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Catalepsy, Fos Protein, and Dopamine Receptor Occupancy After Long-Term Haloperidol Treatment

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COPPENS, H. J., J. B. SEBENS AND J. KORF. *Catalepsy, Fos protein, and dopamine receptor occupancy after long-term haloperidol treatment.* PHARMACOL BIOCHEM BEHAV 51(2/3) 175–182, 1995.—During 12-week haloperidol treatment of rats, the cataleptic effect of an additional challenge dose becomes gradually weaker. We studied whether such a tolerance phenomenon is related to receptor supersensitivity—thus leaving more spare receptors—to a shift in affinity of the receptors towards agonist binding or to an attenuation of a postsynaptic response to dopamine (D_2-type) receptor blockade in the rat basal ganglia. Receptor occupancy was studied with the radioactive agonist [^{3}H]*N*-propylapomorphine (NPA) and antagonist [^{3}H]*N*-methylspiperone (MSPIP) to label free dopamine D_2 receptors in vivo. Fos protein served as an index of the postsynaptic response, which was histochemically quantified. This study does not support the concept that dopamine receptor supersensitivity may overcome neuroleptic receptor blockade, but there may be a shift towards higher agonist binding over time. The attenuation of Fos protein expression in the basal ganglia precedes the development of behavioral tolerance.

Catalepsy Fos protein Dopamine receptor occupancy Receptor supersensitivity Spiperone *N*-Propyl-apomorphine Haloperidol Chronic treatment

BLOCKADE of cerebral D_2-type dopamine receptors is considered crucial both for the therapeutic and the extrapyramidal side effects (drug-induced Parkinsonism) of typical antipsychotics (9,10,15,16,33,36,41,43). Parkinsonism becomes less prominent after chronic treatment and may eventually disappear (4,28). The animal equivalent of Parkinsonism is catalepsy (14), and tolerance develops after long-term treatment as well (3,12,14). Such a tolerance has been attributed to compensatory mechanisms, thereby restoring cerebral dopaminergic neurotransmission, at least in part. According to one hypothesis, the increased receptor density (B_{max}) of D_2 receptors may leave an increasing number of these receptors unoccupied during long-term neuroleptic treatment (22). A second hypothesis states that neurons postsynaptic to the dopaminergic neurons may adapt to chronic receptor blockade, as has already been shown in cholinergic neurons of the basal ganglia (25). When administered acutely, typical antipsychotics induce Fos protein (derived from an immediate early gene) in presumably dopamine-innervated neurons of the basal ganglia (13, 31,37). We considered the possibility that such a response attenuates in rats chronically exposed to antipsychotic drugs. According to a third hypothesis, behavioral tolerance towards antipsychotic drugs is the result of a higher affinity of the dopamine receptors towards agonist binding (1,35).

In the present study, rats were treated with haloperidol up to 12 weeks to induce behavioral tolerance. Subsequently, the in vivo binding to D_2 receptors was assessed in the caudate nucleus, nucleus accumbens, frontal cortex, and olfactory tubercle with an agonist or an antagonist, to differentiate between agonist and antagonist binding sites, respectively, and to study the possible emergence of free receptors. Of the dopamine agonist [^{3}H]*N*-propyl-norapomorphine (NPA) and the antagonist [^{3}H]*N*-methyl-spiperone (MSPIP) used here, the in vivo binding characteristics have previously been described in

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detail, which show the potency to detect increased B_{max} in vivo (25,47–50). Fos protein expression was immunocytochemically quantified in separate groups of rats (40).

