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A paradoxical regulation of the dopamine D_3 receptor expression suggests the involvement of an anterograde factor from dopamine neurons

(antipsychotics/6-hydroxydopamine/nucleus accumbens shell/D_3 receptor mRNA/7-[^{3}H]hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin)

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ABSTRACT **The effects of interruption of dopaminergic transmission or sustained blockade of dopamine receptors by neuroleptics on the dopamine D_3 receptor in the shell of the nucleus accumbens were investigated in rats. In this brain area the D_3 receptor is abundant and may mediate antipsychotic drug effects. The D_3 receptor density and mRNA abundance were evaluated with 7-[^{3}H]hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin and by quantitative PCR or image analysis of *in situ* hybridization signals, respectively. Unilateral dopamine neuron degeneration by 6-hydroxydopamine or sections triggered, after a few days, a marked decrease (up to 50%) in D_3 receptor binding and mRNA in the nucleus accumbens. In contrast, a 2-week treatment with the neuroleptic haloperidol (20 mg/kg) had no effect on D_3 receptor density and mRNA but enhanced D_2 receptor density and mRNA level by >50%. In addition, tolerance to the haloperidol-induced change of neurotensin mRNA mediated by the D_2 receptor developed, but there was no tolerance to the opposite change mediated by the D_3 receptor. Reserpine, a monoamine-depleting drug with antipsychotic activity, did not modify D_3 receptor mRNA. These observations reinforce the idea that the D_3 receptor may be an important target for neuroleptics whose antipsychotic actions, but not extrapyramidal motor actions, do not display tolerance. The D_3 receptor mRNA level was also decreased by a unilateral injection in dopamine cell body areas of colchicine, a drug blocking the anterograde axonal transport, or by baclofen, a type A γ-aminobutyric acid receptor agonist reducing dopamine neuron activity, but not by sustained blockade of D_1-like and D_2-like, neurotensin, or cholecystokinin receptors. We therefore propose that an anterograde factor present in mesolimbic dopaminergic neurons, but distinct from dopamine and known peptide cotransmitters, plays a positive role on transcription of the D_3 receptor gene.**

The dopamine (DA) D_3 and D_4 receptors, two receptors displaying similarities with the DA D_2 receptor, have been recently identified. Their high sequence homology (1, 2), similar coupling to guanine nucleotide binding proteins (3–5), and recognition by antipsychotic drugs (1, 2, 6, 7) have raised questions regarding the functional significance of this diversity.

Receptor diversity may allow a single neurotransmitter to exert distinct influences on various target neurons. In this respect, D_3 and, even more, D_4 receptors seem to be expressed in much more restricted brain areas and in distinct neuronal populations as compared to the D_2 receptor (1, 2, 8). The D_4 receptor, however, seems to mediate, like the D_2 receptor, inhibition of adenylyl cyclase (5). In the case of the D_3 receptor, the signaling system in cerebral neurons remains to be identified but its stimulation may trigger responses opposite to those mediated by the D_2 receptor. Thus, in the nucleus accumbens, blockade of the two receptor subtypes by neuroleptics enhances neurotensin/neuromedin (NT) gene expression in D_2 receptor-rich areas such as the cone part (also called septal pole) of the shell but nearly abolishes it in neurons of the ventromedial part of the shell expressing exclusively the D_3 receptor (9).

In addition, receptor diversity may allow a differential modulation of the efficiency of dopaminergic transmission in distinct neuronal populations if distinct mechanisms regulate the expression of the various receptor subtypes. The behavioral and biochemical supersensitivity to DA agonists that occurs after impairment of DA neurotransmission and tolerance to DA antagonists are accompanied by an upregulation of D_2 receptor expression in brain (reviewed in refs. 10–12). The process of neuroleptic-induced upregulation in receptor density has recently been shown to be associated with an increased D_2 receptor mRNA level (13–16). In contrast, no tolerance to the antipsychotic effects of these drugs seems to develop (17), suggesting that a subpopulation of D_2-like receptors subserving the antipsychotic effects is not subject to antagonist-induced upregulation.

