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# $D_2$ Dopamine Receptor Antisense Oligodeoxynucleotide Inhibits the Synthesis of a Functional Pool of $D_2$ Dopamine Receptors

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## SUMMARY

*In vivo* administration of an antisense oligonucleotide targeted toward the $D_2$ dopamine (DA) receptor mRNA ($D_2$ AS) markedly inhibited $D_2$ receptor-mediated behaviors but produced only a relatively small reduction in the levels of $D_2$ DA receptors in mouse striatum. This apparent dissociation between DA receptor-mediated behaviors and the levels of $D_2$ DA receptors was addressed by inhibiting the total number of $D_2$ DA receptors by intraperitoneal administration of the selective, irreversibly acting $D_2$ DA receptor antagonist fluphenazine-*N*-mustard (FNM) and then determining the effects of $D_2$ AS, administered intracerebroventricularly, on the rate of synthesis of $D_2$ DA receptors and on the recovery of $D_2$ receptor-mediated behaviors. FNM inactivated ~90% of $D_2$ DA receptors within 4 hr of treatment, after which the receptors returned to normal levels by ~8 days. $D_2$ AS treatment significantly inhibited the rate of recovery of $D_2$ DA receptors in striatum of FNM-treated mice. FNM treatment also produced a number of behavioral alterations, including catalepsy, and the inhibition of stereotypic behavior induced by the $D_2/D_3$ DA receptor agonist quinpirole. Both of these behaviors returned to normal within 8 days after FNM treatment. $D_2$ AS treatment delayed the restoration of these FNM-induced behaviors. Thus, it reduced the rate of disappearance of the cataleptic behavior induced by FNM and significantly delayed the restoration of the stereotypic behavior induced by quinpirole. The changes induced by $D_2$ AS on $D_2$ receptor-mediated behaviors were reversed on cessation of $D_2$ AS treatment. A random oligomer given in the same amount and for the same length of time as that of the $D_2$ AS had no significant effects on either $D_2$ DA receptor synthesis or DA receptor-mediated behaviors. These studies demonstrate that *in vivo* administration of $D_2$ AS decreased the rate of recovery of $D_2$ DA receptors and inhibited the recovery of $D_2$ DA receptor-mediated behaviors after irreversible receptor inactivation and suggest that $D_2$ AS treatment inhibits the synthesis of a functional pool of $D_2$ DA receptors.

Decreasing the input to postsynaptic aminergic receptors often results in up-regulation of these receptors (1). Up-regulation of dopaminergic receptors induced by DA receptor antagonists such as the neuroleptics may be the result of an increased expression of postsynaptic $D_2$ DA receptors (2–6). This up-regulation of DA receptors may be responsible for the loss of effectiveness of neuroleptic drugs and to certain dyskinesias associated with their chronic use (7). Ideally, one would like to reduce the activity of neurotransmitter receptors without inducing a compensatory increase in their levels. One possible method of achieving this goal is to develop a way to inhibit the synthesis of these receptors.

As the result of the rapid advances in molecular biology and molecular pharmacology, this goal may now be within reach as it is possible to develop new agents that interfere with the function of neurotransmitter receptors by acting at the level of gene expression. Among the many attractive strategies, the use of AS to inhibit the expression of neurotransmitter receptors offers some unique advantages over other forms of gene therapy, such as convenience, economy, and ease of use (8). Although the exact mechanisms by which AS inhibit specific gene expression remain to be determined (9–12), the molecular and biological effects of AS compounds directed toward the mRNAs encoding neurotransmitter receptors have been demonstrated in a wide variety of *in vitro* and *in vivo* studies. Thus, AS have been shown to inhibit the expression of the receptors and the behaviors mediated by the receptors for neuropeptide Y (13), enkephalin (14), glu-

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**ABBREVIATIONS:** DA, dopamine; AS, antisense oligodeoxynucleotide(s); FNM, fluphenazine-*N*-mustard; quinpirole, *trans*-(−)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-(or 2*H*)-pyrazolo-(3,4g)-quinoline dihydrochloride; (±)-SKF 38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine HCl; SCH 23390,(*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline.

tamate (15), acetylcholine (16), and DA (17–21). In several instances, however, the changes in the density of the specific receptors did not correlate well with the changes in the behavioral events mediated by these receptors (14, 17, 18, 20, 21).

For example, *in vivo* administration of an AS directed to the $D_2$ DA receptor mRNA ($D_2$ AS) inhibited behaviors induced by $D_2$ DA receptor agonists but not those induced by $D_1$ DA receptor agonists or by cholinergic muscarinic agonists (17, 19, 21). Conversely, administration of an AS directed to the $D_1$ DA receptor mRNA ($D_1$ AS) inhibited behaviors induced by $D_1$ DA receptor agonists but not those induced by $D_2$ DA receptor agonists or cholinergic agonists (20). However, despite the fact that $D_2$ AS treatment caused marked inhibition of $D_2$ DA receptor-mediated behaviors, it produced only a modest decrease in the density of $D_2$ DA receptors. This raised the question of how the relatively small reduction in the density of $D_2$ DA receptors induced by $D_2$ AS treatment can result in a profound inhibition of $D_2$ DA receptor-mediated events.

Because animal behavior is the final output of multiple neuronal systems, often involving several different neurotransmitters, the magnitude of changes in a specific behavior may not always correlate quantitatively with the magnitude of changes in the levels of a specific type of receptor. Insofar as the dopaminergic system is concerned, this apparent discrepancy has been explained by the existence of "spare" DA receptors (22, 23) that may be nonfunctional. In the context of the present study, we define nonfunctional receptors as $D_2$ DA receptors that are not functionally coupled to the G-proteins. Anatomically, these nonfunctional receptors may be either in the plasma membrane or internalized in the cell. In earlier studies (18, 21), we hypothesized that $D_2$ AS treatment may be acting by inhibiting a relatively small pool of functional $D_2$ DA receptors and that the reduction in the levels of these functional receptors may be responsible for the observed behavioral changes. Accordingly, a better correlation between the reduction in the levels of $D_2$ DA receptors and the reduction in the $D_2$ DA receptor-mediated behaviors may be revealed if the nonfunctional receptors were eliminated.

