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RAPID COMMUNICATION

Enhanced Pilocarpine-Induced Oral Activity Responses in Neonatal 6-OHDA Treated Rats

RICHARD M. KOSTRZEWA[1] AND DAVID NEELY

Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614-0577

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KOSTRZEWA, R. M. AND D. NEELY. *Enhanced pilocarpine-induced oral activity responses in neonatal 6-OHDA treated rats.* PHARMACOL BIOCHEM BEHAV **45**(3) 737–740, 1993.—Neonatal destruction of rat nigrostriatal dopaminergic fibers results in an enhanced oral activity response to both dopamine (DA) D_1 and serotonin (5-HT) agonists. Because cholinergic systems represent another one of the neural circuits involved in oral behavior, it was of interest to determine whether muscarinic receptors might also be sensitized in the lesioned rats. At 3 days after birth, rats were pretreated with desipramine HCl (20 mg/kg, IP) 1 h before 6-hydroxydopamine (6-OHDA) HBr (100 μg in each lateral ventricle) or saline-ascorbic acid (0.1%) vehicle. Between 2 and 4 months, behavioral supersensitivity to a D_1 agonist (SK&F 38393) and 5-HT agonist (*m*-chlorophenylpiperazine; *m*-CPP) was established before rats were challenged with the muscarinic receptor agonist, pilocarpine HCl (0.125 to 10.0 mg/kg, IP). The pilocarpine dose-effect curve was shifted to the left, with a maximal effect of 63.7 ± 8.6 oral movements being produced by a 1.0 mg/kg pilocarpine HCl dose in the 6-OHDA lesioned rats, versus 15.0 ± 2.4 oral movements in the control group ($p < 0.001$). The enhanced response to pilocarpine was attenuated by the muscarinic receptor antagonist, scopolamine HCl (0.1 mg/kg IP). These findings indicate that neonatal 6-OHDA treatment produces supersensitization of muscarinic receptors in rats.

6-Hydroxydopamine Muscarinic receptor Oral activity Pilocarpine Supersensitization Ontogeny

NEONATAL 6-hydroxydopamine (6-OHDA) destruction of dopaminergic neurons is associated with enhanced dopamine (DA) D_1agonist-induced stereotyped and locomotor activities (2,3). Oral activity represents one of the stereotyped behaviors that are enhanced by D_1 agonists in these rats (9,10).

Although serotonin (5-HT) fiber sprouting occurs consequent to the destruction of DA fibers in the striatum (1,13), most behavioral effects of 5-HT agonists are not modified in these rats (11,14). One exception is the enhancement of *m*-chlorophenylpiperazine (*m*-CPP)-induced oral activity in the lesioned rat (6), which appears to be due to supersensitization of 5-HT_{1C} receptors (7).

Striatal DA fibers are known to exert some of their effects through cholinergic fibers (5). Because of this fact, as well as the finding of co-sensitization of D_1 and 5-HT_{1C} receptors in the 6-OHDA-lesioned rat, the present study was performed to determine if cholinergic receptors also may be cosensitized with those of the monoaminergic neurochemical systems.

METHOD

Timed pregnant Sprague-Dawley albino rats (Charles River Labs, Research Triangle Park, NC) were housed at 22 ± 1°C under a 12 L : 12 D cycle (light on at 0700 h) and given free access to food and water. Pups were treated at 3 days after birth with desipramine HCl (20 mg/kg, IP, base form, 1 h; Sigma Chemical Co., St. Louis, MO), followed by 6-OHDA HBr (100 μg in each lateral ventricle; Regis Chemical Co., Chicago, IL) or saline-ascorbic acid (0.1%) vehicle. This procedure has been described in detail (9). Rats were weaned at about 28 days and group housed by sex in wire cages.

Rats were observed for oral responses to test agents between 2 and 4 months of age. For each test session, rats were placed in individual clear plastic cages (48 × 26 × 36 cm) with steel grid floors, in a quiet, well-ventilated and well-lighted room. After an acclimation period of at least 1 h, each rat was challenged with a single dose of agonist or vehicle. Starting 10 min later, each animal was observed for 1 min

[1] To whom requests for reprints should be addressed.

every 10 min over a 60-min period. Numbers of oral movements were thus counted over a cumulative time of 6 min. This procedure has been described in detail (9).

