Pharmacology Biochemistry & Behavior, Vol. 40, pp. 881–886. Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Long-Lasting Dopamine Receptor Up-Regulation in Amphetamine-Treated Rats Following Amphetamine Neurotoxicity

JEREMY Z. FIELDS, LAWRENCE WICHLINSKI, GEORGE E. DRUCKER, KEVIN ENGH AND JOHN H. GORDON

Research Service (151), Hines V. A. Hospital, Hines, IL 60141
and Department of Pharmacology, Loyola University Stritch School of Medicine
2160 S. First Ave., Maywood, IL 60153

Received 4 June 1991

FIELDS, J. Z., L. WICHLINSKI, G. DRUCKER, K. ENGH AND J. H. GORDON. *Long-lasting dopamine receptor up-regulation in amphetamine-treated rats following amphetamine neurotoxicity.* PHARMACOL BIOCHEM BEHAV **40**(4) 881–886, 1991.— Amphetamine (A) (9.2 mg/kg, IP), in combination with iprindole (I) (10.0 mg/kg, IP), caused long-lasting dopamine (DA) depletions in striatum (−49%, 4 weeks) but not in nucleus accumbens following one A/I injection. Striatal DA had recovered by 4 months. DA receptors (DAr) were up-regulated: 1) behavioral responses to a DA receptor agonist (apomorphine) were significantly elevated. These included apomorphine-induced locomotor activity (+103% and +160%, on weeks 3 and 10) and apomorphine-induced stereotypy (day 10). 2) B_{max} for [^{3}H]spiroperidol binding to striatal D_2 DAr (12 weeks) increased (+53%, week 12). Injection of the DAr neuromodulator cyclo(leucyl-glycyl) (8 mg/kg/day × 4 days, SC) reversed the B_{max} increase. Thus toxicity (DA depletion) following high-dose amphetamine appears to induce compensatory changes in DAr. This DAr upregulation may explain the lack of abnormal movements despite enduring DA depletion. Additionally, the A/I paradigm as an animal model of long-lasting DAr up-regulation, could be used to screen neuromodulatory agents, like CLG, that might treat disorders (e.g., tardive dyskinesia and schizophrenia) thought to involve up-regulated DAr.

Amphetamine Iprindole Dopamine receptor supersensitivity Locomotor activity Cyclo(leucyl-glycyl)

IN most currently available rodent models of dopamine (DA) receptor supersensitivity, DA receptor (DAr) up-regulation is only transient. Such models include chronic treatment with haloperidol (2) and morphine (16). In contrast, humans suffering from disorders thought to involve DAr supersensitivity (e.g., tardive dyskinesia and schizophrenia) often show symptoms that are, for all intents and purposes, permanent (19). Thus there is a need for animal models wherein the DAr up-regulation is very long-lasting or permanent.

We recently developed a permanent model of DAr supersensitivity, the ovariectomized (OVX) rat (11). In this model the supersensitivity develops slowly, appearing by 3 months postovariectomy, and the effect persists for at least 16 months. Another available rodent model of permanent DAr supersensitivity is the 6-OHDA lesion model (15). Both of these models, however, suffer from the drawback that surgery is required to produce the desired effect and, in the 6-OHDA model, presynaptic neurons are destroyed. Thus there is a need to develop less traumatic, more rapidly developed, and less costly models that can achieve the same result—permanent DAr up-regulation. Moreover, the ideal model should be applicable to both males and females, and be one in which interactions of presynaptic DA terminals with up-regulated DAr can be investigated.

Accordingly, in the present study, we developed a new model of permanent DAr up-regulation, namely, acute treatment of rats with a single high dose of amphetamine in combination with iprindole. Iprindole prevents the para-hydroxylation of amphetamine, thus prolonging its half-life (8).

Early work using the A/I paradigm was an extension of a series of reports showing that one or repeated injections of high doses of methamphetamine (M-AMPH) depleted both dopamine and serotonin in several brain areas [see (21)]. Fuller and Hemrick-Luecke showed that a single injection of the A/I combination produced a similar decrease (−30%) in brain DA levels measured in the cerebral hemispheres 1 week later (9,10). A comparable decrease in brain DA levels was observed in rat striatum as early as 12 hours later, while DOPAC and HVA levels were also reduced 1 week after treatment (22).

