ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1992, 52: 31-41

Serotonin (5-HT) systems mediate dopamine (DA) receptor supersensitivity

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Abstract. To study interactions between DA and 5-HT neurochemical systems in the DA D_1 supersensitized induction of oral activity in neonatal 6-hydroxydopamine (6-OHDA) lesioned rats, the effects of a variety of 5-HT receptor agonists and antagonists were determined. At 3 days after birth rats were treated with desipramine HCl (20 mg/kg i.p., base form) 1 h before 6-OHDA HBr (100 µg, salt form, in each lateral ventricle). When these rats were studied as adults it was determined that the striatal content of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was reduced by 98%, while the striatal content of 5-HT was elevated by 75%. The B_{max} and K_d for [^{3}H]SCH 23390 and [^{3}H]spiperone binding to striatal homogenates was unaltered in the lesioned rats. However, oral activity responses to a D_1 agonist (SKF 38393), D_2 antagonist (spiperone) and 5-HT_{1C} agonist [1-(3-chlorophenyl)piperazine] were enhanced several fold in the lesioned rats. Several other agonists and antagonists that act at 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors did not produce an altered response in the lesioned rats, nor were these substances effective in attenuating *m*-CPP-enhanced oral activity responses. The DA D_1 receptor antagonist, SCH 23390 HCl (0.30 mg/kg i.p.), did not attenuate the response to *m*-CPP 2HCl (1.0 mg/kg i.p.). However, the 5-HT receptor antagonist, mianserin HCl (1.0 mg/kg s.c.) did effectively attenuate the oral activity response to SKF 38393 HCl (1.0 mg/kg i.p.). These findings indicate that there is supersensitization of both DA D_1 and 5-HT_{1C} receptors in neonatal 6-OHDA-lesioned rats, and that a D_1 agonist acts via the 5-HT_{1C} receptors. Therefore, induction of oral activity by DA agonists occurs through a serotoninergic neurochemical system.

Key words: 5-HT receptor, 6-hydroxydopamine, D_1 receptor, D_2 receptor, dopamine, oral activity, serotonin, SKF 38393, supersensitization

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INTRODUCTION

Long-term treatment of rats with neuroleptics results in the development of spontaneous oral activity (Clow et al. 1979, Waddington and Gamble 1980). Acute treatment of rats with dopamine (DA) D_2 receptor antagonists or D_1 receptor agonists, also results in the production of oral activity. The balance between D_1 and D_2 receptor responsiveness has been suggested as an important factor in the induction of oral activity (Rosengarten et al. 1983). This hypothesis is supported by the fact that there is a greater incidence of oral activity in rats when there is a functional or overt increase in the D_1/D_2 receptor ratio, as occurs (1) after treatment with a D_2 receptor antagonist (Rosengarten et al. 1983, Arnt et al. 1987, Koshikawa et al. 1987), (2) after treatment with a D_1 receptor agonist (Rosengarten et al. 1983, Molloy and Waddington 1987, 1988, Murray and Waddington 1989), (3) after prenatal neuroleptic treatment which reduces D_2 receptor number, (4) in certain strains of rats that have reduced numbers of D_2 receptors (Rosengarten et al. 1986a), or (5) as a consequence of aging (Rosengarten et al. 1986a, Molloy and Waddington 1988). A reduction in oral activity following inactivation of D_1 receptors is compatible with this view (Rosengarten et al. 1986b).

Breese and co-workers showed that rats lesioned as neonates with the catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA), can become sensitized to agonists acting at DA D_1 receptors (Breese et al. 1984, 1985a, b, 1987, Criswell et al. 1989). The ability to sensitize D_1 receptors appears to be related to immaturity of the DA system at the time of lesioning, since 6-OHDA treatment of adult rats is not associated with such receptor sensitization (Breese et al. 1984).

The above factors led to a series of studies that have established the neonatal 6-OHDA lesioned rat as a useful model for determining the involvement of DA systems in the induction of oral activity. Moreover, the interaction between DA and other neurochemical systems in the genesis of oral activity is now being realized. It is suggested that this animal model is one that is suitable for studying mechanisms that may be involved in dyskinetic activity in humans with tardive dyskinesia - a long-lived motor abnormaility that arises after excessive use of neuroleptic agents.

METHODS

Animal treatment

Timed pregnant Sprague Dawley albino rats were treated at 3 days after birth with desipramine hydrochloride (20 mg/kg i.p., base form) 1 h before bilateral intracerebroventricular (i.c.v.) injections of 6-OHDA hydrobromide (100 µg, salt form, on each side). Control rats received the same dose of desipramine, but in combination with the vehicle, saline (0.85%) containing ascorbic acid (0.1%), in place of 6-OHDA. After weaning at 28 days, rats were housed by sex and studied when at least 6 weeks old.

Animal testing

To observe oral activity rats were placed in individual clear plastic cages (48 x 26 x 18 cm or 48 x 26 x 36 cm, depending on the size of the rat) in a quiet, well-ventilated and well-lighted room. In some cases there was a steel grid floor. Cages of the same height were used for any single test session.