METHOD

Design of the Study

A dose–response curve was made to determine the optimal dose of haloperidol (1 mg/kg) to achieve maximal catalepsy scores. Next, the long-term experiment was started. Accordingly, rats were daily injected with haloperidol and catalepsy was scored twice weekly after the last (challenge) dose. Because of the duration of the investigation, aging effects had to be considered. Two control groups were used to correct for, and to differentiate between, age and drug effects. One group of rats remained untreated (blanks), whereas another group was treated daily with haloperidol, but a wash-out period of 5 days was allowed, to show the development of receptor supersensitivity, as demonstrated with increased specific in vivo binding. Cerebral D_2 receptor binding was estimated in vivo with [^{3}H]NPA and [^{3}H]MSPIP similar to our previous studies (25,47–50). Specificity of the in vivo binding was demonstrated by pretreatment with antagonists of the D_1 receptor (SCH 23390) or of the serotonin$_{2A}$ receptor (ritanserin) to prevent specific binding by either ligand. Fos protein expression in the rat brain was studied 3 weeks after the start of the medication. To avoid stress effects due to handling and injection, control rats received saline during the same period of time but the last injection was either saline ("blank") or haloperidol ("acute"). For immunohistochemistry the animals were deeply anaesthetized with sodium pentobarbital (100 mg/kg, IP) and perfused transcardially with a fixative 2 h after the last injection.

Materials

Haloperidol (Janssen Pharmaceutica, Beerse, Belgium), obtained in a commercial form for intravenous use, was diluted with saline. Ritanserin was a gift of Janssen Pharmaceutica. SCH 23390 was obtained from Schering (Weesp, The Netherlands). [^{3}H]MSPIP (specific activity: 60 Ci/mmol = 2.2 TBq) and [^{3}H]NPA (specific activity: 51.5 Ci/mmol = 1.9 TBq) were from NEN Research Products (Dupont de Nemours, Dreiech, Germany). Sources of the other reagents are mentioned in the appropriate sections. All other reagents were of pro analyse quality and purchased from Merck (Darmstadt, Germany).

Rats and Treatment

Male Albino rats (180–200 g) of a locally bred Wistar-derived strain (Centraal Proefdieren Lab., Groningen) were housed in a constant temperature of 22°C. with a 12L : 12D schedule and free access to food and water. At the onset of the experiment, rats were divided into two groups: haloperidol- and saline-treated rats. Haloperidol (1 mg/kg in 0.5 ml) or saline (0.5 ml) was injected intraperitoneally (IP) between 0900 and 1000 h 6 days a week.

Catalepsy Test

Onset, time course, and intensity of catalepsy were tested 0, 15, 30, 60, 100, 120, and 180 min after IP injection of either saline or haloperidol. Standardization of the catalepsy test is important to achieve reproducible results (38,39). To minimize the effects of arousal and stress, rats were gently handled and exposed to the testing site several times before catalepsy measurements were started (52). Catalepsy was measured according to the standard bar-hanging procedure (14). Rats were placed in a card box of 30 × 35 × 15 cm, in which the forepaws were gently placed over a horizontal wooden bar (1.1 cm diameter at 12 cm height). Catalepsy was considered finished when the forepaw touched the bottom or the wall of the box, or when the rat climbed upon the bar. If the rats did not move, they were allowed to remain for 60 s (maximal score) in the cataleptic position, whereafter they were gently replaced on the bottom of the test box and retested 1 min later. Four attempts were made and the mean of the three longest recordings was noted as catalepsy score.

Tissue Preparation and Radioactivity Measurements

The ligands [^{3}H]NPA (5 μCi = 185 kBq) or [^{3}H]MSPIP (2.5 μCi = 92.5 kBq) were given intravenously 80 min after the last haloperidol or saline injection and rats were killed by decapitation 60 or 100 min thereafter. In control experiments it was shown that in the striatum and nucleus accumbens MSPIP and NPA bind virtually exclusively to D_2 receptors (47–50). To determine whether this is still the case after 12 weeks of haloperidol treatment, two additional series of experiments were performed to check for possible binding to the serotonin type 2A or the dopamine type 1 receptors. Accordingly, rats were pretreated with the highly selective agents ritanserin (2.5 mg/kg; the serotonin antagonist) and SCH 23390 (0.3 mg/kg, given twice because of the short half-life; the dopamine antagonist), respectively, to prevent specific binding.

