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Serotonin$_{2A}$ Receptor Modulation of D_1 Dopamine Receptor-Mediated Grooming Behavior

J. M. SCALZITTI,[1] L. S. CERVERA, C. SMITH AND J. G. HENSLER[2]

Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7764

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SCALZITTI, J. M., L. S. CERVERA, C. SMITH AND J. G. HENSLER. *Serotonin$_{2A}$ receptor modulation of D_1 dopamine receptor-mediated grooming behavior*. PHARMACOL BIOCHEM BEHAV **63**(2) 279–284, 1999.—We have previously observed that intracerebroventricular infusion of a 5-HT$_{2A}$ receptor antisense oligonucleotide for 8 days results in an increase in cortical 5-HT$_{2A}$ receptor sites and an increase in central 5-HT$_{2A}$ receptor function as measured by quantitation of 5-HT$_{2A}$ receptor-mediated headshake behavior (28). Because lesioning serotonergic neurons or chronic administration of 5-HT$_{2A}$ receptor antagonists does not result in an increase in 5-HT$_{2A}$ receptor density or function in the brain, we have taken advantage of this unique upregulation of 5-HT$_{2A}$ receptors following 5-HT$_{2A}$ receptor antisense oligonucleotide infusion to study the modulation of D_1 receptor-mediated behaviors by 5-HT$_{2A}$ receptors. Grooming behavior, elicited by acute injection of SKF 38393, was attenuated after chronic ICV infusion of a 5-HT$_{2A}$ receptor antisense oligonucleotide. There was also a reduction in vacuous chewing behavior induced by SKF 38393, which did not reach statistical significance. Other oral behaviors (i.e., tongue protrusions and gnawing at the cage bottom) were not attenuated. An increase in the density of cortical, as well as striatal 5-HT$_{2A}$ receptor sites was observed following chronic antisense oligonucleotide administration. There was no change in striatal D_1 dopamine receptors following 5-HT$_{2A}$ receptor antisense oligonucleotide administration. SKF 38393-induced grooming behavior was also attenuated in naive rats pretreated acutely with the 5-HT$_2$ receptor agonist DOI. These results suggest a role for the 5-HT$_{2A}$ receptor in the modulation of D_1 receptor function. © 1999 Elsevier Science Inc.

5-HT$_{2A}$ receptors D_1 dopamine receptors Antisense oligonucleotide Vacuous chewing Grooming behavior

THERE have been identified numerous subtypes of receptor for the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). These receptors, located in the central nervous system and in the periphery, mediate a wide variety of behavioral responses and physiological processes. Serotonin$_{2A}$ (5-HT$_{2A}$) receptors in the central nervous system have been implicated in the regulation of neuroendocrine function and behavior, and in certain psychiatric disorders. The 5-HT$_{2A}$ receptor has high affinity for psychoactive drugs such as hallucinogens, atypical antipsychotics, and many antidepressants (4,7,14).

The 5-HT$_{2A}$ receptor appears to be regulated in an anomalous manner in response to drug treatment or changes in neurotransmitter exposure. The expected response to receptor blockade or removal of neurotransmitter input to a receptor is supersensitivity and receptor upregulation (i.e., an increase in receptor number) (5). However, most investigators have not found any change in 5-HT$_{2A}$ receptors in any area of brain after lesioning central serotonergic neurons (2,3,5). Furthermore, chronic administration of 5-HT$_2$ receptor antagonists, including atypical neuroleptics and many antidepressant drugs, results in a paradoxical decrease in the density of 5-HT$_{2A}$ receptors (3–6,11,15). The downregulation of 5-HT$_{2A}$ receptors observed following chronic administration of many antidepressants appears to be due to 5-HT$_{2A}$ receptor occupancy or blockade, and not due to an increase in the exposure of this receptor to 5-HT, in that it is not prevented by the destruction of serotonergic neurons (3,5).

