^{3}H]-Muscimol Binding (DPM/mg Protein)			
	Control	0 Day Withdrawal	4 Day Withdrawal	8 Day Withdrawal
Substantia Nigra	7,211 ± 765	10,445 ± 995*	9,926 ± 835*	7,649 ± 735
Striatum	8,734 ± 2,122	7,503 ± 1,850	6,684 ± 1,950	8,910 ± 1,080
Pons-Medulla	2,886 ± 309	3,015 ± 273	3,100 ± 419	2,804 ± 308
Cerebellum	20,111 ± 954	22,742 ± 1,180	20,339 ± 787	23,832 ± 1,099*
Spinal Cord	2,175 ± 289	2,105 ± 428	1,966 ± 399	2,351 ± 306

Alliquots of brain regions were incubated with 10 nM [^{3}H]-muscimol (10.3 Ci/mmol) and assayed by centrifugation, as described in the text. The values are the mean ± S.D. of a single experiment (done in triplicate). Significance was determined by ANOVA and Duncan Multiple Range Tests (*$p<0.01$).

suggests that the dopaminergic nigrostriatal terminals within the striatum functionally modulate the activity of postsynaptic GABAergic striatal neurons [1, 20, 25, 28, 32].

In the present study we have shown that chronic haloperidol administration results in an increase in the binding of the GABA agonist [^{3}H]-muscimol in the substantia nigra, but not in the striatum, the area which receives the massive dopaminergic input. This increased GABA receptor binding that resulted from chronic haloperidol administration was maximal prior to withdrawal from haloperidol when the responsiveness of the dopaminergic system was maximally reduced by the presence of haloperidol within the CNS. Following withdrawal from haloperidol, the responsiveness of the dopaminergic system gradually increased and was significantly increased at 4 days and peaked at 8–9 days. The muscimol binding studies demonstrated just the opposite trend for GABA binding. Thus the number of GABA binding sites was maximally increased prior to withdrawal from haloperidol; and although still significantly elevated at 4 days following haloperidol withdrawal, the number of GABA binding sites had begun to decrease. By 8 days of withdrawal from haloperidol, when the responsiveness of the dopaminergic system was maximal, the number of GABA binding sites in the substantia nigra had declined and was not different from that observed for the control group. The time course of decline in the number of GABA binding sites within the substantia nigra following withdrawal from chronic haloperidol treatment observed in this study correlates well with the time course of decline in the supersensitivity to the behavioral effects of intranigral injections of muscimol reported for rats withdrawn from chronic haloperidol treatment [2].

Additional evidence in support of increased GABA binding sites in the substantia nigra was obtained from the 10 point Scatchard analysis of [^{3}H]-GABA binding. The observed increase in GABA receptor binding within the substantia nigra was due to an increase in the number (B_{max}) of high-affinity GABA binding sites and not due to a change in affinity (K_D) of this binding site for GABA. Deafferentation of the substantia nigra, either by sectioning the strionigral pathways or by injecting kainic acid into the striatum, results in a similar alteration in nigral GABA receptors. There is no change in K_D, but there is a significant increase in the number of high-affinity [^{3}H]-GABA binding sites [12,33].

Our findings are in agreement with those of Gale [8] who also observed an increase in GABA binding sites in the substantia nigra, but not in the striatum, of rats that had been withdrawn from haloperidol or chlorpromazine for 5 days. Thus it appears that blockade of the dopamine receptors results in an increase in GABA receptor binding in the substantia nigra; and as this blockade is eliminated and dopamine is again able to interact with its binding sites, there is a gradual reversal of this increase in GABA binding leading to a normalization in the number of binding sites at the time that the responsiveness of the dopaminergic system is approaching a maximum. Recent behavioral evidence suggests that this increase in GABA binding following chronic haloperidol administration is associated with an increased responsiveness in these animals to the GABA agonist muscimol [16].

