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Intranigral Administration of D_2 Dopamine Receptor Antisense Oligodeoxynucleotides Establishes a Role for Nigrostriatal D_2 Autoreceptors in the Motor Actions of Cocaine

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SUMMARY

Dopamine D_2 autoreceptors found on nigrostriatal dopaminergic neurons are thought to inhibit dopamine release, tyrosine hydroxylase activation, and spontaneous firing rate. It is likely that these receptors play an important role in moderating the behavioral response to cocaine, but the lack of potent selective autoreceptor ligands has made it difficult to assess this contribution. We have developed an antisense phosphorothioate oligodeoxynucleotide (ODN) against D_2 receptor mRNA, which was used to reduce levels of D_2 receptors *in vitro* and *in vivo*. Unilateral administration of antisense ODN, via intracerebral cannula, into the substantia nigra of rats for several days caused dramatic contralateral rotational behavior in response to a subcutaneous injection of cocaine. This effect was maximal by 10 min after injection of cocaine and lasted for >30 min; without cocaine, no spontaneous rotational behavior was noted. In striatal slices, the potency of sulpiride, a D_2 antagonist, in enhancing electrically stimulated dopamine release was significantly reduced on the antisense-treated side; this is consistent with a decrease in the striatal D_2 autoreceptor population. As measured by quantitative autoradiography, administration of antisense ODN caused a loss of approximately 40% of nigral D_2 receptor [^{125}I]iodosulpride binding, compared with the untreated side. *In vitro*, treatment of WERI-27 retinoblastoma cells with D_2 antisense ODN at a concentration of 1 μM reduced D_2 receptor levels by 57% after 3 days. The robustness of cocaine-induced rotation and the impaired ability of sulpiride to enhance dopamine release from slices suggest that nigrostriatal D_2 autoreceptors play a direct role in reducing the motor response to cocaine administration. Furthermore, the absence of spontaneous rotation in antisense ODN-treated animals suggests that autoreceptor effects are masked by compensatory mechanisms during normal behavior.

Autoreceptors found on axon terminals and soma/dendrites of dopaminergic neurons originating in the substantia nigra appear to inhibit dopamine release, tyrosine hydroxylase activity, and neuronal firing rate in response to dopamine agonists, and these receptors have been pharmacologically characterized as the D_2 dopamine receptor subtype. Behaviorally, motor activity is decreased by low doses of the mixed D_1/D_2 agonist apomorphine *in vivo* (1), an effect thought to be due to activation of the higher affinity D_2 autoreceptors. In superfusion studies D_2 agonists and antagonists decrease and enhance, respectively, dopamine release from electrically stimulated striatal slices (2). This finding also has been extended to autoreceptor control of *in vivo* dopamine release, as measured by microdialysis (3). In synaptosomal preparations and in striatal minces, D_2 agonists reduce the activity of tyrosine hydroxylase, the rate-limiting enzyme of the catecholamine synthetic pathway, via interaction with the autoreceptor (4). Electrophysiologically, application of low dose apomorphine directly to nigral slices or systemically in intact animals results in a depression of nigral firing rates (5, 6).

Although the data reported to date document aspects of the existence and function of the D_2 autoreceptor, there remains considerable doubt as to the behavioral function of these receptors in situations where dopaminergic activity is high, such as in the response to stimulant drugs such as cocaine (7, 8). Cocaine blockade of dopamine transporters inhibits dopamine reuptake and enhances dopamine overflow from nigrostriatal neurons, yet D_2 autoreceptors may play an important role in controlling this release. The best way to establish a role for the D_2 autoreceptor in the action of cocaine would be the use of autoreceptor-selective antagonists. However, currently available compounds are only partially selective for the D_2 autoreceptor, and their effects on autoreceptor-mediated events have complex profiles that depend on the dose, system (*in vitro* versus *in vivo*), specific behavior, and species studied (8–11).

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ABBREVIATION: ODN, oligodeoxynucleotide.

One recent development that may obviate the need for specific antagonists is the use of specific antisense ODNs, which has recently been advanced as a method for investigating specific functions of the central nervous system. Exposure of cells to antisense ODNs leads to hybridization of the ODN with cognate mRNA and subsequently to interference with protein expression (12). This technique offers the possibility of directly determining a role for key proteins in the neuronal signal transduction cascade, and it has the potential to be selective and reversible. Antisense constructs and ODNs have been used *in vitro* to reduce the expression of a number of neuronal proteins, including neurotransmitter receptors (13–15). More recently, antisense ODNs have been injected intracerebroventricularly to alter brain neuropeptide Y receptors (16) and *N*-methyl-D-aspartate receptors (17) *in vivo*, resulting in behavioral and biochemical changes in the animals. In contrast to the approach of injecting large amounts of ODNs intracerebroventricularly, the localized injection of ODNs into specific bed nuclei provides a means to determine the function of a particular receptor in the terminal region of an anatomically distant site. This procedure clearly segregates presynaptic from postsynaptic receptor effects. We have used the unilateral intranigral injection of antisense ODNs to examine the contribution of nigrostriatal D_2 autoreceptors to dopaminergic neuronal function elicited by the psychostimulant cocaine. The results indicate in a direct fashion that the D_2 autoreceptor plays an important role in the control of stimulated dopamine release from striatal slices and of motor behavior elicited by cocaine in intact rats.