We have previously observed that intracerebroventricular (ICV) infusion of an antisense oligonucleotide for 8 days results in a marked increase in cortical 5-HT$_{2A}$ receptor sites, as

[1]Current address: Department of Pharmacology, New York University Medical School, 550 First Avenue, New York, NY 10016.
[2]Requests for reprints should be addressed to J. G. Hensler, Ph.D., Department of Pharmacology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284-7764.

measured by the binding of [^{3}H]-ketanserin, and in an increase in central 5-HT_{2A} receptor-mediated headshake behavior induced by acute injection of the 5-HT_2 receptor agonist DOI (28). In that report, we hypothesize that this antisense oligonucleotide, which is complementary to the coding region of rat 5-HT_{2A} receptor mRNA, relieves some form of translational suppression, resulting in an increase in 5-HT_{2A} receptor expression (28). The inhibition of gene expression by antisense oligonucleotides complementary to the coding region of target mRNA appears in many cases to be dependent on RNase H activity. However, the stability of mRNA and unmodified oligonucleotides in the brain, and the effectiveness of unmodified antisense oligonucleotides, are consistent with low activity of nucleases in the brain [(28), and references therein]. Computer analysis (GCG Sequence Analysis Software package) of 5-HT_{2A} receptor mRNA indicates the potential existence of a stem loop structure in the region of 5-HT_{2A} receptor mRNA targeted by our antisense oligonucleotide. We speculate that the binding of our antisense oligonucleotide may interfere with formation of this secondary structure or inhibit the interaction of 5-HT_{2A} receptor mRNA with regulatory proteins (28). Disruption of such a regulatory mechanism may relieve suppression of translation resulting in an increase in 5-HT_{2A} receptor expression observed in vivo.

Because lesioning serotonergic neurons or chronic administration of 5-HT_{2A} receptor antagonists does not result in an increase in 5-HT_{2A} receptor density or function in brain, we have taken advantage of this unique upregulation of 5-HT_{2A} receptors following chronic ICV infusion of 5-HT_{2A} receptor antisense oligonucleotide to study the modulation of central dopaminergic D_1 receptor function by 5-HT_{2A} receptors. Serotonergic modulation of D_1 receptor-mediated grooming behavior has been previously reported by Lucki and Kucharik (16), who showed an enhancement of SKF 38393-induced grooming behavior following 5,7-dihydroxytryptamine (5,7-DHT) lesion of central serotonergic neurons. We hypothesized that if removal of serotonergic input resulted in an enhancement of D_1 receptor-mediated grooming behavior, then an upregulation of central 5-HT_{2A} receptors may attenuate this behavioral response.

In the current study, grooming and oral behaviors induced by acute injection of the D_1 receptor agonist SKF 38393 were quantitated following infusion of antisense or mismatch oligonucleotide, or vehicle into the lateral cerebral ventricle of rats for 8 days. An increase in 5-HT_{2A} receptors following antisense oligonucleotide administration was confirmed by the binding of [^{3}H]-ketanserin in homogenates of the cortex and striatum. We also investigated the effect of acute pretreatment of naive rats with the 5-HT_2 receptor agonist DOI on SKF 38393-induced grooming and oral behaviors. The findings of these studies suggest a role for the 5-HT_{2A} receptor in the modulation D_1 receptor-mediated behaviors.

METHOD

Oligodeoxynucleotides

The following sequences, corresponding the rat 5-HT_{2A} receptor cDNA in the coding region (+57 to +74) (10), were chosen for these studies (5′ to 3′): Antisense-GGGCCATCACCTAATTGC; Mismatch-GG**A**CCATC**G**CCTA**G**TT**A**C. Oligonucleotides were synthesized by Oligos Etc. Inc. (Wilsonville, OR) as chimeras with the outer three 5′ and 3′ bonds phosphorothioated; the 11 inner bonds were phosphodiester bonds.

