D2 Dopamine Antisense RNA Expression Vector, Unlike Haloperidol, Produces Long-term Inhibition of D2 Dopamine-Mediated Behaviors without Causing Up-regulation of D2 Dopamine Receptors

GENOVEVA DAVIDKOVA, LONG-WU ZHOU, MARK MORABITO, SUI-PO ZHANG and BENJAMIN WEISS

Department of Pharmacology, MCP-Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, Pennsylvania

ABSTRACT

Long-term inhibition of D2 dopamine receptors using classic D2 dopamine receptor antagonists such as haloperidol often causes a compensatory up-regulation of the D2 dopamine receptors. We investigated whether the long-term inhibition of D2 dopamine receptors using an eukaryotic expression vector housing a cDNA sequence encoding an antisense RNA directed to the D2 dopamine receptor transcript (D2 antisense vector) would also produce up-regulation of the D2 receptors. Single, bilateral injections of the D2 antisense vector into the corpora striata of mice inhibited the stereotypy induced by acute challenge injections with the D2/D3 dopamine receptor agonist quinpirole but did not inhibit the grooming induced by acute challenge injections with the D1 agonist SKF 38393. Similar treatment with the D2 antisense vector produced a long-term (>1 month) cataleptic response without producing tolerance to challenge injections with haloperidol. By contrast, catalepsy induced by a single injection of haloperidol lasted only ~2 days, and tolerance developed to its effects after long-term treatment. Repeated treatment of mice with haloperidol resulted in an inhibition of apomorphine-induced climbing behavior throughout the time of treatment with haloperidol, but the climbing behavior markedly increased to levels significantly higher than that of the control mice immediately after withdrawal from haloperidol treatment. This increased climbing was accompanied by increased levels of D2 dopamine receptors in the striatum. By contrast, single, bilateral intrastriatal injections of the D2 antisense vector significantly inhibited apomorphine-induced climbing for ~30 days but failed to increase the climbing behavior or the levels of D2 dopamine receptors in striatum over those of the control values. These results suggest that a single injection of a D2 antisense RNA expression vector into mouse striatum produces specific, long-term inhibition of D2 dopamine receptor behaviors without causing a compensatory increase in the levels or function of D2 dopamine receptors.

It is well established that receptors for catecholamine neurotransmitters, such as norepinephrine and dopamine, can be up- or down-regulated in response to physiological and pharmacological changes in neuronal input (Tarsy and Baldessarini, 1974; Weiss and Costa, 1967; Weiss et al., 1984; Filtz et al., 1994). For example, decreases in dopamine receptor input produced by the administration of dopamine receptor antagonists, such as haloperidol, have been shown to increase the levels D2 dopamine receptors (Creese et al., 1976; Flemingier et al., 1983) and D2 receptor mRNA (Rogue et al., 1991) and produce dopaminergic supersensitive behaviors (Tarsy and Baldessarini, 1974). Similarly, lesioning dopaminergic terminals with neurotoxins causes an up-regulation of dopamine receptors (Zhou et al., 1994). These findings are supported by clinical data showing that long-term treatment with most antipsychotic agents causes an up-regulation of D2 dopamine receptors, an effect that may result in the production of irreversible motor side effects such as tardive dyskinesia (Seeman, 1987).

There are several possible mechanisms by which the number or function of dopamine receptors may increase in the face of long-term reductions in the stimulation of these receptors; these include a decrease in their rate of destruction (Merlo Pich et al., 1987), an increase in their rate of synthesis (Lisovosky et al., 1992; Rogue et al., 1991) or an increase in the coupling between the receptors and G proteins (Butkerait et al., 1994; Marcotte et al., 1994). Moreover, although it is generally recognized that there is not a direct correspondence between the number of D2 receptors and the behaviors they subserve (Mileson et al., 1991) and that complex interactions exist between the activation of receptors and specific behav-

ABBRVIATIONS: CSF, cerebrospinal fluid; DOTAP, 1,2-dioleolyl-3-trimethylammonium propane; SKF 38393, (±)-SKF 38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine, HCl; FNM, fluphenazine-N-mustard.
ors (Fleminger et al., 1983; Owen et al., 1980), most of the evidence supports the view that increases in the levels of D2 receptors are associated with increases in D2 dopamine receptor-mediated behaviors (Fleminger et al., 1983; Zhou et al., 1994). Therefore, regardless of the specific mechanisms that may be involved in the production of dopaminergic supersensitivity, by inhibiting the synthesis of D2 dopamine receptors, one should be able to reduce dopaminergic function. Furthermore, by inhibiting dopamine receptor synthesis, it may be possible to reduce dopamine-mediated behaviors without the concomitant development of dopaminergically supersensitive responses.