Experimental Procedures

ODNs. Antisense (5′-AGATTCAGTGGATCCAT-3′), sense (5′-ATGGATCCACTGAATCT-3′), and missense (5′-AGCATTGAACA-AGCCAT-3′) phosphorothioate ODNs were purified by precipitation and were resuspended in sterile artificial cerebrospinal fluid (124 mM NaCl, 1 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 10 mM glucose) at a concentration of 50 μM. The antisense ODN was complementary to the first 17 nucleotides in the coding block of the human D_2 receptor, and the missense ODN was designed to contain the same G-C content as the antisense ODN.

Cell culture. WERI-27 cells (18, 19) stably expressing dopamine receptors were maintained in RPMI 1640 medium containing 10% horse serum, 5% fetal bovine serum, penicillin 100 μ/ml, and streptomycin 100 μg/ml, at 37°. To determine the effect of various ODNs on D_2 receptor function, cells were pelleted, washed with serum-free RPMI medium without antibiotics, and resuspended in 1 ml of the same medium at a density of 4×10^6 cells/ml. ODNs were prepared in serum-free RPMI medium at a concentration of 2 μM and were complexed with 20 μl/ml Lipofectin by incubation at 37° for 10 min. To each culture, 1 ml of complexed ODN solution was added, and cells were returned to the incubator for 6 hr, with rotation. Ten milliliters of complete RPMI medium were then added and the cells were allowed to grow for an additional 18 hr. The cells were then pelleted and treated again with complexed ODNs for 6 hr, as described above. At the end of the ODN exposure period, complete medium was added as before, and cells were allowed to grow for an additional 12 hr before being counted and harvested for binding.

Receptor binding. D_2 receptor densities were determined with a concentration of 0.5 nM [^{125}I]iodosulpride (Amersham); nonspecific binding was determined in the presence of 1 μM (+)-butaclamol. Briefly, cells were washed with ice-cold phosphate-buffered saline and resuspended in 1 ml of ice-cold lysis buffer (10 mM Tris·HCl, 0.5 mM EDTA, pH 7.5). Cells were then homogenized with a Polytron homogenizer for 15 sec and centrifuged for 20 min at 30,000 × *g* to recover membranes. Pellets were resuspended with a syringe in 500 μl of ice-cold binding buffer (50 mM Tris·HCl, 120 mM NaCl, 0.5 mM EDTA, pH 7.5). Membrane aliquots were incubated for 1 hr at room temperature with radioligand in binding buffer, in the presence or absence of (+)-butaclamol, and bound ligand was separated from free by rapid filtration on a Brandel cell harvester (Gaithersburg, MD). Filters were washed three times with cold binding buffer, and bound radioactivity was measured by γ scintillation counting. Proteins were measured using the Bradford assay, and specifically bound counts were converted to femtomoles bound/milligram of protein.

Animals. Male Sprague-Dawley rats weighing between 150 and 200 g (Charles River Laboratories) were acclimated to the vivarium, on a 12-hr light/dark cycle (light between 7 a.m. and 7 p.m.), for 1 week before surgery. Animals were housed in pairs, with continuous access to food and water.

Cannula implantation and ODN administration. Under pentobarbital anesthesia (65 mg/kg, intraperitoneally), animals were shaved and placed in a Kopf rat stereotaxic device on a warm heating pad. Using sterile technique, the dorsal surface of the skull was exposed and a hole was drilled to yield an implantation site corresponding to −5.8 mm bregma, 1.8 mm lateral, according to the atlas of Paxinos and Watson (20). A sterilized guide cannula (21) was lowered into place, with the tip resting 2–3 mm above the substantia nigra pars compacta, and was fixed in place with dental cement and screws. A stylet was inserted to maintain patency, and the entire structure was covered with a plastic cap. Wounds were closed with surgical clips, and the animals were allowed to recover with tetracycline treatment for 7 days before exposure to experimental conditions. Cannula placement was confirmed by postexperimental dye injection or by direct visualization of the cannula tract after sectioning.

ODN solutions (50 μM) were administered to conscious unrestrained animals via an internal cannula that extended beyond the guide cannula to the substantia nigra. Solutions were administered in a volume of 0.8 μl over 1 min with a Harvard pump, and the internal cannula was left in place for an additional 2 min. ODN treatments began after a 7-day recovery period, and animals received one dose every 12 hr for a total of five doses. Subsequent measurements were performed 4 hr after the last ODN dose.