FNM is a nitrogen mustard analogue of the phenothiazine fluphenazine that has been shown to be a relatively selective, irreversible inhibitor of $D_2$ DA receptors (24–27). Our strategy was to administer FNM to inactivate the total pool of $D_2$ DA receptors and then determine the effects of $D_2$ AS treatment on the rate of recovery of the $D_2$ DA receptors. This would be correlated with the effects of $D_2$ AS treatment on the restoration of $D_2$ DA receptor-mediated behaviors after their inhibition with FNM. The results of these studies showed that *in vivo* administration of $D_2$ AS significantly inhibited the rate of recovery of $D_2$ DA receptors and reduced the rate of recovery of $D_2$ DA-mediated behaviors after their irreversible inactivation, suggesting that $D_2$ AS treatment inhibited the synthesis of a relatively small but functional pool of $D_2$ DA receptors.

## Materials and Methods

**Animals.** Male Swiss Webster mice (22–24 g) were purchased from ACE Animals Inc. (Boyertown, PA). Mice were housed in groups of 10 per cage with wood-chip bedding. The animals were maintained in a room at 21 ± 2° and 50% humidity with a 12-hr light/dark cycle. They had free access to food and water throughout the study.

**Chemicals.** The $D_2$ AS that we used was a 20-mer phosphorothioate oligodeoxynucleotide bridging the start codon (from −10 to +10) of the $D_2$ DA receptor mRNA (18). A randomly placed sequence with the same composition of oligodeoxynucleotides as the $D_2$ AS was used as a negative control. Quinpirole (LY-171555), (±)-SKF 38393, and FNM were purchased from RBI (Natick, MA). [$^3$H]SCH 23390 (specific activity, 86.5 Ci/mmol) and [$^3$H]raclopride (specific activity, 69.5 Ci/mmol) were purchased from New England Nuclear (Boston, MA).

**Administration of FNM and $D_2$ AS.** FNM was administered in a single bolus injection (20 μmol/kg IP). $D_2$ AS was administered into the left lateral cerebral ventricle every 12 hr (2.5 nmol per injection), starting at 4 hr after the injection of FNM. The intraventricular injections were performed with a Hamilton micropipette syringe with the use of a plastic injection mold (28) fitted with a guide cannula positioned above the left cerebral ventricle. Two microliters of the solution containing $D_2$ AS were delivered into the cerebral ventricle over a 2-min period, and the syringe was allowed to remain in place for another minute. Control animals were given either the random oligomer or vehicle. Mice were killed at various times after the injection of FNM.

**Behavioral measurements.** Catalepsy was determined at various times after the injection of FNM according to the method described by Morelli and Chiara (29) with minor modifications (30). The forepaws of the mice were placed on a horizontal metal bar at a height of 6 cm above the table. The time that the mice maintained their forepaws in a stationary position on the bar was recorded, with a cutoff time of 60 sec. The degree of catalepsy was determined at 8–10 hr after the last injection of $D_2$ AS or random oligomer.

Stereotypic behavior and grooming were determined in a single-blind fashion. Mice were transferred to a behavioral testing room and allowed 30 min for adaptation before behavioral evaluation. The mice were placed into a 15 × 25-cm plastic cage with wood-chip bedding, and behavioral measurements were made with a rating scale modified from that of Sturgeon *et al.* (31) as described earlier (32). To record the degree of stereotypy, mice were administered the $D_2$/$D_3$ DA receptor agonist quinpirole (5 μmol/kg SC), and, starting 5 min after the injection, stereotypic behavior was observed for 20 sec at each of 15 4-min intervals for a total of 1 hr. To record grooming behavior, we administered the $D_1$ DA receptor agonist (±)-SKF 38393 (40 μmol/kg SC) to the mice, and, starting 5 min after the injection, grooming was observed for 20 sec at each of 15 4-min intervals for 1 hr. In each case, the maximum behavioral score obtainable by this scoring system was 300 sec. The observations of stereotypy and grooming behaviors were performed 8–10 hr after the last injection of $D_2$ AS or random oligomer.

**Preparation of brain sections and receptor autoradiography.** All animals were killed 2 hr after the last injection of either $D_2$ AS or random oligomer. The brains were sectioned on a cryostat (12 μm) at the level of F 0.8–1.0 according to the method of Slotnick and Leonard (33) and were thaw-mounted onto gelatin-coated microscope slides. Receptor autoradiography was performed as described previously (30). Briefly, vacuum-dried tissue sections were washed twice in ice-cold 50 mM Tris·HCl buffer, pH 7.4, containing 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM KCl, and 120 mM NaCl. To label the $D_1$ DA receptors, the tissue sections were incubated in 50 mM Tris·HCl buffer in the presence of 2 nM [$^3$H]SCH 23390 (specific activity, 86.5 Ci/mmol) for 60 min at room temperature. Nonspecific binding was determined by incubating anatomically adjacent tissue sections in the above solution with 2 μM SCH 23390 and 80 nM ketanserin. To label the $D_2$ DA receptors, the tissue sections were incubated in 50 mM Tris·HCl buffer in the presence of 2 nM [$^3$H]raclopride (specific activity, 69.5 Ci/mmol). Nonspecific binding was determined by incubating anatomically adjacent tissue sections in the above solution with 2 μM sulpiride. The tissue sections were then rinsed, dried, and exposed to tritium-sensitive film (Hyper [$^3$H] sensitive; Amersham) for 1 week (for $D_1$ DA receptors) or 4–6 weeks (for $D_2$ DA receptors). Tritium

standards and brain mash standards were coexposed with the tissue sections. The images of the corpus striatum on the autoradiograms were quantified with a DUMAS image analyzer (34), with the dorsolateral area of the striatum defined as described by Joyce and Marshall (35). The optical densities were converted to receptor densities (femtomoles per milligram of protein) using the tritium and brain mash standards.