Since methamphetamine produces a persistent decrease in brain DA, appearing to last at least months in rodents and years in primates, and yet spontaneous behavior is normal, we reasoned that some sort of compensation had occurred—either DA release had increased or DAr were up-regulated. The goals of this investigation were 1) to test the second possibility—a persistent long-term DAr up-regulation, using both behavioral (locomotion, stereotypy) and biochemical (receptor binding) measures, and 2) to test biochemically (DA levels) whether A/I toxicity is long-lasting.

METHOD

Subjects used were male adult Sprague-Dawley (Harlan) rats at 90 days of age. Rats were kept on a 12:12 light:dark cycle

ABBREVIATIONS

A/I	amphetamine/iprindole
AMPH	amphetamine
APO	apomorphine
B_{max}	maximum receptor density
CLG	cyclo(leu-gly)
DA	dopamine
DAr	dopamine receptors
DHBA	dihydroxybenzoic acid
DOPAC	dihydroxyphenylacetic acid
EC	electrochemical detection
IPRIN	iprindole
M-AMPH	methamphetamine

and given food and water ad lib. Animals were group housed (2–3 per cage) prior to experimental treatment. Following drug administration, animals were housed singly in hanging wire cages. This was essential as the A/I treatment raises the animals' temperature and in a group situation this can result in death (24). Mortality was minimal (<10%) when ambient temperatures were kept below 23°C.

Drug Treatment Protocol

In these experiments the rats received a single "cocktail" injection of A/I composed of iprindole HCl (10.0 mg/kg, IP, Wyeth-Ayerst, Philadelphia, PA) and d-amphetamine sulfate (9.2 mg/kg, IP, Sigma, St. Louis, MO). Controls received a saline or iprindole injection.

Apomorphine-Induced Stereotypy

Ten days after A/I injection, rats were tested for stereotypic sniffing induced by the DA agonist, apomorphine HCl (APO). Animals were placed in wire cages measuring 50 × 20 × 17.5 cm whose mesh measured 2.5 × 2.5 cm. Rats were injected with APO (0.45 mg/kg, IP) or saline, and, starting 10 min later, were monitored for the presence or absence of stereotypic sniffing. Apomorphine is a potent dopamine receptor agonist, and thus provides a tool for assessing the status of DAr function. Rats were observed one at a time for a maximum of 10 seconds. The observer, blind to the animal's treatment condition, recorded whether or not the animal engaged in stereotypic sniffing, and then proceeded to examine the rat in the next wire compartment. After all rats had been scored once, the observer returned to the first rat and commenced scoring again. The procedure continued until a total of ten observations had been made on each rat. All testing took place in a darkened room, with only a red light overhead for illumination. Data were analyzed with chi-square tests.

Apomorphine-Induced Locomotion

Animals were tested for APO-induced locomotor activity changes at 3 and 10 weeks after A/I treatment. The dose of apomorphine HCl (Sigma, St. Louis, MO) administered was 0.3 mg/kg, IP. Rats were placed in Plexiglas cages with an automated activity monitor (Digiscan, Omnitech, Columbus, OH) and activity was electronically recorded. In all cases animals were tested for locomotor effects for 20 min after APO. Scoring was divided into 2-min blocks. The dependent measure was the average horizontal activity score for the five 2-min blocks beginning 10 min after APO administration (i.e., the 10–20-min block after injection). In other words, statistical analysis was done for the group means at 10–12 min, 12–14, etc., following APO injection. Thus, in the table, the mean actually constitutes the mean of the means of each of the five 2-min periods. This procedure allows for a reduction in the error variance. A *t*-test was used to analyze the data for significance.

Apomorphine-Induced Locomotion After Treatment With Cyclo(Leucyl-Glycyl) (CLG)

At 12 weeks after A/I another locomotor activity test was done in which some of the A/I animals received injections of the peptide cyclo(leucyl-glycyl) (CLG) (8 mg/kg in distilled H_2O, SC for the previous 4 days, Bachem, Torrance, CA), a neuromodulator known to down-regulate DAr (1, 2, 5, 16) or distilled H_2O prior to APO. The last dose of CLG was given on the morning of testing. All other procedures were as above. All animals in this study were sacrificed for DA receptor binding at 12 weeks (see below).

