

INCREASED SENSITIVITY OF ADENYLATE CYCLASE ACTIVITY IN THE STRIATUM OF THE RAT TO CALMODULIN AND GppNHp AFTER CHRONIC TREATMENT WITH HALOPERIDOL*

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Summary—Chronic treatment of rats with haloperidol causes behavioral supersensitivity to dopaminergic agonists and an increase in the sensitivity of adenylate cyclase activity in the striatum to stimulation by dopamine. In this study the authors examined whether chronic treatment with haloperidol could elicit a change in sensitivity of adenylate cyclase in the striatum of the rat for guanyl nucleotides and the endogenous Ca^{2+} -binding protein, calmodulin. These agents increase the activation of adenylate cyclase activity by dopamine but act beyond the level of the dopamine receptor. Male, Sprague-Dawley rats were injected subcutaneously with either 0.6 mg/kg haloperidol or vehicle for 14 days. Four days after the last injection, the animals were sacrificed and the activity of adenylate cyclase was measured in a EGTA-washed particulate preparation of the striatum. There was an increase in the activation of adenylate cyclase activity by calmodulin and GppNHp but not by guanosine triphosphate (GTP) in particulate fractions of the striatum from rats treated with haloperidol as compared to controls. The sensitivity of adenylate cyclase to calmodulin was increased 5-fold in particulate fractions from rats treated with haloperidol as opposed to vehicle-treated rats. The lack of change in activation by GTP was not due to an altered activity of GTPase in rats treated with haloperidol. In animals treated for 14 days but not withdrawn from haloperidol there was no statistically significant increase in the sensitivity of adenylate cyclase to calmodulin. There was no change in activation of the enzyme by GppNHp or GTP as compared to control. The activation of adenylate cyclase by calmodulin was not affected when haloperidol was added *in vitro* to the assay or after the acute injection of rats with haloperidol.

These results demonstrate that the development of dopaminergic supersensitivity after withdrawal from chronic blockade of dopamine receptors resulted in increased sensitivity of post-receptor coupling mechanisms, as well as in changes in the receptor-mediated activity. In previous studies, increases in DA-stimulated adenylate cyclase activity were found with this treatment regimen. In this study it was shown that although basal activity was not changed, there was a selective increase in the sensitivity of adenylate cyclase to stimulation by calmodulin and GppNHp after chronic receptor blockade. These findings support previous observations suggesting a regulatory role for calmodulin in dopamine-stimulated adenylate cyclase activity.

Key words: supersensitivity, adenylate cyclase, dopamine, calmodulin, haloperidol.

The purpose of this study was to investigate further the role of calmodulin (CaM) in the regulation of dopamine (DA)-stimulated adenylate cyclase activity. Adenylate cyclase in the brain is activated by calmodulin, a low-molecular weight calcium-binding protein (Brostrom, Brostrom and Wolff, 1978; Cheung Bradham, Lynch, Lin and Tallant, 1975). Several lines of evidence suggest a role for calmodulin in modulating dopaminergic activity (Gnegy, Lau and Treisman, 1980). Striatal membranes from rats exhibiting behavioral supersensitivity to DA after

withdrawal from chronic treatment with haloperidol had an increased content of calmodulin and increased activity of DA-sensitive adenylate cyclase (Gnegy, Lucchelli and Costa, 1977a; Gnegy, Uzunov and Costa, 1977b). Hanbauer, Pradham and Yang (1980) reported that membranes prepared from slices of striatum, treated with amphetamine contained decreased activity of DA-sensitive adenylate cyclase and decreased content of calmodulin as compared to controls. Calmodulin activates basal activity of adenylate cyclase in striatal membranes from the rat and increases the sensitivity of the enzyme for DA 3-4-fold (Gnegy and Treisman, 1981). Several investigators have found two components of the activity of adenylate cyclase in the brain of the rat, including the striatum, one of which is sensitive to stimulation by calmodulin and one of which is insensitive to calmodulin (Brostrom, Brostrom and Wolff,

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1977; Heideman, Wierman and Storm, 1982; Treisman, Bagley and Gnegy, 1983). Previous studies suggested that calmodulin-sensitive adenylate cyclase activity in the striatum of the rat was activated by guanyl nucleotides, preferentially by small concentrations of the non-hydrolyzable analog, guanosine triphosphate (GTP), guanosine-5'-(β,γ -imido)-triphosphate (GppNHp; Treisman *et al.*, 1983).