The effects of $D_2$ AS treatment on the rates of synthesis and degradation of $D_2$ DA receptors after FNM administration were determined with a procedure proposed by Mauger *et al.* (36). This model assumes that the synthesis of receptors is constant and that the degradation of the receptors is proportional to the receptor concentration at any given time during the receptor recovery period after irreversible inactivation. According to this model, the data for the recovery of $D_2$ DA receptors after FNM treatment are plotted with $\ln[R_{ss}]/[R_{ss} - R_t]$ as the vertical axis and time as the horizontal axis, where $R_{ss}$ is receptor concentration at steady state and $R_t$ is receptor concentration at time $t$. The semilogarithmic transformation generates a linear plot with the slope of the regression line representing the rate constant for receptor degradation ($K$). The rate of synthesis of receptors ($r$) can be calculated from $[R_{ss}] = r/K$. To perform statistical analyses of $K$ and $r$, five regression lines were constructed using the data generated from each animal at each time point. This procedure enabled us to perform a two-tailed Student's $t$ test to analyze and derive apparent $K$ and $r$ values.

**Statistical Analysis.** We used a two-way analysis of variance followed by a Newman-Keuls test to compare the effects of different treatments on the recovery of $D_2$ DA receptors and $D_2$ receptor-mediated behaviors. A Student's $t$ test was used to compare the rates of the synthesis of $D_2$ DA receptors and the rate constants for the degradation of $D_2$ DA receptors.

## Results

**Effects of FNM on $D_2$ DA receptors in striatum.** Fig. 1 shows the effects of a single dose of FNM (20 μmol/kg) on the inactivation and recovery of $D_2$ DA receptors in the dorsolateral region of the mouse corpus striatum as determined with receptor autoradiography. At 4 hr after the injection of FNM, the $D_2$ DA receptor binding sites were reduced to ~10% of their control levels. By 8 days after the injection of FNM, the $D_2$ DA receptor binding sites had returned to ~80% of their control levels. As reported previously (24), FNM treatment did not significantly alter the levels of $D_1$ DA receptor binding sites as determined by receptor autoradiography (data not shown).

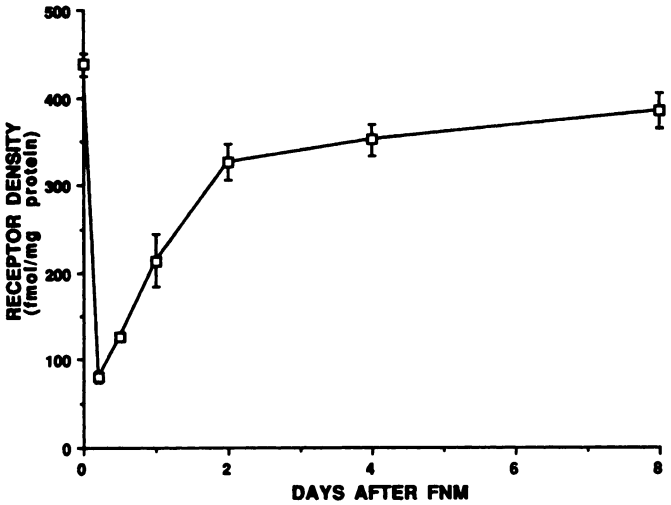


**Fig. 1.** Effect of FNM on the density of $D_2$ DA receptors in dorsolateral striatum of mice. Mice were administered a single injection of FNM (20 μmol/kg IP), and the animals were killed at various times thereafter. The brains were removed and sectioned, and receptor autoradiography was performed with the $D_2$ DA receptor ligand [$^3$H]raclopride (2 nM). Optical densities of the dorsolateral areas of the striatum were analyzed with a DUMAS image analyzer. *Points*, mean values from five mice; *error bars*, standard error.

**Effects of $D_2$ AS on the recovery of $D_2$ DA receptors in striatum after their irreversible inactivation.** In these experiments, mice were administered a single dose of FNM to irreversibly inhibit the $D_2$ DA receptors and then were administered twice-daily intraventricular injections of $D_2$ AS, random oligomer, or vehicle. The densities of $D_2$ DA receptors were determined with receptor autoradiography at different times after treatment with the oligomers. Fig. 2 shows an example of the receptor autoradiographic analysis of $D_2$ DA receptors in striatum of mice 2 days after treatment with FNM. $D_2$ AS treatment resulted in a reduced level of $D_2$ DA receptors in mouse striatum compared with vehicle-treated mice or random oligomer-treated mice. This decrease was particularly evident in the dorsolateral striatum.

Fig. 3 shows a quantitative analysis of the receptor density in dorsolateral striatum from five mice per group plotted as a percentage of recovery, with the densities of $D_2$ DA receptors before FNM treatment representing 100% recovery and the density of receptors 4 hr after FNM treatment representing 0% recovery. Treatment of mice with $D_2$ AS significantly inhibited the recovery of $D_2$ DA receptors in striatum. Statistically significant effects of $D_2$ AS were seen in the dorsolateral striatum at 1, 2, and 4 days after the injection of FNM; the degree of inhibition seen at 1 day was almost 60%, and this degree of inhibition decreased over time. No statis-

