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ORIGINAL INVESTIGATION

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Enhanced oral activity responses to intrastriatal SKF 38393 and *m*-CPP are attenuated by intrastriatal mianserin in neonatal 6-OHDA-lesioned rats

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Abstract Enhanced oral activity is induced in neonatal 6-hydroxydopamine- (6-OHDA-) lesioned rats by systemic administration of the dopamine (DA) D_1 receptor agonist SKF 38393 and serotonin (5-HT) 5-$HT_{2A,2C}$ agonist *m*-chlorophenylpiperazine (*m*-CPP). The DA D_1 receptor antagonist SCH 23390 effectively attenuates the effect of SKF 38393 but not *m*-CPP. The 5-HT_2antagonist mianserin attenuates the effects of both *m*-CPP and SKF 38393, suggesting that DA agonist effects are mediated by 5-HT neurochemical systems. To test whether DA and 5-HT agonist effects and interactions might occur within the neostriatum, rats were implanted with permanent injection cannulae, with tips in the ventral striatum. One group of rats was lesioned at 3 days after birth with 6-OHDA HBr (100 μg salt form, in each lateral ventricle; desipramine HCl pretreatment, 20 mg/kg IP, base form, 1 h), while controls received the vehicle in place of 6-OHDA. Cannulae were implanted when rats weighed 200–250 g. During a 1-h observation session SKF 38393 (5 nmol per side) produced 74.3 ± 19.2 oral movements in intact rats and 310.7 ± 97.0 oral movements in 6-OHDA-lesioned rats. *m*-CPP (10 nmol per side) produced 72.6 ± 15.1 and 274.5 ± 65.0 oral movements in these respective groups. These responses were several-fold greater than the 25.3 ± 7.3 and 41.8 ± 9.5 oral movements in the same groups after saline (0.5 μl per side) ($P < 0.05$). Mianserin (6 nmol per side) alone had no effect on oral activity but attenuated responses to both SKF 38393 and *m*-CPP in intact and 6-OHDA-lesioned rats. These findings demonstrate that enhanced oral activity responses are produced by intrastriatal SKF 38393 and *m*-CPP in neonatal 6-OHDA-lesioned rats. Also, when the 5-HT_2 receptor antagonist mianserin was administered intrastriatally, induction of oral activity by the DA D_1 agonist SKF 38393 was attenuated. These findings indicate that ventral striatum represents at least one brain focus at which DA and 5-HT systems interact to modulate oral activity in rats.

Key words Dopamine D_1 receptor · 5-HT_{2C} receptor · Supersensitivity · SKF 38393 · *m*-Chlorophenylpiperazine · Oral activity · Striatum · 6-Hydroxydopamine

Introduction

Dopamine (DA) and its receptors are thought to have a prominent role in the genesis of oral activity. Such movements have been used in the study of animal models of parkinsonism (Salamone et al. 1990), dystonias (Rupniak et al. 1985, 1986) and dyskinesias (Ellison and See 1989). Intact rats treated acutely with a DA D_1 receptor agonist display an increased incidence of spontaneous oral activity (Rosengarten et al. 1983, 1986; Arnt et al. 1987; Koshikawa et al. 1987; Molloy and Waddington 1988; Levin et al. 1989). The balance between D_1 and D_2 receptor responsiveness has been implicated in this behavior, since the incidence of oral activity is greater when there is a functional or overt increase in the D_1:D_2 receptor ratio (Rosengarten et al. 1983). This occurs in rats with genetic or permanent drug-induced reduction in D_2 receptor number (Rosengarten et al. 1986), after acute treatment with a D_2 receptor antagonist (Rosengarten et al. 1983; Arnt et al. 1987; Koshikawa et al. 1987) or D_1 receptor

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agonist (Rosengarten et al. 1983; Molloy and Waddington 1988; Murray and Waddington 1989) and in aged rats with reduced numbers of DA D_2 receptors (Rosengarten et al. 1986; Molloy and Waddington 1988). Long-term treatment of rats with DA D_2 receptor antagonists results in the development of spontaneous oral activity (Clow et al. 1979; Iversen et al. 1980; Waddington and Gamble 1980). The DA D_1receptor antagonist SCH 23390 [*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine] attenuates oral activity responses to DA D_1 agonists and DA D_2 antagonists (Rosengarten et al. 1983, 1986).