Oligonucleotide Infusions

Adult male Sprague–Dawley rats (200–250 g) were anesthetized and a stainless steel cannula (ALZET brain infusion kits, Alza Corp) stereotaxically implanted into the lateral ventricle: AP, −0.8 mm; ML, 1.4 mm; DV, −3.5 mm from bregma (24). Antisense or mismatch oligonucleotide (250 μg/day) or sterile saline vehicle was administered ICV for 8 days at a rate of 1 μl/h by an osmotic pump (Alza Corp) implanted subcutaneously. Cannula placement was confirmed by dye injection at sacrifice.

Drug Treatments

In some studies, naive rats were pretreated acutely with the 5-HT_2 receptor agonist DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] or chronically with the 5-HT_2 receptor antagonist mianserin. Naive rats were pretreated with the 5-HT_2 receptor agonist DOI (0.5 mg/kg, SC) 10 min before injection of SKF 38393 (2 mg/kg, SC). Grooming behavior was measured after injection of SKF 38393, as described below. In a separate experiment, naive rats were treated for 8 days with the 5-HT_2 receptor antagonist mianserin (5 mg/kg, SC) and SKF 38393-induced grooming behavior was assessed 24 h after the last injection of mianserin. This treatment paradigm has been shown by us previously to downregulate 5-HT_{2A} receptors (9). Control animals received injections of saline vehicle.

D_1 Receptor-Mediated Grooming and Oral Behaviors

Rats were handled and exposed to the testing cage and room each day for 5 days prior to behavioral experiments. For each test session, rats were allowed to acclimate to the testing environment, a quiet well-ventilated room, for at least 1 h. All behavioral testing was done between 1500 and 1800 h. Rats were injected subcutaneously with SKF 38393 or saline vehicle (1 ml/kg) and placed in a Plexiglas cage (29 cm wide × 58 cm long × 41 cm high) with a wire mesh bottom. The amount of time that rats spent grooming themselves was measured continuously for a 30-min period, assessed 5 to 35 min after injection of SKF 38393 (31). We measured total time spent grooming, rather than total number of episodes, because measuring total time spent grooming has been shown to be more reliable (31). Oral behaviors induced by SKF 38393 (2 mg/kg, SC) were also assessed continuously during this 30-min period: (a) the number of tongue protrusions; (b) time spent exhibiting jaw tremor and "vacuous chewing" behavior (i.e., spontaneous chewing that is not directed onto any evident physical material) (32); (c) time spent gnawing at the cage bottom.

Homogenate Binding Experiments

Animals were sacrificed shortly after behavioral testing. Cerebral cortices and striata were dissected on ice, flash frozen, and stored at −80°C. Binding reactions, performed as described below, were terminated by the addition of 5 ml of ice-cold buffer (50 mM Tris pH 7.4 at 4°C). Membranes were collected on glass fiber filters (Schleicher and Schuell, #25) that had been soaked in 0.3% polyethylenimine. Filters were washed four times with ice-cold buffer. Saturation binding data were fit by nonlinear regression to the model: B (B_{max}/$1+(K_d/[D]m_1)$) + (m_2*[D]), where B is the amount of radioligand bound at the radioligand concentration D, B_{max} is the maximal concentration of bound ligand, K_d is the equilibrium dissociation constant, m_1 is the slope of the total binding curve, and m_2 is the slope of the nonspecific binding curve.

Protein concentrations were determined by the method of Bradford (1).

[^{3}H]-Ketanserin Binding

The binding of [^{3}H]-ketanserin (0.1–10 nM; Dupont NEN) was performed in glass test tubes containing 50 mM Tris (pH 7.4 at 3°C), and 100 nM prazosin and 100 nM pyrilamine to prevent the binding of [^{3}H]-ketanserin to α_1-adrenergic and H_1 histamine receptors, respectively as described (28). In experiments measuring the binding of [^{3}H]-ketanserin to striatal homogenates, tetrabenazine (1 μM) was also present in the assay to prevent the binding of [^{3}H]-ketanserin to nonserotonergic sites on dopaminergic nerve endings (13). Nonspecific binding was determined in the presence of 10 μM methysergide. Binding was initiated by the addition of cortical homogenate (150–200 μg protein per assay tube) or striatal homogenate (50–75 μg protein per assay tube). Assay tubes were covered and incubated for 1 h at 37°C.