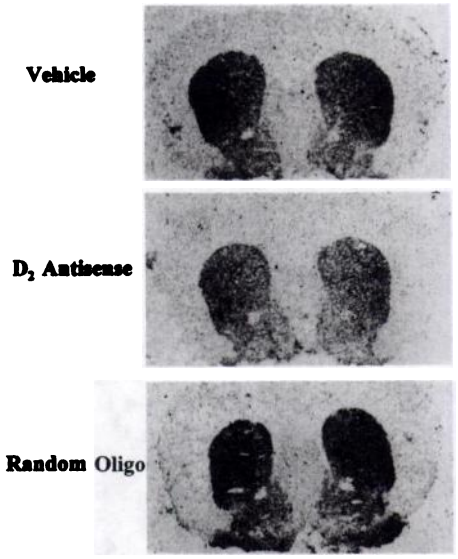


**Fig. 2.** Effect of $D_2$ AS on $D_2$ DA receptors after FNM treatment in mouse brain. Mice were pretreated with a single injection of FNM (20 μmol/kg IP). Starting at 4 hr later, they were administered intraventricular injections of vehicle (2 μl of artificial cerebrospinal fluid), $D_2$ AS (2.5 nmol/2 μl), or random oligodeoxynucleotide (2.5 nmol/2 μl) twice daily for 2 days. The brains were removed 2 hr after the fourth injection of vehicle or oligomer and 48 hr after treatment with FNM. Then, 12-μm coronal sections at the level of the corpus striatum were prepared, the brain sections were incubated with [$^3$H]raclopride (2 nM), and the slides were processed for receptor autoradiography as described in Materials and Methods.

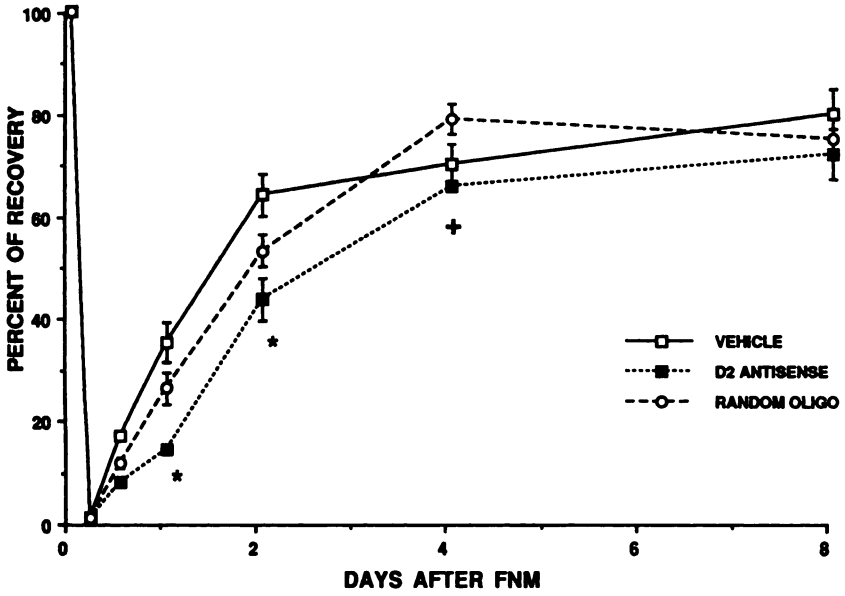


**Fig. 3.** Effect of $D_2$ AS on the recovery of $D_2$ DA receptors in dorsolateral striatum of mice treated with FNM. Mice were pretreated with a single injection of FNM (20 μmol/kg IP). Starting at 4 hr later, they were administered intraventricular injections of vehicle (2 μl of artificial cerebrospinal fluid), $D_2$ AS (2.5 nmol/2 μl), or random oligodeoxynucleotide (2.5 nmol/2 μl) twice daily for 8 days. The brains were removed at various times after treatment with FNM, always 2 hr after the last injection of vehicle or oligomer. Then, 12-μm coronal sections were incubated with [$^3$H]raclopride (2 nM), and the slides were processed for receptor autoradiography. Optical densities of the dorsolateral areas of the striatum were analyzed with a DUMAS image analyzer. *Points*, mean values from five mice; *error bars*, standard error. Statistical comparisons between AS-treated mice with either vehicle-treated or random oligomer-treated mice were carried out with two-way analysis of variance followed by a Newman-Keuls test. *, $p < 0.05$ compared with vehicle- or random oligomer-treated mice; +, $p < 0.05$ compared with random oligomer-treated mice.

tically significant effects of $D_2$ AS were seen at 8 days after FNM treatment. No significant effects of the random oligomer on the recovery of $D_2$ DA receptor were seen at any time point studied. Furthermore, as reported previously (21), $D_2$ AS treatment had no significant effects on the levels of $D_1$ DA receptors at any time period studied (data not shown).

With the use of the data shown in Fig. 3, we calculated the rates of synthesis and degradation of $D_2$ DA receptors. The results of these analyses (Table 1) show that $D_2$ AS treatment significantly decreased the rate of the synthesis of $D_2$ DA receptors. This was accompanied by a decrease in the rate constant for receptor degradation, resulting in an increase in the half-life for the receptors.

**Effect of FNM on $D_2$ DA receptor-mediated behaviors.** Fig. 4 shows that a single intraperitoneal injection of FNM induced a profound cataleptic effect in mice. The catalepsy was most evident at 4 hr after the injection of FNM and was no longer apparent 4 days after FNM treatment. The figure also shows that administration of quinpirole produced stereotypic effects, whereas the administration of (±)-SKF 38393 induced a grooming behavior. The quinpirole-induced stereotypy was inhibited in mice pretreated with FNM. The greatest inhibition was seen during the first 24 hr after FNM administration; this quinpirole-induced stereotypy was restored to normal by ~4 days after FNM treatment. In contrast, FNM had no significant effects on grooming behavior induced by the $D_1$ receptor agonist (±)-SKF 38393 (Fig. 4).

**Effects of $D_2$ AS on DA receptor-mediated behaviors in mice treated with FNM.** Fig. 5 shows the effects of $D_2$ AS treatment on the rate of disappearance of FNM-induced catalepsy in mice. Treatment of mice with $D_2$ AS resulted in a significantly slower rate at which FNM-induced catalepsy disappeared. The random oligomer had no significant effect on the rate of disappearance of FNM-induced catalepsy at any time point examined.