After an accommodation period of at least 1 h, some rats were treated with the DA D_1 receptor agonist, SKF 38393 HCl [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride; 0.3 to 6.0 mg/kg i.p.], or with the DA D_2 receptor-antagonist, spiperone hydrochloride (80 µg/kg i.p.). Other rats received the following 5-HT receptor agonists: (±)-8-hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT HBr; 0.50 mg/kg s.c.), 1-(3-chlorophenyl)piperazine dihydrochloride (*m*-CPP 2HCl; 0.3 to 6.0 mg/kg), 7-trifluoromethyl-4-(4-ethyl-1-piperazinyl)-pyrrolo[1,2-alquinoxaline] 1:2 maleate salt (CGS 12066B maleate; 3.0 mg/kg i.p.); or saline (0.9%) vehicle.

In some studies rats were pretreated with the DA D_1 receptor antagonist, SCH 23390 HCl [R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetra hydro-1H-3-benzazepine hydrochloride; 0.30 mg/kg i.p., 1 h]; or the 5-$HT_{1A,1B}$ receptor antagonist, pindolol (1.0 mg/kg i.p., 30 min); or mixed 5-HT_{1C} and 5-HT_2 receptor antagonist, mianserin HCl (1.0 mg/kg s.c., 30 min); or the 5-HT_2 receptor antagonist, ketanserin tartrate (5.0 mg/kg i.p., 30 min); or 5-HT_3 receptor antagonist, MDL 72222 (3-tropanyl-3,5-dichlorobenzoate; 10.0 mg/kg s.c., 30 min).

Each rat was observed one at a time, for 1 min every 10 min, over a 30 or 60 min period, starting 10 min after an agonist or 60 min after spiperone. Numbers of rapid jaw movements were counted. Oral activity was 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." Oral activity that occurred in eating, grooming or taffy pulling (coordinated movement of the forepaws toward the mouth and then away from the body) was not counted.

Neurochemical analysis

After completion of oral testing, at least 3 days intervened before rats were decapitated. Each brain was rapidly removed and the striata were dissected free, frozen on dry ice and stored at -70° C. Striata were sonicated in 0.10 M trichloroacetic acid containing 0.20 mg/ml of cysteine and the internal standard, 0.20 nmol/ml of 5-hydroxyindole carboxylic acid.

Striatal concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were assessed, using a Bioanalytical Systems liquid chromatograph with an Econosphere C18 analytical column (5 micron, 4.6 x 150 mm; Alltech Associates) having a mobile phase of 0.10 M monochloroacetic acid, 1 mM EDTA, 220 mg/l of sodium octanesulfonic acid and 8% acetonitrile with a pH of 2.6 at a flow rate of 1.3 ml/min and temperature of 40°C. A glassy carbon electrode was used at a potential of +0.75 V (see Gong et al. 1992)

Asessment of DA D_1 and D_2 binding sites

Striata were assessed for dopamine D_1 and D_2 receptor binding activity, respectively, by the methods of Schulz et al. (1985) and Creese and Snyder (1979). Briefly, striata were gently homogenized, using a Teflon on glass mortar and pestle in 50 mM Tris buffer (pH 7.4), to avoid destruction of DA receptors (Norman et al. 1989). For D_1 receptor binding the ligand [^{3}H]SCH 23390 (50 to 2500 pM, final conc.) was used for *in vitro* incubations with tissue homogenates. For D_2 receptor binding the ligand [^{3}H]spiperone (25 to 1500 pM) was used (see Kostrzewa and Hamdi 1991).

Statistics

Behavioural and biochemical data of treated and control groups were compared by an analysis of variance (ANOVA), followed by the post-ANOVA test of Newman-Keuls.

RESULTS

Induction of oral activity by a DA D_1 receptor agonist or D_2 receptor antagonist; supersensitized responses in lesioned rats

In both intact and neonatal 6-OHDA lesioned rats, an acute saline vehicle injection was associated with less than 5 oral movements during a 1 h observation session. The D_1 receptor agonist, SKF 38393 HCl (3.0 mg/kg i.p.), produced 40 oral movements in the 6-OHDA group, nearly a 4-fold greater effect than in controls. The D_2 receptor antagonist, spiperone (80 µg/kg i.p.), produced an effect similar to that of SKF 38393 (Fig. 1). Although not shown, the dose of each agent corresponds to maximal potency. These findings indicate that (1) D_1 receptor activation and D_2 receptor antagonism produce oral activity in rats, while (2) neonatal 6-OHDA treatment

markedly enhances the effects of these agents (Kostrzewa and Hamdi 1991).