[^{3}H]-SCH 23390 Binding

The binding of [^{3}H]-SCH 23390 (0.1–10 nM; Dupont NEN) was performed in glass test tubes containing 50 mM Tris buffer (pH 7.4 at 37°C) with 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 100 nM mianserin to prevent the binding of [^{3}H]-SCH 23390 to 5-HT_2 receptors as previously described (12). Nonspecific binding was determined in the presence of 1 μM cis-flupenthixol. Binding was initiated by the addition of striatal homogenate (50–75 μg protein per tube). Assay tubes were incubated for 20 min at 37°C.

Statistical Analysis

Data were analyzed by ANOVA (factorial design) and significant *F*-values were evaluated by Fisher's Least Significant Difference post hoc test.

RESULTS

Acute administration of the D_1 dopamine receptor agonist SKF 38393 (0.5–8 mg/kg, SC) produced a dose-dependent-increase in grooming behavior, assessed as total time spent grooming in a 30-min period (Fig. 1). Baseline grooming, total time spent grooming in a 30-min period after subcutaneous injection of saline, was 113 ± 11 s ($n = 5$).

Grooming behavior induced by acute injection of SKF 38393 (2 mg/kg, SC) was assessed after infusion of vehicle, mismatch, or antisense oligonucleotide (250 μg/day) into the lateral ventricle for 8 days. SKF 38393-induced grooming behavior was significantly attenuated in animals that had received 5-HT_{2A} receptor antisense oligonucleotide infusion [time spent grooming (seconds) vehicle: 372 ± 18; mismatch: 348 ± 13; antisense: 262 ± 13] $F(2, 12) = 14.7$, $p = 0.0006$ (Fig. 2). SKF 38393-induced vacuous chewing behavior was also reduced [time spent exhibiting behavior (seconds) vehicle: 110 ± 23; mismatch: 119 ± 40; antisense: 45 ± 11, but this reduction did not reach statistical significance, $F(2, 12) = 2.11$, $p = 0.16$. The other oral behaviors induced by acute injection of SKF 38393 (i.e., tongue protrusions and gnawing at the cage bottom) were not attenuated as a result of antisense oligonucleotide administration (SKF 38393-induced tongue protrusions: $F(2, 12) = 0.44$, $p = 0.66$; time spent gnawing on cage bottom: $F(2, 12) = 0.06$, $p = 0.94$.

The effect of ICV infusion of 5-HT_{2A} receptor antisense oligonucleotide for 8 days on D_1 receptors was determined by measuring the binding of the antagonist radioligand [^{3}H]-SCH 23390 in striatal homogenates. There was no change in the density of D_1 receptor sites [B_{max} (fmol/mg protein) vehicle: 943 ± 78; antisense: 873 ± 86] or in the affinity of the receptor for radioligand [K_d (nM) vehicle: 1.29 ± 0.22; antisense; 1.05 ± 0.15] ($n = 5$), as a result of chronic ICV infusion of 5-HT_{2A} receptor antisense oligonucleotide. These data suggest that the attenuation of D_1 receptor-mediated grooming behavior was not due to changes in D_1 receptor number or affinity. In confirmation of an earlier study (28), ICV infusion of antisense oligonucleotide for 8 days resulted in an increase in 5-HT_{2A} receptor sites as measured by the binding of [^{3}H]-ketanserin in cortical homogenates (Table 1). Intracerebroventricular infusion of antisense oligonucleotide for 8 days also resulted in an increase in striatal 5-HT_{2A} receptor sits (Table 2).