Fig. 6 shows the effects of $D_2$ AS treatment on the recovery of stereotypy induced by quinpirole (Fig. 6A) and of grooming behavior induced by (±)-SKF 38393 (Fig. 6B) in mice pretreated with a single acute injection of FNM. As was seen in Fig. 4, the quinpirole-induced stereotypic behavior was completely inhibited by 24 hr after FNM treatment but was rapidly restored thereafter. Treatment of mice with $D_2$ AS markedly reduced the rate at which the stereotypic effects of quinpirole recovered. Mice that were administered the random oligomer exhibited no significant effect on the restoration of quinpirole-induced stereotypic behavior. Repeated treatment with either the $D_2$ AS or random oligomer failed to significantly alter (±)-SKF 38393-induced grooming behavior.

**Reversibility of the effects of $D_2$ AS on $D_2$ DA receptor-mediated behaviors.** To determine whether the effects of $D_2$ AS treatment were reversible, $D_2$ DA receptor-mediated behaviors were examined at 1, 2, 4, and 8 days after cessation of the $D_2$ AS injections. The results showed that the inhibitory effects of $D_2$ AS on the disappearance of catalepsy induced by FNM were still evident at 1 day after cessation of $D_2$ AS treatment (Fig. 7) but not at 2 and 4 days. Similarly, the stereotypy induced by quinpirole in FNM-treated mice was restored to normal after cessation of $D_2$ AS treatment (Fig. 8). In mice that had been administered $D_2$ AS, quinpirole-induced stereotypy was still inhibited during the first

TABLE 1

**Effect of $D_2$ AS on the synthesis and degradation of $D_2$ DA receptors in dorsolateral striatum after treatment with FNM**

Mice were pretreated with a single injection of FNM (20 μmol/kg IP). Starting at 4 hr later, they were administered intraventricular injections of vehicle (2 μl of artificial cerebrospinal fluid), $D_2$ AS (2.5 nmol/2 μl), or random oligomer (2.5 nmol/2 μl) twice daily for 8 days. The brains were removed 4, 12, 24, 48, 96, and 192 hr after treatment of FNM, always 2 hr after the last injection of vehicle or oligomer. $D_2$ DA receptors were measured in dorsolateral striatum with receptor autoradiography, and calculations of the rates of synthesis, degradation, and half-lives of the receptors were performed as described in Materials and methods. The data represent the mean ± standard error for five mice per group. Statistical analyses were carried out with a paired *t* test.

| Treatment | Synthesis | Degradation | Half-life |
|---|---|---|---|
| | *fmol/mg protein/hr* | $K^{-h}$ | *hr* |
| Vehicle | 5.2 ± 0.9 | 0.014 ± 0.001 | 57 ± 9 |
| $D_2$ AS | 3.0 ± 0.2[a,c] | 0.008 ± 0.002[b] | 85 ± 4[a,c] |
| Random | 5.4 ± 0.5 | 0.014 ± 0.002 | 49 ± 4 |

[a] $p < 0.05$ compared with values from vehicle-treated mice.
[b] $p < 0.05$, [c] $p < 0.01$ compared with values from random oligomer-treated mice.

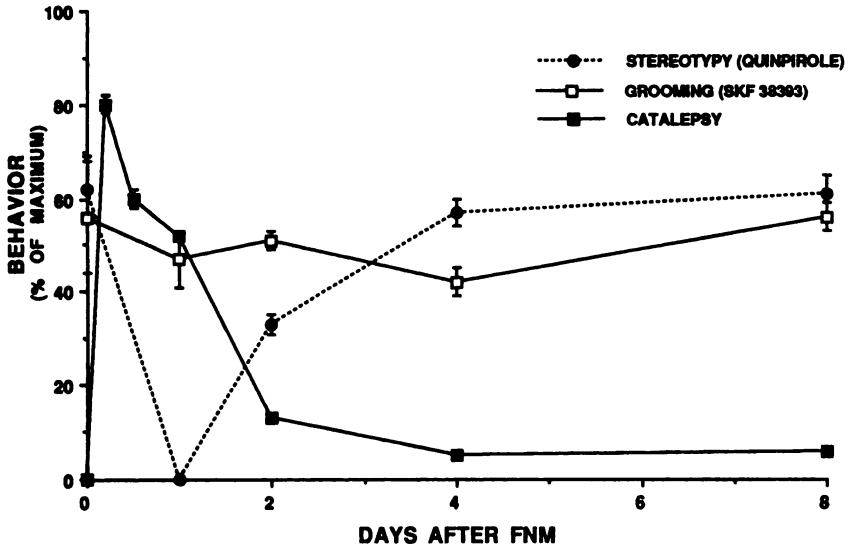


**Fig. 4.** Effect of FNM on DA receptor-mediated behaviors in mice. Mice were administered a single injection of FNM (20 μmol/kg IP), and a series of DA receptor-mediated behaviors were measured at 4 and 12 hr and at 1, 2, 4, and 8 days afterward. The behaviors measured were catalepsy, stereotyped behavior induced by the $D_2$ DA receptor agonist quinpirole (5 μmol/kg SC), and grooming behavior induced by the $D_1$ DA receptor agonist SFK 38393 (40 μmol/kg SC). *Points*, mean values for 5–15 mice calculated as the percentage of the maximum behavioral score; *error bars*, standard error.