In the case of the D_4 receptor, the extremely low abundance of its gene transcripts in brain and the lack of a selective receptor ligand still make it difficult to study regulation of its expression. This does not apply, however, to the D_3 receptor, whose gene transcripts can be rather easily detected by *in situ* hybridization—namely, in nucleus accumbens (8, 9)—and that can be reliably labeled in membrane binding or autoradiographic studies using 7-[^{3}H]hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin ([^{3}H]7-OH-DPAT) (18). We have studied here changes in D_3 receptor mRNA and binding sites occurring mainly as a result of sustained interruption of DA transmission elicited by 6-hydroxydopamine (6-OHDA), a catecholamine neuron-ablating neurotoxin, by DA receptor antagonists or by reserpine, a monoamine-depleting agent.

MATERIALS AND METHODS

Treatments. Groups of five to eight male Sprague–Dawley rats (180–200 g; Iffa Credo) received twice daily i.p. injections

Abbreviations: DA, dopamine; [^{3}H]7-OH-DPAT, 7-[^{3}H]hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin; 6-OHDA, 6-hydroxydopamine; NT, neurotensin/neuromedin.

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of haloperidol (5 or 20 mg per kg of body weight) alone or in various combinations with SCH 23390 (0.5 mg/kg); devazepide (0.5 mg/kg), a cholecystokinin type A receptor antagonist; and L-365,260 (0.5 mg/kg), a gastrin cholecystokinin type B receptor antagonist; or with SR 48692 (1 mg/kg), a nonpeptide neurotensin receptor antagonist. Other drugs used were reserpine (1 mg/kg) or baclofen (30 mg/kg). Colchicine (20 μg in 0.5 μl of distilled water) was injected unilaterally in the substantia nigra/ventral tegmental area complex at coordinates as follows: A, 3.7 mm; L, 2.0 mm; H, 1.5 mm (19, 20).

Lesions. Rats were anesthetized with chloral hydrate (350 mg/kg, i.p.), and were unilaterally injected with 6-OHDA (8 μg/4 μl in 0.05% ascorbic acid) at the following coordinates: A, 4.7 mm; L, 1.5 mm; H, 1.0 mm (19). After 10 days, rats displaying contralateral turning after subcutaneous administration of apomorphine (0.25 mg/kg) were selected and killed 10–12 days later unless otherwise stated. For hemisections, a razor blade (1 mm wide, 0.1 mm thick) was introduced through the open skull at the same coordinates as for 6-OHDA lesions.

Binding Assays. D_2 receptor binding (21) was measured in establishing saturation isotherms on particulate tissue fractions from three to five rats with [^{125}I]iodosulpride (Amersham; 2000 Ci/mmol; 1 Ci = 37 GBq). Nonspecific binding was evaluated in the presence of 1 μM raclopride. D_3 receptor binding (18) was measured with [^{3}H]7-OH-DPAT (158 Ci/mmol; custom synthesized at Centre d'Études Nucléaires, Saclay, France) either in saturation isotherms established with a particulate fraction from pooled tissues of 5–10 rats or at a single ligand concentration (0.4–0.5 nM) in tissue from one to three rats. Nonspecific binding was evaluated in the presence of 1 μM DA.