The observed attenuation of D_1 receptor-mediated grooming behavior following infusion 5-HT_{2A} receptor antisense oligonucleotide suggested that central 5-HT_{2A} receptors may

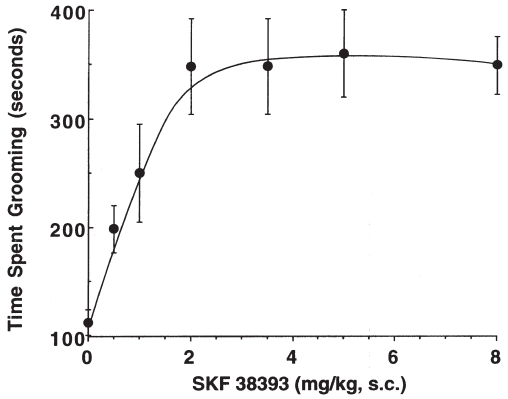

FIG. 1. Grooming behavior induced by the D_1 receptor agonist SKF 38393. The total time spent grooming in a 30-min period was measured for each animal beginning 5 min after injection of SKF 38393. Plotted are the mean ± SEM of four to six animals.

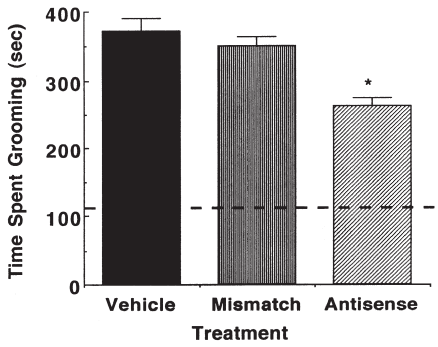

FIG. 2. Effect of chronic ICV infusion of 5-HT_{2A} receptor antisense oligodeoxynucleotide on D_1 receptor-mediated grooming behavior. SKF 38393-induced grooming behavior was assessed after infusion of vehicle, mismatch, or antisense oligodeoxynucleotide (250 μg/day) into the lateral ventricle for 8 days. The total time spent grooming in a 30-min period was measured for each animal beginning 5 min after injection of SKF 38393 (2 mg/kg). (- - -), time spent grooming in naive animals injected with saline: 113 ± 11.5 s ($n = 5$). Plotted are mean ± SEM of $n = 5$ animals per experimental group, *$p < 0.01$, when compared to vehicle or mismatch.

TABLE 1

EFFECT ON INTRACEREBROVENTRICULAR INFUSION OF 5-HT_{2A} RECEPTOR ANTISENSE OLIGONUCLEOTIDE ON CORTICAL 5-HT_{2A} RECEPTOR SITES AS MEASURED BY THE BINDING OF [^{3}H]-KETANSERIN (0.1–10 nM)

Treatment	n	K_d (nM)	B_{max} (fmol/mg protein)
Vehicle	5	1.35 ± 0.16	201 ± 13
Antisense	5	1.79 ± 0.22	302 ± 20*
Mismatch	5	1.15 ± 0.16	156 ± 11

Data shown are mean ± SEM.
*$p < 0.01$ when compared to vehicle or mismatch.

modulate this D_1 receptor-mediated behavior. We postulated that perhaps stimulation of central 5-HT_{2A} receptors by an agonist in naive rats would also attenuate D_1-mediated grooming behavior. To test this hypothesis, naive rats were pretreated with the 5-HT_2 receptor agonist DOI (0.5 mg/kg, SC) 10 min before injection of SKF 38393 (2 mg/kg, SC). Grooming behavior, measured for a 30-min period 5 min after injection of SKF 38393, was reduced by pretreatment of rats with DOI (Fig. 3A). By contrast, DOI pretreatment of rats significantly increased vacuous chewing/jaw tremor induced by SKF 38393 (Fig. 3B). There was no significant difference in other oral behaviors, i.e., the number of tongue protrusions, $F(2, 8) = 1.32$, $p = 0.30$, or time spent gnawing on cage bottom, $F(2, 8) = 1.07$, $p = 0.37$, when comparing DOI-pretreated animals with saline-pretreated animals.