Receptor autoradiography. Four hours after completion of ODN treatment, brains were removed rapidly after sacrifice and were frozen by being gently lowered into −40° isopentane. Frozen brains were stored at −80° in sealed tubes until processing. Brains were brought to −14° in a cryostat, and 20-μm sections were made and thaw-mounted on subbed glass slides. Slides were stored at −80°, with desiccant capsules, until processing for autoradiography. D_2 receptor autoradiography was performed according to the method of Bouthenet *et al.* (22), with modifications. Slides were brought to room temperature and fixed with 0.5% formaldehyde in phosphate buffer, pH 7.4, for 10 min. After being dipped briefly in binding buffer (50 mM Tris·HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5.7 mM ascorbate, pH 7.4), slides were incubated for 30 min at room temperature with 0.25 nM [^{125}I]iodosulpride in binding buffer. After labeling, sections were briefly washed with ice-cold binding buffer and dried under a stream of desiccated cold air. Autoradiograms were made by exposure of the sections for 2 days to Hyperfilm-βmax (Amersham), with ^{125}I-polymer standards (1.25–640 nCi/mg of polymer; Amersham) included for calibration. Autoradiograms were developed with Kodak D_{19} developer (25°, 5 min), fixed in Kodak Rapid Fixative (8 min), and rinsed with water (15 min). Autoradiograms were scanned on a Howtek scanner in transmission mode at 400 dpi and were analyzed on a Macintosh computer using the public domain program NIH Image (written by Wayne Rasband and available via Internet by anonymous FTP from zippy.nimh.gov or from Library 9 of the MacApp forum on CompuServe). Images were calibrated in nanocuries/milligram using a standard curve fitted to the average densities of the ^{125}I-polymer standard (23). Background binding in white matter was subtracted from the image, the substantia nigra compacta was outlined on each side, and

its mean radioactivity was measured. To ensure a representative sample, each brain was measured at four different sections taken over a 200-μm interval and the results were averaged.

For measurement of [^{3}H]mazindol binding to striatal homogenates, brains were obtained as described above and kept frozen until analysis. Brains were warmed to −15° and a 2-mm section was obtained by making sagittal cuts with a razor blade at the optic chiasm and 2 mm rostral to the first cut. Each striatum was dissected from the slice and homogenized in 5 ml of ice-cold lysis buffer (50 mM Tris·HCl, pH 7.9) with a Polytron homogenizer. Homogenates were brought to 10 ml with lysis buffer and centrifuged at 575 × *g* for 10 min at 4°. The pellet was discarded and the supernatant was centrifuged at 18,000 × *g* for 30 min at 4°. Pellets were resuspended in 3 ml of ice-cold incubation buffer (50 mM Tris·HCl, 300 mM NaCl, 5 mM KCl, pH 7.9) with a Polytron homogenizer. Binding of [^{3}H]mazindol was determined by incubation of membranes with 100 nM [^{3}H]mazindol (24 Ci/mmol; DuPont-NEN) at 0° for 60 min. Nonspecific binding was defined in the presence of 10 μM nomifensine. Incubations were terminated by addition of 5 ml of ice-cold binding buffer and rapid filtration onto a Whatman GF-B filter, followed by two rinses with cold buffer. Filters were then placed in liquid scintillation vials and counted. Protein concentrations were determined using the Bio-Rad assay, and results are stated in picomoles specifically bound/milligram of protein.

Rotational behavior. Animals were tested for rotational behavior as described (24), with modifications. One week after cannula implantation, animals were acclimated to cylindrical test chambers (37-cm diameter) for 15 min and the number of 360-degree rotations was then counted for an additional 30 min, to ensure that the surgery had no effect on rotational behavior. The next day, animals were acclimated as before and a test dose of cocaine (10 mg/kg) was given subcutaneously, followed by a 30-min observation period. On the next day, animals began receiving either antisense, sense, or missense ODNs or vehicle every 12 hr. Four hours after the fifth dose, animals were acclimated to the test chambers as before and were observed for 15 min before a second dose of cocaine (10 mg/kg) was given. Animals were then observed for 30 min after the cocaine dose.

Dopamine release. Animals were treated with antisense ODN unilaterally in the substantia nigra as described above, and stimulated dopamine release from superfused striatal slices was measured by high performance liquid chromatography. Four hours after the final ODN dose, animals were sacrificed by decapitation and 1-mm sections of striatum were prepared in a chilled slice block. Two slices were then transferred to a glass superfusion chamber and equilibrated in superfusion buffer (124 mM NaCl, 1 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 10 mM glucose), which had been saturated with 95% O_2/5% CO_2 and warmed to 35°. Chambers were equipped with platinum electrodes 2 cm apart. After 1 hr of equilibration at 1 ml/min, superfusion buffer containing 3 μM nomifensine was substituted as the buffer, and the tissue was allowed to equilibrate for an additional 30 min. After five consecutive base-line effluent samples were collected, slices were electrically stimulated with square-wave pulses of 2-msec duration, at a frequency of 10 Hz, for 1 min. Effluents were collected during the stimulation period and for an additional 9 min after the stimulation. After samples were collected, slices were perfused for an additional 15 min. Superfusion buffer containing 3 μM nomifensine and 40 μM sulpiride was then substituted as the buffer, and the tissue was allowed to equilibrate for an additional 30 min. The sampling procedure was repeated as before. An aliquot of each perfusate sample (800 μl) was mixed with 200 μl of stabilizing buffer to final concentrations of 0.5 mM EDTA and 0.1% sodium azide, with 50 pg/ml epinine as the internal standard.