Apomorphine-Induced Locomotion and Dopamine Levels

APO-induced locomotion tests were conducted as above. Separate groups of animals were tested for APO-induced locomotor activity at 3 or 10 weeks. The group tested at 3 weeks was sacrificed 4 weeks after the initial A/I treatment, and was then examined for brain DA-DOPAC content using high-performance liquid chromatography (HPLC) with electrochemical detection (EC). The group tested at 10 weeks was sacrificed at 16 weeks and examined for brain DA-DOPAC content by HPLC-EC.

Following sacrifice, brains were quickly removed and frozen in beakers containing methanol surrounded by acetone and solid CO_2. The striata and nucleus accumbens were excised and stored at −70°C. For tissue preparation for HPLC runs, samples were thawed, weighed, and homogenized in 10× volume with 0.3 M perchloric acid [containing 2.5 ng/ml dihydroxybenzoic acid (DHBA)]. The homogenate was centrifuged at 18,000 rpm for 5 minutes and then filtered using microfilterfuge tubes (0.2 micron Nylon-66 membrane filters) for an additional 5 minutes. Samples required 16 minutes retention time at a flow rate of 1 ml/min using a Waters 501 HPLC Pump. The mobile phase consisted of 0.1 M citric acid, 0.2 M dibasic sodium phosphate, 70 mg/l 1-octanesulfonic acid (sodium salt), and 10% acetonitrile adjusted to a pH of 4.0. Aliquots (10 μl) of each sample were injected onto a 8 mm × 10 cm Resolve C18 5 μ Radial-Pak Cartridge with a Waters 712 WISP. DA and DOPAC were reduced utilizing a series of detectors (ESA Coulochem Model 5100A) designed to provide voltage windows to exclude unwanted compounds, thus eliminating the need for an extraction procedure. Samples were oxidized (+0.40 V) at an initial guard cell, partially reduced at detector 1 (−0.06 V), and then fully reduced by detector 2 (−0.40 V). The peak heights of interest, DA (retention time 5.84 minutes) and DOPAC (retention time 8.86 minutes), were compared to the internal standard DHBA (retention time 4.67 minutes). The DA and DOPAC concentrations in ng/mg tissue were recorded from detector 2 and calculated by a PE Nelson OMEGA Analytical Workstation run on an Epson Equity 1+ computer. DA and DOPAC concentrations were analyzed using a one-way ANOVA with post hoc Scheffe's tests.

D_2 DA Receptor Binding

For the receptor binding experiments, the procedure was as follows: Animals were sacrificed 24 hours after the last behav-

TABLE 1

EFFECTS OF AMPHETAMINE/IPRINDOLE ON STRIATAL DA MEASURES

	Control	AMPH/IPRIN	% Change
Dopamine (ng/mg ± SEM)			
4 Weeks	14.1 ± 1.0 (n=8)	7.2 ± 1.1* (n=6)	−49
16 Weeks	17.7 ± 1.4 (n=9)	15.3 ± 1.6 (n=9)	−14
DOPAC (ng/mg ± SEM)			
4 Weeks	1.3 ± 0.2 (n=8)	0.8 ± 0.2† (n=6)	−38
16 Weeks	1.3 ± 0.2 (n=9)	1.0 ± 0.1 (n=9)	−23

*$p<0.001$ vs. control. †$p<0.05$ vs. control. Measurements were obtained by HPLC with ECD.

TABLE 2

EFFECTS OF AMPHETAMINE/IPRINDOLE ON NUCLEUS ACCUMBENS DA MEASURES

	Control	AMPH/IPRIN	% Change
Dopamine (ng/mg ± SEM)			
4 Weeks	6.6 ± 0.8 (n=6)	6.2 ± 0.5 (n=5)	−6
16 weeks	6.1 ± 0.6 (n=9)	5.2 ± 0.6 (n=9)	−15
DOPAC (ng/mg ± SEM)			
4 Weeks	0.7 ± 0.1 (n=6)	0.8 ± 0.1 (n=5)	+ 14
16 Weeks	0.6 ± 0.1 (n=9)	0.6 ± 0.1 (n=9)	0

Measurements were obtained by HPLC with ECD. No significant differences were found.

ioral test was completed (12 weeks after drug treatment). Brains were rapidly removed and placed on solid CO_2. Frozen brains were wrapped in aluminum foil and stored frozen (−70°C) until dissection (12) and assay. The corpus striatum was removed and used for all DA receptor binding. Tissue samples were homogenized in 100 volumes of phosphate buffer (100 mM, pH 7.4) using a Brinkmann polytron. The homogenate was centrifuged at 3000 × g for 10 min and the supernatant removed. The supernatant was then spun again for 10 min at 18,000 × g for 10 min. This time the supernatant was discarded and the pellet preserved. The pellet was resuspended in 100 volumes of buffer and centrifuged once more at 18,000 × g for 10 min. The final pellet was resuspended in 100 volumes (corresponding to original tissue weight) of phosphate buffer and 200 µl was added to each tube of the binding assay.