Recently we found that the DA D_2 receptor antagonist spiperone and DA D_1 receptor agonists SKF 38393 [(±)-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine-7,8-diol] and A77636 [(1*R*, 3*S*)-3-(1′-adamantyl)-1-aminomethyl-3, 4-dihydro-5, 6-dihydroxy-1H-2-benzopyran) produced a 2.5- to 8-fold increase in oral activity responses of rats in which DA neurons in the brain were destroyed neonatally with 6-hydroxydopamine (6-OHDA) (Kostrzewa and Hamdi 1991; Huang and Kostrzewa 1994). The oral activity effects in these rats is accompanied by a 95–99% reduction in neostriatal DA content, but with no change in the B_{max} or K_d for DA D_1 or D_2 receptors (Kostrzewa and Hamdi 1991). In neonatal 6-OHDA-lesioned rats there is a marked increase in D_1 receptor agonist-induced stereotyped and locomotor activities (Breese et al. 1985a, b, 1987; Hamdi and Kostrzewa 1991; Gong et al. 1993a). DA D_1 receptor supersensitization seemingly accounts for the enhanced oral activity response to D_1 agonists in neonatal 6-OHDA-lesioned rats (Kostrzewa and Gong 1991).

In neonatal 6-OHDA-treated rats there is reactive proliferative sprouting of serotonin (5-HT) fibers in the neostriatum (Stachowiak et al. 1984; Berger et al. 1985; Snyder et al. 1986; Descarries et al. 1992). Accompanying this change is an enhanced oral activity response to the 5-$HT_{2A,2C}$ agonist, *m*-chlorophenylpiperazine (*m*-CPP) (Gong and Kostrzewa 1992), but no change in the response to the respective 5-HT_{1A} and 5-HT_{1B} agonists, 8-hydroxydipropylaminotetralin and CGS 12066B (7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1, 2-alquinoxaline]) (Arvidsson et al. 1981; Middlemiss and Fozard 1983; Neale et al. 1987; Gong et al. 1992). Pindolol (Hoyer et al. 1985; Glennon 1987), ketanserin (Leysen et al. 1981, 1982) and MDL 72222 (3-tropanyl-3,5-dichlorobenzoate) (Fozard 1984), antagonists with high affinity for 5-$HT_{1A,1B}$, 5-HT_{2A} and 5-HT_3 receptors, respectively, did not attenuate *m*-CPP actions. However, mianserin, an antagonist with high affinity for 5-$HT_{2A,2C}$ receptors (Glennon 1987), attenuated the oral response to *m*-CPP. Therefore, 5-HT_{2C} receptors seem to be associated with the *m*-CPP-induced oral activity responses in intact and DA-lesioned rats (Gong et al. 1992). [Nomenclature for 5-HT receptors follows the recommendations of the Serotonin Club Receptor Nomenclature Committee. The 5-HT_{2A} receptor was formerly the 5-HT_2 receptor. The 5-HT_{2C} receptor was formerly the 5-HT_{1C} receptor (Humphrey et al. 1993)].

By varying the age at which 6-OHDA was administered to rats and by varying the dose of 6-OHDA, it was found that 5-HT receptor supersensitization can occur in the absence of DA D_1 receptor supersensitization, but D_1 receptor supersensitization does not occur in the absence of 5-HT_{2C} receptor supersensitization in neonatal 6-OHDA-lesioned rats (Gong et al. 1993b; Kostrzewa et al. 1993). In fact, enhanced oral activity responses to a D_1 agonist seem to be mediated via 5-HT_{2C} receptors, since the 5-HT receptor antagonist mianserin attenuates oral activity responses to a D_1 agonist. Conversely, the D_1 receptor antagonist SCH 23390 does not attenuate *m*-CPP-induced oral activity in neonatal 6-OHDA-lesioned rats (Gong et al. 1992). Partial 5,7-dihydroxytryptamine (5,7-DHT) destruction of 5-HT fibers in neonatal rats also prevents development of enhanced oral activity responses to a D_1 agonist in neonatal 6-OHDA-lesioned rats (Brus et al. 1994). These series of studies indicate that 5-HT fibers and receptors have a role in mediating oral activity responses to a DA D_1 receptor agonist.