One possible way to achieve this goal is through the use of antisense oligodeoxynucleotides (Weiss, 1997). These agents, which have several advantages over the traditional pharmacological drugs, including their ability to bind to individual receptor subtypes with high selectivity, have been used with success in vivo to block the expression of several different receptors for neurotransmitters (Weiss et al., 1997a). We have previously presented evidence showing that repeated intraventricular administration of an antisense oligodeoxynucleotide directed to the D2 dopamine receptor transcript reduces the formation of a relatively small pool of newly synthesized, functional D2 dopamine receptors (Qin et al., 1995). One of the main disadvantages of using antisense oligodeoxynucleotides in brain, however, is their relatively short duration of action (Zhang et al., 1994; Zhou et al., 1994). To effectively reduce D2 dopaminergic responses, antisense oligodeoxynucleotides have had to be administered either repeatedly (Zhang et al., 1994; Zhou et al., 1994) or continuously (Zhang and Creese, 1993; Weiss et al., 1996) into the brain, because the responses return to normal within a few days after cessation of the antisense treatment (Zhang and Creese, 1993; Zhang et al., 1994; Zhou et al., 1994). It clearly would be desirable to develop an agent that would produce long-term inhibition of the synthesis of dopamine receptors after a single injection.

Recent results from our laboratory have suggested that it is possible to produce long-term, selective inhibition of D2 dopamine-mediated behaviors through the use of plasmid vectors into which has been engineered a DNA sequence encoding an antisense RNA to the D2 dopamine receptor transcript (D2 antisense vector). We have shown that such D2 antisense-generating vectors reduce the levels of D2 dopamine receptors both in vitro in human embryonic kidney cells expressing the D2 dopamine receptors (Davidkova et al., 1998), and in vivo in mouse corpus striatum (Weiss et al., 1997b). In the in vivo studies, a single injection of a D2 antisense vector into the corpus striatum produced selective, long-term inhibition of D2 dopamine-mediated responses in dopaminergically supersensitive mice. These behavioral changes were accompanied by molecular changes that provided evidence of the presence of the D2 antisense vector in mouse striatum for an extended period of time after a single intrastriatal administration and of the presence of D2 antisense RNA in the injected striatum. In the present study, normosensitive animals were used to determine whether this D2 antisense vector can produce long-term reductions in D2 dopamine receptor-mediated behaviors without inducing a compensatory supersensitive behavioral response or an up-regulation of D2 dopamine receptors.

### Methods

#### Animals

Male Swiss-Webster mice (20–24 g), purchased from Ace Animals (Boyertown, PA), were used throughout these studies. The mice were housed in groups of 10 in plastic cages with wood-chip bedding and were provided free access to food and water. All mice were maintained in temperature- and humidity-controlled rooms with a 12-hr light/dark cycle.

#### Construction of a D2 Antisense Vector

The D2 antisense vector used throughout these studies was constructed by PCR cloning using total striatal RNA isolated from mouse brain and the eucaryotic expression vector pCR3 (Invitrogen, San Diego, CA), as described in detail previously (Weiss et al., 1997b). Briefly, the D2 antisense vector contains a 337-bp DNA sequence encoding an antisense RNA directed to a portion of the D2 dopamine receptor transcript. This portion of the transcript encodes a section of the third cytoplasmic loop of the long isoform of the D2 dopamine receptor. This D2 dopamine receptor cDNA sequence (nucleotides, +636 to +972 of the mouse cDNA) was chosen because it shares the least degree of homology with the remaining dopamine receptor subtypes (Mack et al., 1991). Comparative analysis of the D2 antisense sequence with all the sequences currently in the GenBank Database showed no significant homology. The cloning vector pCR3 without the PCR insert, referred to as the empty vector, was used as a negative control throughout the studies.