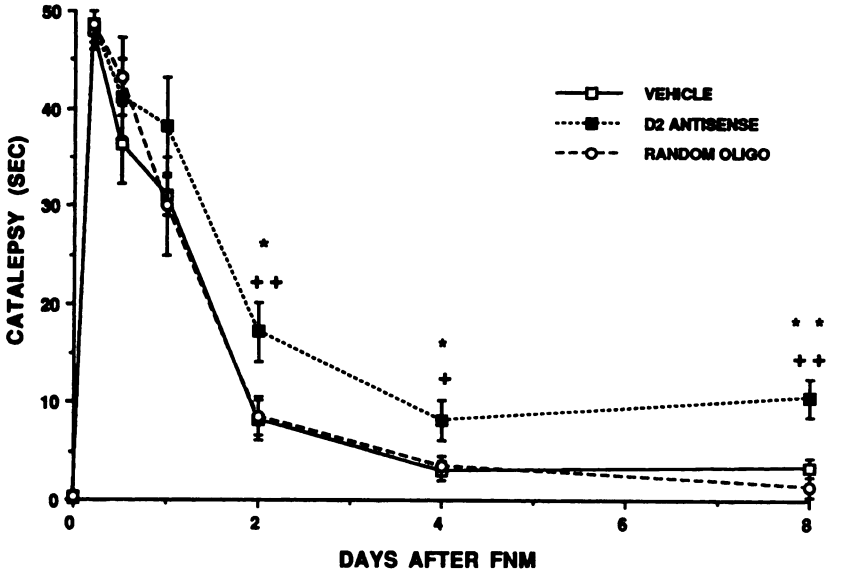


**Fig. 5.** Effect of $D_2$ AS on catalepsy induced by FNM in mice. Mice were pretreated with a single injection of FNM (20 μmol/kg IP). Starting at 4 hr later, they were administered intraventricular injections of vehicle (2 μl of artificial cerebrospinal fluid), $D_2$ AS (2.5 nmol/2 μl), or random oligodeoxynucleotide (2.5 nmol/2 μl) twice daily for 8 days. Catalepsy was measured at various times after the administration of FNM, always 10 hr after the last injection of vehicle or oligomer. *Points*, mean Catalepsy scores ± standard error for 5–15 mice. Maximum attainable score was 60 sec. Control animals had a catalepsy score of 0. Statistical analyses were performed with a two-way analysis of variance followed by a Newman-Keuls test. *, $p < 0.05$; **, $p < 0.01$ compared with values from vehicle-treated mice measured at the same time points; +, $p < 0.05$; ++, $p < 0.01$ compared with values from random oligomer-treated mice.

and second days after cessation of $D_2$ AS treatment but was completely restored by 8 days.

## Discussion

The *in vivo* administration of AS directed to the messenger RNAs encoding a number of receptors has been shown to selectively and reversibly reduce the expression of these receptors and to inhibit the biological functions that they mediate (13–15, 17–19, 21). These studies suggest that an AS strategy offers another powerful pharmacological tool with which to alter biological activity and to uncover specific physiological and biochemical functions that newly discovered receptor subtypes subserve. However, these studies also revealed a long-standing pharmacological dilemma, i.e., functional activity of a neurotransmitter system does not always correlate directly with the density of the target receptors. With regard to the dopaminergic system, dissociations between the density of DA receptors and DA-mediated behaviors have been known for many years; small changes in the density of DA receptors are often associated with profound changes in DA-mediated behavioral responses (37–40). Thus, unilateral denervation of the dopaminergic neurons innervating the corpus striatum results in a profound rotational behavior in response to the administration of $D_2$ DA receptor agonists but a relatively small increase in $D_2$ DA receptors (41, 42). Studies from our laboratory showed that after the administration of EEDQ or FNM, which irreversibly inhibit DA receptors, recovery of behavioral effects induced by DA agonists was more rapid than the recovery of $D_2$ DA receptors. Rotational behavior induced by quinpirole was restored to normal by 48 hr after EEDQ treatment (43), a time at which the $D_2$ DA receptors had recovered by only 20% over their lowest values seen 4 hr after EEDQ treatment (30). Similar conclusions were reached in studies in which mice were treated with FNM (26). Taken together, these results show that the restoration of only a portion of the total pool of DA receptors is sufficient for the restoration of full dopaminergic function, results that suggest that there is a small pool of functional receptors that, when present, are sufficient to completely restore to normal the impaired function mediated by these receptors. The corollary of these experiments is that if the synthesis of this functional pool of receptors were