To understand better the interactive nature between DA and 5-HT neurochemical systems in the genesis of oral activity, it is important to know whether these systems might be interacting within the same region or in different foci in the brain. The ventral neostriatum represents a brain region in which DA agonists and amphetamine induce oral behavioral responses of rats (Arnt 1985; Scheel-Krüger and Arnt 1985; Kelley et al. 1988; Koshikawa et al. 1989). Enhanced oral activity produced by apomorphine in this site is attenuated by microinjections of SCH 23390 and numerous DA receptor antagonists at this site (Arnt 1985). Similarly, oral activity induced by systemic apomorphine is attenuated by local injection of SCH 23390 and sulpiride into ventral neostriatum (Scheel-Krüger and Arnt 1985; Koshikawa et al. 1989). When administered into ventrolateral but not dorsolateral neostriatum, amphetamine induces oral activity (Kelley et al. 1988). Although a 6-OHDA lesion of the ventral neostriatum attenuates the oral activity response of amphetamine microinjected into the ventrolateral neostriatum (Scheel-Krüger and Arnt 1985), the 6-OHDA-lesion per se induces oral activity (Jicha and Salamone 1991). Other neurochemical systems in ventral or ventrolateral neostriatum similarly appear to be involved in the production of oral activity of rats (Pisa 1988; Pisa and Schranz 1988; Kelley et al. 1989; Salamone et al. 1990). Because of the extensive findings implicating ventral striatum as a focus of dopaminergic and other neuronal interactions in the induction of oral activity of rats, we studied the possible interaction between DA and 5-HT systems at this site. Rats were chronically implanted with cannulae so that DA and 5-HT receptor agonists

and antagonists could be locally administered into the ventral neostriatum.

Materials and methods

Animals

Timed-pregnant CD albino rats were obtained from Charles River Laboratories (Research Triangle, N.C.). Animals were housed at 22 ± 1°C, under a 12:12-h schedule (lights on 0700–1900 hours). At birth, rat pups from all litters were grouped together and then randomly assigned to dams, with a maximum new litter size of ten pups. Thus, each dam would have had rats from several litters.

Neonatal treatment

At 3 days after birth rats were pretreated with desipramine HCl (20 mg/kg IP, base form), 1 h before bilateral intracerebroventricular (ICV) injection of 6-OHDA HBr (100 µg, salt form, in each lateral ventricle) or the vehicle, saline-ascorbic acid (0.1%). This procedure has been described in detail (Kostrzewa and Gong 1991).

Neonatal 6-OHDA treatment is known to impair growth and development of rats (Smith et al. 1973; Bruno et al. 1987; Gong et al. 1992). In litters with both 6-OHDA-lesioned and intact rats, lesioned rats are less competitive for the dam's nipples, so that the disparity in body weight between intact and lesioned rats becomes increasingly greater during development. From experience, we have found that there is more uniformity in weight within groups of intact rats and groups of 6-OHDA-lesioned rats, when rats of only a single treatment are present within a litter. This means of rearing also produces less disparity in body weight between intact and lesioned rats. For this reason, whole litters were constituted of either 6-OHDA- or vehicle-treated rats. Rats were weaned at 28 days and were then group housed by sex in wire cages. Rats of both sexes were used in this study.

Surgery

Guide cannulae (C 315/26 gauge; Plastics One, Roanoke, Va.) were implanted when rats had a body mass of 200–250 g. Each rat was anesthetized with sodium pentothal (Nembutal, 55 mg/kg IP; Abbott, North Chicago, Ill.) prior to incising the dermis overlying the skullplate. Burr holes were made on each side of the interaural line, using coordinatesof A 2.2 mm and L 3.0 mm from Bregma; V 5.0 mm below the dura (Pellegrino et al. 1979). The guide cannulae were attached to the skull by screws and cranioplastic cement (Plastics One) and positioned so that the tips resided 1 mm above the site of injection.