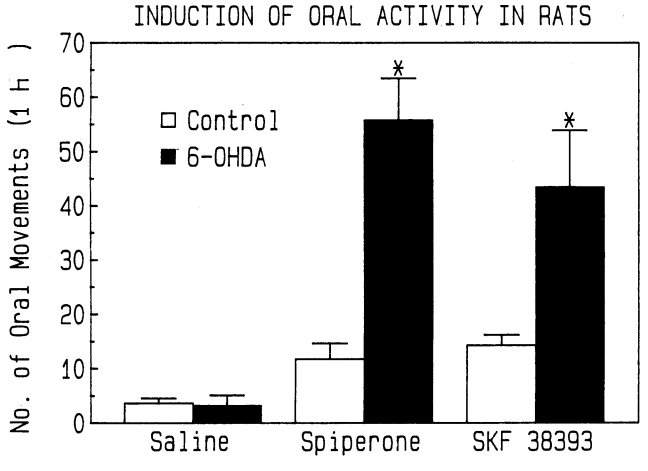

Fig. 1. Enhanced induction of oral activity by SKF 38393 and spiperone in neonatal 6-OHDA-lesioned rats. At 3 days after birth rats were treated with desipramine HCl (20 mg/kg i.p., base form) 1 h prior to 6-OHDA HBr (100 μg in each lateral ventricle). Numbers of oral movements were determined in these rats in adulthood, by counting the chewing movements for one min every 10 min over a 60 min period, beginning 10 min after SKF 38393 HCl (3.0 mg/kg i.p.) or 60 min after spiperone HCl (80 μg/kg i.p.). Each group is the mean (± SEM) of 4 to 8 rats. *indicates $P<0.005$ vs. respective control group. (Reprinted with permission from Pergamon Press Ltd., Kostrzewa and Gong, 1991).

Destruction of dopaminergic fibres by neonatal 6-OHDA treatment

Marked depletion (98%) of striatal DA, DOPAC and HVA was produced by neonatal 6-OHDA treatment and this was accompanied by a marked increase (75%) of striatal 5-HT content (Table I). These changes are compatible with the findings of others, reflecting destruction of DA fibres (Breese and Traylor 1971, 1972, Breese et al. 1984, Stachowiak et al. 1984) and sprouting of 5-HT fibres (Stachowiak et al. 1984, Berger et al. 1985, Luthman et al. 1987, Towle et al. 1989). Either or both events are seemingly responsible for the supersensitized responses to SKF 38393 and spiperone in the neonatal 6-OHDA-lesioned rats (Gong et al. 1992).

Effect of neonatal 6-OHDA treatment on striatal D_1 and D_2 binding sites

Neonatal 6-OHDA treatment did not alter binding of ligands to striatal D_1 and D_2 sites. The B_{max} for [^{3}H]SCH 23390 *in vitro* binding was 1254 ± 49 in striatum from control rats and 1203 ± 69 pmol/g protein in striatum from 6-OHDA-treated rats; K_d was 371± 12 and 359 ± 15 pM, respectively. The B_{max} for [^{3}H]spiperone *in vitro* binding was 505 ± 43 in striatum from control rats and 517 ± 64

TABLE I

Effect of neonatal 6-OHDA treatment of concentrations of DA, 5-HT and their metabolites in the striatum of rats. Values are mean nanomoles per gram of tissue ± SEM. Numbers in parentheses, numbers of samples per group. Rats were treated at 3 days after birth with 6-OHDA HBr(200 μg i.c.v.) or vehicle. Striata were removed for assay at 9 months. (Reprinted with permission from the Williams and Wilkins Company, Gong et al. 1992)

	Treatment		
Monoamine or metabolite	Vehicle	6-OHDA	% of control
DA	67.83 ± 1.8(6)	0.95 ± 0.16(9)***	1.4
HVA	7.50 ± 0.53(6)	0.14 ± 0.04(9)***	1.9
DOPAC	14.45 ± 0.87(6)	0.27 ± 0.05(9)***	1.9
5-HT	3.52 ± 0.15(6)	6.23 ± 0.55(9)***	177
5-HIAA	6.53 ± 0.30(6)	7.50 ± (9)	115

***$P<0.001$ when compared to vehicle control group

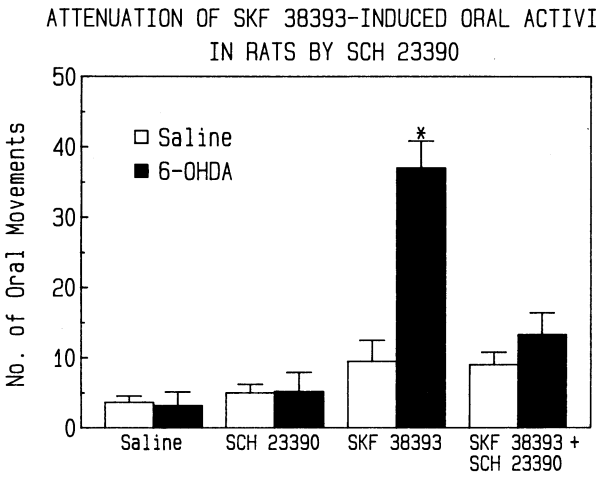

Fig. 2. Attenuation of SKF 38393-induced oral activity in rats by SCH 23390. Rats were treated neonatally and observed for oral activity as in Fig. 1. SCH 23390 HCl (0.30 mg/kg i.p.) or saline was administered 1 hr prior to SKF 38393 HCl (0.30 mg/kg i.p.). Each group is the mean (± SEM) of 4 to 6 rats. *indicates $P<0.005$ vs. other 6-OHDA groups. (Reprinted with permission from Pergamon Press Ltd., Kostrzewa and Gong 1991).