Chronic treatment of rats with a DA antagonist such as haloperidol has been used as a model for Parkinson's disease (Hornykiewicz, 1973, 1975). After withdrawal from chronic treatment with haloperidol, rats exhibit an increased behavioral sensitivity to DA agonists (Christensen, Fjalland and Møller Nielsen, 1976; Klawans and Rubovits, 1972; Sayers, Bürki, Ruch and Asper, 1975; Tarsy and Baldessarini, 1974). In striatal membranes from rats treated chronically with haloperidol, increases in the binding of [3 H]haloperidol, a measure of binding to DA D-2 receptors (Burt, Creese and Snyder, 1977) and the activity of DA-stimulated adenylate cyclase, a measure of the activity of DA D-1 receptors (Marshall and Mishra, 1980; Gnegy *et al.*, 1977a, b; Iwatsubo and Clouet, 1975) have been reported. These changes reflect a change in dopaminergic activity at the level of the receptor.

In this study, the authors wished to determine whether the supersensitivity could be expressed through agents which affect dopaminergic stimulation, yet activate adenylate cyclase at a level beyond the receptor. The effect of chronic treatment with haloperidol on the activation of adenylate cyclase by calmodulin, as well as by GTP and GppNHp, which activate adenylate cyclase by binding to an activating guanyl nucleotide-binding protein termed N_s (Rodbell, 1980; Limbird, 1981) was examined. It was found that the activity of adenylate cyclase had an increased sensitivity to both calmodulin and GppNHp but no change in sensitivity to GTP as compared to vehicle-treated controls. The lack of change in stimulation by GTP was not due to an increase in hydrolysis of GTP elicited by the treatment with haloperidol. These results demonstrate that chronic treatment with haloperidol can increase the sensitivity of adenylate cyclase to factors which affect the coupling of the DA receptor to the catalytic subunit.

METHODS

Drug treatments

Male, Sprague-Dawley rats Strain Crl: CD(SD)BR (200–250 g) were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts, U.S.A. The rats were injected for 14 days with 0.6 mg/kg (s.c.) of haloperidol or 0.1 ml/0.1 kg of a vehicle consisting of 0.9% saline solution containing 5 μ l/ml of ethanol and 5 μ l/ml glacial acetic acid. A similar regimen has been shown to result in increased stereotyped behavior in rats after a challenge with

apomorphine (Gnegy *et al.*, 1977a). In some experiments, rats were withdrawn from the drug for 4 days before sacrifice. Rats not withdrawn from the drug were killed 1 hr after the last injection. Acute treatments consisted of a single injection of the above solutions 1.5 hr prior to decapitation.

Preparation of particulate fraction

Particulate fractions of striatum were prepared as described by Brostrom *et al.* (1978). Briefly, rats were killed by decapitation, the striata were removed and homogenized in 9 vol of 10 mM Tris maleate buffer, pH 7.5 containing 1 mM $MgSO_4$ and 1.2 mM ethylene glycol bis (β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA). The homogenate fraction was centrifuged at 27,000 g for 20 min and the resulting pellet was resuspended in 9 vol of the above buffer and centrifuged a second time. The pellet resulting from the final centrifugation was resuspended in the above buffer to a concentration of approx. 5 mg of protein/ml. Protein content was determined by the method of Lowry, Rosebrough, Farr and Randall (1951).

Assay of adenylate cyclase

The activity of adenylate cyclase was measured in an assay (200 μ l volume) containing: 80 mM Tris maleate buffer, pH 7.5, 5 mM $MgSO_4$, 2 mM cAMP, 4 mM phosphoenolpyruvate, 20 μ g pyruvate kinase, 0.12 mM isobutylmethylxanthine, 100–150 μ g of particulate membrane protein, 0.15 mM EGTA and 1 mM [α - 32 P]ATP (1 μ Ci per assay) with or without additions such as GppNHp, GTP, $CaCl_2$ and calmodulin. Assays were incubated for 10 min and the reaction was stopped by heating for 1 min at 95°C. A solution (200 μ l) containing 20 mM ATP and 0.7 mM cAMP was then added to the tubes. The particulate material was centrifuged and 32 P-labelled cAMP in the supernatant fraction was determined by the method of Krishna, Weiss and Brodie (1968). Recovery of the cAMP was measured using the [3 H]cAMP and was usually 80–90%. Free or effective concentrations of Ca^{2+} were calculated using a dissociation constant for Ca^{2+} EGTA of 4.08×10^{-8} M according to the method of Nanninga and Kempen (1971).