For determining the dose-response relationship for pilocarpine-induced oral activity, a pilocarpine HCl (Sigma) dose range of 0.125–10 mg/kg IP was chosen. Because salivation was prominent at the 10-mg/kg dose, higher doses of pilocarpine were not used for fear of compromising respiration. To test for muscarinic receptor involvement in the pilocarpine action, rats were pretreated with (−)scopolamine HCl (0.1 mg/kg IP, salt form, 1 h; Sigma). The initial effect of pilocarpine was obtained with the observer being "blinded." The remainder of the study was not able to be done in a blind manner because of permanent identification markings and size differences between control and 6-OHDA rats. Because the numbers of oral movements after saline injection were similar during the initial three sessions (observation in the first 30 min) and latter three sessions (observations in last 30 min), it seems that rats may have been habituated to the presence of the observer. The observer had no experience prior to this study. The results have been verified in a separate study by someone that has had extensive experience in scoring oral activity of rats.

To test for D_1 agonist and 5-HT agonist effects, SK&F 38393 HCl [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride; 1.0 mg/kg, IP; Research Biochemicals Inc., Natick, MA] and *m*-CPP [1-(3-chlorophenyl)piperazine dihydrochloride; 1.0 mg/kg, IP; Research Biochemicals Inc.] were respectively administered to rats 10 min before observation. To test for effects of antagonists to D_1, 5-HT and muscarinic receptors, SCH 23390 HCl [R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; 0.30 mg/kg, IP; Research Bio-

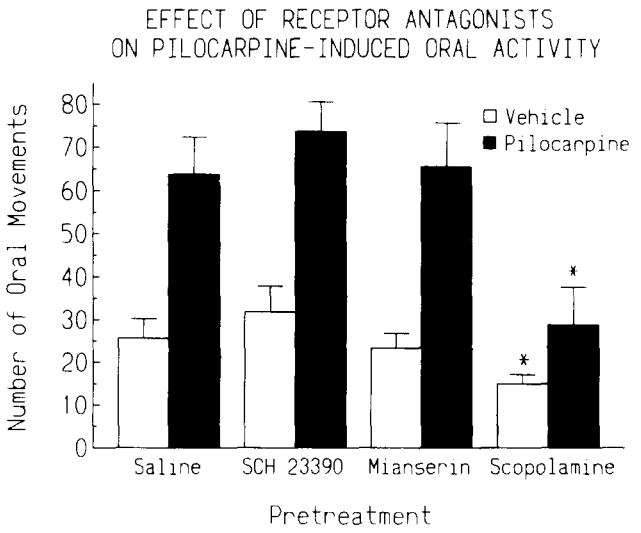

FIG. 2. Effect of receptor antagonists on pilocarpine-induced oral activity in adult rats that were treated neonatally with 6-OHDA. Rats were treated neonatally as in Fig. 1. Prior to testing for pilocarpine (1.0 mg/kg, IP) induced oral activity the neonatal 6-OHDA lesioned rats were pretreated with SCH 23390 (0.3 mg/kg, IP; 1 h), mianserin (1.0 mg/kg, IP; 1 h), or scopolamine (0.1 mg/kg, IP). Each group is the mean of nine rats. SCH 23390 and mianserin failed to attenuate the response to pilocarpine. *indicates that scopolamine attenuated the response to pilocarpine ($p < 0.01$).