#### Injection of Vectors

The expression plasmids were purified using Endo-free Qiagen (Valencia, CA) columns according to standard methodology and were dissolved in artificial CSF at a concentration of 10 μg/μl. The plasmids were transfected into mouse brain with the lipid DOTAP (Avanti Polar Lipids, Birmingham, AL). DOTAP was suspended at a concentration of 10 μg/μl in CSF and sonicated in a bath-type sonicator. Plasmid DNA-DOTAP complexes were prepared by mixing the plasmid DNA and DOTAP at a ratio of 2.5:1 and incubating at 37°C before injection. Before the intracerebral injections, the mice were anesthetized with halothane. The injections were made bilaterally into the corpora striata with a Hamilton syringe using a plastic injection mold fitted with a guide cannula (Groodale et al., 1985). The plasmid DNA-DOTAP injections (25 μg DNA and 10 μg DOTAP in a final injection volume of 5 μl/striatum) were administered over a 10-min period.

#### Administration of Drugs

Drugs were freshly prepared and administered in a dose volume of 10 ml/kg. Quinpirole, SKF 38393, apomorphine and FNM (all purchased from Research Biochemicals, Natick, MA) were dissolved in 0.1% ascorbic acid. Haloperidol (Sigma Chemical, St. Louis, MO) was dissolved in 0.1% acetic acid. [3H]Raclopride (specific activity, 69.5 Ci/mmol) was purchased from Dupont-New England Nuclear (Boston, MA).

#### D2 Dopamine Receptor Binding Assay

The density of D2 receptors was assessed in washed striatal membranes from the binding of [3H]Raclopride (2 nM), with specific binding defined as that displaced by 10 μM sulpiride. Striatal membranes were prepared and receptor binding analyses performed as previously described (Winkler et al., 1987).

#### Behavioral Measurements

**Stereotyped and grooming behaviors.** Mice were transferred to individual plastic cages (17 × 28 cm) in a behavioral testing room and were allowed 20 min to adapt to this environment. Stereotyped and grooming behaviors were measured as described earlier (Zhou et al., 1991; Zhang et al., 1994). For the measurement of stereotypy,
mice were administered quinpirole (5 μmol/kg s.c.), and 5 min later, stereotyped behavior was measured for 20 sec at 4-min intervals for a total period of 60 min (i.e., 15 times during the 60-min observation period, with the maximum possible score being 45). To determine grooming behavior, mice were administered SKF 38393 (40 μmol/kg s.c.), and 5 min later, grooming was measured for 20 sec at 4-min intervals for a total period of 60 min (maximum score, 300 sec).

Catalepsy. On the day of testing, animals were moved to the testing room for 30 min. Catalepsy was determined by placing the forepaws of the animals over a metal horizontal bar that was 0.3 cm in diameter, 30 cm long and at a height of 6.5 cm above the working surface. The time that the animals maintained their forepaws in a stationary position on the bar was taken as the cataleptic score.

Climbing behavior. A modification of the basic procedure of Protas et al. (1976) was used to assess apomorphine-induced climbing behavior. The animals were placed individually into cylindrical wire mesh cages that were closed at the top and had a diameter of 15 cm and a height of 15 cm. After a 1-hr habituation period, the animals were given apomorphine (4 or 16 μmol/kg s.c.) and placed back into the cage. Beginning 10 min after the injection of apomorphine, climbing behavior was scored for 20 sec every 4 min for a total period of 1 hr, according to the following scale: 0 = four paws on the floor, 1 = two or three paws holding the cage wire and one or two paws on the floor, 2 = intermittent climbing up the side of the cage, 3 = continuous climbing up the side of the cage, 4 = two paws holding the top of the cage, and 5 = four paws holding the top of the cage. The scores obtained during the entire 60 min were determined, with the maximal possible score being 75.

**Statistical Analyses**

Single statistical comparisons of a drug-treated group to its control group were performed using an independent Student’s t test. Comparisons of two groups were performed using a two-way analysis of variance followed by a Newman-Keuls test.

**Results**

**D₂ antisense vector inhibits quinpirole-induced stereotypy but not SKF 38393-induced grooming.** To determine the specificity by which the D₂ antisense vector can inhibit dopamine receptor-mediated behaviors, mice were administered single, bilateral injections of the D₂ antisense vector or a control, empty vector into the striata, after which stereotyped behavior induced by acute challenge injections of quinpirole or grooming behavior induced by SKF 38393 were measured. Figure 1 shows that in mice injected with the D₂ antisense vector, there was a statistically significant inhibition of the stereotypic behavior induced by the D₂ agonist quinpirole (fig. 1A) but no significant change in the grooming behavior induced by the D₁ agonist SKF 38393 (fig. 1B). Mice treated with the empty vector evidenced no significant changes in either quinpirole-induced stereotypy or SKF 38393-induced grooming.