Assay of GTPase

The activity of GTPase was assayed using [γ - 32 P]GTP according to the method of Cassel, Levkovitz and Selinger (1977). Assay contents, modified to be more similar to those of the adenylate cyclase assay in a volume of 100 μ l, were: 12.5 mM Tris HCl buffer, pH 7.5, 5 mM $MgCl_2$, 1 mM ATP, 1 mM adenosine-5'-(β,γ -imido)triphosphate (AppNHp), 1 mM creatine phosphate, 5 U creatine phosphokinase, 1 mM ouabain, 2 mM dithiothreitol, 5 μ g membrane protein, 150 μ M and 50,000 cpm of [γ - 32 P]GTP plus various additions such as nonradioactive GTP, Ca^{2+} and CaM. Incubations were conducted for 10 min at 37°C. Assays were

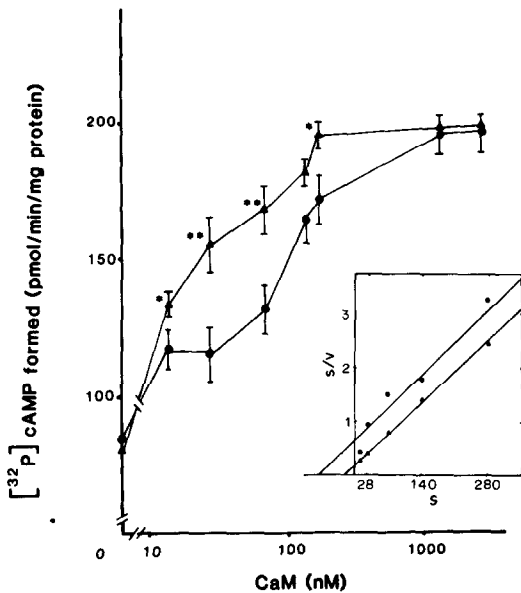


Fig. 1. Activation of the activity of adenylate cyclase by calmodulin (CaM) in particulate fractions from the striatum of rats chronically treated with haloperidol (▲) or vehicle (●) and withdrawn for 4 days. Activity of adenylate cyclase was measured in EGTA-washed striatal particulate fractions as described in Methods. Assays contained $0.12 \mu\text{M}$ free Ca^{2+} ($100 \mu\text{M}$ added CaCl_2). Each point represents the average value from five different animals \pm SEM. * $P \leq 0.05$; ** $P \leq 0.02$. Inset: Results expressed as a Hanes plot with substrate (s) on the abscissa and substrate/velocity (s/v) on the ordinate. Kinetic constants from this form of analysis are shown in Table 1.

terminated and free ^{32}P determined as described by Cassel *et al.* (1977). The activity of the low K_m GTPase can be closely approximated by correcting for the hydrolysis of GTP by a high K_m activity of nucleoside triphosphate (Cassel *et al.*, 1977). In the present experiments, low K_m activity of GTPase was calculated as the difference between the number of counts per minute of ^{32}P released in the absence and presence of $100 \mu\text{M}$ GTP.

Preparation and measurement of calmodulin

Calmodulin was purified from bovine brain using phenylsepharose chromatography and DEAE-cellulose chromatography by a modification of the methods of Dedman, Potter and Means (1977) and of Gopalkrishna and Anderson (1982). The calmodulin demonstrated a single band on disc gel electrophoresis containing 10% polyacrylamide. Calmodulin was prepared in the presence of millimolar concentrations of EGTA, dialyzed against 0.05 M $(\text{NH}_4)_2\text{HCO}_3$, and lyophilized. The calmodulin was redissolved in 10 mM Tris maleate buffer, pH 7.5. The M_r determined by slab gel electrophoresis using standards of known molecular weight was 16,200 daltons. Calmodulin was measured by its ability to activate calmodulin-depleted preparations of bovine brain 3'5'-cyclic-AMP phosphodiesterase

(PDE) using purified calmodulin as a standard (Gnagy, Nathanson and Uzunov, 1977c). Calmodulin-activated PDE was prepared as described by Klee and Krinks (1978).

Statistics

Statistical differences were analyzed using an unpaired Student's *t*-test.

Materials

Haloperidol was a generous gift from McNeil Laboratories, Inc. (Fort Washington, Pennsylvania, U.S.A.); [α - ^{32}P]ATP (sp. act. 10–30 Ci/mmol), [γ - ^{32}P]GTP (sp. act. 20–40 Ci/mmol) and [^3H]cAMP (sp. act. 20–30 Ci/mmol) were purchased from Amersham Searle. Phosphoenolpyruvate, AppNHp, AMP and ATP were obtained from Sigma Chemical Co.; pyruvate kinase was from Boehringer Mannheim. Both GTP and GppNHp (high pressure liquid chromatography purified) were purchased from International Chemical and Nuclear Corp. All other materials and reagents were the highest commercially available grade produced.