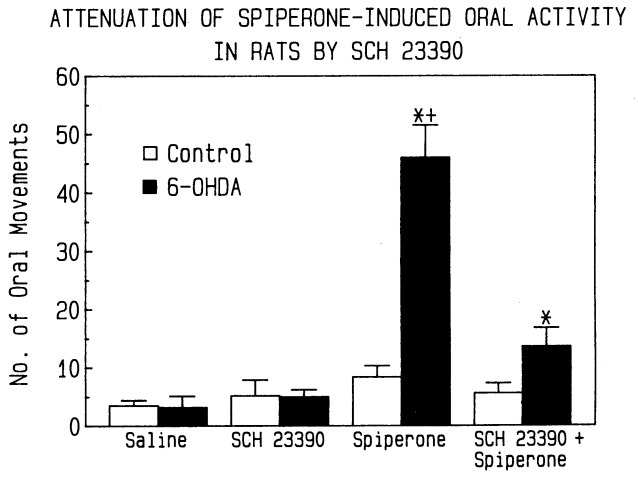

Fig. 3. Attenuation of spiperone-induced oral activity in rats by SCH 23390. Rats were treated neonatally and observed for oral activity as in Fig. 1, except that the observation session for oral activity was only 30 min. SCH 23390 HCl (0.30 mg/kg i.p.) or saline was administered 1 h prior to spiperone HCl (80 μg/kg i.p.). Each group is the mean (± SEM) of 5 to 11 rats. *indicates $P<0.001$ vs. respective saline control group. $^{+}$indicates $P<0.005$ vs. 6-OHDA groups treated with SCH 23390 alone or with SCH 23390 plus spiperone. (Reprinted with permission from Pergamon Press Ltd., Kostrzewa and Gong 1991).

pmol/μg protein in striatum from 6-OHDA-treated rats; K_d was 88 ± 6 and 86 ± 9 pM, respectively (Kostrzewa and Hamdi 1991). These findings indicate that the marked reduction of striatal DA content and loss of DA fibers was not associated with a change in binding parameters of D_1 and D_2 sites.

Antagonism of SKF 38393- and spiperone-induced oral activity by SCH 23390

In rats pretreated with the DA D_1 receptor antagonist, SCH 23390 (0.30 mg/kg i.p.), the oral activity response to SKF 38393 HCl (0.30 mg/kg i.p.) was attenuated in both control and neonatal 6-OHDA-lesioned rats (Fig. 2). Similarly, the oral activity response of both groups of rats to the DA D_2 receptor antagonist, spiperone, was attenuated by SCH 23390 (Fig. 3) (Kostrzewa and Gong 1991). These findings indicate that the DA D_1 receptor is ultimately responsible for oral activity induced by both SKF 38393 and spiperone. As previously stated, the balance between D_1 and D_2 receptor activation is crucial for oral activity responses.

Induction of 5-HT receptor supersensitivity in neonatal 6-OHDA-lesioned rats

Because 5-HT fibre hyperinnervation of the striatum occurs in the neonatal 6-OHDA-lesioned rat (Stachowiak et al. 1984, Berger et al. 1985) and because a 5-HT agonist is capable of inducing oral activity (Stewart et al. 1989), it was of interest to determine whether there was a change in the efficacy and potency of a 5-HT agonist in the lesioned rat. The respective 5-HT_{1A} and 5-HT_{1B} agonists, 8-OH-DPAT HBr (0.50 mg/kg s.c.) and CGS 12066B maleate (3.0 mg/kg i.p.), did not have augmented oral activity effects in the neonatal 6-OHDA-lesioned rat (not shown).

However, *m*-CPP, an agonist that acts at 5-HT_{1C} and 5-HT_2 receptors, produced a markedly enhanced oral activity response in the neonatal 6-

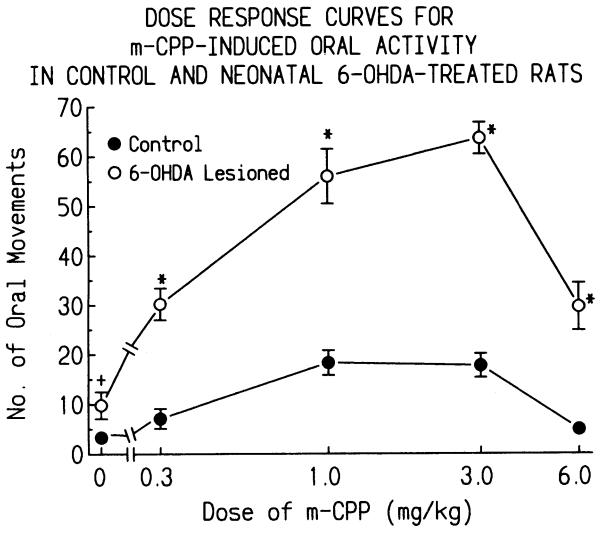

Fig. 4. Dose-response curve of *m*-CPP-induced oral activity in adult rats. Rats were treated neonatally and observed for oral activity as in Fig. 1. Each group is the mean (± SEM) of 6 or 7 rats. **P*<0.001 vs. vehicle group challenged with the same dose of *m*-CPP 2HCl; ^{+}P=0.024 vs. saline group. (Reprinted with permission from Pergamon Press Ltd., Gong and Kostrzewa 1992).