Brains were taken out of the skull and the striatum, nucleus accumbens, cerebellum, frontal and prefrontal cortex, and the olfactory tubercle were dissected, immediately frozen on dry ice, and weighed. Blood samples were taken after decapitation from the trunk. To measure radioactivity, 0.5 ml distilled water and 1 ml solubilizer (NCS, Amersham, Buckinghamshire, UK) were added to the brain tissue samples and to the 0.1-ml blood samples. The samples were incubated at 50°C until all the tissue was dissolved and 10 ml Plasmasol (Packard, Brussels, Belgium) was added. After 24 h, liquid scintillation spectrometry was performed (Isocap 300, Searle) in the dark. Radioactivity was expressed in dpm/mg. Details of the procedures have previously been described (47–49).

Tissue Preparation and Fos Protein Immunocytochemistry

Fixation was performed by perfusion with saline (100 ml) followed by 300 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Brains were removed and postfixed overnight at 4°C in 4% paraformaldehyde containing 0.05% glutaraldehyde, before being stored in 50 mM Tris-HCl buffer, pH 7.4, containing 0.1% Na-azide. Coronal sections of 50 μm were cut from the postfixed brains, using a Vibratome. Immunostaining was performed on free-floating sections with a polyclonal antiserum (Oncogene Science, Ab-2, Uniondale, NY), raised in rabbit against Fos peptide (4–17 amino acids of human Fos). Briefly, sections were rinsed twice with 50 mM Tris-buffered saline, pH 7.4, exposed to the same buffer containing 0.3% hydrogen peroxide for 10 min at room temperature, subsequently rinsed five times in the Tris-buffer, followed by incubation with the Fos primary antiserum (diluted 1 : 250) in the incubation medium containing 1% bovine serum albumin (BDH Laboratory Supplies, Poole, UK) and 0.5% Triton X-100 (Baker Grade) in Tris-buffered saline for 72 h at 4°C. Sections were rinsed with Tris-buffered saline (3 × 10 min each) and incubated with a biotinylated rabbit

anti-goat secondary antibody (diluted 1 : 400, Vector Laboratories, Burlingame, CA) in the incubation medium for 1 h at room temperature. After rinsing in the Tris-buffer (2 × 15 min each), sections were incubated with avidin-biotinylated horseradish-peroxidase complex (diluted 1 : 125, Vector Laboratories) in the incubation medium for 1 h at room temperature. Both the second and the third incubation steps were repeated. After the last incubation, sections were rinsed twice with Tris-buffered saline and 50 mM Tris-HCl buffer, pH 7.4. Staining was revealed by the addition of a solution containing 0.05% 3,3'-diaminobenzidine (Pierce Chemical Company, Rockford, IL), 0.2% ammonium nickel sulphate (BDH), and 0.01% H_2O_2 and the reaction was stopped by rinsing in Tris-buffered saline. Sections were mounted on gelatin/chrome alum-coated slides, air dried, dehydrated, and coverslipped with DePeX mounting medium (BDH). Sections from control and experimental groups were processed at the same time using the same solutions. Other details have been published recently (40).

Data Presentation and Statistics

Receptor binding of the tracer was expressed as the ratio of dpm/mg tissue to dpm/μl serum. In previous studies we have also used cerebellum as a reference tissue, instead of blood. In the present study we chose blood because it appeared to reflect more precisely the bioavailability of the injected dose, because blood is the central pool in pharmacokinetics. Conclusions drawn in the present study were irrespective of the reference tissue. Catalepsy was expressed as the mean score in seconds (mean ± SEM). Fos-like-immunoreactive nuclei were counted within a 400 × 400 μm grid at a magnification of 125×. Cell counts were done in two different areas of the lateral striatum and in the nucleus accumbens; the mean value of bilaterally cell counts was used, and for all the groups (control, acute, and chronic haloperidol) mean values and SEM were calculated. For related data Wilcoxon's matched pairs signed ranks test was used. To test for significant differences in radioactivity and cell count data the Wilcoxon-Mann-Whitney Test was used; catalepsy scores were compared with the Wilcoxon Signed Ranks Test (42).