It is unlikely that the increase in GABA binding sites in the substantia nigra observed following chronic haloperidol administration is a result of a direct blockade of these binding sites by haloperidol since, as a class, the neuroleptics have little or no affinity for GABA receptors in the brain [26]. Instead, the increased GABA receptor binding concurrent with blockade of DA receptors by haloperidol suggests that prolonged neuroleptic treatment may result in a reduced

TABLE 2

EFFECT OF CHRONIC HALOPERIDOL TREATMENT ON THE BINDING CONSTANTS OF [^{3}H]-GABA IN SUBSTANTIA NIGRA

	Specific [^{3}H]-GABA	
	K_D(nM)	B_{max} (fmol/mg protein)
Control	38	904
0 Day Withdrawal	50	2527
4 Day Withdrawal	40	2682
8 Day Withdrawal	45	923

For each treatment group, substantia nigra were pooled from eight rat brains and processed for tissue preparation. Scatchard plots of GABA were generated using 10 concentrations of [^{3}H]-GABA (0.25–504 nM). The Scatchard data could be best fit to a single site. The K_D and B_{max} values were obtained by the linear regression of the binding data.

GABA synaptic activity which then leads to a compensatory increase in GABA receptors. If this hypothesis is correct, one would expect that stimulation of striatal DA-receptors increases the release of GABA in the nigra and that facilitation of nigral GABAergic transmission would potentiate the behavioral effects of striatal DA-receptor stimulation. In agreement with this expectation, it has been found that systemic administration of apomorphine stimulates GABA-turnover in the substantia nigra [27] and that increasing nigral GABA-levels result in potentiation of the stereotypies induced by apomorphine [10]. Interaction studies with neuroleptics and GABAmimetics also provide support for this hypothesis. Thus coadministration of GABAmimetics has been shown to potentiate catalepsy resulting from acute injections of haloperidol and to prevent the development of tolerance to its cataleptogenic actions that normally occurs with chronic administration of haloperidol [18]. This suggests that stimulation of GABA receptors concomitant to the blockade of DA receptors prevents the development of tolerance to catalepsy that results from DA receptor blockade. This might imply that alterations in GABAergic transmission are involved in the expression of at least some DA receptor-mediated events.

Gale *et al.* [9] have provided the most convincing evidence to date that neuroleptic blockade of striatal postsynaptic dopamine receptors results in a decrease in GABA activity in the substantia nigra. The allosteric activation of striatal tyrosine hydroxylase induced by systemic administration of haloperidol was abolished by discrete electrolytic lesions of strionigral pathways, antagonized by systemic administration of GABA agonists and potentiated by administration of GABA antagonists. Direct injection of muscimol into the substantia nigra resulted in a bicuculline-reversible blockade of the haloperidol-induced activation of striatal tyrosine hydroxylase. They have also shown that dopamine autoreceptors on nigral neurons are not critical to this action of haloperidol. The critical site for haloperidol-induced alteration in GABAergic synaptic function appears to be the postsynaptic dopamine receptors in the striatum which are able to influence the activity of strionigral GABAergic pathways.

The failure to observe changes in GABA receptor binding in the striatum suggests that dopamine may not play an important role in regulating GABAergic transmission in this region although the striatum receives a major dopaminergic input and there are thought to be GABAergic intrinsic neurons and possibly collaterals from GABAergic projection neurons that terminate in the striatum. Although the hippocampus, cerebellum and spinal cord contain GABAergic neurons, they receive very little dopaminergic input and presumably have few dopamine receptors, thus we did not expect to see alterations in GABA binding correlated with altered dopaminergic responsiveness in these brain regions. Although other investigators have reported a decrease in GABA binding in the cerebellum following chronic neuroleptic treatment [15,24]; we observed an increase in GABA binding, but this increase was variable and did not correlate well with changes in responsiveness of the dopaminergic system. The lack of significant dopaminergic input to the cerebellum makes it difficult to attach any significance to such changes relative to blockade of dopamine receptors.