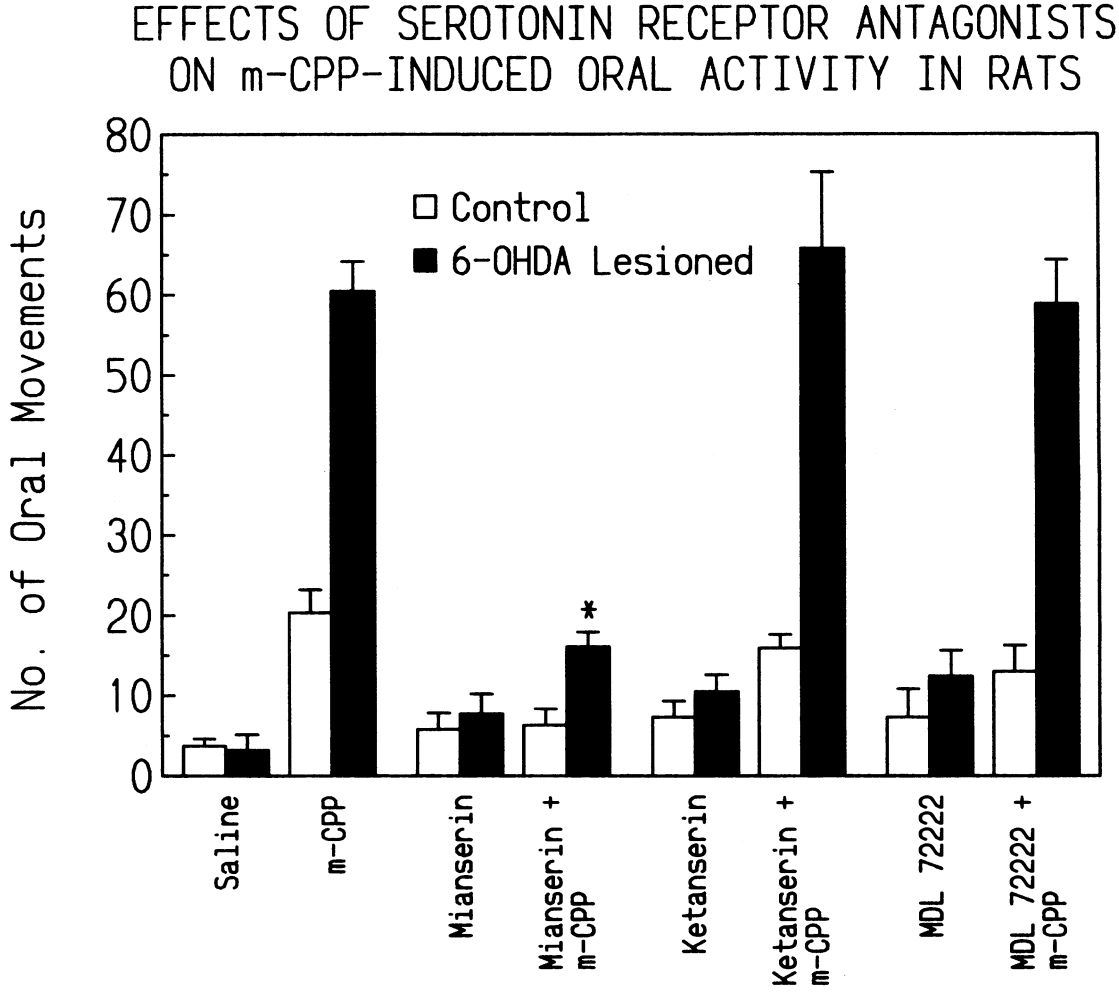

Fig. 5. Effects of 5-HT receptor antagonists on *m*-CPP-induced oral activity in control and 6-OHDA-lesioned rats. Rats were treated neonatally and observed for oral activity as in Fig. 1. Mianserin HCl (1.0 mg/kg s.c.), ketanserin tartrate (5.0 mg/kg i.p.) and MDL 72222 (10.0 mg/kg s.c.) were administered 30 min before *m*-CPP 2HCl (1.0 mg/kg i.p.) or its vehicle. Each group is the mean (± SEM) of 6 to 11 rats. **P*<0.001 vs. *m*-CPP effect in the 6-OHDA-lesioned rats. (Reprinted with permission from the Williams and Wilkins Company, Gong et al. 1992).