RESULTS

Catalepsy

Maximal catalepsy scores were reached 60 min after the haloperidol injection and remained constant for a few hours. Alterations of the catalepsy scores after the challenge dose of haloperidol during the long-term haloperidol treatment varied over time of haloperidol treatment. First, maximal catalepsy scores were reached significantly faster after 4, 8, and 12 weeks of the treatment. Second, the maximal scores and duration of the catalepsy progressively decreased over the 12-week treatment period. Thus, after the 8 and 12 weeks of haloperidol treatment, rats become cataleptic more quickly when exposed to a challenge dose of haloperidol, but the intensity of catalepsy was less than that of saline or shorter neuroleptic-treated rats. The time courses of the catalepsy scores are shown in Fig. 1.

Ligand Binding

Control experiments. Results of the in vivo ligand binding studies are summarized in Fig. 2 (striatum and nucleus accumbens) and Table 1 (cerebral cortex and olfactory tubercle). In the saline-treated rats (blanks), receptor binding of

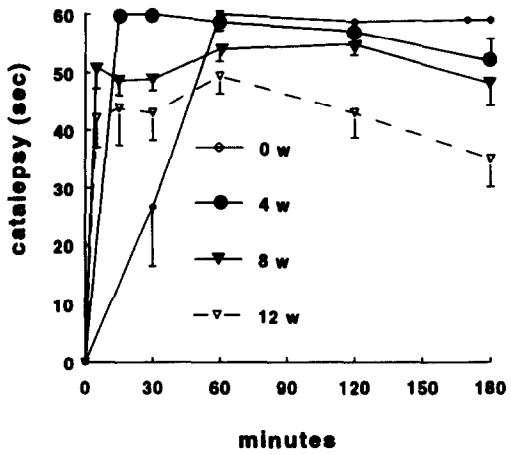

FIG. 1. Catalepsy scores (between 0 and 180 min after IP haloperidol) during long-term treatment with haloperidol (1 mg/kg/day) up to 12 weeks. The complete curves of the scores are shown at week 0 (first dose), 4, 8, and 12. The rate of onset of catalepsy was faster at 4, 8, and 12 weeks compared to the first dose ($p < 0.03$ at 30 min), whereas the maximal scores were significantly lower than those at the first dose (between 60 and 180 min; $p < 0.02$) only at week 8 and 12. Bars are mean ± SEM (number of rats scored) at week 0 (10); week 4 (15); week 8 (15), and week 12 (15).

[^{3}H]MSPIP in the striatum decreased significantly from 15.37 to 7.56 over the 12-week period. During that period the specific [^{3}H]NPA binding in the striatum remained almost unchanged (being 2.39 at the beginning and 2.21 at 12 weeks, not significantly different). In the nucleus accumbens and the olfactory tubercle, but not in the cerebral cortex (where D_2 receptor density is less pronounced), we found a significant decrease of the [^{3}H]MSPIP binding, over the same 3-month test period without significant change of the specific [^{3}H]NPA binding. Plasma concentrations of the radioactivities ([^{3}H]MSPIP and [^{3}H]NPA) remained unchanged with age, suggesting that there was little, if any, change in the bioavailability of the ligands.

Of the rats treated for 12 weeks with haloperidol and investigated 5 days after a wash-out period, the striatal [^{3}H]MSPIP binding ratio was 14.4, which is not significantly different from that of the young untreated control animals, but is almost twice as high (90% increase) as that of the age-matched, saline-treated rats (blanks). The striatal [^{3}H]NPA binding of such rats was 3.08, which is an increase of about 40% compared to the appropriate control rats. The nucleus accumbens and the olfactory tubercle exhibited a similar course of in vivo binding. In the frontal cortex there was no increased binding.

Haloperidol-exposed rats. Over the 12-week haloperidol treatment and testing after the last challenge, the residual striatal [^{3}H]MSPIP binding slowly decreased from 2.84 to 1.65. The residual radioactivity of [^{3}H]NPA binding, however, did not significantly change (remained about 0.87). Preventing tests with ritanserin (blocking serotonin type 2A receptors) and with SCH 23390 (blocking D_1 receptors) showed that most of this residual radioactivity after the long-term haloperidol treatment cannot be attributed to either D_1 receptor binding or serotonin type 2A receptor binding, although in the frontal cortex binding to these receptors may contribute (Fig. 2, Table 1). In the other aspects, changes in specific binding in the frontal cortex and olfactory tubercle were rather similar to those in the striatum and the nucleus accumbens. The effects