The total number of receptors (B_{max}) and affinity ($1/K_d$) (for antagonists) were evaluated from saturation isotherms for D_2 DA receptor binding using the radioligand [^{3}H]spiroperidol, as described previously (11), and the cold ligand, (10^{-5} M) sulpiride, to further define specific binding. After appropriate amounts of drugs were added to each tube, all tubes were incubated for 45 min at 37°C in a final volume of 3.0 ml of phosphate buffer, pH 7.4. A total of 12 concentrations of hot ligand (range 5–500 pM) were used to bracket the predicted K_d value (50 pM).

The interaction of DA agonists with D_2 DA receptors was evaluated from agonist competition assays which were also performed as described previously (3). Briefly, assay parameters were: 2 mg tissue (original wt.) incubated in 2.3 ml phosphate buffer containing 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA and 90–100 pM [^{3}H]spiroperidol. Unlabeled displacer (DA) concentrations ranged from 10^{-10} to 10^{-4} M. After all incubations were completed, the contents of each tube were suctioned through Whatman GF/B filter paper (Brandel). Filter paper containing the trapped radioactive membranes were placed in 20 ml scintillation vials and 4.5 ml of Scintiverse was added to each vial. Vials were then counted on a Beckman Liquid Scintillation Counter (Model 3081).

Binding parameters (B_{max} and K_d) were estimated from saturation isotherms for [^{3}H]spiroperidol binding to D_2 DA receptors in striatal membranes using a nonlinear least-squares regression analysis program (18) based on the independent site models and assumptions of Feldman (4). All values were corrected for protein loss during preparation of the membranes. Post hoc Scheffe's tests were used for experimental group comparisons where appropriate. Values of p less than or equal to 0.05 were considered significant; values of p greater than 0.05 were considered not significant (ns).

This computer program allows the data on specific binding [total − (nonspecific)] to be further resolved into one saturable component (the D_2 DAr) and one nonsaturable component (unknown identity). B_{max} and K_d values were tabulated only for the saturable component. It is unlikely that [^{3}H]spiroperidol binding to $5HT_2$ receptors confounded our results since: 1) Sulpiride has extremely weak potency at $5HT_2$-r, 2) striata have about 10-fold more D_2 than $5HT_2$ receptors, 3) the concentrations of [^{3}H]spiroperidol were enough to exclude most $5HT_2$ binding ($K_d = 3$ nM), 4) the computer program "peeled out" low affinity binding (see above) and 5) we have consistently seen a robust positive correlation ($p>0.9$) between changes in our B_{max} values for striatal D_2 DAr and changes in APO-induced stereotypy (data not shown).

RESULTS

Striatal and N. Accumbens Dopamine Levels Post A/I Treatment

Results of neurochemical experiments using the A/I paradigm can be found in Tables 1 and 2. Rats sacrificed 4 weeks following A/I treatment, one week after demonstration of APO-induced hyperlocomotion (see below), showed a significant depletion of striatal DA, $t(11) = 4.47$, $p<0.001$. However, rats sacrificed 16 weeks after A/I, 4 weeks after demonstration of APO-induced hyperlocomotion, showed no significant reduction in striatal DA

TABLE 3

APOMORPHINE-INDUCED STEREOTYPY

Control	AMPH/IPRIN
43/120	45/60
36%	75%*

*$p<0.005$ vs. control group. Data indicate No. of observations where stereotypic sniffing was found divided by the total number of observations. Stereotypy tests took place 10 days after initial A/I injection. Test dose of APO was 0.45 mg/kg, IP. Testing was done 10 min after APO injection. The number of rats in each group was 12, 6 and 6, respectively.