Dopamine content of the perfusate samples was quantitated by separation on a Keystone Octyl/B 5-μm reverse phase high performance liquid chromatography column (150 × 4.6 mm) and detection with an ESA Coulochem II detector equipped with a 5201 conditioning cell and a 5011 analytical cell. The conditioning cell was maintained at +450 mV, and the analytical cell was set at +100 mV (E1) and −340 mV (E2). The mobile phase consisted of 0.1 M potassium acetate, 0.05 M citric acid, 0.5 mM EDTA, 0.8 mM sodium octyl sulfate, 3% glacial acetic acid, and 2% acetonitrile. Chromatograms were recorded with a SpectraPhysics SP 4270 integrator. Peak height ratios were calculated against an internal standard (epinine), and the amount of dopamine in each sample was calculated by comparison with a standard curve.

Data analysis. Results were compared by standard analysis of variance, with *post hoc t* tests if the analysis of variance revealed significant differences among treatment groups. Autoradiographic data were analyzed with nonparametric Mann-Whitney tests.

Results

***In vitro* and *in vivo* effects of ODNs on D_2 receptor numbers.** Initially, antisense, sense, and missense ODN constructs were tested *in vitro* to determine their putative efficacy. Seventeen-mers were tested in WERI-27 retinoblastoma cells, which normally express the D_2 receptor. An ODN corresponding to the amino terminus of the D_2 receptor was found to suppress receptor binding by 57%, compared with G-C content-matched missense ODN-treated or untreated cells, after 2 days of intermittent ODN exposure *in vitro* (Fig. 1).

If D_2 receptors are decreased by antisense ODN treatment *in vitro*, then local administration of ODN *in vivo*, via an implanted cannula, should decrease D_2 receptor numbers at the injected site and at distal presynaptic sites on the same neurons as well. Therefore, the ability of antisense ODN to decrease D_2 receptors *in vivo* was determined by measuring autoradiographic changes in D_2 receptor levels after intranigral injection of ODNs. Administration of ODN directly into the substantia nigra compacta on one side of the rat brain, as shown in Fig. 2, caused a 40% decrease in [I^{125}]iodosulpride labeling of the treated substantia nigra compacta, compared with the untreated side. In contrast, only a 9% decrease was found in the animals treated with missense ODN. Antisense treatments did not appear to influence other markers of dopaminergic neuronal