RESULTS

Activation of the activity of adenylate cyclase by calmodulin in particulate fractions of the striatum from rats withdrawn from chronic treatment with vehicle or haloperidol

Activation of the activity of adenylate cyclase by various concentrations of calmodulin was measured in particulate fractions of the striatum from rats treated with haloperidol or vehicle. As shown on the ordinate of Fig. 1, basal activity of adenylate cyclase did not significantly differ in the two groups. Calmodulin stimulated formation of cAMP to a greater extent in particulate fractions from animals treated with haloperidol than from controls at concentrations up to 250 nM (Fig. 1). The activation of adenylate cyclase by calmodulin, however, did not differ significantly between the two groups at concentrations greater than $1 \mu\text{M}$ calmodulin. The activity of adenylate cyclase from rats treated with haloperidol, however, had a greater sensitivity to calmodulin than did that from vehicle-treated rats. Kinetic analysis of the data using a Hanes plot (insert Fig. 1) revealed a 4.7-fold increase in sensitivity of adenylate cyclase for calmodulin in membranes from rats treated with haloperidol as compared to vehicle with no change in maximum response. The apparent kinetic constants determined using Hanes analysis for the two groups are given in Table 1. The apparent K_a for calmodulin was significantly decreased almost 5-fold from 76 nM in fractions from vehicle-treated rats to 16 nM in fractions from rats treated with haloperidol. The apparent V_{max} did not change as a result of treatment with drug and was 116 and $115 \text{ pmol/min per mg protein}$ in fractions from vehicle and haloperidol-treated rats, respectively.

Table 1. Apparent kinetic constants for activation of adenylate cyclase by calmodulin (CaM) in rats treated chronically with haloperidol or vehicle

Treatment†	Apparent kinetic constants*			
	Withdrawn		Non-withdrawn	
	K_a^{app}	V_{max}^{app}	K_a^{app}	V_{max}^{app}
Vehicle	76 ± 13	116 ± 15	60 ± 12	131 ± 11
Haloperidol	16 ± 3‡	115 ± 10	33 ± 6	110 ± 10

*Apparent kinetic constants (app) were determined by Hanes analysis of the data presented in Fig. 1 (withdrawn from drugs) and Fig. 3 (non-withdrawn from drugs). The apparent K_a 's are given in nM and apparent V_{max} 's in pmol/min per mg protein. Values are given ± SEM for 5 different animals in each group.

†Rats were injected for 14 days with 0.6 mg/kg of haloperidol (s.c.), or 0.1 ml/0.1 kg of a vehicle consisting of 0.9% vehicle solution containing 5 µl/ml of ethanol and 5 µl/ml glacial acetic acid. Rats were withdrawn from the drug for 4 days before sacrifice. Rats not withdrawn from the drug were killed 1 hr after the last injection.

‡ $P < 0.001$ as compared to value for vehicle-treated animals.

The change in sensitivity to calmodulin was not due to a difference in endogenous content of calmodulin in the particulate fractions of striatum from rats treated with haloperidol or vehicle. The calmodulin content in the EGTA-washed particulate fractions of striatum from rats treated with vehicle and haloperidol was 2.5 ± 0.4 and 2.5 ± 0.3 ng CaM/µg membrane protein, respectively; EGTA reduced the concentration of calmodulin in striatal membranes by over 50% but did not remove all of the endogenous calmodulin.

Activation of adenylate cyclase by GppNHp and GTP in particulate fractions of striatum from rats withdrawn from chronic treatment with vehicle or haloperidol

The stimulation of the activity of adenylate cyclase from the striatum by GTP and the non-hydrolyzable analog of GTP, GppNHp was examined. As shown in Fig. 2, the activity of adenylate cyclase in striatal membranes from rats treated chronically with haloperidol was significantly more sensitive to stimulation by GppNHp than that from controls. Maximal stimulation by the nucleotide occurred at 100 µM GppNHp and was not different in fractions from animals treated with vehicle or haloperidol. The EC_{50} for GppNHp, the effective concentration which provided 50% of the maximal activation, was 0.5 µM in the fractions from rats treated with vehicle and 0.08 µM in fractions from rats treated with haloperidol. On the contrary, the results in Table 2 demonstrate that no differences were found in the response of adenylate cyclase to GTP in striatal fractions from animals treated with vehicle or haloperidol. The response to GTP was biphasic and a maximal response occurred at 1 µM GTP, as shown previously (Treisman *et al.*, 1983).