TABLE 4

APOMORPHINE STIMULATION OF LOCOMOTOR ACTIVITY IN AMPHETAMINE/IPRINDOLE-TREATED RATS

	Control	AMPH/IPRIN
A		
3 Weeks		
	156 ± 16 (n=7)	317 ± 6* (n=11)
10 Weeks		
	89 ± 14 (n=7)	231 ± 11† (n=11)
B		
3 Weeks after A/I		
	132 ± 33 (n=6)	257 ± 33‡ (n=6)
10 Weeks after A/I		
	124 ± 10 (n=8)	227 ± 13§ (n=10)

*$p<0.0005$ vs. controls. †$p<0.0001$ vs. controls. ‡$p<0.05$ vs. control group. §$p<0.0005$ vs. control group. Values are mean activity counts (± SEM) from 10–20 min after APO (0.3 mg/kg, IP) injection.

levels, $t(16)=1.17$, n.s.

At 4 weeks, DOPAC levels were lower in the striatum of A/I-treated rats, $t(16)=2.17$, $p<0.05$. At 16 weeks striatal DOPAC levels were not significantly different between A/I rats and controls, $t(16)=1.33$, n.s.

In nucleus accumbens (Table 2), a single A/I treatment failed to significantly decrease DA levels at either 4 weeks, $t(9)=0.39$, n.s., or 16 weeks, $t(16)=0.87$, n.s., post A/I treatment. Nor did A/I treatment alter accumbens levels of DOPAC at either 4 weeks, $t(9)=0.49$, n.s., or 16 weeks later, $t(16)=0.62$, n.s.

Stereotypic Sniffing

Table 3 shows that stereotypic sniffing was significantly enhanced in the group, $\chi^2(1)=24.6$, $p<0.05$. A similar effect was observed 6 weeks after 3 A/I injections (data not shown).

APO-Induced Hyperlocomotion

A single A/I treatment produced a significant increase in APO-induced locomotor activity at 3 weeks, $t(8)=9.61$, $p<0.0005$, and at 10 weeks as well, $t(8)=9.51$, $p<0.0001$ (Table 4, panel A).

This experiment was also repeated on rats whose DA levels were determined (Table 4, panel B). Results of the repeat behavioral experiments on rats 3 weeks postinjection showed a significant elevation in APO-induced locomotor activity, measured 10–20 min after APO injection, $t(8)=2.67$, $p<0.05$. Rats tested with APO 10 weeks postinjection also showed a significant enhancement of locomotor activity, $t(8)=6.36$, $p<0.0005$.

TABLE 5

EFFECTS OF CLG ON APOMORPHINE STIMULATION OF LOCOMOTOR ACTIVITY IN RATS TREATED WITH AMPHETAMINE/IPRINDOLE

AMPH + Vehicle	AMPH + CLG
236 ± 9 (n=5)	177 ± 9* (n=6)

*$p<0.005$ vs. AMPH + Vehicle. Values are mean locomotor activity counts (± SEM) 10–20 min following APO (0.3 mg/kg, IP) administration. Behavioral tests were done 12 weeks after A/I treatment.

TABLE 6

CLG ATTENUATION OF INCREASED STRIATAL [^{3}H]SPIROPERIDOL BINDING IN RATS TREATED WITH AMPHETAMINE/IPRINDOLE

	B_{max}	K_d	% of Control
Control	17.4 ± 1.4	78 ± 8	100
AMPH + Vehicle	26.6 ± 1.3*	95 ± 16	153
AMPH + CLG	21.6 ± 1.6*	74 ± 12	124

*$p<0.05$. For B_{max}, all groups are significantly different from each other. B_{max} values are in fmol/mg tissue, K_d values are in pM. Each value represents the mean (± SEM) of 4 individual animals. Binding assays were done on rats sacrificed 12 weeks after A/I treatment and 24 h after behavioral testing.

Apomorphine-Induced Locomotion After Treatment With Cyclo(Leucyl-Glycyl) (CLG)

In Table 5 it can be seen that CLG significantly reduced the locomotor activity of A/I-treated rats, $t(8)=4.81$, $p<0.005$, compared to the corresponding control group of rats.

D_2 DA Receptor Binding With and Without CLG Treatment

Receptor binding data following A/I with or without exposure to CLG are found in Table 6. Data were analyzed using a one-way ANOVA. The overall ANOVA for striatal receptor number (B_{max}) was significant, $F(2,10)=10.75$, $p<0.005$, while the overall ANOVA for receptor affinity (K_d) was not, $F(2,10)=0.87$, n.s. For the B_{max} data, post hoc tests indicated that all groups were significantly different from each other. Thus A/I treatment resulted in an increase in B_{max} for striatal D_2 DAr and subsequent CLG treatment partly reversed this DAr upregulation (to 45% of the maximum A/I effect). This was similar to CLG's reversal of behavioral supersensitivity (data from Tables 2 and 3).