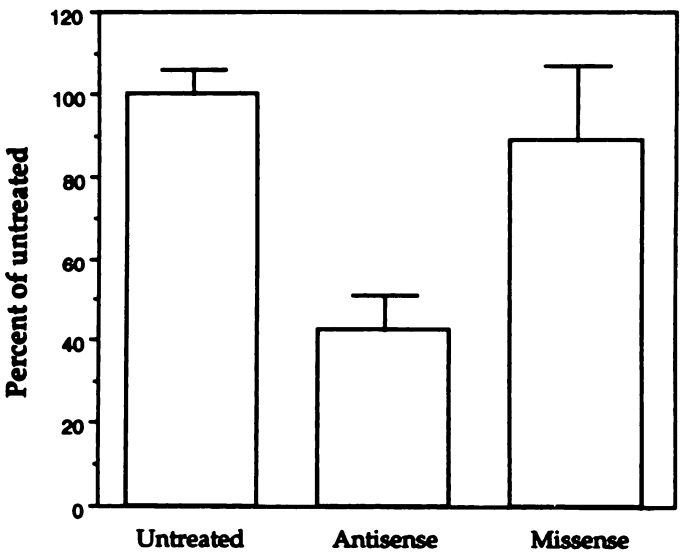
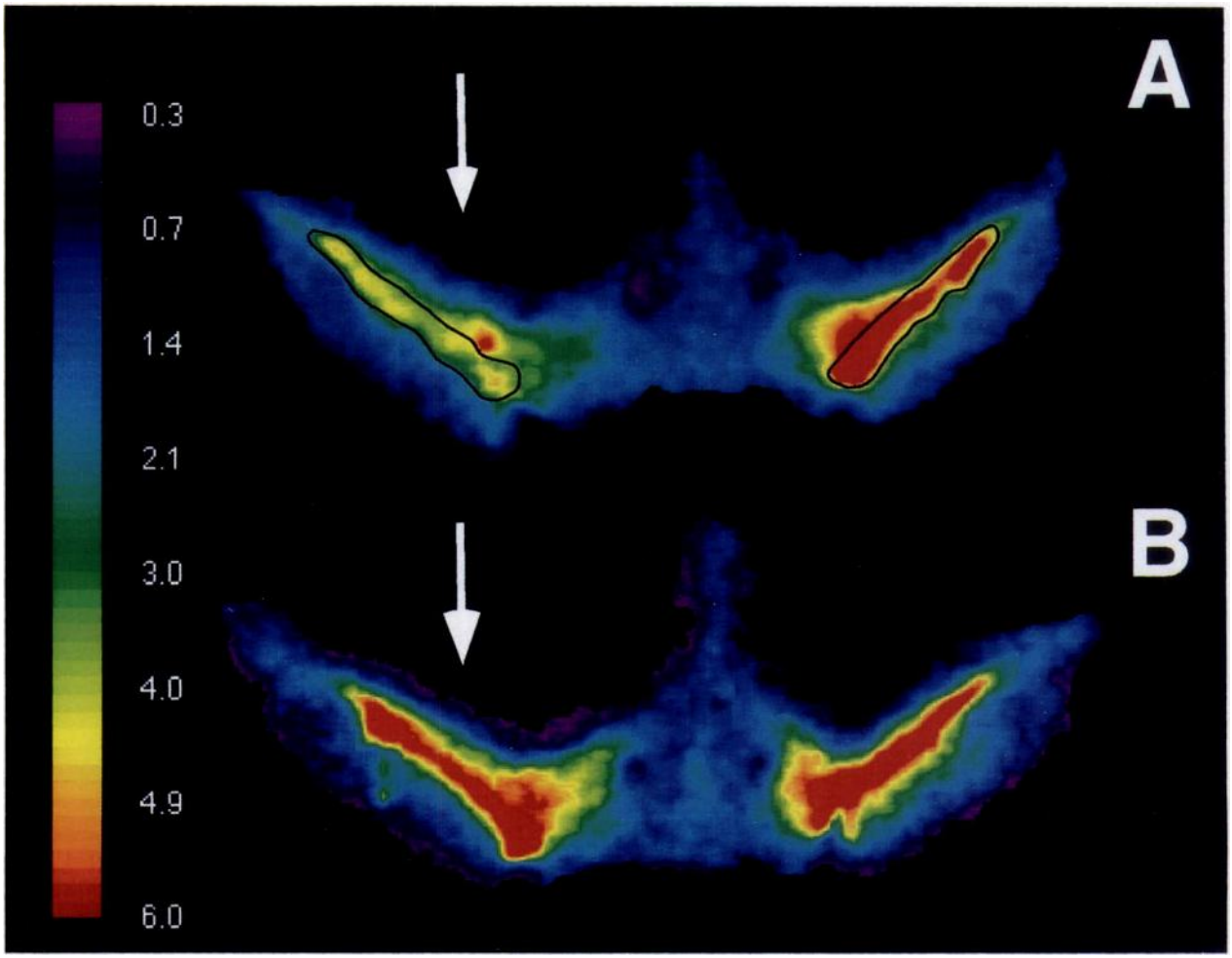
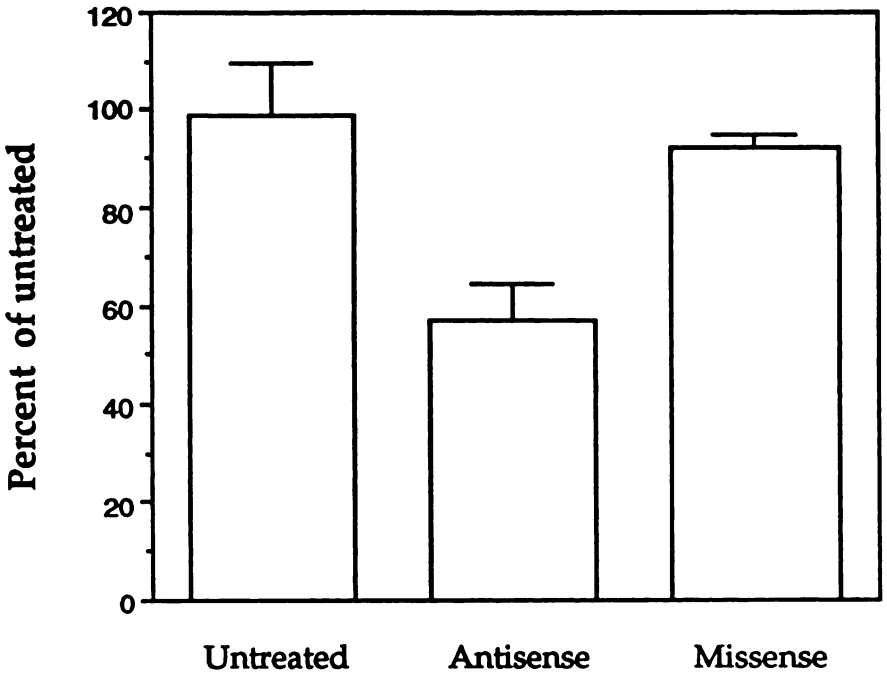


Fig. 1. Effect of ODN treatment on D_2 receptor levels in WERI-27 retinoblastoma cells. Cells were incubated with 1 μM ODN as indicated in Experimental Procedures. Twelve hours after the last treatment, cells were harvested and D_2 receptor binding was determined. Nonspecific binding was defined with 1 μM (+)-butaclamol. Antisense treatment led to a 57% decrease in D_2 receptor binding ($p < 0.05$, Student's *t* test), compared with control or missense ODN-treated groups. There was no significant difference between control and missense ODN-treated groups. Results are from four determinations per group.