**D₂ antisense vector produces a long-term cataleptic response.** To determine whether the D₂ antisense vector could produce a cataleptic response similar to that induced by selective D₂ dopamine receptor antagonists, mice were given single, bilateral intrastratal injections of the D₂ antisense vector, empty vector or DOTAP, and catalepsy was measured at various times thereafter. Figure 2 shows that a significant degree of catalepsy was apparent as early as 1 day after treatment with the D₂ antisense vector and that this behavior persisted for up to 34 days. Mice treated with the empty vector or DOTAP alone failed to induce significant catalepsy at any time point studied.

**Haloperidol produces a short-term cataleptic response.** To compare the duration and other properties of the cataleptic response induced by the D₂ antisense vector with that induced by a classic D₂ dopamine receptor antagonist,
mice were administered single or repeated injections of haloperidol, and catalepsy was measured. Figure 3A shows the dose-response curve for acute injections of haloperidol in producing catalepsy. A significant cataleptic response was seen with doses as low as 0.6 μmol/kg. Figure 3B shows the time course of the catalepsy induced by acute administration of haloperidol (0.6 μmol/kg) in mice. As may be seen, a significant degree of catalepsy appeared at the earliest time point measured (40 min) and then rapidly disappeared so that there was no significant effect beyond 2 days after treatment. Based on this experiment, two doses were selected for subsequent studies: a submaximal dose (0.6 μmol/kg), which produced a degree of catalepsy similar to that induced by treatment with the D2 antisense vector (see fig. 2), and a dose of haloperidol (3 μmol/kg) that produced a near-maximum cataleptic response.

Figure 4 shows the time course of the catalepsy induced by repeated, daily administration of haloperidol (0.6 μmol/kg). As may be seen, the catalepsy lasted throughout the entire period of treatment but rapidly disappeared on cessation of the haloperidol injections.

**Pretreatment with haloperidol, but not with the D2 antisense vector, produces tolerance to catalepsy induced by acute challenge injections of haloperidol.** Long-term inhibition of D2 dopamine receptors often leads to tolerance to these receptor-mediated responses, presumably because chronic reduction in D2 receptor function may cause an up-regulation of these receptors. To compare the development of tolerance in mice chronically treated with haloperidol with that produced by the D2 antisense vector, mice were administered either single, bilateral intrastriatal injections of the D2 antisense vector (or empty vector) or repeated, daily injections of haloperidol (or vehicle) for 26 days. At 26 days after treatment with the vectors or 1 day after the last injection of haloperidol, the mice were administered an acute challenge injection of haloperidol, and the degree of catalepsy was determined. Figure 5 shows that pretreatment of mice either with single injections of the D2 antisense vector or with repeated injections of haloperidol induced a statistically significant cataleptic response compared with the responses seen in animals pretreated with the empty vector or with the haloperidol vehicle, indicating that both of these treatments produced long-term inhibition of this D2 dopamine receptor-mediated response (open bars; no challenge). However, there was a different response to acute challenge injections of haloperidol (hatched bars); mice chronically pretreated with haloperidol showed a significantly smaller cataleptic response than those pretreated with the D2 antisense vector, the haloperidol vehicle or the empty vector, whereas mice pretreated with single injections of the D2 antisense vector showed a normal cataleptic response to acute challenge injections of haloperidol.

**Time course of the effect of the D2 antisense vector on apomorphine-induced climbing.** Figure 6 shows the dose-response curve of apomorphine-induced climbing in...
mice. As can be seen, apomorphine produced a dose-related increase in climbing, with an ED_{50} value of ~6 μmol/kg. To determine whether the D_2 antisense vector can persistently inhibit a dopamine receptor-mediated behavior without inducing a supersensitive dopaminergic response, mice were administered single, bilateral intrastriatal injections of the D_2 antisense vector or the empty vector, and apomorphine-induced climbing behavior was measured at various times thereafter. Figure 7 shows that the D_2 antisense vector significantly inhibited this behavior for up to 28 days, after which the climbing behavior gradually recovered to the levels measured before injecting the D_2 antisense vector. The D_2 antisense vector failed to induce an increase in apomorphine-induced climbing at any time-point measured (up to 56 days), even though relatively high doses of apomorphine (16 μmol/kg) were used.