DISCUSSION

DA Depletion

The most consistent finding reported following high doses of AMPH (in iprindole-treated rats) or M-AMPH is a decrease in synaptic DA and a loss of DA terminals (21). Our DA depletion data are consistent with these observations.

The apparent normalcy of spontaneous behaviors of A/I rats despite DA depletion suggests a compensatory response has occurred. We hypothesized that it was likely that such compensation occurred at the DA synapse itself although compensation by other neural systems is also possible. This could take the form of an increase in DA synthesis or release (per impulse or per surviving nerve terminal); it could also take the form of an up-regulation of postsynaptic DA receptors. In this study, therefore, we sought to determine, both behaviorally and biochemically, whether there is a compensatory DAr up-regulation.

Occurrence of Compensation—DAr Up-Regulation

A long-term up-regulation of DAr was found after a single A/I injection, using both behavioral (Tables 3 and 4) and bio-

chemical (Table 6) measures. Behaviorally, increases in APO-induced locomotor activity were shown in rats up to 10 weeks after A/I treatment and were duplicated in rats whose DA depletions were then verified. In addition, an increase in APO-induced stereotypic sniffing was observed 10 days following A/I treatment. Biochemically, an increase in striatal receptor binding was shown (B_{max}: +53%) 12 weeks after A/I treatment. Thus evidence is provided here of long-term, DA receptor up-regulation in both mesolimbic and nigrostriatal tracts following acute administration of A/I.

Since the DA depletions can be quite long-lasting in these AMPH models, and since the DAr up-regulation we found lasts at least 12 weeks, it is possible that the DAr up-regulation is permanent. Even the apparent recovery of DA levels might not lead to DAr down-regulation if release of DA does not reach original levels. Similarly, the lack of change in gross DA levels in the n. accumbens after A/I treatment did not prevent DAr up-regulation in that area. The up-regulation of mesolimbic DAr suggests a decrease in levels of DA in the mesolimbic synaptic cleft had occurred. Our data, however, indicate that any such decrease is not due to an overall DA depletion in the accumbens. It is possible that an increase in DA synthesis per surviving nerve terminal could have occurred, restoring total DA levels, but DA release per terminal remained constant. This should result in a decreased amount of DA in the synaptic cleft, which would lead to DAr up-regulation.

Reversal of DAr Up-Regulation

One advantage of having developed a model of long-term DAr up-regulation is the availability of a screening assay to look for agents that down-regulate DAr since such agents could be useful in treating schizophrenia, tardive dyskinesia, and L-DOPA-induced dyskinesias. CLG may be a prototype of such agents. CLG is a DA neuromodulator, and has been shown to down-regulate DA receptors in a number of models of DA receptor supersensitivity (2, 5, 6, 20). The A/I model thus provides yet another example of the utility of this neuropeptide since CLG was found here to also reverse an already established DAr supersensitivity.

Our data, using both behavioral and biochemical techniques for both nigrostriatal and mesolimbic DA tracts, support our hypothesis that there is an up-regulation of DAr but our data do not rule out a possible increase in release which could also compensate for DA depletion.

Previous reports on changes following high doses of M-AMPH indicate a down-regulation of DAr as assessed by behaviors induced by a direct-acting DA receptor agonist, APO (17,25). This discrepancy may be due to the time at which our biochemical and binding assays were done post A/I treatment. Our data were obtained 2 to 12 weeks following A/I administration, whereas previous reports were all done at relatively early time points [two weeks or earlier after the M-AMPH injections (17,25)]. In fact we did observe a Parkinsonian-like phase during the first few weeks (data not shown) which was probably a response to the overstimulation of DA tracts by A/I. At the same time, it is well known that DAr up-regulation can require a minimum amount of time to become expressed following various drug or toxin treatments. For example, there is a well-established finding that DAr up-regulation in the chronic neuroleptic model requires a minimum of several weeks before it appears (23). Thus the DAr up-regulation or behavioral supersensitivity might only be seen when time points longer than 2 weeks are examined.