Acute injections

Drugs were administered bilaterally into the ventral neostriatum via the guide cannulae, starting 1 week after implantation. Briefly, the dummy cannulae were removed from the guide cannulae, and internal cannulae (C 315/33 gauge; Plastics One) were inserted. The internal cannulae were connected by polyethylene tubes with Hamilton microsyringes (Hamilton; Reno, Nev.), which delivered each agent in a volume of 0.5 µl on each side. Each injection was made manually over an interval of 30 s. Cannulae remained in place for an additional 30 s. After removal, the dummy cannulae were reinserted. Rats were awake for these injections. No sedative or anesthesia was required.

Phenomenology of oral activity

Oral activity is of the type described by Waddington (1990) as "vacuous (or abortive or spontaneous) chewing, whereby what appear to be robust chewing sequences are manifested, but are not directed onto any evident physical material." No differentiation was made between lateral and vertical jaw movements. There was also occasional tongue thrusting. Oral activity that may have occurred in eating or grooming was not counted.

Observation of oral activity

For each test session rats were placed in individual clear plastic cages (48 × 24 × 36 cm) with steel grid floors in a quiet, well-ventilated and well-lighted room. Rats were allowed to acclimate to this environment for at least 1 h prior to intrastriatal injections. Each rat was then observed one at a time, continuously for the following 60 min. A single observer who was experienced with the procedure determined the numbers of oral movements for each rat. Generally, there was an average of one or two oral movements per min. When a relatively high dose of SKF 38393 (5 nmol per striatum) or *m*-CPP (10 nmol per striatum) was used (Fig. 1), there was an average of five oral movements per min, sometimes occurring in bouts. In this study another oral movement would be counted only if there was an interval of > 2 s between adjoining chewing episodes. However, a single oral movement could have persisted for several seconds.

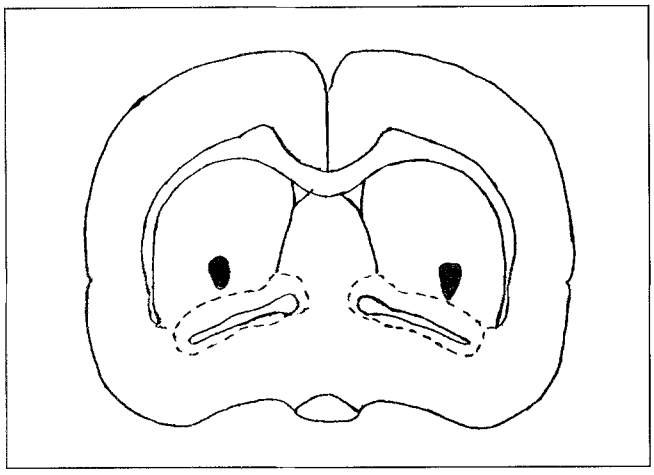
Because of the inherent error that arises when the magnitude of the oral activity response is great, we used relatively low doses of SKF 38393 and *m*-CPP after the first study. However, these low doses of SKF 38393 and *m*-CPP would have minimal or negligible effects on oral activity in intact rats. Because we wished to demonstrate that intact rats would also display oral behavior with the same agonists administered at the same site, it was necessary to use a different dose of the agonists in intact and lesioned rats. Even with this "bias" against showing a difference in the oral activity response between intact and lesioned rats, the findings clearly demonstrate such a difference – reflecting the receptor supersensitization that occurs in neonatal 6-OHDA-lesioned rats.

After each session, rats were placed in other cages and observed at intervals for the next few hours, so that abnormal or repetitive behaviors could be detected if they occurred. No abnormal behaviors were observed. Most rats were tested more than once, but with an interval of at least 5 days between sessions.

A total of 36 rats were used in this study, with each group consisting of four to nine rats. The majority of rats received three intrastriatal microinjections or less. Only three rats received as many as three injections with SKF 38393. All groups consisted of two orthree rats that were being tested for the first time. The number of oral movements after a replicate injection of SKF 38393 was virtually identical to that produced by the first injection of SKF 38393 (10 nmol) in the two control and one 6-OHDA-lesioned rats that were tested in this way. After saline administration there also was no change in the number of oral movements in each of six 10-min segments of the 1-h observation session, for either intact or 6-OHDA-lesioned rats.