Withdrawal from chronic administration of haloperidol produces a supersensitive climbing response to apomorphine. To compare the effect of the D_2 antisense vector on a dopamine-mediated behavior with that produced by a classic D_2 receptor antagonist, mice were pretreated with haloperidol once daily for 21 days and apomorphine-induced climbing was measured at various times: before injecting haloperidol, during chronic treatment with haloperidol and after withdrawal from haloperidol treatment. Two doses of haloperidol and apomorphine were used: one dose of haloperidol (0.6 μmol/kg/day) produced the same degree of catalepsy as did the D_2 antisense (compare figs. 2 and 3), and the other (3 μmol/kg/day) was similar to that used by other investigators in studies of haloperidol-induced dopaminergic supersensitivity (Protais et al., 1976; Fields et al., 1990). A relatively high dose of apomorphine (16 μmol/kg) was chosen because this was the one used in the previous experiment on the effects of pretreatment with the D_2 antisense on apomorphine-induced climbing (fig. 7). A lower dose of apomorphine (4 μmol/kg) was chosen to determine whether haloperidol could produce a supersensitive response even to low doses of this dopamine receptor agonist. The results showed that when mice were given repeated injections of low doses of haloperidol, the climbing behavior induced by relatively high doses of apomorphine was significantly decreased throughout the entire period of haloperidol treatment but was significantly increased at 1, 2, 4 and 8 days after withdrawal...
from the haloperidol treatment (fig. 8A). Figure 8B shows that in mice given repeated injections of higher doses of haloperidol, the climbing behavior induced by a lower dose of apomorphine also was significantly inhibited during chronic haloperidol treatment and significantly increased at 1, 2, 4 and 8 days after withdrawal from the treatment with haloperidol.

**Treatment with haloperidol, but not with the D2 antisense vector, produces an up-regulation of D2 dopamine receptors.** To determine whether the relative abilities of haloperidol and the D2 antisense vector to produce tolerance to catalepsy or to induce an increase in apomorphine-induced climbing may be related to their relative abilities to produce an up-regulation of the D2 receptors, the density of D2 dopamine receptors in striatum was measured in mice pretreated with haloperidol, the D2 antisense vector or vehicle. In these studies, one group of mice was administered haloperidol or vehicle once daily for 21 days, whereas another group of mice received single, bilateral intrastriatal injections of the D2 antisense vector. The density of D2 dopamine receptors in the striatum was measured 3 days after haloperidol was withdrawn (i.e., at 24 days after the initial dose of haloperidol) and 26 days after the single treatment with the D2 antisense vector. Figure 9 shows that 3 days after withdrawal from chronic administration of haloperidol (the time at which there was a significant increase in the climbing response to apomorphine; see fig. 8), there was a significant increase in the levels of striatal D2 dopamine receptors. In contrast, single, bilateral intrastriatal administration of the D2 antisense vector failed to increase the levels of striatal D2 dopamine receptors.

**Effect of D2 antisense vector on the time course of the catalepsy produced by FNMe.** To determine whether the catalepsy induced by the D2 antisense vector may be associated with the inhibition of a relatively small pool of functional D2 receptors, mice treated with the D2 antisense vector were administered a single dose of FNMe, a nitrogen mustard analog of the phenothiazine fluphenazine that has been shown to be a relatively selective, irreversible inhibitor of D2 dopamine receptors (Qin et al., 1995). Our goal in administering FNMe was to inactivate the total pool of D2 dopamine receptors and then to determine the effects of treatment with the D2 antisense vector on the disappearance of FNMe-induced catalepsy. In these experiments, mice were given bilateral intrastratal injections of the D2 antisense vector (or empty vector). Five days after the vector treatment, the mice were administered a single injection of FNMe, and the recovery from FNMe-induced catalepsy was measured. As may be seen (fig. 10), treatment with the D2 antisense vector slowed the disappearance of FNMe-induced catalepsy compared with the empty vector-treated group. Statistically significant differences were seen for as long 16

![Fig. 8. Time course of the effects of chronic administration of haloperidol on apomorphine-induced climbing. Mice were administered haloperidol (either 0.6 μmol/kg i.p. (A) or 3 μmol/kg i.p. (B)) or vehicle once daily for 21 days. The animals were challenged with apomorphine (either 16 μmol/kg s.c. (A) or 4 μmol/kg s.c. (B)) at 3, 6, 12, 18 and 21 days during haloperidol treatment (the challenge injections of apomorphine were given and climbing behavior was measured before the subsequent injections of haloperidol) and at 1, 2, 4, and 16 days after withdrawal from the haloperidol treatment. Apomorphine-induced climbing behavior was determined as described in the legend to figure 6. Each point represents the mean values from 5 mice. Vertical brackets indicate the S.E. *P < .05; **P < .01; ***P < .001 compared with values from mice treated with vehicle.