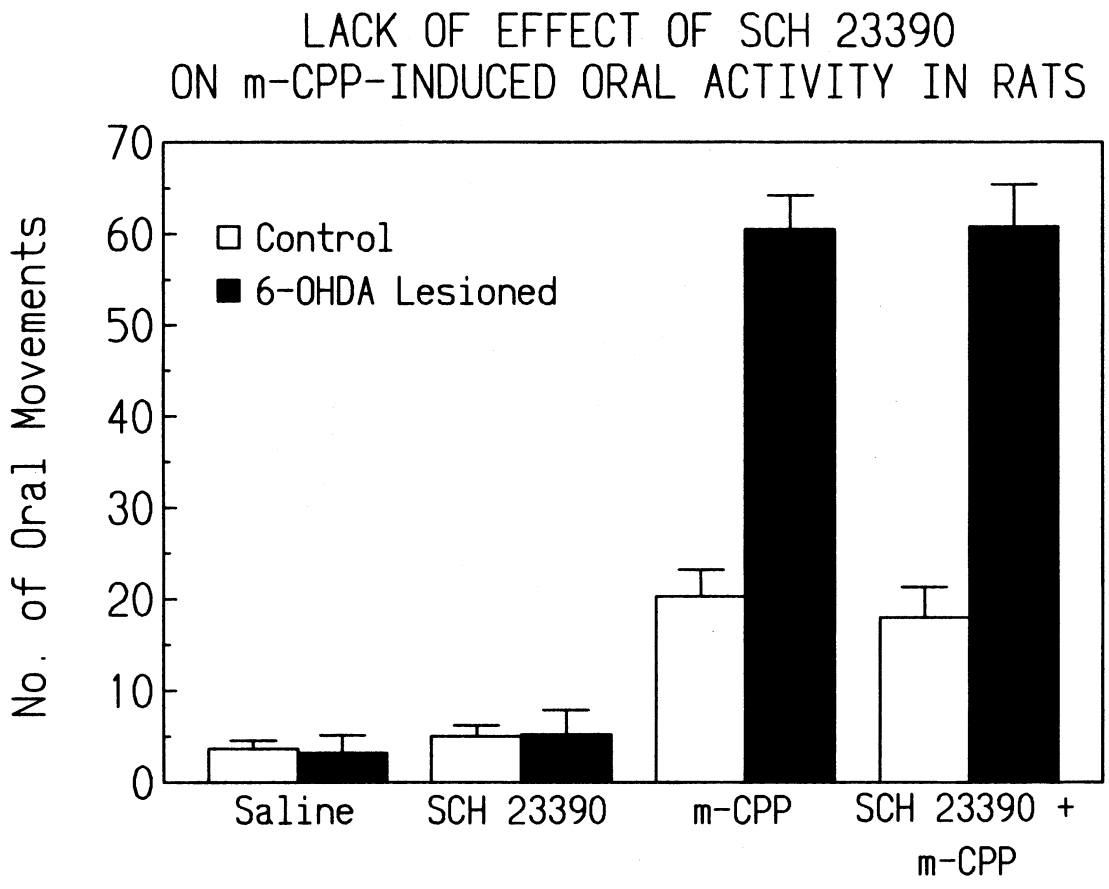

Fig. 6. Lack of effect of SCH 23390 on *m*-CPP-induced oral activity in control and 6-OHDA-lesioned rats. Rats were treated neonatally and observed for oral activity as in Fig. 1. SCH 23390 HCl (0.30 mg/kg i.p.) or saline was administered 1 hr before *m*-CPP 2HCl (1.0 mg/kg i.p.) or its vehicle. Each group is the mean (± S.E.M.) of 4 to 11 rats. An increase in oral activity was produced by *m*-CPP in intact rats ($P<0.01$) and in 6-OHDA-lesioned rats ($P<0.001$). SCH 23390 did not attenuate the response to *m*-CPP. (Reprinted with permission from the Williams and Wilkins Company, modified from Gong et al. 1992).

OHDA-lesioned rat. In the control group a maximal response of 18.3 ± 2.5 oral movements was produced by an *m*-CPP 2HCl dose of 1.0 mg/kg. In the neonatal 6-OHDA group the maximal response was 63.6 ± 3.2 oral movements, occurring with an *m*-CPP 2HCl dose of 3.0 mg/kg (Fig. 4).

Pindolol (1.0 mg/kg i.p., 30 min), a mixed 5-HT_{1A} and 5-HT_{1B} receptor antagonist, did not attenuate the response to *m*-CPP (not shown). Nor did ketanserin tartrate (5.0 mg/kg i.p.), a 5-HT_2 receptor antagonist; nor did MDL 72222(10 mg/kg s.c., 30 min), a 5-HT_3 receptor antagonist. Mianserin HCl (1.0 mg/kg s.c., 30 min), a mixed 5-HT_{1C} and 5-HT_2 receptor antagonist, did attenuate the response to *m*-CPP (Fig. 5).

These findings indicate that 5-HT_{1C} receptors are supersensitized, along with DA D_1 receptors, in neonatal 6-OHDA-lesioned rats.

Effect of a DA D_1 receptor antagonist, SCH 23390, on *m*-CPP-induced oral activity

To determine whether a DA D_1 receptor antagonist would attenuate the oral activity response to a 5-HT receptor agonist, rats were treated with SCH 23390 HCl (0.30 mg/kg i.p.) 1 hr before *m*-CPP 2HCl (1.0 mg/kg i.p.). As shown in (Fig. 6), the *m*-CPP response was unaltered by SCH 23390. This finding indicates that 5-HT agonist-induced oral activity is not mediated via a DA D_1 site.