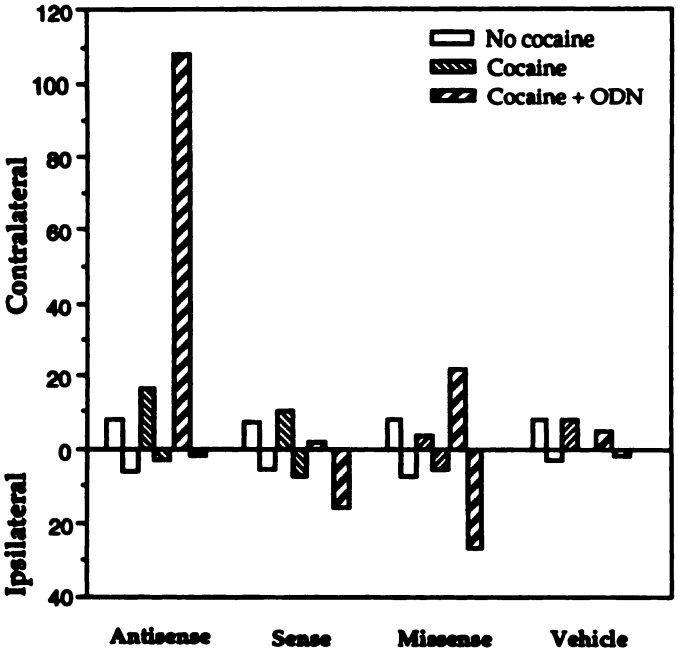


Fig. 3. Effect of ODN treatment on cocaine-induced rotational behavior. Animals were prepared and treated with ODNs as described in Experimental Procedures. Base-line complete (360-degree) rotations in the absence of cocaine were determined 7 days after surgery and after a 10 mg/kg dose of cocaine. Vehicle or antisense, sense, or missense ODNs were then administered intranigrally, as noted in the text, and 4 hr after the last dose animals were injected with 10 mg/kg cocaine and rotational behavior was measured. Results are the means of seven determinations.

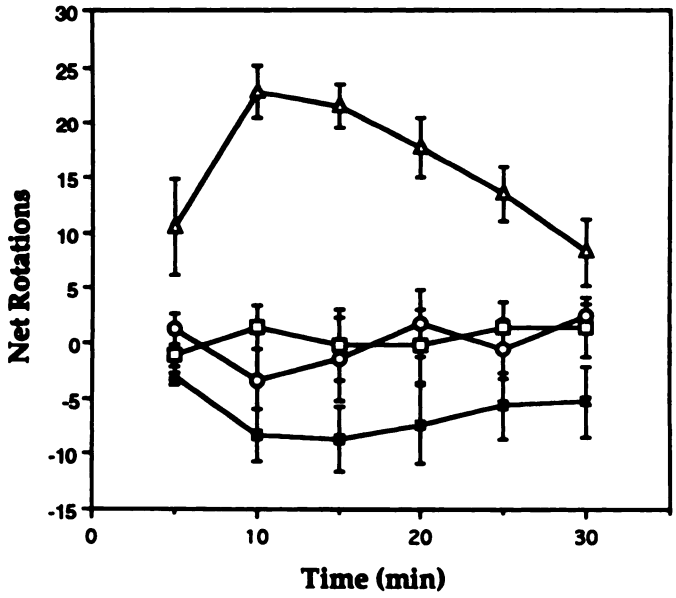


Fig. 4. Time course of cocaine-induced rotational behavior in intranigrally treated rats. Animals were prepared as before, and rotational behavior in response to cocaine was measured 4 hr after the last ODN dose. Net complete rotations (contralateral − ipsilateral) were determined for antisense ODN (△), missense ODN (○), vehicle (□), and sense ODN (×) treatments. Results are the means ± standard errors of seven determinations.

viability. No changes in striatal D_2 receptor density were found, and [^{3}H]mazindol binding was unchanged in the antisense-treated striatum, compared with the untreated side (treated, 7.24 ± 0.9 pmol/mg; untreated, 6.97 ± 0.2; mean ± standard error). Histological examination of the nigral area 10 days after the final ODN administration revealed no pathological changes as a result of intranigral injection (data not shown). Therefore, intranigral D_2 antisense ODN reduces nigral D_2 receptor binding site density directly.

Effect of ODNs on cocaine-induced motor behavior. Because direct administration of antisense ODN into the substantia nigra results in decreases in nigral and possibly striatal D_2 autoreceptor numbers, a unilateral reduction of these inhibitory receptors should facilitate dopamine release from these neurons in response to cocaine if presynaptic receptors play a significant role in the control of release at high levels of dopamine. With a unilateral nigral antisense treatment, this would result in more dopamine release on the treated side and thus rotation away from that side. Therefore, to determine the behavioral effect of reduction in D_2 autoreceptors, animals previously implanted with nigral cannulas were initially tested for base-line rotational behavior, with a single test dose of cocaine (10 mg/kg), before intranigral injections. No directed spontaneous rotation was seen in any treatment group before cocaine administration, and the test dose of cocaine caused only a slight, bidirectional, rotational behavior in all groups (Fig. 3). Antisense, missense, or sense ODN or vehicle was administered via cannula every 12 hr for five doses. Four hours after the last ODN administration, animals were injected with a second dose of cocaine and rotational behavior was measured. Animals receiving D_2 antisense ODN rotated significantly more, contralaterally to the treated side, whereas control groups showed only a slight increase in rotations under the same conditions (Fig. 3). The time course of the rotational behavior revealed that in antisense-treated animals net rotations were highest at 10 min after administration of cocaine and had not returned to base-line levels by 30 min, whereas no laterality of rotation was seen in saline- or missense ODN-treated animals over the same period (Fig. 4). Interestingly, sense ODN treatment produced a tendency to rotate ipsilaterally to the treated side. The response to antisense ODN treatment strongly supports a significant role for D_2 autoreceptors in reducing the motor response to cocaine.