Quantitative Analysis of mRNA. Total RNAs prepared (22) from dissected nucleus accumbens tissues of individual animals were reverse transcribed and amplified by PCR with specific primers for β-actin and D_2 receptor mRNAs as described (13). For D_3 receptor mRNA, primers corresponding to amino acids 343–350 and to a sequence located 21–45 nucleotides downstream from the TGA stop codon were used. Reverse transcription PCR was performed in the presence of four dilutions of specific synthetic RNA standards containing target sequence for respective sets of primers and deleted by $\approx$100 bases to allow electrophoretic separation (23). cDNAs were then amplified for 18, 25, and 29 cycles for β-actin, D_2 receptor, and D_3 receptor, respectively, in the presence of [α-^{32}P]dATP. DA transporter mRNA was measured by reverse transcription PCR (without internal standards) in the presence of [α-^{32}P]dATP with primers corresponding to amino acids 167–174 and 271–277 from the sequence of rat DA transporter (24). PCR products were electrophoresed in 2.5% agarose gels, which were autoradiographed and scanned. RNA levels were expressed as equivalent standard amounts and D_2 receptor/β-actin, D_3 receptor/β-actin, and DA transporter/β-actin ratios were calculated.

***In Situ* Hybridization and Receptor Autoradiography.** *In situ* hybridization was performed with ^{32}P-, ^{33}P-, or ^{35}S-labeled complementary RNA probes for D_3 receptor and NT mRNAs as described (8, 9). Quantifications of autoradiograms were performed on a Biocom 2000 apparatus (Les Ulis, France) and gray values obtained were transformed into nCi/ng equivalents with a ^{14}C standard stripe (Amersham). For autoradiography on dipped emulsion, slides were dipped in LM-1 photographic emulsion (Amersham) melted at 43°C, air-dried for at least 3 h, loaded into plastic slide boxes, and stored at 4°C in the dark for 6 weeks. The emulsion was then developed in D-19 developer (15°C) for 4 min, rinsed rapidly in deionized water (15°C, three times), and fixed in 30% sodium thiosulfate (15°C) for 10 min. After rinsing in distilled water for 1 h, the sections were counterstained with Mayer's hemalum/eosin, dehydrated, and then coversliped with Permount. Receptor autoradiography with [^{3}H]7-OH-DPAT was performed as described (18).

RESULTS

Effects of 6-OHDA Lesion and Hemisection on D_3 and D_2 Receptor Expression. Three weeks after unilateral 6-OHDA infusion, the extent of the lesion was assessed on selected rats displaying apomorphine-induced contralateral rotations. The lesion produced a loss of 90% ± 5% and 84% ± 5% (n = 12; P < 0.01 by Mann–Whitney U test) of the DA transporter mRNA abundance in the substantia nigra and ventral tegmental areas, respectively. In the ipsilateral ventromedial shell of the nucleus accumbens, there were concomitant reductions of D_3 receptor binding and mRNA and of NT mRNA (Fig. 1). Analysis of autoradiograms indicated that D_3 receptor and NT mRNAs, a putative index of D_3 receptor function (9), decreased by 26% ± 2% (Table 1) and 38% ± 5% (n = 6; P < 0.005 by Mann–Whitney U test), respectively, as compared to the contralateral side. In contrast, NT mRNA expression was unchanged in the septum or cone subdivision of nucleus accumbens (Fig. 1*C*) and was instead increased ipsilaterally in dorsal striatum (data not shown; see also ref. 25). Hybridization signals generated from slides dipped into a liquid emulsion (Fig. 1 *D* and *E*) showed the decrease in D_3 receptor mRNA signals to result from a decrease in the number of silver grains per cell with no apparent loss in the labeled or total neuronal population: 49% vs. 48% of cells were positive on the ipsilateral and contralateral sides, respectively, whereas corresponding numbers of neurons per mm^2 were 2470 versus 2716 (over a total of 1838 cells counted on slices from three different lesioned animals).