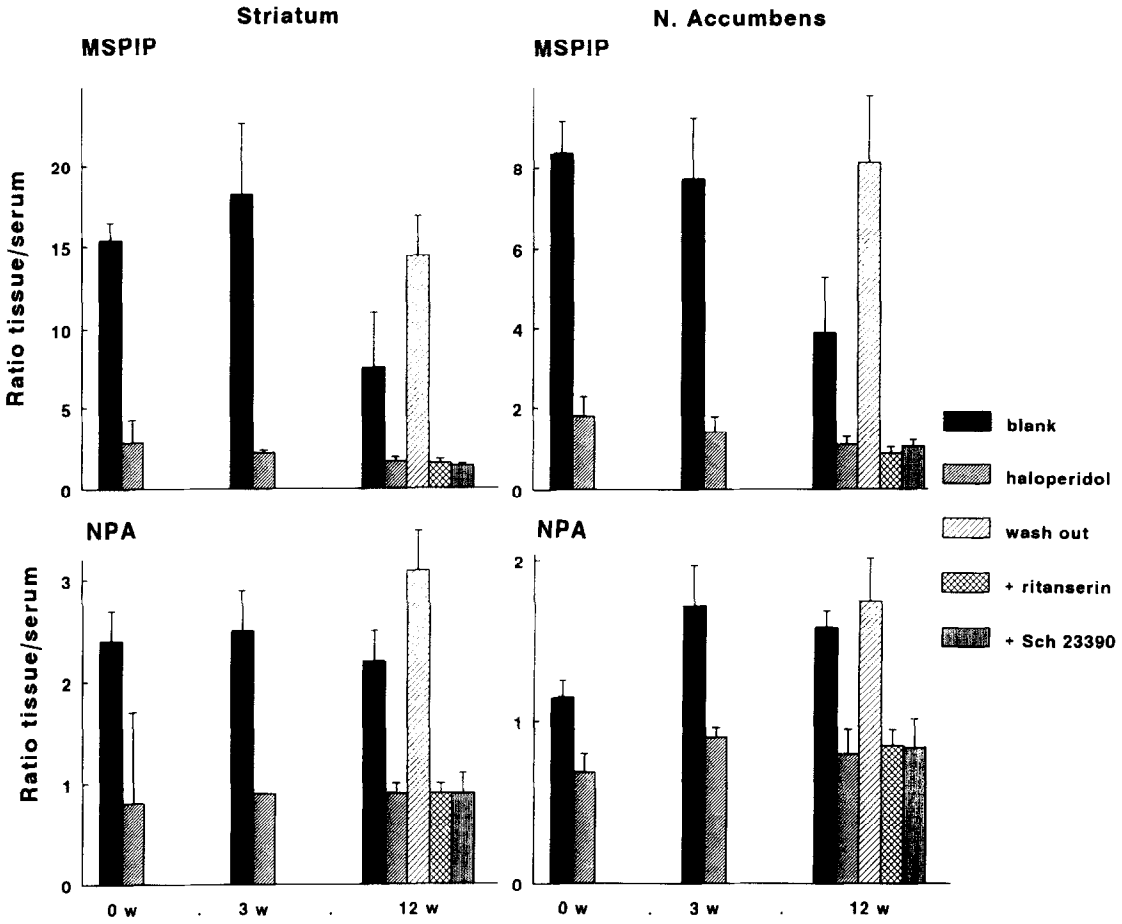

FIG. 2. In vivo ligand binding of MSPIP and NPA in the striatum and the nucleus accumbens (expressed as ratio radioactivity tissue/serum). Rats were treated with haloperidol (IP, 1 mg/kg/day) for 0 (first dose), 3, and 12 weeks before the administration of either tracer. Blanks were age-matched rats that were never treated with haloperidol, but only with saline. Ratio binding after haloperidol treatment was always significantly lower than the time-matched blanks ($p < 0.01$). After the last challenge dose of haloperidol, the residual binding was not significantly different in the various time-matched groups; when ritanserin was coadministered with the last dose of haloperidol, the ratios obtained with NPA or MSPIP were not significantly decreased in the accumbens. Only in the striatum was the MSPIP ratio somewhat decreased ($p < 0.05$) after the coadministration of the D_1 receptor blocker SCH 23390. After 12 weeks of haloperidol treatment, some rats had a 5-day wash-out period; they show significantly higher MSPIP and NPA ratio than the time-matched control group (blanks; $p < 0.02$; both striatum and n.accumbens). Bars (mean ± SEM) are based on experiments with five to seven rats; in some cases the SEM was too small to be presented in the figure.

on dopamine receptor binding in the frontal cortex were smaller than those in the other brain regions studied.