To determine the effect of 5-HT_{2A} receptor downregulation on D_1 receptor-mediated behaviors, naive rats were treated for 8 days with the 5-HT_2 receptor antagonist mianserin (5 mg/kg, SC) and SKF 38393-induced grooming behavior was assessed 24 h after the last injection of mianserin. Mianserin treatment resulted in a marked decrease in the density of 5-HT_{2A} receptors as measured by the binding of [^{3}H]-ketanserin in cortical homogenates (B_{max} (fmol/mg protein) vehicle: 256 ± 14; mianserin: 95 ± 13) without a change in the affinity of the receptor for radioligand [K_d (nM) vehicle: 1.2 ± 0.3; mianserin: 1.2 ± 0.3] ($n = 6$ per experimental group). Downregulation of cortical 5-HT_{2A} receptors as a result of chronic administration of mianserin did not alter grooming behavior induced by SKF 38393 (2 mg/kg, SC) (time spent grooming (seconds) vehicle-treated: 333 ± 32; chronic mianserin-treated: 315 ± 38).

DISCUSSION

We have previously observed that infusion of a 5-HT_{2A} receptor antisense oligonucleotide into the lateral cerebral ventricle of rats for 8 days results in an increase in cortical 5-HT_{2A} receptor sites and an increase in central 5-HT_{2A} receptor function as measured by quantitation of 5-HT_{2A} receptor-mediated headshake behavior (28). In the current report we have examined the effect of this novel upregulation of 5-HT_{2A} receptors following chronic ICV infusion of 5-HT_{2A} receptor antisense oligonucleotide on dopamine D_1 receptor-mediated behaviors. An increase in the density of cortical, as well as striatal 5-HT_{2A} receptor sites was observed following chronic antisense oligonucleotide administration. D_1 receptor-mediated grooming behavior, elicited by acute injection of SKF 38393, was attenuated after chronic ICV infusion of 5-HT_{2A} receptor antisense oligonucleotide. SKF 38393-induced vacuous chewing behavior was also reduced, although this did not reach statistical significance. There was no change in striatal D_1 dopamine receptors to accompany this attenuation in D_1 receptor-mediated behavior. In naive rats, acute pretreatment with the 5-HT_2 receptor agonist DOI reduced SKF 38393-induced grooming behavior. Taken together these results suggest a role for the 5-HT_{2A} receptor in the modulation of D_1 receptor-mediated behaviors.

TABLE 2

EFFECT OF INTRACEREBROVENTRICULAR INFUSION OF 5-HT_{2A} RECEPTOR ANTISENSE OLIGONUCLEOTIDE ON STRIATAL 5-HT_{2A} RECEPTOR SITES AS MEASURED BY THE BINDING OF [^{3}H]-KETANSERIN (0.1–10 nM)

Treatment	n	K_d (nM)	B_{max} (fmol/mg protein)
Vehicle	5	2.01 ± 0.17	258 ± 19
Antisense	5	2.24 ± 0.40	368 ± 23*
Mismatch	5	2.21 ± 0.33	269 ± 12

Data shown are mean ± SEM.
*$p < 0.01$ when compared to vehicle or mismatch.