Determination of cannula placement

At the end of the experiment 2 µl of a methylene blue marker in gelatin was injected via the cannulae. Rats were decapitated and brains were removed and stored in 10% formalin for 3–4 days. The gelatin would have impeded dye diffusion during this stage (see Myers 1971). Alternate transverse frozen sections (50 µm) were stained with cresyl violet so that the placement site of the guide cannulae could be determined in the anterior plane.



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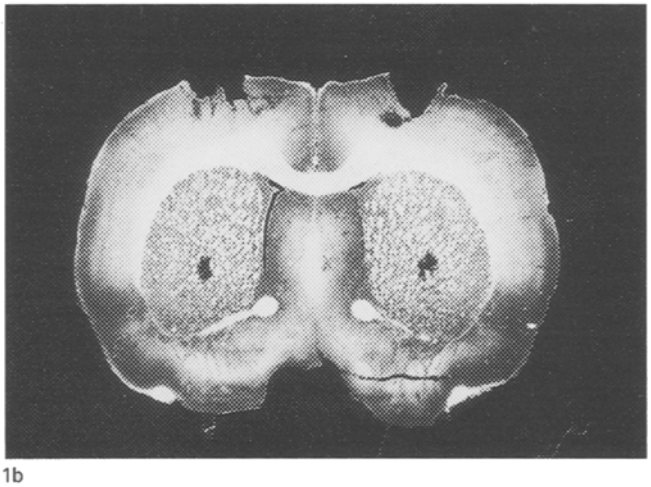

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Fig. 1a Schematic illustration (*top*) of a coronal section of rat brain corresponding to an A-P level of 8.0 from the atlas of Pellegrino et al. (1979). The *shaded area* indicates the site of cannula tips in cannula-implanted rats. **b** Cresyl violet-stained coronal section of rat brain corresponding to the identical A-P level as in **a**. The site of methylene blue injection (2 µl) corresponds to the site of cannula tips

Data analysis

For each of the intact and neonatal 6-OHDA-lesioned rat groups, measurements of oral activity were analyzed for statistical effects of different experimental test conditions (various DA and 5-HT agonist and antagonist treatments) with analysis of variance using the general linear models procedure in the SAS-PC software package.

Data were additionally analyzed on the log-transform scale due to heterogeneous variances of original measurements. For each study group the mean and standard deviation were used to summarize measurements of oral activity, while the geometric mean was used to summarize the log-transformed measurements for each study condition.

Since the *F*-test for experimental conditions was statistically significant for the lesioned versus non-lesioned groups, the least significant difference procedure was used to assess pairwise comparisons of interest (Milliken and Johnson 1984). Probability levels of 0.05 or smaller were used to indicate statistical significance.

Drugs

SKF 38393 HCl, SCH 23390 HCl, *m*-CPP 2HCl and mianserin HCl were obtained from Research Biochemicals (Natick, Mass.). Desipramine HCl was from Sigma (St Louis, Mo.) and 6-OHDA HBr was from Regis Chemical (Chicago, Ill.). Except for desipramine, doses of all drugs refer to the salt form in this study. Also, except for 6-OHDA HBr, all drugs were dissolved in saline (0.9%).

Results

Cannula placement

The tips of implanted cannulae were located in ventral striatum, at sites shown in the cresyl violet-stained sections and in the illustration of the corresponding panel from the Pellegrino and Cushman stereotaxic atlas (Pelligrino et al. 1979). Nearly all rats had such cannula placement. In this study two rats were excluded because the cannulae were improperly positioned. In addition, two rats were excluded, due to their losing the cap with the guide cannula.

Effects of intrastriatal SKF 38393 and *m*-CPP on oral activity of intact and 6-OHDA-lesioned rats

Following bilateral intrastriatal saline injection, control rats had 25.3 ± 7.3 (mean ± SEM) oral movements during a 1-h observation period. Intrastriatal SKF 38393 HCl (5 nmol per side) and *m*-CPP 2 HCl (10 nmol per side) increased oral activity in these rats about 3-fold (versus saline injection) during the 1-h session (Table 1).