Activation of adenylate cyclase by calmodulin, GTP and GppNHp in animals treated chronically with haloperidol or vehicle and not withdrawn from the drugs before sacrifice

In order to assess whether the increased response

to calmodulin and GppNHp in membranes from the striatum of rats withdrawn from treatment with haloperidol, as opposed to vehicle, was due to a compensatory response to continued receptor blockade, the activation of adenylate cyclase by calmodulin and by guanyl nucleotides in striata from rats not withdrawn from the drugs was examined. Male, Sprague-Dawley rats were treated for 14 days with haloperidol or vehicle as described in Methods but were sacrificed 1 hr after the last injection. As shown in Fig. 3, the activity of adenylate cyclase in particulate fractions from the striatum of animals chronically treated with haloperidol was not significantly

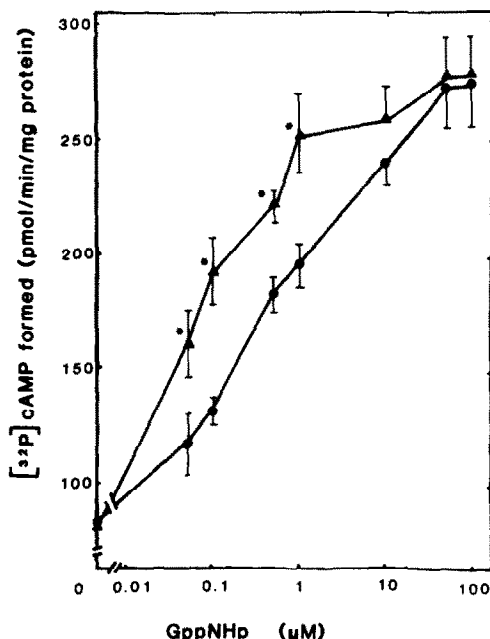


Fig. 2. Activation of the activity of adenylate cyclase by GppNHp in particulate fractions from the striatum of rats chronically treated with haloperidol (▲) or vehicle (●) and withdrawn for 4 days. The activity of adenylate cyclase was measured in EGTA-washed striatal particulate fractions as described in Methods. Each point represents the average value from five different animals ± SEM. * $P \leq 0.05$.

Table 2. Activation of adenylate cyclase by GTP in particulate fractions from the striatum of animals chronically treated with haloperidol or vehicle and withdrawn for 4 days

GTP concentration (μM)	Adenylate cyclase activity	
	Vehicle (pmol/min per mg protein)	Haloperidol* (pmol/min per mg protein)
None	83 \pm 3	75 \pm 3
0.1	123 \pm 7	116 \pm 8
1.0	179 \pm 9	176 \pm 10
10.0	156 \pm 9	158 \pm 9
100.0	158 \pm 23	151 \pm 14

*Rats were treated with haloperidol or vehicle as described in Table 1. Results are given \pm SEM for $N = 5$.

different from controls. The overall activity of adenylate cyclase was slightly but not significantly decreased in the particulate fractions from rats treated, but not withdrawn from, haloperidol as compared to controls. Although the absolute response values were slightly decreased with calmodulin, there was a 2-fold increase in sensitivity to calmodulin in the particulate fractions from rats treated with haloperidol as compared to controls. Apparent kinetic constants for the activation of adenylate cyclase by calmodulin in the two groups are given in Table 1. The change in sensitivity for calmodulin in the non-withdrawn animals was not statistically significant nor as great as for those

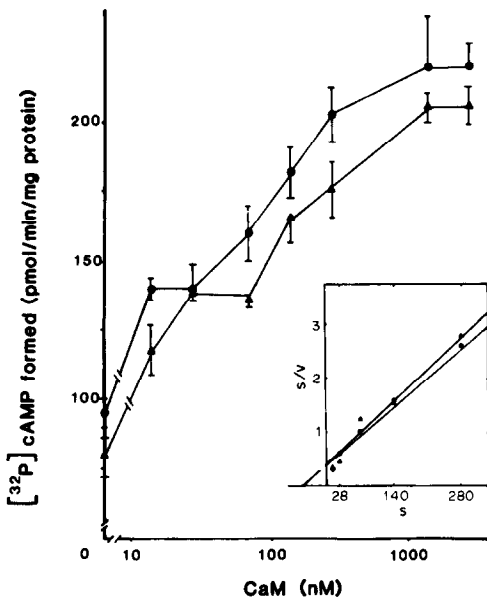


Fig. 3. Activation of the activity of adenylate cyclase by calmodulin (CaM) in animals treated chronically with haloperidol (▲) or vehicle (●) and sacrificed 1 hr after the last injection. The activity of adenylate cyclase was measured in EGTA-washed particulate fractions as described in Methods. Assays contained $0.12 \mu\text{M}$ free Ca^{2+} ($100 \mu\text{M}$ added CaCl_2). Each point represents the average value from five different animals \pm SEM. Inset: Results expressed as a Hanes plot with substrate (s) on the abscissa and substrate/velocity (s/v) on the ordinate. Kinetic constants from this form of analysis are shown in Table 1.