By competitive reverse transcription PCR, we confirmed the ipsilateral decrease in D_3 receptor mRNA in the nucleus accumbens induced by 6-OHDA (Figs. 2 and 3 *Left*) and showed that a brain hemisection at the level of the medial forebrain bundle produced a similar effect that was maximal after 5 days (Fig. 2). In contrast, D_2 receptor mRNA increased

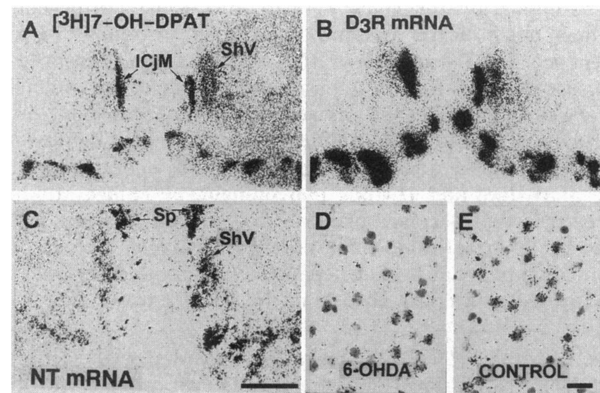

FIG. 1. Effects of a unilateral 6-OHDA injection on dopamine D_3 receptor and NT mRNA expression in nucleus accumbens. Rats injected with 6-OHDA into the left medial forebrain bundle were sacrificed 21 days later and adjacent coronal brain sections at bregma 1.2 mm (19) were used to generate autoradiograms of D_3 receptor binding using [^{3}H]7-OH-DPAT (0.5 nM) (*A*), of D_3 receptor (D_3R) mRNA using a ^{32}P-labeled complementary RNA probe (*B*), or of NT mRNA using a ^{35}S-labeled complementary RNA probe (*C*). Note the reductions in [^{3}H]7-OH-DPAT, D_3 receptor, and NT mRNAs in the ventromedial shell subdivision (ShV) on the left side close to the Island of Calleja major (ICjM). Sp, septum. (*D* and *E*) Dark-field photomicrographs obtained after dipping into liquid emulsion of a section taken at the level of the ventromedial shell subdivision in the lesioned side (*D*) or contralateral side (*E*) and hybridized with the D_3 receptor complementary RNA probe. Note that the lesion mainly affected the number of grains per cell rather than the number of positive cells. (*A–C*, bar = 500 μm; *D* and *E*, bar = 20 μm.)

Table 1. Changes in D_3 receptor mRNA expression in nucleus accumbens shell after various drug treatments

Treatment(s)	D_3 receptor mRNA, % change
6-OHDA	−26 ± 2**
Colchicine	−22 ± 4**
Baclofen	−48 ± 3**
Reserpine	+5 ± 8 (NS)
Haloperidol + SCH 23390	+6 ± 5 (NS)
Haloperidol + SCH 23390 + SR 48692	+10 ± 4 (NS)
Haloperidol + SCH 23390 + devazepide + L-365,260	+36 ± 10*

Injections of 6-OHDA (4 μg) were into the medial forebrain bundle, whereas colchicine injections (20 μg) were into the substantia nigra/ventral tegmental area complex 21 and 9 days, respectively, before sacrifice. Other treatments consisted of twice daily i.p. injections for 5 days of baclofen (30 mg/kg), reserpine (1 mg/kg), or haloperidol (5 mg/kg) plus SCH 23390 (0.5 mg/kg) with or without addition of SR 48692 (1 mg/kg), a neurotensin receptor antagonist, or devazepide (0.5 mg/kg) plus L-365,260 (0.5 mg/kg), two cholecystokinin receptor antagonists. D_3 receptor mRNA signals in the ventromedial shell subdivision of the nucleus accumbens were measured by quantitative *in situ* hybridization. Values are expressed as % change compared to either the contralateral side, for 6-OHDA and colchicine injections, or the respective vehicle-treated group for other treatments. Values are means ± SEM from three to five animals with at least three brain sections analyzed per animal. NS, not significant. *, $P < 0.05$; **, $P < 0.01$ according to unpaired Student's t test. A mean value of 81 nCi/g for signal intensity in controls was estimated by comparison with ^{14}C standard stripes.

by 48% ipsilaterally in nucleus accumbens of 6-OHDA-lesioned rats (Fig. 3 *Left*).