![Fig. 9. Pretreatment with haloperidol, but not with the D2 antisense vector, produces an up-regulation of D2 dopamine receptors. Four groups of mice were studied. In the first two groups, mice were injected with vehicle or haloperidol (0.6 μmol/kg i.p.) once daily for 21 days. In the other two groups, mice were injected bilaterally into each corpus striatum with either the D2 antisense vector or empty vector (25 μg each). Three days after the last injection of haloperidol and 26 days after the single, bilateral injections of the D2 antisense vector, the striata were removed, and the density of D2 dopamine receptors was measured as described in Methods. Each column represents the mean value from 5 mice. Vertical brackets indicate the S.E. *P < .05 compared with values from vehicle-, D2 antisense vector- or empty vector-treated mice.
of D2 dopamine receptors after their irreversible inhibition the recovery of D2 dopamine receptors in the striatum after the receptors were inhibited with FNM. Mice were administered a single injection of FNM (20 μmol/kg i.p.), and catalepsy was determined at various times thereafter. Each point represents the mean value from 8 to 14 mice. Vertical brackets indicate the S.E. *P < .05; **P < .01; ***P < .001 compared with corresponding values from the empty vector-treated mice.

Effect of D2 antisense vector on the rate of recovery of D2 dopamine receptors after their irreversible blockade with FNM. To determine whether the slower disappearance of FNM-induced catalepsy produced by the D2 antisense vector may be related to the inhibition of the synthesis of D2 dopamine receptors, the density of D2 dopamine receptors was measured at various time points after a single dose of FNM in mice treated with the D2 antisense vector. Fig. 11 shows that treatment with FNM greatly reduced the levels of D2 dopamine receptors in both the antisense vector- and empty vector-treated animals at 4 hr after treatment. Treatment of mice with the D2 antisense vector significantly inhibited the recovery of D2 dopamine receptors in the striatum after the receptors were inhibited with FNM. As in the previous experiment, statistically significant effects of the D2 antisense vector on the density of dopamine receptors (as compared to the effects of the empty vector) were seen for as long as 16 days after the injection of FNM (i.e., 21 days after the antisense treatment).

Discussion

Recent studies from our laboratory showed that a single, unilateral intrastriatal injection of an expression vector that generates antisense RNA to the transcript encoding the D2 dopamine receptor produced selective, long-term inhibition of the rotational behavior mediated by D2 dopamine agonists in 6-hydroxydopamine-lesioned mice (Weiss et al., 1997b). In the present study, we investigated further the specificity by which this vector inhibits dopamine receptor-mediated behaviors in normal animals. We also addressed whether the D2 antisense RNA produced by the D2 antisense vector can produce long-term inhibition of D2 dopamine receptors in brain without inducing D2 dopamine receptor up-regulation.

The results showing that single, bilateral injections of the D2 antisense vector into the corpora striata of mice inhibited the stereotyped behavior induced by the D2 receptor agonist quinpirole but not the grooming behavior induced by the D1 receptor agonist SKF 38393 provided further evidence for the specificity of this vector. These data are in agreement with other results showing that bilateral injections of the D2 antisense vector into the corpora striata of mice decreased the levels of D2, but not D1, dopamine receptors (Weiss et al., 1997b).