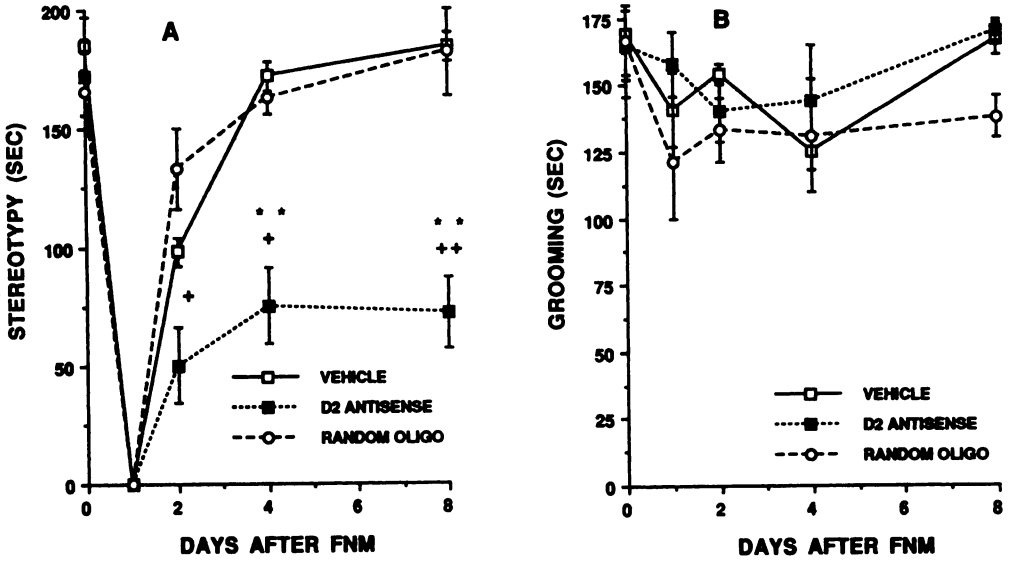


**Fig. 6.** Effect of $D_2$ AS on quinpirole-induced stereotypy and SKF 38393-induced grooming in mice treated with FNM. Mice were pretreated with a single injection of FNM (20 $\mu$mol/kg IP). Starting at 4 hr later, they were administered intraventricular injections of vehicle (2 $\mu$l of artificial cerebrospinal fluid), $D_2$ AS (2.5 nmol/2 $\mu$l), or random oligodeoxynucleotide (2.5 nmol/2 $\mu$l) twice daily for 8 days. Behaviors were measured in separate animal groups at 1, 2, 4, and 8 days after treatment of FNM, always 10 hr after the last injection of vehicle or oligomer. A, Stereotyped behavior induced by quinpirole (5 $\mu$mol/kg SC). B, Grooming behavior induced by SKF 38393 (40 $\mu$mol/kg SC). In each case, the maximum attainable score was 300 sec. In the absence of FNM treatment, vehicle-treated mice had stereotypy scores of 0 and grooming scores of 40 sec. *Points*, mean values ± standard error for five mice. Statistical analyses were performed with a two-way analysis of variance followed by a Newman-Keuls test. **, $p < 0.01$ compared with values from vehicle-treated mice; +, $p < 0.05$; ++, $p < 0.01$ compared with values from random oligomer-treated mice.

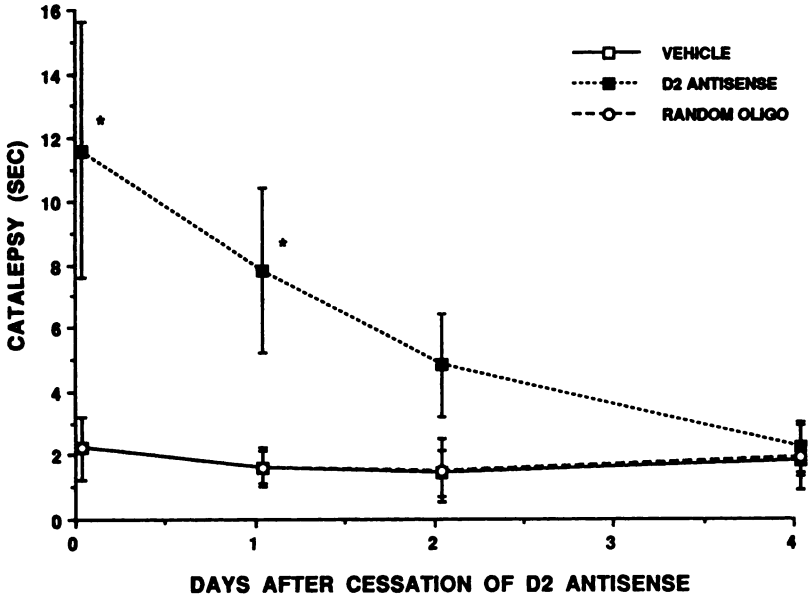


**Fig. 7.** Recovery from FNM-induced catalepsy after cessation of $D_2$ AS treatment in mice. Mice were pretreated with a single injection of FNM (20 $\mu$mol/kg IP). Starting at 4 hr later, they were administered intraventricular injections of vehicle (2 $\mu$l of artificial cerebrospinal fluid), $D_2$ AS (2.5 nmol/2 $\mu$l), or random oligodeoxynucleotide (2.5 nmol/2 $\mu$l) twice daily for 8 days. Catalepsy was measured 10 hr after the last injection of vehicle or oligomer (0 day) and at 1, 2, and 4 days after cessation of treatment with vehicle or oligomer. *Points*, mean values ± standard error for five mice. Statistical analyses were performed with a two-way analysis of variance followed by a Newman-Keuls test. *, $p < 0.05$ compared with values from vehicle- or oligomer-treated mice.

inhibited, the result would be a profound reduction in behaviors mediated by these receptors.

In previous studies (18, 21) on the effects of AS on the $D_2$ DA receptor mRNA, we found that $D_2$ AS administered *in vivo* to mice with unilateral 6-hydroxydopamine-induced lesions of the corpus striatum dramatically inhibited $D_2$ receptor-mediated behaviors but produced only relatively small effects on $D_2$ DA receptors. This led to the suggestion that the newly synthesized pool of $D_2$ DA receptors that was inhibited by $D_2$ AS treatment accounted for a relatively small proportion of the total receptor population but that this pool of receptors was functionally active (18, 21). Therefore, the effects of $D_2$ AS on the density of $D_2$ DA receptors were not easily detected because of the presence of a relatively large pool of nonfunctional receptors that are indistinguishable from functional receptors in conventional DA receptor binding assays. We reasoned that we might more readily observe changes in DA receptors after $D_2$ AS treatment if the total pool (functional and nonfunctional pool) of $D_2$ DA receptors was reduced before we administered the $D_2$ AS. Accordingly, in the present study, we used an irreversible $D_2$ receptor antagonist, FNM, to inactivate the total pool of $D_2$ DA receptors before $D_2$ AS treatment. FNM preferentially inactivates $D_2$ DA receptors with little or no effect on $D_1$ DA receptors (24, 25, 27, 30, 44), making FNM a valuable pharmacological tool with which to study the synthesis of $D_2$ DA receptors and