Table 3. Activation of adenylate cyclase by guanyl nucleotides and DA in striata from rats chronically treated with vehicle or haloperidol and sacrificed 1 hr later

Addition to assay	Concentration (μM)	Adenylate cyclase activity	
		Vehicle (pmol/min per mg protein)†	Haloperidol* (pmol/min per mg protein)†
None		95 \pm 5	91 \pm 8
GppNHp	0.1	128 \pm 12	131 \pm 12
	1.0	189 \pm 12	196 \pm 12
	10.0	267 \pm 6	272 \pm 16
	100.0	311 \pm 11	309 \pm 12
GTP	0.1	121 \pm 9	131 \pm 12
	1.0	215 \pm 9	207 \pm 13
DA‡	1	27 \pm 2	7 \pm 1§
	50	90 \pm 9	108 \pm 9

*Rats were treated for 14 days with vehicle or haloperidol as described in Methods and Table 1 and killed 1 hr after the last injection. Adenylate cyclase activity was measured in EGTA-washed particulate fractions as described in Methods.

†Results are given \pm SEM for $N = 5$.

‡Assays containing DA also contained $1 \mu\text{M}$ GTP since GTP is required to detect activation by DA in these washed fractions. Results are given as the pmol of cAMP/min per mg protein produced by DA above that produced by $1 \mu\text{M}$ GTP.

§ $P \leq 0.05$ as compared to the value for vehicle-treated rats.

withdrawn from haloperidol. These results suggest that, in the same manner as the change in sensitivity to DA, the increased activation by calmodulin became more apparent after withdrawal of the drug.

Table 3 shows the data for activation of adenylate cyclase from the striatum by GTP, GppNHp and DA in fractions from the striatum of rats chronically treated with vehicle or haloperidol, but not withdrawn from the drugs before sacrifice. No significant differences in activation by GTP or GppNHp were found between the two groups. Activation of adenylate cyclase by a submaximal concentration of DA was decreased in striatal particulate fractions from rats treated with haloperidol as compared to controls, suggesting that haloperidol was still present in the membranes and blocking activation by DA.

Effect of haloperidol, administered acutely, on the activation of adenylate cyclase from the striatum of the rat by calmodulin

The effect of an acute treatment with haloperidol on the activity of adenylate cyclase and the activation by calmodulin was examined. The authors wished to determine further whether treatment with haloperidol directly affected the activation of adenylate cyclase by calmodulin. Rats were treated with either 0.6 mg/kg haloperidol subcutaneously or an equal volume of vehicle and sacrificed 1.5 hr later. The activation of adenylate cyclase by calmodulin and DA was determined in particulate fractions from the striatum of rats treated with haloperidol or vehicle. As shown in Table 4, there was no significant difference in the activity of adenylate cyclase between fractions from rats treated with haloperidol or vehicle. Similarly, the responsiveness to calmodulin in terms of percentage stimulation of basal activity was not significantly different between the two groups. The haloperidol present in the striatum after injection did block DA

Table 4. Activation of adenylate cyclase by calmodulin (CaM) and DA in striata from rats treated acutely with haloperidol or vehicle

Addition to assay	Concentration	Adenylate cyclase activity	
		Vehicle (pmol/min per mg protein)†	Haloperidol* (pmol/min per mg protein)†
None		117 ± 9	114 ± 13
CaM	28 nM	158 ± 14	135 ± 16
	140 nM	194 ± 19	167 ± 14
	280 nM	215 ± 23	192 ± 32
	1400 nM	226 ± 30	230 ± 20
DA‡	1 µM	47 ± 7	8 ± 8§
	50 µM	93 ± 8	118 ± 11

*Male Sprague-Dawley rats were injected with either 0.6 mg/kg (s.c.) haloperidol or an equal volume of vehicle and sacrificed 1.5 h later. The activity of adenylate cyclase was measured in EGTA-washed striatal particulate fractions as described in Methods. Assays containing calmodulin (CaM) also contained 100 µM CaCl₂ (0.12 µM free Ca²⁺).

†Results are given ± standard error of the mean, N = 3.

‡Dopamine (DA)-stimulated activity was determined as described in Table 1. Adenylate cyclase activity in the presence of 1 µM GTP was 285 ± 30 and 220 ± 9 pmol/min per mg protein in particulate fractions from the striatum from vehicle and haloperidol-treated rats, respectively.

§P < 0.01 as compared to the value for vehicle-treated rats.

receptors as shown by the decrease in activation of adenylate cyclase by 1 µM DA in striata from rats treated with haloperidol as compared to controls (Table 4).

Haloperidol added to particulate fractions of striatum *in vitro* did not decrease the basal activity of adenylate cyclase until concentrations of 10 µM were attained (data not shown). Similarly, haloperidol *in vitro* did not inhibit activation by calmodulin until concentrations of 10 µM were reached, even when haloperidol was preincubated with the membranes for 5–10 min at 37°C.