Another possible explanation for previous failures to find up-regulation of receptor binding in AMPH models may be due to the use by the other investigators of relatively high concentrations of [^{3}H]spiroperidol and d-(+)-butaclamol to define specific binding such that nondopaminergic receptors, especially $5HT_2$ and alpha-adrenergic receptors, may have also been labelled by the radioligand. Such nondopaminergic binding could mask any D_2 receptor changes.

Cause of the DAr Up-Regulation

We believe nigrostriatal DAr up-regulation represents a compensatory response to diminished levels of DA. We assume a similar mechanism for DAr up-regulation in the n. accumbens. Indeed, we observed a significant loss of DA in accumbens (−29%) after 3 A/I injections (one per day) at doses equal to those used here (unpublished results). This suggests that DA terminals even in this area are susceptible to the toxicity of amphetamines. Explaining the DAr up-regulation in the accumbens/mesolimbic system in animals that received a single A/I injection is more difficult because mesolimbic DA levels were unchanged. DA levels in this tract appear to be critical for locomotor activity (13,14). It may be that a) there is a loss of DA terminals, b) surviving terminals have increased DA synthesis, but c) total DA release is still low leading to DAr up-regulation.

Important studies that remain to be done in the A/I model, especially after a single A/I dose, are direct counting of limbic DA terminals and/or other parameters that would each partially verify a loss of DA terminals. These could include V_{max} for the reuptake of [^{3}H]DA, B_{max} for [^{3}H]GBR-12935 binding to DA reuptake sites, and V_{max} for tyrosine hydroxylase activity.

Alternatively, DA depletion in rats treated with A/I only once might have occurred in a subset of nucleus accumbens neurons, and this depletion may have been too subtle to detect with our methods. Additionally, a role for the nigrostriatal DA tract in the suppression of spontaneous locomotor activity after A/I cannot be excluded (7) since striatal DA in our experiments was depleted.

Recovery of DA Depletion

We also observed a recovery of striatal DA levels after 4 months after use of the A/I paradigm. It is possible that DA terminals have indeed been lost but that the surviving terminals have increased DA synthesis sufficiently to bring total striatal DA back to control values. Alternatively, sprouting may have led to creation of new DA terminals. Our finding of long-term partial recovery of DA levels appears to be the first instance reported of recovery of DA levels in rodents subjected to amphetamine-induced DA depletion (21).

Conclusions

In summary, A/I elicits a novel animal model of long-term, and possibly permanent, up-regulation of DAr and behavioral supersensitivity. The development of suitable models of enduring DAr supersensitivity is important, because in several major diseases which are thought to involve a DAr up-regulation, such as schizophrenia (up-regulated limbic DAr) and tardive dyskinesia (up-regulated striatal DAr), DAr up-regulation is thought to be long-lasting (19). This A/I model can also be used to screen for agents that down-regulate D_2 DAr.

ACKNOWLEDGEMENTS

Supported in part by the Veterans Administration, NIH (#NS-26449), Tourette's Syndrome Association, and the Scottish Rite Schizophrenia Research Program, N.M.J., USA. We gratefully acknowledge Wyeth-Ayerst Laboratories, Philadelphia, PA for the iprindole used in the course of these studies.