In neonatal 6-OHDA-lesioned rats, bilateral intrastriatal saline injection was associated with 41.8 ± 9.4 oral movements. Intrastriatal SKF 38393 HCl (1 or 5 nmol per side) increased oral activity about 2- or 8-fold (versus saline injection), respectively. Intrastriatal *m*-CPP, 5 or 10 nmol per side, increased oral activity 3-fold and 6-fold, respectively (Table 1).

Effects of intrastriatal SCH 23390 and mianserin on agonist-induced oral activity

In control rats, SCH 23390 (10 nmol in each striatum) attenuated the oral activity response to SKF 38393 (5 nmol in each striatum) (Table 1). A dose of mianserin (6 nmol in each striatum) that effectively attenuated *m*-CPP-induced (10 nmol in each striatum) oral activity also attenuated SKF 38393-induced (5 nmol in each striatum) oral activity.

In neonatal 6-OHDA-lesioned rats the enhanced oral activity response to SKF 38393 (1 nmol in each striatum) was attenuated by both SCH 23390 (2 nmol in each striatum) and mianserin (6 nmol in

Table 1 Oral activity responses of intact and neonatal 6-OHDA-lesioned rats after various DA and 5-HT agonists and antagonists. Each value is the mean (± SEM) number of oral movements of rats that were treated at 3 days after birth with 6-OHDA (134 μg, ICV; desipramine pretreatment, 20 mg/kg IP, 1 h). Numbers of rats per groups are indicated in parentheses. When intact rats were treated with both SCH 23390 and SKF 38393, doses of 20 nmol and 10 nmol were used, respectively; with 6-OHDA-lesioned rats, doses of 4 nmol and 2 nmol were used respectively. Also, when rats were treated with both mianserin and *m*-CPP, 20 nmol and 10 nmol doses of *m*-CPP were used in intact and 6-OHDA-lesioned rats, respectively

Treatment	Arithmetic means		Geometric means	
	Intact rats	6-OHDA-lesioned rats	Intact rats	6-OHDA-lesioned rats
Saline	25.3 ± 7.3 (6)	41.8 ± 9.4 (5)	16.4	48.0^{+}
SKF 38393, 2 nmol	37.5 ± 9.0 (8)	85.3 ± 14.8 (9)$^{++}$	22.9	90.9^{***+}
SKF 38393, 10 nmol	74.3 ± 19.2 (4)**	310.7 ± 97.0 (5)$^{**++}$	32.7^{*}	139.8^{***+}
m-CPP, 10 nmol	32.2 ± 9.7 (5)	137.6 ± 23.0 (7)$^{**++}$	21.6	85.6^{***+}
m-CPP, 20 nmol	72.6 ± 15.1 (5)**	274.5 ± 65.0 (6)$^{**++}$	46.9^{*}	119.8^{***+}
SCH 23390				
$^{+}$Saline	46.0 ± 12.9 (4)	44.8 ± 9.7 (4)	17.3	35.9
$^{+}$SKF 38393	12.8 ± 1.7 (5)$^{\#}$	47.2 ± 13.9 (5)$^{\#}$	$18.7^{\#}$	$24.6^{\#\#}$
Mianserin, 12 nmol				
$^{+}$Saline	20.4 ± 3.6 (5)	18.0 ± 5.6 (5)	12.9	18.7
$^{+}$SKF 38393, 10 nmol	23.8 ± 6.8 (4)$^{\#}$	132.9 ± 49.0 (7)$^{\#}$	$36.5^{\#}$	$70.3^{\#\#}$
$^{+}$*m*-CPP	15.6 ± 4.8 (5)$^{\#}$	68.3 ± 47.3 (4)$^{\#}$	$17.0^{\#}$	$22.6^{\#\#}$

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus saline treatment of same group; $^{+}P = 0.03$, $^{++}P < 0.01$ versus intact rats treated with same challenge agent; $^{\#}P < 0.01$, $^{\#\#}P < 0.001$, versus group treated with same agonist (SKF 38393 or *m*-CPP) in the absence of the antagonist (SCH 23390 or mianserin)

each striatum). Mianserin also attenuated the enhanced oral activity response to *m*-CPP (5 nmol in each striatum).