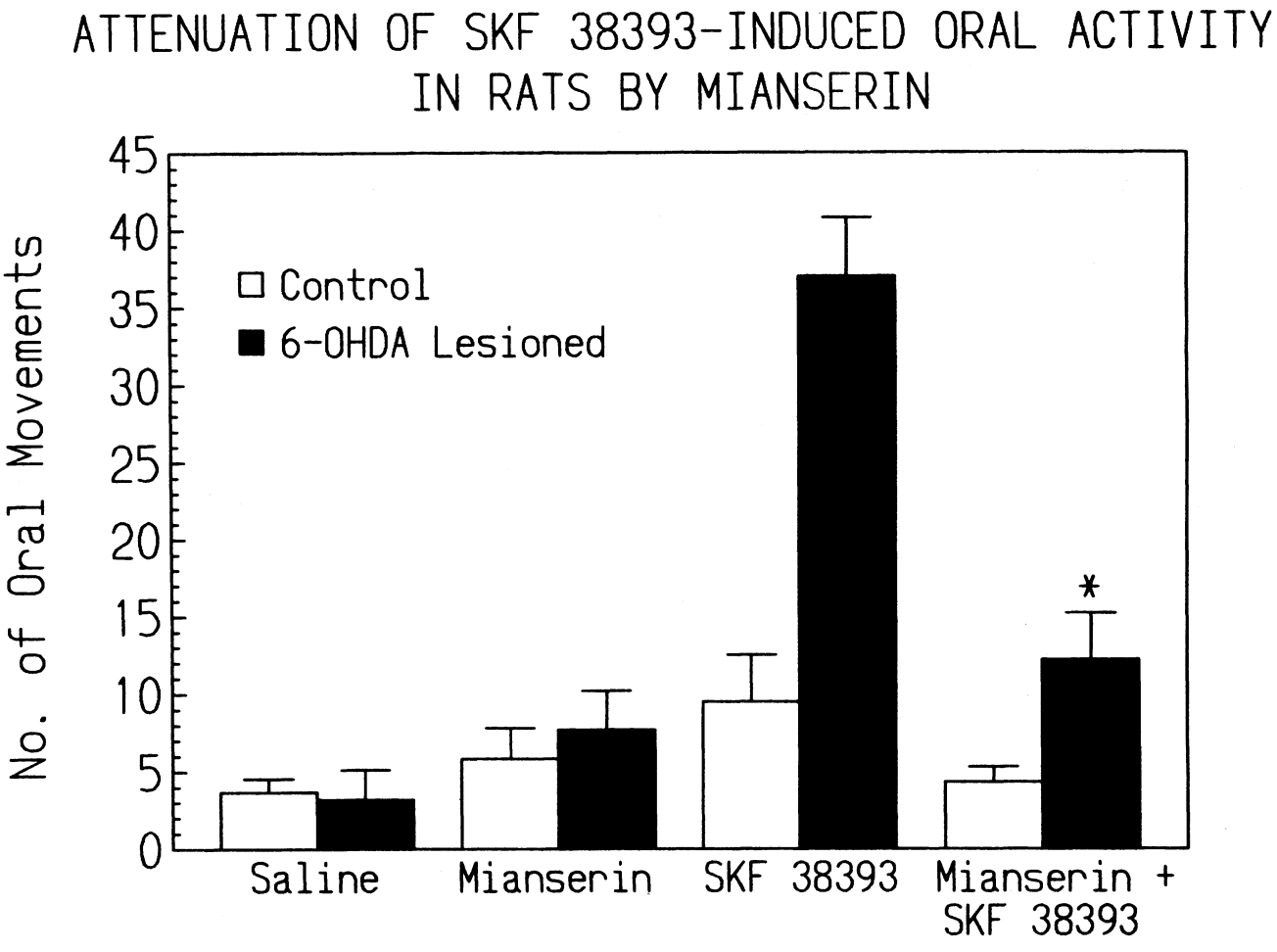

Fig. 7. Attenuation of SKF 38393-induced oral activity in control and 6-OHDA-lesioned rats by mianserin. Rats were treated neonatally and observed for oral activity as in Fig. 1. Mianserin HCl (1.0 mg/kg i.p.) or saline was administered 30 min before SKF 38393 HCl (1.0 mg/kg i.p.) or its vehicle. Each group is the mean (± SEM) of 4 to 11 rats. An increase in oral activity was produced by SKF 38393 in the neonatal 6-OHDA-lesioned rats ($P<0.001$) and this response was attenuated by mianserin (*$P<0.001$ vs. SKF 38393 effect in the 6-OHDA-lesioned group of rats. (Reprinted with permission from the Williams and Wilkins Company, modified from Gong et al. 1992).

Effect of a 5-HT$_{1C}$ receptor antagonist, mianserin, on SKF 38393-induced oral activity

To determine whether a 5-HT$_{1C}$ receptor antagonist would attenuate the oral activity response to a DA D$_1$ receptor agonist, rats were treated with mianserin HCl (1.0 mg/kg s.c.) 30 min before SKF 38393 HCl (1.0 mg/kg i.p.). As shown in Fig. 7, the SKF 38393 response was attenuated by mianserin. This finding indicates that DA D$_1$ agonist-induced oral activity is mediated via a 5-HT$_{1C}$ receptor.

DISCUSSION

Neonatal 6-OHDA treatment produces a near-total destruction of DA fibres in rat brain, and consequent marked reduction of striatal levels of DA and its metabolites (Breese and Traylor 1971, 1972, Breese et al. 1984). In association with this change there is an elevation in striatal levels of 5-HT and 5-HIAA (Breese et al. 1984, Stachowiak et al. 1984), consequent to sprouting and ensuing hyperinnervation by 5-HT-containing fibres (Stachowiak et al. 1984, Berger et al. 1985, Luthman et al. 1987, Towle et al. 1989).

Neonatal 6-OHDA-lesioned rats are known to be supersensitized to DA agonists. That is, doses of L-dihydroxyphenylalanine (L-DOPA), apomorphine or SKF 38393 which have little effect in intact rats, produce marked changes in stereotyped and locomotor behaviours in the lesioned rats (Breese et al. 1984, 1985a,b, 1987, Hamdi and Kostrzewa 1991). This change in sensitivity is a latent one, which must be 'unmasked' by repeated doses of DA agonists. This has been termed a 'priming' phenomenon (Criswell et al. 1989).