In 6-OHDA-lesioned rats (Fig. 3 *Left*), the binding of [^{3}H]7-OH-DPAT, a D_3 receptor ligand (18), was decreased ipsilaterally in the nucleus accumbens to the same extent as D_3 receptor mRNA (−54%); the decrease was slightly less pronounced in the olfactory tubercle (−33%) and was insignificant in the striatum (−4%). By comparison, D_2 receptor binding (Fig. 3 *Left*), evaluated with [^{125}I]iodosulpride which essentially labels this receptor subtype (18), was strongly enhanced in nucleus accumbens (+42%). D_2 receptor mRNA (Fig. 3 *Left*) was also enhanced significantly in the ipsilateral accumbens (+48%).

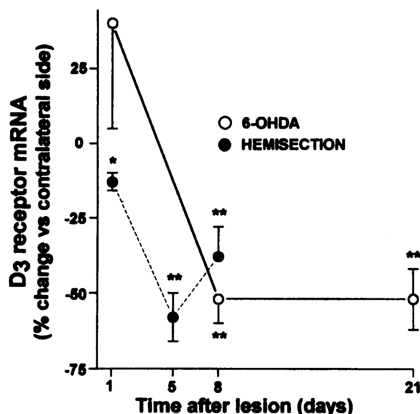

FIG. 2. Effect of unilateral lesions on D_3 receptor mRNA in nucleus accumbens. Animals were lesioned either by injection of 6-OHDA or by hemisection at the level of the medial forebrain bundle and D_3 receptor, and β-actin mRNAs were measured by competitive reverse transcription PCR. Results are expressed as % changes of D_3 receptor/β-actin ratios compared to the contralateral side. Means ± SEM of values obtained from six to eight separate determinations. Lesioned and contralateral sides of the same animals were compared by paired Student's t test. *, $P < 0.05$; **, $P < 0.01$ vs. contralateral side.

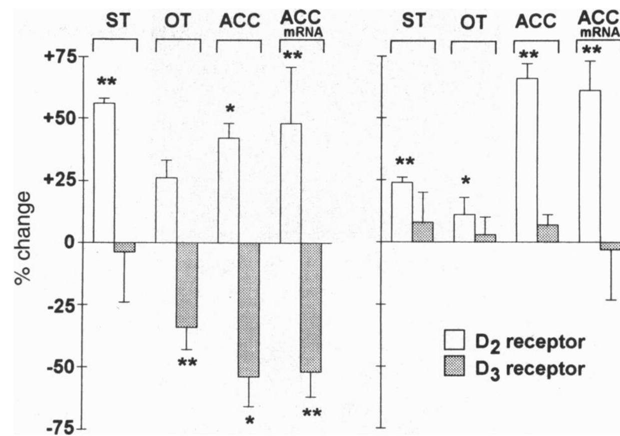

FIG. 3. Effects of 6-OHDA and chronic haloperidol treatment on D_2 and D_3 receptor expression in various rat brain regions. D_2 receptor and D_3 receptor binding in tissues from animals treated with 6-OHDA 3 weeks before sacrifice (*Left*) or with haloperidol (20 mg/kg, i.p.) for 2 weeks (*Right*) were measured with [^{125}I]iodosulpride and [^{3}H]7-OH-DPAT, respectively. Maximal receptor densities (B_{max}) after haloperidol treatment were established from saturation isotherms. For vehicle-treated rats, mean B_{max} (fmol per mg of protein ± SEM) were 73 ± 5 in nucleus accumbens (ACC), 120 ± 6 in olfactory tubercle (OT), and 342 ± 5 in striatum (ST) for D_2 receptor (n = 3), whereas corresponding values for D_3 receptor (n = 5–6) were 14 ± 1, 29 ± 2, and 7.0 ± 1.0. In these animals, K_d values were ≈0.6 nM for [^{125}I]iodosulpride and ≈0.8 nM for [^{3}H]7-OH-DPAT in all three regions. In lesioned animals B_{max} values (fmol per mg of protein ± SEM; n = 3) at the D_2 receptor were 80 ± 15 in nucleus accumbens, 106 ± 23 in olfactory tubercle, and 237 ± 17 in striatum on the contralateral side. On the contralateral side of other lesioned animals, D_3 receptor binding evaluated at 0.4–0.5 nM [^{3}H]7-OH-DPAT from pooled tissues from three to five animals was (fmol per mg of protein ± SEM; n = 8–10) 3.8 ± 0.7 in nucleus accumbens, 5.3 ± 0.4 in olfactory tubercle, and 0.7 ± 0.1 in striatum. Changes in mRNAs, evaluated as in Fig. 2, are expressed as % change over contralateral side for 6-OHDA and over vehicle-treated rats for haloperidol. *, $P < 0.05$; **, $P < 0.01$ according to a paired (6-OHDA) or unpaired (haloperidol) Student's t test.