Discussion

It is well established that DA D_1 receptors become supersensitized in rats that are lesioned neonatally with the neurotoxin, 6-OHDA (Breese et al. 1984, 1985a, b, 1987). The DA D_1 receptor supersensitization is associated with prolonged depletion of endogenous striatal DA content (Breese et al. 1984, 1985a, b, 1987; Gong et al. 1992), but without a change in the B_{max} or K_d for DA D_1 receptors (Breese et al. 1987; Kostrzewa and Hamdi 1991). In earlier studies in this laboratory we have found that the dose of 6-OHDA used in the present study is associated with a 98–99% reduction in DA content of the neostriatum (Gong et al. 1992, 1993a, b; Kostrzewa et al. 1993; Brus et al. 1994). The loss of DA fiber input to the striatum is accompanied by proliferative sprouting of 5-HT fibers which ultimately hyperinnervate the striatum (Stachowiak et al. 1984; Berger et al. 1985; Snyder et al. 1986; Luthman et al. 1987; Towle et al. 1989; Descarries et al. 1992).

While DA D_1 and 5-HT receptor agonists induce oral activity in intact rats (Rosengarten et al. 1983, 1986; Arnt et al. 1987; Molloy and Waddington 1988; Murray and Waddington 1989; Stewart et al. 1989), the effects of these substances are potentiated in neonatal 6-OHDA-lesioned rats studied as adults (Kostrzewa and Gong 1991; Gong and Kostrzewa 1992). The 5-HT_{2C} receptor seems to be of particular importance in mediating the oral activity responses of DA D_1 and 5-HT receptor agonists (Gong et al. 1992). Although it was proposed that DA systems acted via 5-HT fibers and receptors (Kostrzewa 1993), it is possible that these neural systems could act in different parts of the brain and influence a common site without directly interacting. For that reason, it was important to study the effect of DA and 5-HT receptor agonists and antagonists in an area known to play an important role in the genesis of oral activity, specifically the ventral striatum. Although we focused on this single locus in the present study, it is recognized that DA and 5-HT neurochemical systems can interact at many other sites in the brain.

Our findings demonstrate that the 5-HT receptor agonist, *m*-CPP, produces oral activity following its administration directly into ventral neostriatum. This effect is believed to be due to an agonistic action of *m*-CPP at 5-HT_{2C} receptors (Pazos et al. 1984; Glennon 1987; Curzon and Kennett 1990; Gong et al. 1992). Moreover, the responses to intrastriatal SKF 38393 and *m*-CPP are enhanced in the neonatal 6-OHDA-lesioned rats, as is the case following their peripheral administration (Kostrzewa and Gong 1991; Gong and Kostrzewa 1992). The 5-HT_{2A} receptor antagonist, ketanserin does not attenuate SKF 38393- and *m*-CPP-induced oral activity (Gong et al. 1992). However, because the 5-$HT_{2A,2C}$ antagonist mianserin attenuates the oral activity responses to both SKF 38393 and *m*-CPP, it appears that intrastriatal 5-HT_{2C} receptors are specifically involved in the mediation of DA and 5-HT agonist effects. This pharmacological interaction reinforces the suggestion that 5-HT fibers are in a neural circuit with DA fibers in the induction of oral behavior in rats. Moreover, since the 5-HT neurotoxin, 5,7-DHT, eliminates enhanced oral activity responses to a DA D_1 agonist (Brus et al. 1994; Kostrzewa et al.

1994), it may be that DA fibers actually make synaptic contact with 5-HT axons or nerve endings. Despite the fact that the DA D_1 receptor agonist SKF 38393 is able to act at 5-HT_{2C} receptors (Setler et al. 1978; Briggs et al. 1991), it has been determined from a study with the highly selective full DA D_1receptor agonist, A77636 ([1*R*, 3*S*]-3-[1′-adamantyl]-1-aminomethyl-3,4-dihydro-5,6-dihydroxy-1H-2-benzopyran), that SKF 38393 produces oral activity responses via DA D_1, not 5-HT_{2C} receptors (Huang and Kostrzewa 1994).