The cataleptic response induced by typical neuroleptics, such as haloperidol and phenothiazines, has been attributed to blockade of dopamine receptors in the striatum or in the nucleus accumbens (Sanberg, 1980; Ossowska et al., 1990) and has been found to correlate positively with their binding to D2, but not D1, dopamine receptors (Hyttel, 1986; Fleming et al., 1983). To study further the biological effects of the D2 antisense vector, we determined the cataleptic effect of the D2 antisense vector and compared it with that of the traditional neuroleptic drug haloperidol. The results showed that both treatments produced catalepsy. However, whereas the cataleptic behavior induced by acute or chronic injections of haloperidol quickly disappeared by 2 to 3 days after cessation of treatment, the catalepsy produced by single, bilateral intrastriatal injections of the D2 antisense vector lasted ~34 days. The intensity of the catalepsy induced by treatment with the D2 antisense vector was equal to that induced by an acute injection of 0.6 μmol/kg of haloperidol, a dose that has been shown to inhibit apomorphine-induced behaviors (Costall et al., 1978; Starr and Starr, 1986). Compared with the effect of a D2 antisense oligodeoxynucleotide (Qin et al., 1995), the degree of catalepsy induced by the D2 antisense vector was much higher and of longer duration.
The inhibition of apomorphine-induced climbing behavior in mice is widely used as a model for the detection of neuroleptic activity (Costall et al., 1978; Wilcox et al., 1980). This test is of particular value because both typical and atypical neuroleptics antagonize this response (Costall et al., 1978; Martres et al., 1977) and because this behavior is mediated through a striatal pathway (Martres et al., 1977; Protais et al., 1976). The results showing that single, bilateral intrastriatal injections of the D₂ antisense vector in normal mice inhibited apomorphine-induced climbing behavior and that this effect lasted ~1 month indicated again that the D₂ antisense vector produced specific, long-term inhibition of the function of D₂ dopamine receptors, inhibition comparable to that induced by classic D₂ receptor antagonists, but of far longer duration.

Most traditional antipsychotic agents are thought to exert their therapeutic effects in neuropsychiatric illnesses through blockade of the D₂ dopamine receptors (Seeman, 1992; Creese et al., 1976). However, these drugs are relatively nonselective in that they block several dopamine receptor subtypes (Schoots et al., 1995; Sumiyoshi et al., 1994) as well as the receptors for a number of other neurotransmitters (See et al., 1990; Vasar et al., 1990). Furthermore, they often up-regulate the very receptors they are designed to inhibit, resulting in undesirable and often irreversible motor disturbances (Chouinard, 1991). For example, continuous administration of D₂ dopamine receptor antagonists such as haloperidol has been shown to induce dopamine receptor up-regulation and concomitant supersensitive behaviors in response to dopamine receptor agonists (Fleminger et al., 1983). These supersensitive dopaminergic responses in the nigrostriatal pathway are thought to be responsible for the motor side effects produced by neuroleptic drugs (Chouinard, 1991). Although the biochemical and molecular mechanisms involved are complex (Coppens et al., 1995; Doucet et al., 1996), chronic treatment with haloperidol is almost always accompanied by dopamine receptor-mediated behavioral supersensitivity and an up-regulation of D₂ dopamine receptors (Fleminger et al., 1983; Hyttel, 1986). The present data suggest that by reducing the synthesis of the pool of functional D₂ dopamine receptors with a plasmid vector that generates an antisense RNA directed to the D₂ dopamine receptor transcript, one can reduce dopaminergic function without inducing a dopaminergically supersensitive response. Thus, we showed that administering the D₂ antisense vector produced a prolonged inhibition of D₂ dopamine receptor-mediated behaviors without causing a subsequent increase in dopamine receptors or an increase in dopamine agonist-mediated behaviors. This is in contrast to the effects produced by haloperidol. For example, the chronic administration of haloperidol resulted in a significant increase in apomorphine-induced climbing after withdrawal from haloperidol treatment. These results are in agreement with those of several investigators who reported a supersensitive response to dopamine agonists in spiroperidol- or haloperidol-treated mice (Wilcox et al., 1980; Grebb et al., 1997). In contrast, single, bilateral intrastriatal injections of the D₂ antisense vector, which like repeated administration of haloperidol also inhibited apomorphine-induced climbing behavior, failed to produce an increase in apomorphine-induced climbing at any time point studied. These results suggest that treatment with the D₂ antisense vector significantly inhibited a dopaminergic-mediated behavior without causing a compensatory supersensitive D₂ dopaminergic behavioral response.

To determine further whether treatment with the D₂ antisense vector produces dopaminergic supersensitivity, we measured the development of tolerance to catalepsy in mice treated with single, bilateral injections of the D₂ antisense vector and compared this effect with that seen in mice chronically treated with haloperidol. We found that the cataleptic response induced by acute challenge injections of haloperidol was significantly decreased at 1 day after repeated daily injections of haloperidol for 26 days. In contrast, the catalepsy induced by acute challenge injections of haloperidol did not decrease at 26 days after single, bilateral intrastriatal treatment with the D₂ antisense vector. These results indicate that unlike treatment with haloperidol, treatment with the D₂ antisense vector produced catalepsy but did not produce tolerance to the catalepsy induced by acute challenges with haloperidol. Because there appears to be a correlation between the time course of the development of tolerance to the effects of a neuroleptic with the rate of development of dopaminergic supersensitivity (Asper et al., 1973; Muller and Seeman, 1978), we suggest that the inability of the D₂ antisense vector to induce tolerance to the catalepsy induced by acute injections of haloperidol resulted from its inability to cause dopaminergic supersensitivity.