REFERENCES

1. Bean, A. J.; Elgin, R. J.; Cooper, D. M.; Martin, G. E. Cyclo(leu-gly) + haloperidol: Effects on dopamine receptors and conditioned avoidance responding. Peptides 8:39–44; 1987.
2. Bhargava, H. N.; Ritzmann, R. F. Inhibition of neuroleptic induced supersensitivity by cyclo(Leu-Gly). Pharmacol. Biochem. Behav. 13:633–636; 1980.
3. Clopton, J. K.; Gordon, J. H. In vivo effects of estrogen and 2-hydroxyestradiol on D_2 receptor agonist affinity states in rat striatum. J. Neural Transm. 66:13–20; 1986.
4. Feldman, H. A. Mathematical theory of complex ligand-binding systems at equilibrium: Some methods of parameter fitting. Anal. Biochem. 48:317–338; 1972.
5. Fields, J. Z.; Gonzalez, L.; Meyerson, L. R.; Lieber, P.; Lee, J. M.; Steece, K. A.; DeLeon-Jones, F. A.; Ritzmann, R. F. Radiofrequency analysis of the effects of haloperidol and cyclo(leucylglycyl) on stereotypy in the rat. Pharmacol. Biochem. Behav. 25: 1279–1284; 1986.
6. Fields, J. Z.; Lee, J. M.; Gordon, J. H.; Wichlinski, L. J.; Ritzmann, R. F. The effects of cyclo(leu-gly) (CLG) on nigrostriatal dopaminergic supersensitivity—Inhibition of apomorphine-induced climbing. Neuropeptides 16:207–211; 1990.
7. Fishman, R. H. B.; Feigenbaum, J. J.; Yanai, J.; Klawans, H. L. The relative importance of dopamine and norepinephrine in mediating locomotor activity. Prog. Neurobiol. 20:55–88; 1983.
8. Freeman, J. J.; Sulser, F. Iprindole-amphetamine interactions in the rat: The role of aromatic hydroxylation of amphetamine in its mode of action. J. Pharmacol. Exp. Ther. 183:307–315; 1972.
9. Fuller, R. W.; Hemrick-Luecke, S. Further studies on the long-term depletion of striatal dopamine in iprindole-treated rats by amphetamine. Neuropharmacology 21:433–438; 1982.
10. Fuller, R. W.; Hemrick-Luecke, S. Long-lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole-treated rats. Science 209:305–306; 1980.
11. Gordon, J. H.; Fields, J. Z. A permanent dopamine receptor upregulation in the ovariectomized rat. Pharmacol. Biochem. Behav. 33:123–125; 1989.
12. Gordon, J. H.; Nance, D. M.; Wallis, C. J.; Gorski, R. A. Effect of septal lesions and chronic estrogen treatment on dopamine, GABA, and lordosis behavior in male rats. Brain Res. 4:341–346; 1979.
13. Kelly, P. H.; Iversen, S. Selective 6-OHDA-induced destruction of mesolimbic dopamine neuron: abolition of psychostimulant induced locomotor activity in rats. Eur. J. Pharmacol. 40:45–56; 1976.
14. Kelly, P. H.; Seviour, P. W.; Iversen, S. D. Amphetamine and apomorphine responses in the rats following 6-OHDA-lesions of the nucleus accumbens and corpus striatum. Brain Res. 94:507–522; 1975.
15. Kostrzewa, R. M.; Jacobowitz, D. M. Pharmacological actions of 6-hydroxydopamine. Pharmacol. Rev. 26:199–288; 1974.
16. Lee, J. M.; DeLeon-Jones, F.; Fields, J. Z.; Ritzmann, R. F. Cyclo(leu-gly) attenuates the striatal dopaminergic supersensitivity induced by chronic morphine. Alcohol Drug Res. 7:1–10; 1986.
17. Lucot, J. B.; Wagner, G. C.; Schuster, C. R.; Seiden, L. S. The effects of dopaminergic agents on the locomotor activity of rats after high doses of methylamphetamine. Pharmacol. Biochem. Behav. 13:409–413; 1980.
18. Lundeen, J. E.; Gordon, J. H. Computer analysis of ligand binding data. In: O'Brian, R. A., ed. Receptor binding in drug research. New York: Pergamon; 1986:31–49.
19. Miller, R. Schizophrenia as a progressive disorder: Relations to EEG, CT, neuropathological and other evidence. Prog. Neurobiol. 33:17–44; 1989.
20. Ritzmann, R. F.; Bhargava, H. N. The effect of cyclo(leu-gly) on chemical denervation supersensitivity of dopamine receptors induced by intracerebroventricular injection of 6-hydroxydopamine in mice. Life Sci. 27:2075–2080; 1980.
21. Seiden, L. S.; Ricaurte, G. A. Neurotoxicity of methamphetamine and related drugs. In Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:359–366.
22. Steranka, L. R. Long-term decreases in striatal dopamine, 3,4-dihydroxy-phenylacetic acid, and homovanillic acid after a single injection of amphetamine in iprindole-treated rats: Time-course and time-dependent interactions with amfonelic acid. Brain Res. 234:123–126; 1982.
23. Tarsy, D.; Baldessarini, R. J. Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. Neuropharmacology 13:927–940; 1974.
24. Wagner, G. C.; Lucot, J. B.; Schuster, C. R.; Seiden, L. S. Ontogeny of aggregation enhanced toxicity. Psychopharmacology (Berlin) 75:92–93; 1981.
25. Yamanaka, Y.; Takano, R.; Egashira, T. Methamphetamine induced behavioral alterations following repeated administration of methylamphetamine. Jpn. J. Pharmacol. 41:147–154; 1986.