In summary, the chronic administration of haloperidol resulted in a significant increase in the number of GABA binding sites within the substantia nigra, but did not affect GABA binding sites in other regions of the brain or spinal cord. The increase in GABA binding sites in the substantia nigra was evident for at least 4 days following termination of haloperidol treatment; however, 8 days following withdrawal from haloperidol when dopaminergic supersensitivity had become maximally expressed, there was a gradual reversal of this increase in GABA binding leading to a normalization in the number of binding sites at a time when the responsiveness of the dopaminergic system was approaching a maximum. These observations suggest that blockade of dopamine receptors by haloperidol results in a compensatory decrease in the activity of the GABAergic strionigral system that results in an increase in the number of GABA binding sites within the substantia nigra. This increase in the number of GABA binding sites can gradually be reversed if dopamine is once again allowed to interact with its receptors.

ACKNOWLEDGEMENTS

We wish to thank Troie P. Burch and Betty Yau for their technical assistance. This work was supported by NIH NS15339 Grant and NSF Grant BNS-77-24774.

REFERENCES

1. Costa, E., D. L. Cheney, C. C. Mao and F. Moroni. Action of antischizophrenic drugs on the metabolism of gamma-aminobutyric acid and acetylcholine in globus pallidus, striatum and n. accumbens. *Fed Proc* **37:** 2408–2414, 1978.
2. Coward, D. M. Classical and non-classical neuroleptics induce supersensitivity of nigral GABA-ergic mechanisms in rat. *Psychopharmacology (Berlin)* **78:** 180–184, 1982.
3. Creese, I. and S. H. Snyder. Chronic neuroleptic treatment and dopamine receptor regulation. *Adv Biochem Psychopharmacol* **24:** 89–94, 1980.
4. DeFeudis, F. V. Binding studies with muscimol; relation to synaptic γ-aminobutyrate receptors. *Neuroscience* **5:** 675–688, 1980.
5. Dray, A. The physiology and pharmacology of mammalian basal ganglia. *Prog Neurobiol* **14:** 221–335, 1980.
6. Enna, S. J. and S. H. Snyder. Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions. *Brain Res* **100:** 81–97, 1975.
7. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–323, 1975.
8. Gale, K. Chronic blockade of dopamine receptors by antipsychotic drugs enhances GABA binding in substantia nigra. *Nature* **283:** 569–570, 1980.
9. Gale, K., E. Costa, G. Toffano, J.-S. Hong and G. Guidotti. Evidence for a role of nigral γ-aminobutyric acid and substance P in the haloperidol-induced activation of striatal tyrosine hydroxylase. *J Pharmacol Exp Ther* **206:** 29–37, 1978.
10. Garcia-Munoz, M., M. N. Nicolaou, I. F. Tullock, A. K. Wright and G. W. Arbuthnott. Feedback loop or output pathway in striato-nigral fibers. *Nature* **265:** 363–365, 1977.
11. Greenlee, D. V., P. C. Van Ness and R. W. Olsen. Endogenous inhibitor of GABA binding in mammalian brain. *Life Sci* **22:** 1653–1662, 1978.
12. Guidotti, A., K. Gale, A. Suria and G. Toffano. Biochemical evidence for two classes of GABA receptors in rat brain. *Brain Res* **172:** 566–571, 1979.