The activity of GTPase in particulate fractions from the striatum of rats treated chronically with haloperidol or vehicle

The results have shown that adenylate cyclase in particulate fractions from the striatum of rats withdrawn from chronic treatment with haloperidol had an increased sensitivity to GppNHp but not to GTP. An increase in the activity of GTPase in striatal particulate fractions from rats treated with haloperidol could negate possible increases in activation of GTP. The hydrolysis of 10⁻⁷ M GTP was measured in particulate fractions from the striatum of animals treated with drug and control animals. This concentration was near the apparent K_a for the low K_m for GTPase related to adenylate cyclase in membranes (Cassel and Selinger, 1976; Koski and Klee, 1981). No change was found in activity of GTPase in particulate fractions from the striatum of rats treated with vehicle or haloperidol withdrawn from treatment 4 days, being 24.2 ± 1.5 and 23.7 ± 1.5 pmol/min per mg protein, respectively. Similarly, there was no significant change in hydrolysis of 10⁻⁷ M GTP in particulate fractions from the striatum of rats not withdrawn from vehicle and halo-

peridol, being 24.3 ± 1.7 and 21.7 ± 1.0 pmol/min per mg protein, respectively.

Activation of adenylate cyclase by dopamine in particulate fractions from the striatum of rats withdrawn from chronic treatment with vehicle or haloperidol

It has previously been demonstrated that activity of adenylate cyclase in particulate fractions from the striatum of rats chronically treated with haloperidol was 3–4-fold more sensitive to DA than that from controls (Gnegy *et al.*, 1977b). To confirm that the animals used in the present studies were supersensitive to DA, the activation of adenylate cyclase by 1 and 50 µM DA was examined in the striatum from drug-treated and control animals. The apparent K_a of adenylate cyclase for DA in this particulate preparations was approx. 1 µM. Fifty micromoles of DA represents a maximal activating concentration (Gnegy and Treisman, 1981). The activation of adenylate cyclase by 1 µM DA was significantly greater in particulate fractions from rats treated with haloperidol as compared to controls, being 40 ± 6 and 24 ± 3 pmol/min per mg protein, respectively (N = 5, P < 0.05 for haloperidol-treated as compared to vehicle-treated fractions). The amount of cAMP produced by 50 µM DA was the same in fractions from rats treated with vehicle and haloperidol, 93 ± 7 and 88 ± 11 pmol/min per mg protein, respectively.

DISCUSSION

Rats withdrawn from chronic treatment with neuroleptic drugs, such as haloperidol, exhibit behavioral supersensitivity to DA agonists. Increased activity of DA receptors has been demonstrated neurochemically in the striatum of the rat by an increase in the binding of [³H]haloperidol (Burt *et al.*, 1977) as well as an increase in the sensitivity of adenylate cyclase to DA (Gnegy *et al.*, 1977a, b). The results of this study demonstrate that adenylate cyclase in the striatum developed a heightened sensitivity for agents acting beyond the receptor as a result of chronic treatment with antipsychotic drugs. Previous studies demonstrated that there was a 3–4-fold decrease in the apparent K_a for activation by DA of adenylate cyclase in membranes from the striatum of rats treated chronically and withdrawn from haloperidol as compared to vehicle-treated controls (Gnegy *et al.*, 1977a). A 5-fold increase in the sensitivity of adenylate cyclase in the striatum for calmodulin and a 6-fold increase in sensitivity for GppNHp in membranes from the striatum of rats chronically treated with haloperidol has now been demonstrated. This represents a large kinetic change as a result of chronic treatment with neuroleptics and would result in a large increase in the activation of adenylate cyclase by these agents *in vivo*.

Calmodulin has been shown to increase the sensitivity of adenylate cyclase in the striatum for DA and thus to play a role in regulating sensitivity to DA

(Gnegy and Treisman, 1981). The exact mechanism by which calmodulin activates adenylylase is not known. Studies have suggested that it acts directly at the catalytic subunit (Salter, Krinks, Klee and Neer, 1981) and does not require GTP for its activity (Heideman *et al.*, 1982; Seamon and Daly, 1982). Calmodulin has been shown to increase the activation of adenylylase by DA in the striatum (Gnegy and Treisman, 1981) and retina (Gnegy, Muirhead and Harrison, 1984) and β -adrenergic receptor activation of adenylylase in the cerebellum (Malnoë, Stein, and Cox, 1983). Calmodulin can increase the sensitivity for guanyl nucleotides of the calmodulin-sensitive adenylylase in the cerebral cortex (Brostrom *et al.*, 1978). It is possible that calmodulin could act at the catalytic subunit in such a manner as to increase the association between the guanyl nucleotide binding protein and the catalytic subunit.