Both DA D_1 and 5-HT_{2C} receptor supersensitization occurred in the rats in this study. However, by varying the dose of 6-OHDA to neonatal rats, it is possible to produce 5-HT_{2C} receptor supersensitization in the absence of DA D_1 receptor supersensitization. The opposite is not so (Kostrzewa et al. 1993). By administering 6-OHDA to rats of different ages, it is also possible to induce 5-HT_{2C} receptor supersensitization in the absence of DA D_1 receptor supersensitization (Gong et al. 1993b, Kostrzewa et al. 1993). Therefore, DA D_1 receptor sensitivity seems to be secondary to changes in 5-HT_{2C} receptor sensitivity. When DA neurons are destroyed during postnatal ontogeny, as in the present study, DA D_1 receptor proliferation does not accompany D_1 receptor supersensitization. Nor does DA D_1 receptor affinity change (Breese et al. 1987; Kostrzewa and Hamdi 1991). It now appears that specific postsynaptic effects or altered neural conduction in downstream pathways may account for the phenomenon of receptor sensitization (Kostrzewa 1994).

The somatotopic organization of rat striatum has recently been a lively area of investigation. The lateral striatum (Pisa 1988) and more specifically the ventrolateral striatum has been found to be associated with motor control of oral activity (Kelley et al. 1988, 1989; Pisa and Schranz 1988; Salamone et al. 1990). In contrast, both the ventrolateral and ventromedial striatum are implicated in effecting tongue protrusions (Salamone et al. 1990). When DA is depleted from the ventrolateral, but not anterior ventromedial or dorsolateral striatum, spontaneous oral activity is produced in rats (Jicha and Salamone 1991). In the present study the lesion site is approximately midway between medial and lateral borders of the ventral striatum. In effect, we show that a site within the striatum that is more centrally located in ventral striatum is also involved in oral activity control. It cannot be determined whether this would represent a broadening of the ventrolateral region of striatum that is involved in motor control of the buccal region, or whether this is a functionally separate area.

Microinjections of high dose *R*- or *S*-SKF 38393 into rat striatum (30 μg/0.5 μl) and nucleus accumbens (3 or 30 μg/0.5 μl) can produce extensive tissue damage at the injection site (Delfs and Kelley 1990; Kelley et al. 1990). Also, starting 2–4 h after large SKF 38393 injections, these rats demonstrate increased oral stereotypies, mainly self-biting of the forelimbs or flanks (Hartgraves and Randall 1986; Delfs and Kelley 1990; Kelley et al. 1990) or increased locomotor activity, peaking as late as 6–7 h (Fletcher and Starr 1987; Dreher and Jackson 1989). However, Isaac et al., (1992) did not alter glutamic acid decarboxylase or acetylcholinesterase activities after microinjections of high dose SKF 38393 (30 μg/0.5 μl) into the striatum and concluded that local cellular damage was not produced in this study. The authors suggested that local tissue injury may have been produced in the earlier studies as a consequence of hyperosmolality, possibly even precipitation of SKF 38393 from the hypertonic solution. In the present study the dose of SKF 38393 was > 20-fold lower than doses reported to produce local tissue damage. There would have been little effect of SKF 38393 on osmolality of the injectate in the present study. At the conclusion of this study, the striatum of every animal was cresyl violet-stained. In no instance was there any evidence of tissue injury, except from the needle. Also, we observed no bizarre behaviors in rats in ≥ 2 h of observation following each injection. Therefore, we believe that the results of SKF 38393 in particular, and other drugs in general, represent receptor-mediated effects. This is supported by the fact that SCH 23390 effectively attenuated SKF 38393-induced oral activity.

In summary, the present study demonstrates that oral activity responses of neonatal 6-OHDA-lesioned rats are enhanced by intrastriatal SKF 38393 and *m*-CPP injections and that these effects are effectively attenuated by intrastriatal administration of the 5-HT receptor antagonist, mianserin. It is possible that DA and 5-HT interactions occur in other brain regions as well.

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