Effects of Chronic Drug Treatments. Haloperidol, a D_2-like receptor antagonist, administered i.p. for 2 weeks, did not modify significantly either D_3 receptor binding in any of the analyzed brain areas or D_3 receptor mRNA level in the ventromedial part of the nucleus accumbens shell (Fig. 3 *Right*). In contrast, [^{125}I]iodosulpride binding was significantly enhanced in all areas, particularly in the nucleus accumbens where the increase was by 66% and the D_2 receptor mRNA was similarly enhanced by 61% (Fig. 3 *Right*).

D_3 receptor mRNA level in the same area of nucleus accumbens (Table 1) was not significantly modified by 5-day treatments combining haloperidol and the D_1-like receptor antagonist SCH 23390 alone or in combination with SR 48692, a neurotensin receptor antagonist (26). A 5-day treatment was used in this series of experiments because lesion studies have shown maximal effects on D_3 receptor expression at this time (Fig. 2). In animals receiving for 5 days the two cholecystokinin receptor antagonists devazepide (27) and L-365,260 (28), in addition to the two DA receptor antagonists, D_3 receptor mRNA was elevated but reserpine was without effect (Table 1). In contrast, unilateral injection into the substantia nigra/ventral tegmental area complex of colchicine, an inhibitor of axonal transport, or a 5-day systemic administration of baclofen, a type A γ-aminobutyric acid receptor agonist, decreased significantly D_3 receptor mRNA (Table 1; Fig. 4). In the ventromedial part of the nucleus accumbens shell, which expresses selectively the D_3 receptor (9), NT gene expression was decreased approximately to the same extent after single or repeated administration of haloperidol (−49% and −58%,

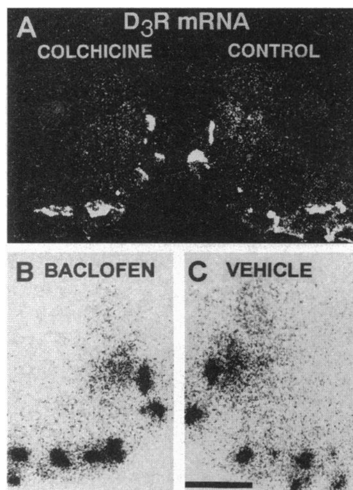

FIG. 4. Effects of unilateral injection of colchicine into the substantia nigra–ventrotegmental area complex (*A*) or baclofen administration (*B*) on D_3 receptor (D_3R) mRNA expression in nucleus accumbens. Coronal sections taken at bregman 1.2 mm were hybridized with a ^{33}P-labeled (*A*) or a ^{32}P-labeled (*B*) complementary RNA probe. In *A*, the slice was dipped into the emulsion and the photograph was taken under dark-field illumination. (Bar = 500 μm.)

respectively), whereas in the cone, which expresses selectively the D_2 receptor (9), the NT receptor mRNA level was enhanced by 568% after the single and by 273% after the repeated haloperidol administration (Fig. 5).