Activation of adenylylase by hormones is mediated by a GTP-binding protein termed N_s , consisting of α , β and γ subunits. Guanyl nucleotides bind to the α subunit of N_s , which is a 45,000 dalton protein that mediates the activation of adenylylase (Manning and Gilman, 1983). An increase in sensitivity of adenylylase to GppNHp suggests that there could be a change in affinity of N_{sz} for guanyl nucleotides or a higher affinity for coupling of N_{sz} with the catalytic subunit as a consequence of supersensitivity. A decrease in coupling of receptor-adenylylase systems through the guanyl nucleotide binding protein has been reported to result from ligand-mediated desensitization in cell lines (Harden, Su and Perkins, 1979). Receptor-coupled inhibition of the activity of adenylylase is mediated by another GTP-binding protein, N_i , that also consists of α , β and γ subunits (Murayama and Ui, 1983; Hildebrandt, Sekura, Codina, Iyengar, Manclark and Birnbaumer, 1983). Activation of DA D-2 receptors has been shown to be mediated through N_i and results in attenuation of adenylylase activity in the pituitary gland (Cote, Grewe and Kebejian, 1981) and striatum (Stoof and Kebejian, 1981). Although the present studies have been directed toward investigating the activation of adenylylase by DA (D-1 receptor activity) through N_s , the increased GppNHp activation could represent a decrease in N_i activity.

However, chronic treatment with haloperidol did not alter the response of adenylylase in the striatum to GTP. The lack of change in stimulation by GTP could not be attributed to a significant alteration in GTPase activity in the membranes from the striatum of rats treated with haloperidol. Previous work suggests that GTP, in the absence of DA, is activating a separate component of adenylylase from that of small concentrations of GppNHp (Treisman *et al.*, 1983). A kinetic analysis of the data demonstrated two components of the activity of adenylylase in the striatum of the rat. One

component was activated by calmodulin and concentrations of GppNHp below $1 \mu\text{M}$. The other component was activated by GTP and larger concentrations of GppNHp. The results attained in this study suggest that chronic treatment with haloperidol induces an alteration in the calmodulin-sensitive component of adenylylase which may be independent of the GTP-activated component. It has similarly been demonstrated that when the sensitivity of adenylylase to DA in retina is modified by light, then the sensitivity of the enzyme to calmodulin and GppNHp, but not to GTP, is altered (Gnegy *et al.*, 1984).

The increase in sensitivity to calmodulin and GppNHp is most likely to be a compensatory supersensitivity resulting from chronic receptor blockade. There was no change in stimulation by GppNHp or GTP and no significant change in activation by calmodulin in particulate fractions from the striatum of rats treated with haloperidol and not withdrawn from the drug, as compared to control. The increase in sensitivity to calmodulin could develop during chronic treatment with drugs but is most apparent when the drug is withdrawn. The reason that the sensitivity to GppNHp and calmodulin did not change prior to withdrawal is not clear. It is possible that the interaction between the dopamine receptor and N_s is altered during chronic blockade, and it is only after withdrawal that the increased sensitivity of adenylylase to actions mediated by N_s or calmodulin are seen. The changes in sensitivity to calmodulin parallel the response of adenylylase to DA. Results from the present studies, using acute treatment with haloperidol or *in vitro* addition to striatal membranes demonstrated that haloperidol did not directly inhibit activation of adenylylase by calmodulin. Antipsychotic drugs, such as trifluoperazine, have been shown to bind to calmodulin and prevent it from activating target enzymes (Levin and Weiss, 1977). Haloperidol is much weaker in its binding to calmodulin than trifluoperazine and might not be expected to inhibit the activity of calmodulin by directly inhibiting activation of adenylylase at smaller concentrations (Weiss, Prozialeck, Cimino, Barnette and Wallace, 1980). The present results suggest that the increased responsiveness to calmodulin after withdrawal from chronic treatment with haloperidol is a consequence of chronic blockade of DA receptors as opposed to a release from continued direct inhibition of calmodulin.

In conclusion, the sensitivity of adenylylase from the striatum of the rat to stimulation by calmodulin and GppNHp was increased after withdrawal from chronic treatment with haloperidol suggesting an increase in the coupling of DA receptors to adenylylase. The supersensitivity, developed to these agents, may be a compensatory response to continued receptor blockade since the increased response was not detected in rats treated acutely with

the drug. The increase in sensitivity to calmodulin and GppNHp correlated with an increase in sensitivity of adenylate cyclase to DA and appears to reflect an increase in the calmodulin-sensitive component of adenylate cyclase. These results demonstrate that neurochemical changes occurring as a result of chronic blockade of DA receptors can develop at a level beyond that of the receptor itself and can be reflected in the coupling mechanism of the receptor as well as at the receptor itself.

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