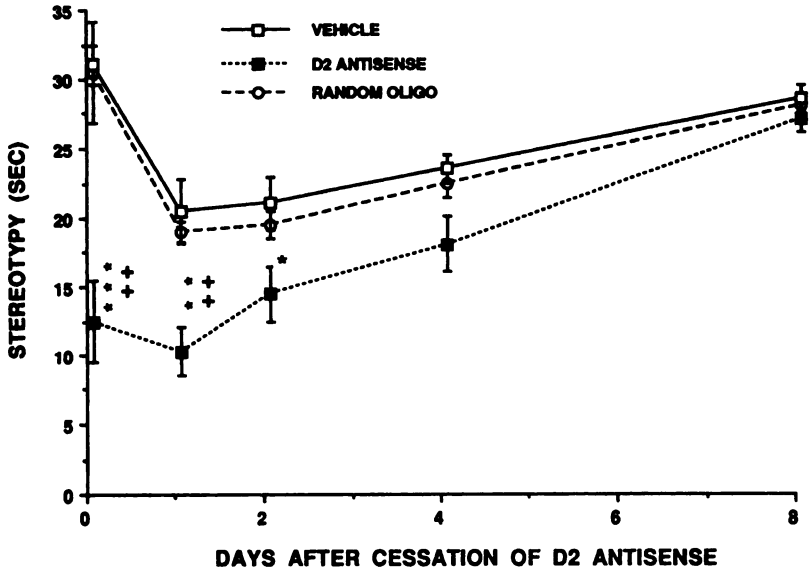


**Fig. 8.** Recovery of quinpirole-induced stereotypy after cessation of $D_2$ AS in mice treated with FNM. Mice were pretreated with a single injection of FNM (20 μmol/kg IP). Starting 4 hr later, they were administered intraventricular injections of vehicle (2 μl of artificial cerebrospinal fluid), $D_2$ AS (2.5 nmol/2 μl), or random oligodeoxynucleotide (2.5 nmol/2 μl) twice daily for 8 days. Stereotyped behavior induced by quinpirole (5 μmol/kg SC) was measured 10 hr after the last injection of vehicle or oligomer (0 day) and at 1, 2, 4, and 8 days after cessation of treatment with vehicle or oligomer. *Points*, mean values ± standard error for five mice. Statistical analyses were performed with a two-way analysis of variance followed by a Newman-Keuls test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with values from vehicle-treated mice at the same time point; ++, $p < 0.01$ compared with values from random oligomer-treated mice.

the recovery of $D_2$ DA receptor-mediated behaviors after treatment with $D_2$ AS.

The results showed that administration of $D_2$ AS into the lateral cerebral ventricle of mice treated with FNM significantly inhibited the rate of recovery of $D_2$ DA receptors in striatum. An analysis of the kinetics of the recovery of $D_2$ DA receptors after FNM treatment showed that $D_2$ AS treatment significantly inhibited the rate of synthesis of $D_2$ DA receptors, presumably by interfering with the translation of $D_2$ receptor mRNA. This is in agreement with previous studies showing that the recovery of $D_2$ DA receptor binding sites after irreversible inactivation requires the synthesis of new receptor proteins (45).

The effects of $D_2$ AS on the recovery of $D_2$ DA receptors after FNM treatment were greater at early stages of the recovery period than at later stages. This may be explained by the accumulation of nonfunctional $D_2$ receptors at the later stages. Some receptors, after being activated by an agonist, are believed to become nonfunctional, either by becoming uncoupled from the G protein (46) or by invaginating into the interior of the cell (47).

$D_2$ AS treatment also produced a decrease in the rate constant for receptor degradation. This could be explained by a mass action mechanism resulting from a reduction in the synthesis and, therefore, a reduction in the levels of $D_2$ DA receptors. Reduced levels of $D_2$ DA receptors would result in a decrease in the rate at which the receptors are degraded.

In confirmation of an earlier study (26), we found that FNM produced behaviors characteristic of $D_2$ DA receptor blockade with no significant effects on $D_1$ DA receptor-mediated behaviors. The recovery of $D_2$ DA receptor-mediated behaviors was accompanied by a recovery of $D_2$ DA receptors. $D_2$ AS treatment significantly delayed the rate of disappearance of FNM-induced catalepsy and delayed the rate of recovery of quinpirole-induced stereotypy. The magnitude of the inhibition of the recovery of $D_2$ receptor-mediated behaviors induced by $D_2$ AS treatment was comparable to the inhibition of the recovery of $D_2$ DA receptors at the early stages of the recovery period after FNM treatment. These studies may explain why inhibition of the synthesis of receptors (e.g., with AS compounds) produces large changes in function without a proportionally large percentage reduction in the total pool of receptors. The results may also help explain why after receptor inactivation, only a relatively small number of newly synthesized receptors must be restored to restore function to normal (22, 26, 39, 40).

In contrast to most classic pharmacological agonists and antagonists, the dose-response curve for AS is relatively steep; in the *in vivo* studies of AS that have been reported, there was only ~1 order of magnitude between the dose that had little or no effect and the dose that induced nonspecific or toxic effects (20, 21, 48–51). Therefore, when studying AS oligodeoxynucleotides, proper controls, such as the inclusion of studies on the effects of random oligomers, are more important than ever. In the present study, the specificity and selectivity of $D_2$ AS were demonstrated at several levels, including the demonstration of nonsignificant effects of $D_2$ AS on $D_1$ DA receptors and on $D_1$ DA receptor-mediated behaviors and of nonsignificant effects of a random oligomer on any parameter examined. We also showed that the effects of $D_2$ AS on these parameters were reversed on cessation of $D_2$ AS treatment, further supporting the specificity of $D_2$ AS.

In summary, the present study demonstrates that *in vivo* administration of $D_2$ AS in mice inhibited the recovery of $D_2$ DA receptors by decreasing their rate of synthesis. $D_2$ AS treatment also inhibited the recovery of $D_2$ DA receptor-mediated behaviors after irreversible receptor inactivation. The degree of $D_2$ AS-induced inhibition of $D_2$ DA receptor production was comparable to the degree of inhibition of $D_2$ receptor-mediated behaviors at the early, but not later, stages of receptor recovery. The effects of $D_2$ AS on $D_2$ DA receptors and $D_2$ receptor-mediated behaviors were selective and reversible. These studies lend further support to the suggestion that $D_2$ AS is a useful pharmacological tool with which to intervene in specific receptor functions and support the notion that the newly synthesized $D_2$ DA receptors constitute a small functional pool of receptors.

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