Effect of ODNs on striatal dopamine release. To directly test whether antisense ODN in the substantia nigra could reduce release-modulating striatal D_2 autoreceptors and cause enhanced dopamine release in the terminal region of the striatum, striatal slices from unilaterally treated rats were stimu-

Fig. 2. Effect of intranigral ODN treatment *in vivo* on nigral D_2 receptor binding, as measured by quantitative autoradiography. Animals were prepared and treated with ODNs as described in Experimental Procedures; ODNs were administered into the left substantia nigra only. Four hours after the last ODN dose, animals were sacrificed and brains were processed for autoradiography with [^{125}I]iodosulpride. After 2 days of exposure, films were developed. *Top*, pseudocolored images derived from typical autoradiographic results in antisense or missense ODN-treated rat brains. Scale is in nCi/mg of polymer standard. *Arrows*, guide cannula placement. A, Section from an antisense ODN-treated animal. *Circled areas*, regions typically selected for measurement. B, Similar section from a missense ODN-treated animal. *Bottom*, results from computer-assisted densitometry of [^{125}I] iodosulpride autoradiograms. Antisense treatment led to a 40% reduction in labeling of the substantia nigra ($p < 0.05$, Mann-Whitney test), compared with the untreated side or missense ODN-treated substantia nigras, with no difference between untreated and missense ODN-treated substantia nigras. Results are the mean of four determinations.

lated electrically and the dopamine released in the perfusate was quantitated by high performance liquid chromatography with coulometric detection. Stimulated dopamine efflux from striata located on the side of the brain that received nigral antisense ODN treatment was compared with that of the untreated side. As shown in Fig. 5, striata from the treated side released more dopamine in response to a train of pulses than did those from the untreated side. In contrast, after preincubation of slices with the D_2 antagonist sulpiride (40 μM), stimulated release from the untreated side was 4 times greater than in the absence of sulpiride, whereas release from the treated side was only twice as high. Basal release of dopamine from the treated side was not different from that from the untreated side, and preincubation with sulpiride resulted in only a slight increase in basal release. This enhanced release of dopamine from nigrally treated striatal slices is consistent with enhanced synaptic release of dopamine on the treated side due to a reduction in release-inhibiting D_2 autoreceptors. Additionally, the observation that sulpiride does not enhance release from nigrally treated slices to the same extent as it does from untreated slices suggests that fewer autoreceptors are available on the treated side for release inhibition.

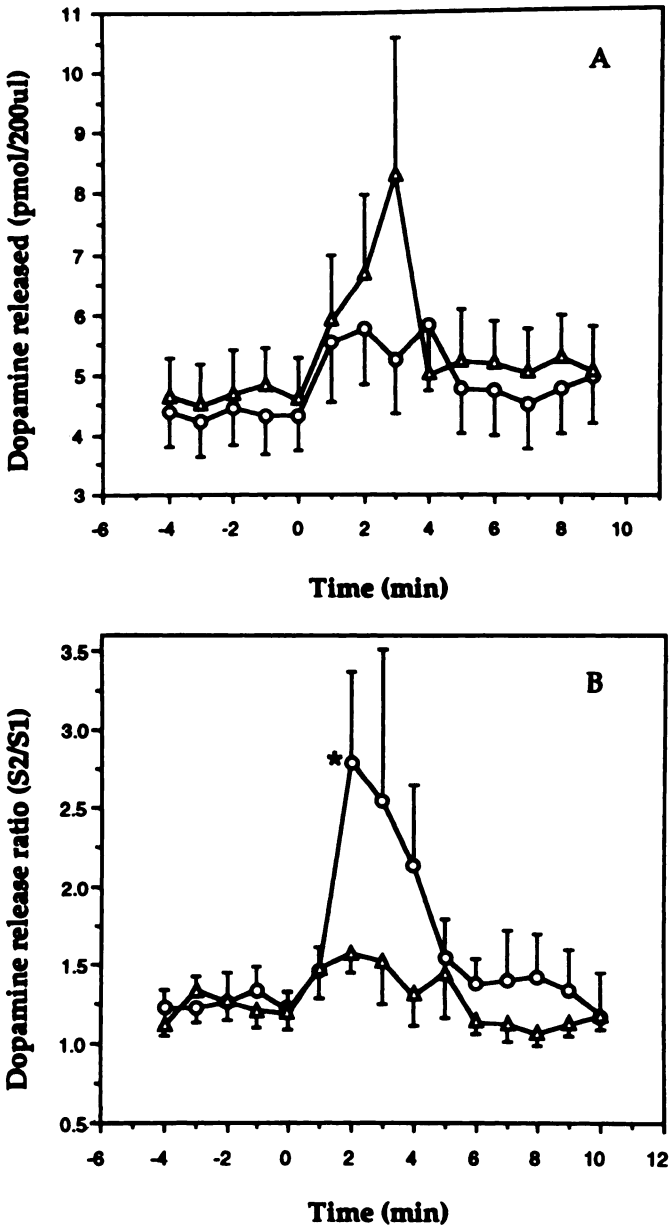


Fig. 5. Dopamine release from striatal slices in response to electrical stimulation. Animals were treated intranigrally with antisense ODN as before, and electrically stimulated dopamine release from striatal slices *in vitro* was measured as outlined in Experimental Procedures. A, Dopamine release from nigrally treated (Δ) or untreated (O) striata in the absence of D_2 receptor antagonist (13 determinations). B, Ratio of electrically stimulated dopamine release in the presence of the D_2 receptor antagonist sulpiride versus release in the absence of sulpiride (S_2/S_1 ratio). The ratio of release was higher in untreated (O) than in treated (Δ) striata by 2 min ($p < 0.05$, Neuman-Keuls t test, seven determinations).