In the presently described studies we have demonstrated that oral activity represents another behaviour that is augmented in neonatal 6-OHDA-lesioned rats (Kostrzewa and Gong 1991, Kostrzewa and Hamdi 1991). In contrast to stereotyped and locomotor responses, oral activity is a behavior that is overtly augmented in the lesioned rats. That is, the first challenge dose of SKF 38393 produces enhanced responses (Gong et al. 1992). In studies by Breese and co-workers (1987) and in our studies the DA D_1 receptor is most closely associated with the enhanced behavioral responses of the neonatal 6-OHDA-lesioned rats. This is so, despite the apparent lack of change in DA D_1 receptor number and affinity (Breese et al. 1985a,b, 1987, Kostrzewa and Hamdi 1991).

The enhanced oral activity responses to *m*-CPP in neonatal 6-OHDA-lesioned rats, demonstrate that 5-HT receptors are sensitized, along with D_1 receptors (Gong and Kostrzewa 1991). This appears to be the most likely explanation for the *m*-CPP effect, since *m*-CPP lacks 5-HT uptake inhibitor properties (Fuller et al. 1981).

The series of studies with 5-HT agonists and antagonists indicate that 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors are not associated with oral activity (Gong et al. 1992). Agonists for these respective receptor sites do not induce substantial numbers of oral movemements in either intact or lesioned rats. Also, antagonists acting at these receptors do not modify the actions of *m*-CPP, which has prominent action at 5-HT_{1C} receptors (Gong et al. 1992). Therefore, the enhanced oral activity effects of *m*-CPP in neonatal 6-OHDA-lesioned rats appear to be related specifically to the 5-HT_{1C} receptor (Gong and Kostrzewa 1992, Gong et al. 1992).

It is conceivable that the process of 5-HT_{1C} receptor supersensitization could be unrelated to simultaneous DA D_1 receptor supersensitization in neonatal 6-OHDA-lesioned rats. Therefore, the studies with the DA and 5-HT receptor antagonists were needed. Since the DA D_1 receptor antagonist, SCH 23390, did not attenuate the action of *m*-CPP, a 5-HT receptor agonist, it seems that the 5-HT agonist acts on neurones which are subsequent to the locus of neurones possessing D_1 receptors. In contrast, since the 5-HT receptor antagonist, mianserin, effectively attenuated the oral activity effect of SKF 38393, a DA D_1 agonist, it appears that neurones possessing 5-HT_{1C} receptors are 'downstream' from those neurones with D_1 receptors.

The above findings and conclusions provide tangible evidence of a correlation between DA D_1 receptor supersensitization and 5-HT receptor supersensitization. It appears that the former process is dependent on occurrence of the latter process. This hypothesis needs to be tested. Because there is prominent proliferation of 5-HT fibers in the striatum of neonatal 6-OHDA-lesioned rats, it is possible that 5-HT_{1C} receptor sensitization is dependent on the occurrence of 5-HT fibre sprouting. Additional study is also needed to test this hypothesis.

Thè noted role of 5-HT fibres and/or receptors in mediating DA D_1 enhanced oral activity responses in neonatal 6-OHDA-lesioned rats is a substantive finding, in that this association represents a functional correlate with 5-HT fibre hyperinnervation of the striatum. Although it was shown by others that 5-HT fiber proliferation correlated with a change in the regulation of acetylcholine-containing neurones *in vitro* (Jackson et al. 1988), there was no behavioural correlate with the sprouted 5-HT fibres until now. That is, 5-HT fibre proliferation was not responsible for augmented stereotyped and locomotor responses to DA agonists in neonatal 6-OHDA-lesioned rats (Towle et al. 1989).

The cellular and biochemical mechanisms involved in receptor supersensitivity states are still

not known. Perhaps there is a change in numbers of high affinity receptors or a change in second messenger production subsequent to receptor complexation with an agonist.

It is felt that the neonatal 6-OHDA lesioned rat is a useful animal model to explore processes and mechanisms which may be involved with the dyskinetic oral activity of humans with tardive dyskinesia. The advantages of this model are as follows: (1) DA D_1 receptor agonists reliably produce an enhanced oral activity response, (2) DA D_2 receptor antagonists have the same effect, as (3) does a 5-HT_{1C} agonist. (4) Cellular events associated with receptor supersensitization can be studied in slices or homogenates of the striatum in either the basal or agonist-stimulated state. (5) Since oral acitivity is low in the unstimulated state, animals at rest in home cages are not distressed with disturbing symptoms. (6) In this model it is possible to investigate the role of 5-HT fibres in dyskinetic oral activity and thereby explore the use of 5-HT receptor antagonists as a class of agents to treat tardive dyskinesia.

ACKNOWLEDGEMENTS

These studies were supported by a grant from the John E. Fogarty International Center Health Scientist Exchange Program with Poland; PHS grant 1 R15 NS 29505 from the National Institute of Neurological Disorders and Stroke; and a grant from the Scottish Rite Schizophrenia Research Program USA (N.M.J.). We thank Ms. Lottie Winters for preparing the manuscript.

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Paper presented at the 1st International Congress of the Polish Neuroscience Society; Session: Neurotransmitters and their receptors