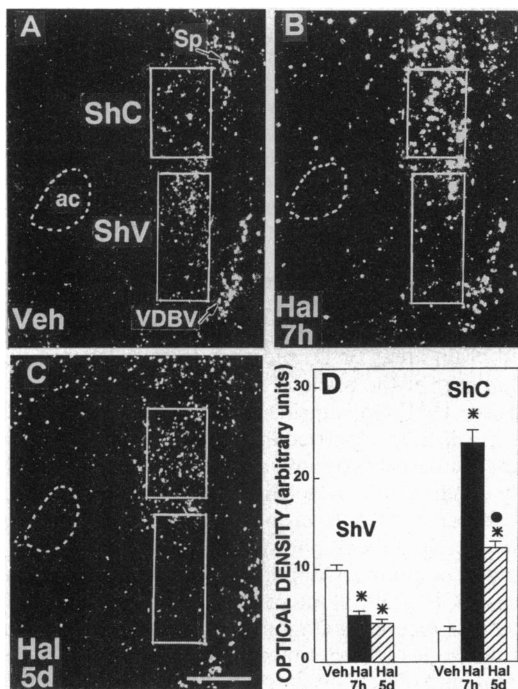

FIG. 5. Effects of haloperidol treatments on NT mRNA expression in subdivisions of the nucleus accumbens. Frontal sections were taken from animals having received vehicle (Veh) (*A*) or haloperidol (Hal) (5 mg/kg, i.p.) (*B*) either by single injection or by repeated injections twice daily for 5 days (5d) (*C*); all animals were killed 7 h after the last injection. Sections were hybridized with a NT ^{35}S-labeled complementary RNA probe and photomicrographs were taken under dark-field illumination. (Bar = 200 μm.) (*D*) Autoradiograms obtained from three to five rats were analyzed in two shell subdivisions of the nucleus accumbens: the ventromedial area (ShV) and the cone (ShC) as delimited in *A–C*. *, $P < 0.05$ vs. vehicle; ●, $P < 0.05$ vs. Hal 7 h according to two-way ANOVA followed by Dunnett's multiple comparison test. ac, Anterior commissura; Sp, septum; VDBV, nucleus of vertical limb of diagonal band, ventral part.

DISCUSSION

The present study shows that the regulation of D_3 receptor gene expression in limbic areas—namely, the shell of nucleus accumbens—after chronic haloperidol treatment or dopaminergic denervation takes place in a manner markedly contrasting that in which regulation of D_1 and D_2 receptor expression occurs. In striatal areas, the progressive increase in the number of D_1 and D_2 receptors, which develops after chronic blockade of these receptors (reviewed in refs. 10–12) or impairment of dopaminergic transmission by reserpine (29), is well substantiated. This process accounts, at least partially, for the behavioral hypersensitivity to DA agonists and the tolerance to some effects of DA antagonists, the latter exemplified here by the impaired D_2 receptor antagonist-mediated stimulation of NT gene expression in the cone subdivision of shell after repeated instead of single haloperidol administration (Fig. 5). Whereas it was suggested earlier that the enhancement in D_2 receptor binding sites could be due to impaired D_2 receptor degradation (30), the parallel elevation of D_2 receptor mRNA and D_2 receptor binding found in several studies (recently reviewed in ref. 12) and confirmed here indicates that an increase in gene transcription rate (or mRNA stability) is involved. In contrast, there was no apparent modification in levels of D_3 receptor binding and mRNA or on a D_3 receptor-mediated response—i.e., inhibition of NT gene expression in the ventromedial subdivision of the shell of the nucleus accumbens (9)—after chronic administration of haloperidol at a dosage high enough to block D_3 receptor. Controversial data were recently reported on the effects of haloperidol on D_3 receptor mRNA in mouse (16) and rat (31, 32) brain. It is known that, whereas tolerance develops to some actions of neuroleptics in humans such as extrapyramidal side effects, this is not the case for the antipsychotic effect of these drugs (17). Hence, the unmodified D_3 receptor expression after haloperidol (or reserpine, another antipsychotic) further supports the hypothesis that the D_3 receptor might represent an important target for antipsychotic drugs (1).