When the rats were divided into subgroups, according to the catalepsy scores, neither the residual specific [^{3}H]MSPIP binding nor that of [^{3}H]NPA significantly covaried with the catalepsy scores (data not shown) in any of the brain regions.

Correlation between catalepsy and agonist/antagonist binding. After the challenge haloperidol injection in control blanks, the striatal MSPIP : NPA ratio progressively decreased from 3.8 to 1.9, as shown in Fig. 3. A correlation between striatal MSPIP : NPA ratio and catalepsy was observed, suggesting a preferential increase of the agonist over the antagonist binding during aging.

Fos Protein Immunohistochemistry

An acute dose of haloperidol induced highly significant increases in Fos protein-positive cells in the striatum (both in the dorsolateral and ventrolateral parts, mean values shown in Fig. 4) and the nucleus accumbens. Already after 3 weeks of treatment there was a highly significant reduction of Fos protein-positive cells in both the striatum and in the nucleus accumbens. The number of Fos-positive cells in these areas was low or almost completely absent in the chronically treated rats compared to chronic saline-treated and haloperidol-challenged controls. Because of such reductions, longer treatment periods were not studied, as a further decrease in Fos protein expression could hardly be distinguished from saline-treated controls.

DISCUSSION

The main observations of the present long-term haloperidol study are: 1) production of a decrease of catalepsy, but an increase of its rate of onset; 2) dopamine receptor supersensitivity develops in these conditions, as was confirmed here with

TABLE 1

LIGAND BINDING IN THE FRONTAL CORTEX AND THE OLFACTORY TUBERCLE DURING LONG-TERM HALOPERIDOL TREATMENT

Treatment (weeks)	Frontal Cortex		Olfactory Tubercle	
	MSPIP	NPA	MSPIP	NPA
Blank (0)	3.97 ± 0.10	0.84 ± 0.06	11.25 ± 1.62	1.57 ± 0.11
Haloperidol (0)	1.79 ± 0.45	0.73 ± 0.08	2.31 ± 0.69	0.66 ± 0.06
Blank (3)	4.02 ± 0.44	1.00 ± 0.09	11.00 ± 1.76	1.85 ± 0.16
Haloperidol (3)	1.69 ± 0.24	0.84 ± 0.07	1.69 ± 0.23	0.94 ± 0.09
Blank (12)	2.62 ± 0.46	0.95 ± 0.07	5.38 ± 2.24	1.62 ± 0.17
Wash out (12)	2.97 ± 0.12	0.85 ± 0.05	10.32 ± 1.92	1.76 ± 0.16
Haloperidol (12)	1.55 ± 0.41	0.85 ± 0.12	1.27 ± 0.31	0.96 ± 0.12
Haloperidol + ritanserin (12)	1.15 ± 0.28	0.81 ± 0.09	1.23 ± 0.23	0.78 ± 0.06
Haloperidol + SCH 23390 (12)	0.94 ± 0.08	0.80 ± 0.21	1.22 ± 0.17	0.87 ± 0.18

These data were obtained from the same animals as presented in Fig. 2. For further details on number of experiments and protocol see legend to Fig. 2.