To determine whether the administration of the D₂ antisense vector produces up-regulation of the D₂ receptors, the density of D₂ receptors in striatum was measured after bilateral intrastriatal injections of the D₂ antisense vectors or after repeated daily injections of haloperidol. We found that daily treatment with haloperidol for 21 days followed by 3 days of withdrawal from haloperidol treatment resulted in a significant increase in the levels of D₂ dopamine receptors in striatum. In contrast, mice treated with the D₂ antisense vector did not evidence any increase in the levels of D₂ receptors when measured 26 days after its single injection. These results suggest that the D₂ antisense vector, unlike haloperidol, did not cause an up-regulation of D₂ dopamine receptors.

The current experiments, as well as earlier studies using D₂ antisense strategies (Weiss et al., 1993, 1997c; Zhou et al., 1994; Davidkova et al., 1996), revealed that treating mice with D₂ antisense oligodeoxynucleotides caused a marked inhibition of D₂ dopamine receptor-mediated behaviors but only a modest decrease in the density of D₂ dopamine receptors. To understand better why the functional activity of a neurotransmitter system does not always correlate directly with the density of the target receptors (see Engber et al., 1993; Staunton et al., 1981; Winkler and Weiss, 1989), we have taken advantage of the selective and irreversibly acting D₂ dopamine receptor antagonist FNM. This compound has been shown to inhibit the total pool of D₂ dopamine receptors but to have little or no effects on D₁ dopamine receptors (Winkler et al., 1987; Chen et al., 1994; Qin and Weiss, 1994). In earlier studies, we found that treatment of mice with a D₂ antisense oligodeoxynucleotide decreased the rate of recovery of D₂ receptors and inhibited the recovery of D₂ dopamine receptor-mediated behaviors after treatment with FNM (Qin et al., 1995). These results suggested that the D₂ antisense oligodeoxynucleotide inhibited a newly synthesized pool of D₂ dopamine receptors, a pool that likely accounted for a relatively small proportion of the total receptor population but
was functionally active (Qin et al., 1995; Weiss et al., 1997c). In the present study, we reasoned that we would more readily observe any subtle inhibitory effect on the levels of D2 dopamine receptors and D2 receptor-mediated behaviors after D2 antisense vector treatment if the total pool of D2 receptors was reduced with FNM after administration of D2 antisense vector. These results showed a significantly slower disappearance of FNM-induced catalepsy and a significantly slower recovery of D2 dopamine receptors in mice that had received bilateral intrastriatal injections of the D2 antisense vector. Both the cataleptic behavior and the density of D2 dopamine receptors recovered to control values at 32 days after the treatment of FNM. These results suggested that a single injection of the D2 antisense vector appears to have a long-term inhibitory effect on the synthesis of a functional pool of D2 dopamine receptors.

In summary, these data showed that a nonviral plasmid vector expressing antisense RNA directed to the mRNA encoding the D2 dopamine receptor can specifically and persistently induce catalepsy and inhibit apomorphine-induced climbing. These behavioral effects are similar to those produced by haloperidol. However, unlike haloperidol, the D2 antisense vector failed to cause either dopaminergic supersensitivity (manifested by either tolerance to catalepsy or an increase in apomorphine-induced climbing) or an up-regulation of D2 dopamine receptors. These studies, combined with those in which the effects of the D2 antisense vector were determined in mice in which the D2 dopamine receptors were inhibited with FNM, suggest that the D2 antisense vector inhibits a pool of functional D2 dopamine receptors and that new receptor synthesis is required for receptor up-regulation to occur. These results may have clinical significance because they suggest the means by which one may overcome the problem of up-regulation of neurotransmitter receptors after their chronic blockade, an effect that may be responsible for the tolerance that develops to these agents and which may be the cause of certain motor side effects. Thus, the results from this study may aid in the development of novel molecular biological approaches to study and treat disorders associated with increased dopaminergic activity in the central nervous system.

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Send reprint requests to: Benjamin Weiss, Ph.D., Department of Pharmacology, MCP-Hahnemann School of Medicine, Allegheny University of the Health Sciences, 3200 Henry Avenue, Philadelphia, PA 19129.