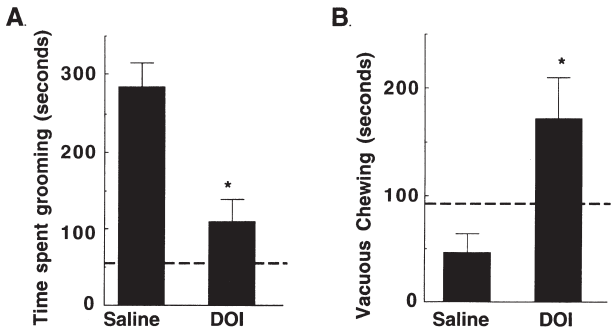

FIG. 3. SKF 38393-induced behaviors following saline or DOI pretreatment. DOI (0.5 mg/kg, SC) or saline were injected 10 min prior to injection of SKF 38393 (2 mg/kg). Grooming (**A**) and vacuous chewing behaviors (**B**) were assessed for a 30-min period beginning 5 min after injection of SKF 38393. (- - -), in the absence of SKF 38393, time spent grooming or exhibiting vacuous chewing after injection of DOI was 54 ± 15 s and 90 ± 16 s, respectively ($n = 5$). Plotted are the mean ± SEM of $n = 5$ animals per experimental group, *$p < = 0.05$.

D_1 dopamine receptor activation induces grooming behavior (19,31) and oral behaviors such as vacuous chewing (32). Inactivation of D_1 dopamine receptors with the irreversible alkylating agent EEDQ prevents grooming behavior induced by SKF 38393; inactivation of D_2 dopamine receptors with EEDQ does not alter SKF 38393-induced grooming behavior, suggesting that grooming behavior appears not to require concurrent stimulation of D_2 dopamine receptors (31). SKF 38393-induced grooming is mediated by activation of D_1 receptors in the CNS, as evidenced by the failure of fenoldopam, a D_1 receptor agonist that does not cross the blood–brain barrier, to increase the amount of time spent grooming compared to saline (31). The lateral aspect of the striatum appears to be primarily involved in SKF 38393-induced grooming behavior (22).

Serotonergic modulation of D_1 receptor-mediated grooming behavior has been previously reported by Lucki and Kucharik (16), who showed an enhancement of SKF 38393-induced grooming behavior following 5,7-DHT lesion of central serotonergic neurons. Lucki and Kucharik (16) found that grooming episodes and total grooming time were increased by 5,7-DHT lesions. The time per grooming episode, however, was significantly decreased; 5,7-DHT–treated rats showed fewer sequential behaviors to complete an episode of grooming (16). As

discussed in the introduction of this report, lesioning serotonergic neurons does not result in an increase in 5-HT_{2A} receptor number in the brain (2,3,5). It is possible that under conditions of normal 5-HT_{2A} receptor expression, serotonergic modulation of D_1 dopamine receptor-mediated grooming behavior is mediated through serotonergic receptors other than the 5-HT_{2A} receptor. Consistent with this are the observations of Molloy and Waddington (19), who have shown that pretreatment of rats with the 5-HT_{2A} receptor antagonist ketanserin does not to alter SKF 38393-induced grooming. The lack of effect of ketanserin on SKF 38393-induced grooming (19) suggests that there is no tonic 5-HT_{2A} receptor input modulating this D_1 receptor-mediated behavior.

In the current study, SKF 38393-induced grooming behavior was attenuated following chronic infusion of 5-HT_{2A} receptor antisense oligonucleotide and, in naive rats, after acute pretreatment with the 5-HT_2 receptor agonist DOI. These observations suggest that activation of 5-HT_{2A} receptors with the agonist DOI, or increasing central 5-HT_{2A} receptor number by antisense administration, reduces SKF 38393-induced grooming behavior. Taken together, these data suggest that although not tonically activated, 5-HT_{2A} receptors are present in the neuronal circuitry mediating this complex behavior to alter its expression. 5-HT_{2A} receptor-mediated modulation of D_1 receptor-mediated grooming behavior becomes apparent when 5-HT_{2A} receptors are upregulated or activated by an exogenous agonist.

Downregulation of 5-HT_{2A} receptors, as a result of chronic administration of the 5-HT_2 receptor antagonist mianserin, did not alter D_1 receptor-mediated grooming behavior. SKF 38393-induced grooming behavior was unchanged in rats treated chronically with mianserin, indicating that decreasing 5-HT_{2A} receptor number does not affect this D_1 receptor-mediated behavior. Our observations that 5-HT_{2A} receptor downregulation does not alter SKF 38393-induced grooming behavior are consistent with the lack of tonic 5-HT_{2A} receptor input modulating this D_1 receptor-mediated behavior. Alternatively, it is possible that an increase in D_1 receptor-mediated grooming behavior could have been observed following chronic administration of the 5-HT_2 receptor antagonist had a lower dose of SKF 38393 (e.g., 0.5 mg/kg) been used. The dose of SKF 38393 used in the current study maximally stimulated grooming behavior (see Fig. 1).