an in vivo method; 3) cerebral D_2 receptors remain virtually completely blocked during haloperidol treatment, so a possible emergence of spare or other receptors was not evident; but 4) attenuation of postsynaptic adaptation mechanisms was found, as indexed with the early gene Fos protein expression, which precedes behavioral tolerance; 5) in vivo binding of a DA agonist was less affected by aging than that of the antagonist; 6) a correlation was observed between the striatal ratio of MSPIP : NPA binding and the development of catalepsy tolerance. The presently observed catalepsy changes following haloperidol in a long-term treatment paradigm may depend upon changes in pharmacokinetics (14); in the development of cerebral regional density (supersensitivity) (21) of a variety of receptors including that of dopamine or serotonin (27) or of their subtypes [e.g., D_1 or D_2 type (32)]; upon the localization of such receptors (cortex or striatum) (29); and upon the agonist or antagonist interaction (35). Changes in pharmacokinetics are of clinical importance because neuroleptics can induce and alter microsomal enzymes (4), hence stimulating their own breakdown. In the present experiments with the radioactive ligands, no differences in serum content of radioactivity were seen after long-term treatment. Thus, our results do not support the idea that tolerance to catalepsy has to be attributed to faster kinetics of the neuroleptic. In vivo binding of both the agonist and the antagonist ligands demonstrates this, despite increased density of the D_2-type receptors during long-term treatment. So, perhaps with the exception of a minor binding in the cortical serotonin type 2 and dopamine type 1 receptor binding, no receptors were significantly labelled. In a recent PET study, high receptor occupancy in the basal ganglia by

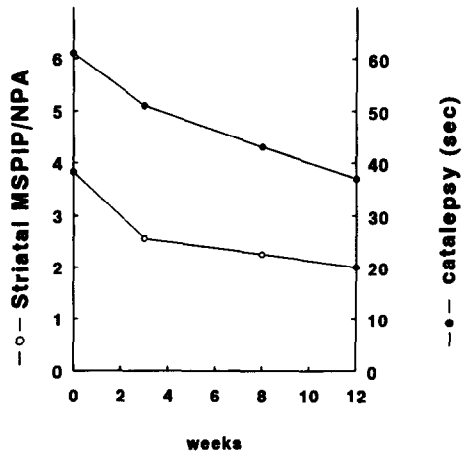

FIG. 3. Ratio radioactive MSPIP : NPA in the striatum and maximal catalepsy scores during the 12-week treatment scores. The ligand binding was always performed in the appropriate blanks. Both maximal catalepsy scores obtained after a challenge dose of haloperidol and the ratio of the antagonist/agonist binding decreased simultaneously and significantly over time and correlated highly to each other (correlation coefficient $r > 0.9$).

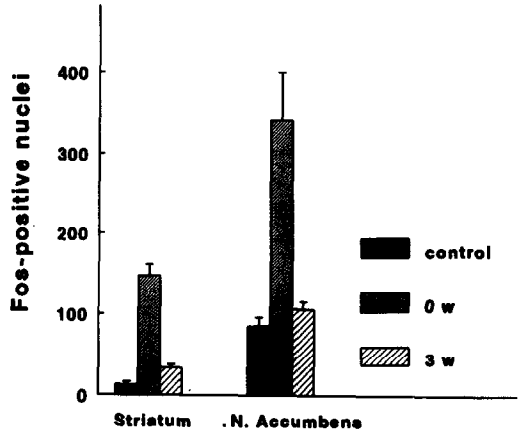

FIG. 4. Fos protein-positive cells in the striatum and the nucleus accumbens after a 3-week treatment with saline (control; black bars), 3-week saline plus a single dose of haloperidol (1 mg/kg; 0 w: intensely hatched bars), or after 3 weeks (18× "acute dosages"; hatched) of treatment. Fos-like-immunoreactive nuclei were counted 2 h after the last injection within a 400 × 400 μm grid. The bars are the mean value (mean ± SEM of three to five rats) of bilaterally cell counts. Acute (0 w) vs. controls or vs. 3 w: in all cases $p < 0.03$; 3 weeks vs. acute in both striatum and nucleus accumbens, $p < 0.05$.

neuroleptics has been associated with extrapyramidal side effects, including Parkinsonism (16), although after long-term treatment such a relation may no longer persist (9). So, we were unable to demonstrate the emergence of free D_2 receptors, as no increased tracer binding in vivo was observed during long-term treatment.