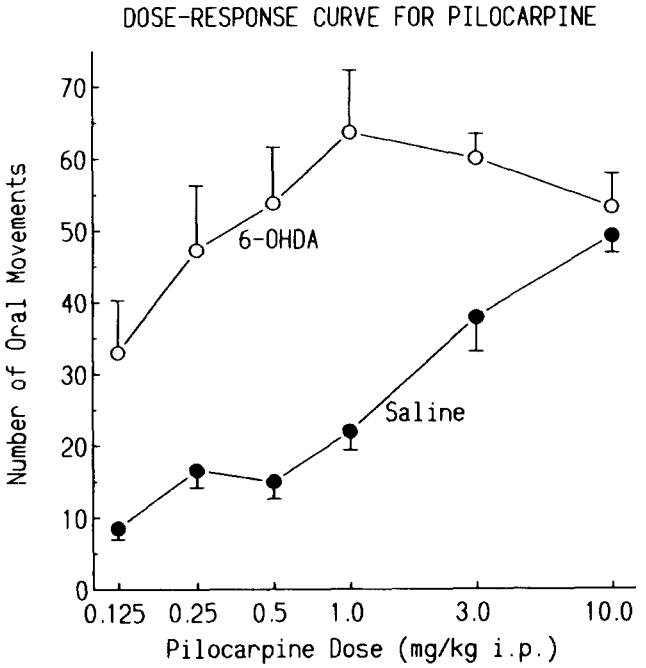

FIG. 1. Dose-response curves for pilocarpine-induced oral activity in adult rats. Rats were treated at 3 days after birth with vehicle or 6-OHDA HBr (100 μg in each lateral ventricle; desipramine pretreatment (20 mg/kg, IP, 1 h). Each rat was observed for 1 min every 10 min over 60 min, starting 10 min after challenge with pilocarpine or saline. Numbers of oral movements were recorded (ordinate) for each dose of pilocarpine (abscissa). Each group is the mean of nine rats. $p < 0.001$ for each pilocarpine dose, except 10 mg/kg.

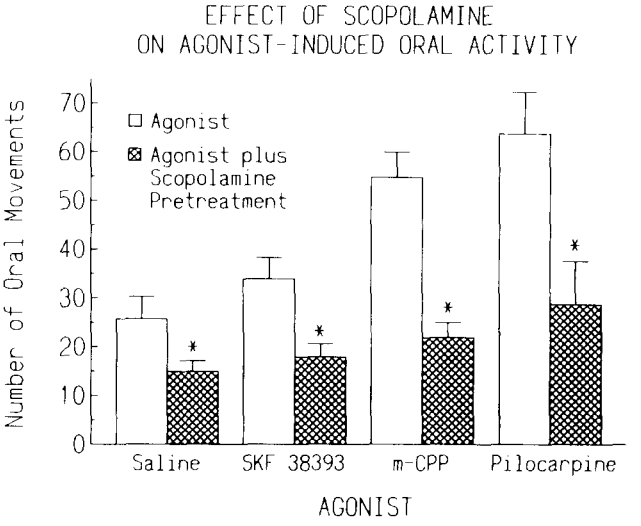

FIG. 3. Effect of scopolamine on agonist-induced oral activity in adult rats that were treated neonatally with 6-OHDA. Rats were treated neonatally and tested in adulthood as in Fig. 1. Scopolamine (0.1 mg/kg, IP) or vehicle was administered to neonatal 6-OHDA-lesioned rats, 1 h prior to saline, SK&F 38393 (1.0 mg/kg, IP), *m*-CPP (1.0 mg/kg, IP), or pilocarpine (1.0 mg/kg, IP). *indicates that scopolamine attenuated the effect of each agonist (i.e., hatched vs. clear bar for each respective pair, $p < 0.01$).

chemicals Inc.], mianserin HCl (1.0 mg/kg, IP; Research Biochemicals Inc.) and scopolamine HCl (0.1 mg/kg, IP), respectively, were administered 30 min before an agonist. Doses of the different agonists and antagonists in this study represent the salt form. An analysis of variance (ANOVA), followed by the post-ANOVA test of Newman-Keuls, was used to test for differences between groups.