Although not statistically significant, D_1 receptor-mediated vacuous chewing behavior, elicited by acute injection of SKF 38393, was also reduced in animals following chronic ICV infusion of 5-HT_{2A} receptor antisense oligonucleotide. Pretreatment of naive rats with the 5-HT_2 receptor agonist DOI, however, resulted in an increase in SKF 38393-induced vacuous chewing. Gong et al. (8) have shown that the 5-HT_{2C} receptor agonist mCPP induces oral activity, specifically vacuous chewing, an effect not attenuated by the 5-HT_1 receptor antagonist pindolol, or by the 5-HT_{2A} receptor antagonist ketanserin. Therefore, the enhancement of SKF 38393-induced vacuous chewing by DOI observed in the current study is most likely due to agonist activity of DOI at 5-HT_{2C} receptors.

In terminal field areas of dopaminergic innervation, 5-HT_{2A} receptors appear to play a role in the modulation of dopaminergic neurotransmission. For example, 5-HT_{2A} receptors are present in moderate densities in the nucleus accumbens and caudate-putamen (18,20,25). In vivo microdialysis studies indicate that activation of 5-HT_{2A} receptors mediate the serotonin-induced increase in dopamine release in nucleus accumbens (23). By contrast, 5-HT_{2A} receptors mediate the serotonin-induced inhibition of dopamine release from rat striatal slices (21). The highest density of 5-HT_{2A} receptors are found in cerebral cortex (18,20,25). In the prefrontal cortex 5-HT_{2A} receptors appear to exert a tonic inhibitory influence on dopamine efflux (26,29). Although there is little evidence from radioligand binding or immunocytochemical studies for the presence of 5-HT_{2A} receptors in dopaminergic cell body areas [i.e., the substantia nigra and ventral tegmental area (VTA)] (20,25), electrophysiological studies indicate that the depolarizing responses of dopamine cells in the VTA to 5-HT are mediated by the 5-HT_{2A} receptor (27).

In the current studies, there was no change in the binding of ^{3}H-SCH 23390 to D_1 receptor sites in striatal homogenates as a result of chronic ICV infusion of 5-HT_{2A} receptor antisense oligonucleotide. These data suggest that the attenuation of D_1 receptor-mediated behaviors was not due to changes in D_1 receptor number or affinity. Because regulatory changes in D_1 receptors occur as a result of changes in agonist exposure or neurotransmitter input to D_1 receptors (17,30), the upregulation of 5-HT_{2A} receptors as a result of chronic 5-HT_{2A} receptor antisense oligonucleotide appears not to have altered dopaminergic neurotransmission.

The upregulation of 5-HT_{2A} receptors in the brain as a result of chronic antisense oligonucleotide infusion offers a unique opportunity to study the role of 5-HT_{2A} receptors in modulating other receptor systems. Chronic ICV administration of 5-HT_{2A} receptor antisense oligonucleotide, which results in the upregulation of cortical 5-HT_{2A} receptor sites and an increase in central 5-HT_{2A} receptor function (28), diminished central D_1 receptor activity, as demonstrated by the attenuation of SKF 38393-induced grooming behavior. In agreement with these data are the observations that SKF 38393-induced grooming behavior was attenuated following acute pretreatment of naive rats with the 5-HT_2 receptor agonist DOI. Localized, site-specific infusion of 5-HT_{2A} receptor antisense oligonucleotide may prove to be a useful approach for future studies examining interactions between 5-HT_{2A} receptors and central dopamine receptors in distinct brain regions.

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