Reports have appeared on behavioral supersensitivity to DA agonists after chronic DA antagonist (neuroleptic) treatment (46). A cross-tolerance for catalepsy (17) and a cross-supersensitivity for apomorphine, induced by different kinds of neuroleptics (1), have been described, suggesting differential behavioral responses to agonists and antagonists. Our study, however, does not support the idea that behavioral tolerance is the result in sensitivity towards agonist binding, as assessed in vivo with NPA binding. This conclusion also refers to dopamine autoreceptors, exhibiting a high affinity for NPA (47). With none of the presently used ligands did these autoreceptors become specifically labelled, and we assume that these receptors are permanently blocked during haloperidol treatment (47,48), as is the case with the postsynaptic dopamine D_2 receptors. Interestingly, we observed an increased rate of onset of the catalepsy scores in the chronic experiment; such an increase may be determined by an elevated number of D_2 receptors in the striatum. We hypothetize that the blockade of a minimal number of receptors is required to evoke catalepsy.

Another possible cause of the behavioral tolerance towards typical neuroleptic drugs is a diminished postsynaptic response. This idea is supported by the present finding of a substantial drop in c-fos labelled cells after a challenge dose of haloperidol in the chronic preparation. The c-fos response, however, was already very low after 3 weeks of treatment, when maximal catalepsy scores were still high. Thus, the attenuation of the c-fos expression precedes tolerance and does not coincide with the decrease in the maximal catalepsy scores. Finally, as an alternative explanation for changes in catalepsy scores following long-term haloperidol treatment, the role of learning processes and conditioning and the emotional stress should be considered (12,21,24,26,34) independent of the presently observed biochemical parameters.

Our study shows that the agonist and antagonist binding in vivo are differently affected by aging. In the saline-treated rats, a reduction of about 50% of the specific MSPIP binding in the striatum was found after 3 months. This is in agreement with other rat studies (20), where a 40% reduction within the first year of life was found, and with positron emission tomography studies in aging men (2,51). It is remarkable that using NPA as tracer no such aging effect was found. These age effects may point to differences in local cerebral kinetics between the two ligands—differences in kinetic behavior have previously been noticed in rats with striatal kainate lesions (49)—or, alternatively, to differences in D_2 receptor properties. Others have also noticed that the efficacy of the neuroleptics may change during aging: there is an increasing response to dopamine agonists (e.g., amphetamine- or apomorphine-induced stereotypy) and a decreasing response to dopamine antagonists [e.g., haloperidol-induced catalepsy (7)], although there is little, if any, age dependent difference in brain concentrations of the neuroleptic (8) and receptor affinity (20). In accordance with another report (30) are the present results with both tracers, as there seems to be little difference in plasma concentration of the drug across age. A possible explanation could be that the agonist and antagonist binding sites are not identical and are differently affected by aging. The differences of the in vivo MSPIP and NPA binding in the striatum correlate well with the development of catalepsy tolerance over the same period.

The acute effects of haloperidol on the cellular Fos protein expression in the striatum and the nucleus accumbens are similar to those seen by other authors (13,31,37). In addition to these studies, we show the rapid development of tolerance to the effects of haloperidol on neuronal Fos protein expression, presumably postsynaptic to dopaminergic neurons. Haloperidol treatment can induce a growth-promoting effect in the rat striatum, leading to some kind of sprouting of the presynaptic neuron, which has been considered as a "compensatory mechanism" (23). The present observations with Fos protein indicate that such possible growth-promoting effects are not due to the continuous involvement of immediate early genes, and favour the idea that after initial triggering, these genes are no longer required for neural plasticity.

The antipsychotic effect of neuroleptics does not exhibit the same tolerance pattern as for Parkinsonism (6), indicating that other receptor subtypes and/or other brain regions are involved, or that the D_2 receptors in the striatum behave differently in this regard from those in, for example, the cortex (5,19,29,44). Moreover, our results suggest that the long-lasting antipsychotic effect is not dependent upon the continuous activation of immediate early genes (as c-fos) in the nucleus accumbens. Rather, the present study supports the idea that—in contrast to the extrapyramidal effects—the therapeutic response to antipsychotic drugs apparently involves other or additional neuronal and/or biochemical systems (11,18,45).

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