13. Hong, J. S., H.-Y. T. Yang, J. C. Gillin and E. Costa. Effects of long-term administration of antipsychotic drugs on enkephalinergic neurons. *Adv Biochem Psychopharmacol* **24:** 223–232, 1980.
14. Iversen, S. D. and I. Creese. Behavioral correlates of dopaminergic supersensitivity. *Adv Neurol* **9:** 81–92, 1975.
15. Kim, J. S. and R. Hassler. Effects of acute haloperidol on the gamma-aminobutyric acid system in rat striatum and substantia nigra. *Brain Res* **88:** 150–153, 1975.
16. Kuruvilla, A., Y. K. Fung and N. J. Uretsky. Behavioral and biochemical changes caused by muscimol in mice withdrawn from haloperidol administration. *Neuropharmacology* **21:** 891–897, 1982.
17. Lloyd, K. G., M. Shibuya, L. Davidson and O. Hornykiewcz. Chronic neuroleptic therapy: Tolerance and GABA systems. *Biochem Psychopharmacol* **16:** 409–415, 1977.
18. Lloyd, K. G. and P. Worms. Sustained gamma-aminobutyric acid receptor stimulation and chronic neuroleptic effects. *Adv Biochem Psychopharmacol* **24:** 253–258, 1980.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
20. Maggi, A., F. Cattabeni, F. Bruno and G. Racagni. Haloperidol and clozapine: specificity of action on GABA in the nigrostriatal system. *Brain Res* **133:** 382–385, 1977.
21. Mao, C. C., D. L. Cheney, E. Marco, A. Revuelta and E. Costa. Turnover times of gamma-aminobutyric acid and acetylcholine in nucleus caudatus, nucleus accumbens, globus pallidus and substantia nigra: effects of repeated administration of haloperidol. *Brain Res* **132:** 375–379, 1977.
22. Marco, E., C. C. Mao, A. Revuelta, E. Peralta and E. Costa. Turnover rates of γ-aminobutyric acid in substantia nigra, N. caudatus, globus pallidus and N. accumbens of rats injected with cataleptogenic and non-cataleptogenic antipsychotics. *Neuropharmacology* **17:** 589–596, 1978.
23. Muller, P. and P. Seeman. Dopaminergic supersensitivity after neuroleptics: time-course and specificity. *Psychopharmacology (Berlin)* **60:** 1–11, 1978.
24. Nicoletti, F., F. Patti, D. J. Condorelli, L. Rampello, G. Giammona, R.-M. Di Giorgio, P. L. Canonico and U. Scapagnini. Comparative effects of chronic haloperidol and sulpiride treatment on nigral and striatal GABA content. *J Neurochem* **37:** 1048–1051, 1981.
25. Olianas, M. C., G. M. DeMontis, G. Mulas and A. Tagliamonte. The striatal dopaminergic function is mediated by the inhibition of a nigral, non-dopaminergic neuronal system via a strio-nigral GABAergic pathway. *Eur J Pharmacol* **49:** 233–241, 1978.
26. Olsen, R. W., M. K. Ticku, P. C. Van Ness and D. Greenlee. Effects of drugs on γ-aminobutyric acid receptors, uptake, release and synthesis in vitro. *Brain Res* **139:** 277–294, 1978.
27. Perez de la Mora, M., K. Fuxe, T. Hokfelt and A. Ljungdahl. Effect of apomorphine on the GABA turnover in the DA cell group rich area of the mesencephalon. Evidence for the involvement of an inhibitory GABAergic feedback control of the ascending DA neurons. *Neurosci Lett* **1:** 109–114, 1975.
28. Scheel-Kruger, J., A. V. Christensen and J. Arnt. Muscimol differentially facilitates stereotypy but antagonizes motility induced by dopaminergic drugs: a complex GABA-dopamine interaction. *Life Sci* **22:** 75–84, 1978.
29. Seeman, P. Brain dopamine receptors. *Pharmacol Rev* **32:** 229–313, 1980.
30. Ticku, M. K., A. Huang and J. L. Barker. Characterization of GABA receptor binding in primary brain cultured cells. *Mol Pharmacol* **17:** 285–289, 1980.
31. Ticku, M. K. and R. D. Huffman. The effects of acute and chronic morphine administration on GABA receptor binding. *Eur J Pharmacol* **68:** 97–106, 1980.
32. Trabucchi, M., S. Govoni, G. C. Tonon and P. F. Spano. Functional interaction between receptors for dopamine antagonists and GABA central receptors. *Life Sci* **23:** 1751–1756, 1978.
33. Waddington, J. L. and A. J. Cross. Denervation supersensitivity in the strionigral GABA pathway. *Nature* **276:** 618–620, 1978.