Discussion

D_2 autoreceptors have been examined extensively in the past, and our understanding of their function has been enhanced by the application of more refined methodologies such as intracerebral microdialysis. However, the direct evidence for an extensive involvement of these autoreceptors in modulating the behavioral response to cocaine is lacking, due in part to a paucity of autoreceptor-selective pharmacological agents with which one could further characterize autoreceptor function in such a model system. The use of antisense ODNs to reduce D_2 receptor density, and thus D_2 receptor function, takes advantage of the observation that the substantia nigra contains the mRNA that codes for nigrostriatal neuronal D_2 receptors (25) and that it is in an anatomically different location than the postsynaptic neurons of the target sites, which also contain D_2 mRNA. This offers the possibility of selectively reducing nigrostriatal autoreceptor populations without significantly altering postsynaptic D_2 receptors in the caudate putamen. The effect of reductions in D_2 autoreceptor density on cocaine-induced behavior may then be measured directly, without altering other aspects of nigrostriatal neuronal function.

In this paper, we show that nigrostriatal D_2 autoreceptors play an important role in the motor response to cocaine administration. After unilateral antisense ODN treatment, cocaine administration resulted in marked rotation contralaterally to the treated side, a finding consistent with loss of autoreceptor-mediated feedback inhibition and subsequently greater dopamine release on that side. In fact, antisense ODN treatment led to a reduction in nigral D_2 autoreceptor binding sites and enhanced striatal release of dopamine in response to electrical stimulation.

The autoradiographic data showed a 40% reduction in D_2 binding sites in substantia nigra compacta that had been treated with antisense ODN for approximately 3 days. No appreciable changes in D_2 receptor density were observed in the striatum of antisense-treated animals. This is consistent with studies using either nigral 6-hydroxydopamine or median forebrain bundle lesions, which show that the number of D_2 binding sites in the striatum does not change or increases after destruction of nigral neurons (26, 27). Thus, even a complete ablation of striatal autoreceptors would lead to undetectable changes in striatal D_2 receptor densities, most likely due to the small percentage of autoreceptors in the striatum, compared with postsynaptic D_2 receptors. On the other hand, studies of nigral D_2 receptor density after nigrostriatal lesions (22, 27) suggest that approximately 70–80% of substantia nigra compacta D_2 receptors originate on nigral cell bodies. Therefore, a complete reduction in nigral D_2 receptor binding would not be

expected even if the antisense ODN treatment was totally effective.

Antisense treatment consistently produced contralateral rotation, compared with the bidirectional rotations of the control groups. Cocaine-induced contralateral rotations in the antisense group were maximal by 10 min, were still present at 30 min, and appeared to have an onset similar to that of the effects of cocaine on dopamine release (28) and other behaviors (29). It is unlikely that the observed behaviors could be due to toxicity of the antisense ODN, because this would reduce dopaminergic innervation, resulting in less dopamine release from the treated side in response to cocaine. This would lead to ipsilateral rotation, rather than the observed contralateral rotation. Additionally, no changes in striatal dopamine transporter density between antisense-treated and untreated sides were noted, as measured by [^{3}H]mazindol binding. It is likely, therefore, that the behaviors resulted from a specific reduction of autoreceptors, rather than a generalized toxic effect.

In contrast to the highly significant rotation after a cocaine challenge, the 40% reduction in nigral D_2 autoreceptor density was insufficient to cause spontaneous rotation. Similarly, this reduction in autoreceptors was associated with an increase in electrically stimulated but not basal striatal dopamine release. The results are consistent with our hypothesis that D_2 autoreceptors play a major role in inhibitory regulation under conditions of high dopamine turnover, whereas during normal behavior or at basal turnover compensatory conditions might mask autoreceptor effects.

In conclusion, D_2 antisense ODNs administered unilaterally into the substantia nigra caused 1) a decrease in nigral D_2 autoreceptor binding site density, 2) markedly stimulated contralateral rotation in response to cocaine, and 3) enhanced release of dopamine from electrically stimulated striatal slices, which was in turn less responsive to the release-enhancing effects of sulpiride. The major implication of these findings is that autoreceptors are robustly functional inhibitors in the high dopamine turnover state. In previous studies, even though low, "autoreceptor-selective" doses of neuroleptics were found to potentiate amphetamine-induced behavior, they blocked only cocaine-induced behavior (30). Similarly, apomorphine failed to inhibit cocaine-induced behavioral hypersensitivity (7). Thus, this study represents the first direct evidence consistent with autoreceptor inhibition of cocaine-induced motor behavior.

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