The D_3 receptor gene expression in limbic areas appears, nevertheless, regulated in a tonic manner by afferent dopaminergic neurons. This is shown by the severe and parallel losses in D_3 receptor binding and mRNA after biochemical or mechanical ablation of these neurons. Significant changes were detected as early as one day postsection and were already maximal 5–8 days after either brain hemisection or unilateral injection of 6-OHDA, indicating that they represent a response to harboring neurons rather than indirect adaptative changes (33). In agreement, visual examination and cell counting showed no apparent cytotoxicity of 6-OHDA in DA projecting areas (Fig. 1 *D* and *E*). The parallel changes in D_3 receptor binding and mRNA after 6-OHDA indicate that the regulation merely affects D_3 receptor postsynaptic to DA axons and does not reflect disappearance of autoreceptors on these axons. This conclusion is consistent with the very limited expression of D_3 receptor mRNA in ventral tegmental DA neurons synthesizing the autoreceptors (34).

What is the messenger present in DA neurons and regulating D_3 receptor expression in projection areas? The lack of effect of D_1- and D_2-like receptors blockaded by haloperidol and SCH 23390 and of reserpine treatment indicates that DA itself, or a factor costored in vesicles, is not involved. Peptide cotransmitters may regulate the expression of receptors for the main transmitter, as shown for the calcitonin gene-related peptide present in motoneurons, which increases the number of muscular nicotinic receptors (35). DA neurons, particularly those projecting to the nucleus accumbens, contain cholecystokinin (36) and neurotensin (37), but the lack of inhibitory effects of antagonists suggests that these putative cotransmitters do not apparently act as activators of D_3 receptor gene expression. This conclusion must be tempered, however, by the

fact that endogenous neurotensin may interact with receptors possibly not blocked by SR 48692 (38). On the contrary, the enhancement of D_3 receptor mRNA by simultaneous blockade of receptors for DA and cholecystokinin suggests that this neuropeptide exerts a negative and not a positive control of D_3 receptor gene expression.

In contrast, the level of D_3 receptor mRNA was decreased after treatments with either colchicine, a drug impairing the fast anterograde axonal transport of macromolecules (namely in DA neurons) (20, 29), or baclofen, a type A γ-aminobutyric acid receptor agonist decreasing DA neuron firing (39). Taken together, these observations indicate that D_3 receptor gene expression is triggered by an anterograde factor originating from DA neurons, distinct from the known transmitters, not stored in reserpine-sensitive vesicles, and whose release depends on neuronal activity. It is not clear, however, why chronic administration of haloperidol, known to result in silence of the DA neurons (40), is not accompanied by reduced D_3 receptor expression as found when DA neuron silence is elicited by baclofen. A similar factor might also have a positive role in the case of D_1 receptor expression, which is decreased in striatum after microinjection of colchicine into the ventral tegmental area (29) or 6-OHDA lesions (41) but enhanced by chronic D_1 receptor blockade (42, 43). The occurrence of both D_1 and D_3 receptors in the same neurons—e.g., in the granule cells of the islands of Calleja (8, 34, 44, 45)—makes it plausible that a single factor regulates the expression of both receptor genes. From a functional point of view, the role of the putative anterograde factor may be a developmental one and/or to regulate the efficiency of transmission in the adult brain. Hence, dopaminergic neurons may affect target NT neurons through the action of the main transmitter, DA, tonically triggering the expression of the NT gene, as well as through that of the anterograde factor regulating the expression of the D_3 receptor gene with a different time scale.

Mesolimbic dopaminergic neurons projecting to the shell of the nucleus accumbens may be implicated in the control of cognitive, emotional, and reward processes (46). Identification of the anterograde factor they release may, therefore, shed some light on a large diversity of clinical conditions such as mood disorders and cognitive deficits, central to schizophrenia or accompanying Parkinson disease, as well as states of drug dependence.

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