RESULTS AND DISCUSSION

It is known that cholinergic agonists induce oral activity of control rats and neuroleptic-treated rats (4,8,12). It is still unsettled as to how cholinergic and dopaminergic neurochemical systems are associated in this behavioral activity. Following an injection of saline, the number of oral movements in control and 6-OHDA-lesioned rats was 6.67 ± 0.88 and 25.7 ± 4.6 (mean and SEM), respectively. In another portion of this study, oral activity following saline injection of 6-OHDA-lesioned rats was 17.0 ± 4.0 versus 2.67 versus 0.87 oral movements in the control group. When the entire study was repeated at a later time, oral activity after saline injection of the lesioned group was only 4.0 ± 0.75. This is in accord with what has been reported in other studies from this lab (6,7,9,10). Therefore, the high activity following saline injection of 6-OHDA-treated rats in the present study, seems to be an aberation.

When rats were treated with pilocarpine, a muscarinic receptor agonist, there was a dose-related increase in oral activity in both control and neonatal 6-OHDA-treated rats (Fig. 1). The lowest dose of pilocarpine, 0.125 mg/kg, produced 33.0 ± 7.3 versus 8.55 ± 1.54 oral movements in the lesioned versus control groups ($p < 0.01$). Through the dose range, up to 3.0 mg/kg, the pilocarpine-induced response was greater in the lesioned group. At the highest dose of pilocarpine, 10 mg/kg, the effects were identical in both groups. These findings indicate a shift in the dose–effect curve to the left in the lesioned group of rats, representing an increase in potency in the absence of a change in efficacy. This sensitization of muscarinic sites is different from that observed for a D_1 agonist (9) and 5-HT agonist (6), wherein the maximal effect is increased along with the change in potency. Pilocarpine-induced oral activity seemed to be similar in form between saline and 6-OHDA-lesioned rats.

To test that the effect of pilocarpine was associated with muscarinic receptors, rats were pretreated with the muscarinic receptor antagonist, scopolamine (0.1 mg/kg, IP). As shown in Fig. 2, scopolamine effectively attenuated the response to pilocarpine (1.0 mg/kg, IP) ($p < 0.001$).

Previously it was shown that the supersensitized response to a DA D_1 receptor agonist could be attenuated by a 5-HT_{1C} receptor antagonist (7). Several agonists and antagonists for the various DA and 5-HT receptors were used in those studies, and current evidence indicates that DA fibers act through 5-HT fibers in the striatum (6,7). The same approach was used in the present study in an attempt to determine the neural circuits involved in the enhanced oral activity responses of the 6-OHDA lesioned rats. Neither SCH 23390 (0.30 mg/kg, IP, 30 min) nor mianserin (1.0 mg/kg, IP, 1 h), antagonists at DA D_1 and 5-HT receptors, attenuated the response to pilocarpine (Fig. 2). However, scopolamine (0.1 mg/kg, IP, 1 h) effectively attenuated the SK&F 38393- and *m*-CPP-enhanced responses in both control (not shown) and 6-OHDA-lesioned rats (Fig. 3).

The present findings demonstrate that oral activity responses to a muscarinic agonist, pilocarpine, are enhanced in neonatal 6-OHDA-treated rats. Therefore, it seems that a DA neurotoxin will supersensitize receptors not only to DA, but those to acetylcholine as well. This conclusion is tempered by the finding of high oral activity after saline injection of the neonatal 6-OHDA-lesioned rats. However, we do find lower oral activity of this group in another phase of the present study (data not shown), and in other studies (6,7,9,10). For these reasons we feel that muscarinic receptors are actually supersensitized in the lesioned rats.

The cosensitization of DA, 5-HT and muscarinic receptor types could conceivably be independent phenomena. The results of this and a previous study (7), however, indicate that these events are corelated. It is known that a 5-HT receptor antagonist, mianserin, will attenuate the D_1 agonist-induced oral activity responses (7). Presently, it is shown that scopolamine, an antagonist for muscarinic receptors, effectively attenuated oral activity responses to a D_1 agonist, 5-HT agonist and muscarinic agonist. Conversely, antagonists to the D_1 and 5-HT receptors did not attenuate the pilocarpine response. It is suggested that the respective DA and 5-HT neurochemical systems may act via the cholinergic system. The findings have implications towards neurochemical mechanisms that may be involved in tardive dyskinesia.

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