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Pharmacology, Biochemistry and Behavior 68 (2001) 347–353

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Increased *m*-CPP-induced oral dyskinesia after lesion of serotonergic neurons

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Received 17 July 2000; received in revised form 18 October 2000; accepted 20 October 2000

Abstract

Peripheral administration of the 5-hydroxytryptamine $(5\text{-HT})_{2C/1B}$ agonist 1-(*m*-chlorophenyl)piperazine (*m*-CPP) produces abnormal orofacial movements in rats. We have previously shown that this behavior is mediated by 5-HT_{2C} receptors in the subthalamic nucleus [Neuroscience 72 (1996) 117]. The present studies examined this effect after serotonin depletion to determine whether removal of endogenous serotonin affected this behavioral response and/or subthalamic 5-HT_{2C} receptors. Rats received an intraventricular infusion of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 100 mg/10 ml) or vehicle after desipramine pretreatment (25 mg/kg ip). The efficacy of serotonin depletion was confirmed by a decrease in serotonin uptake sites measured by autoradiography. Oral dyskinesia induced by peripheral administration of *m*-CPP (1.0 mg/kg ip) was markedly increased in lesioned rats compared to sham-operated controls 4 and 8 but not 12 days after the lesion. A subset of lesioned rats that displayed transient seizures after *m*-CPP injection did not prevent the measurement of oral dyskinesia during the observation period. No differences in 5-HT_{2C} receptor levels were found with ligand-binding autoradiography in the subthalamic nucleus, or in other brain regions that express this receptor, in rats sacrificed 5 days following 5,7-DHT lesions. The data indicate that lesion of serotonergic neurons in adult rats induces a transient increase in motor responses mediated by subthalamic 5-HT_{2C} receptors. These data suggest that functional alterations in serotonergic transmission in the subthalamic nucleus may be involved in the pathophysiology of hyperkinetic movement disorders.

Keywords: 5-HT_{2C}; 1-(*m*-Chlorophenyl)piperazine (*m*-CPP); 5,7-Dihydroxytryptamine (5,7-DHT); 5-Hydroxytryptamine (5-HT); Subthalamic nucleus; Basal ganglia; Tardive dyskinesia; Movement disorders

1. Introduction

The basal ganglia comprise a group of subcortical structures that are known to play a critical role in the control of movement. Dysfunction of the basal ganglia resulting from specific cell loss as a result of neurodegenerative diseases such as Huntington's and Parkinson's diseases leads to severe motor disorders (Albin et al., 1989; Chesselet and Delfs, 1996). Furthermore, administration of pharmacological agents that affect the basal ganglia such as neuroleptics, a type of antipsychotic drug, often produce motor side effects (Tarsy and Baldessarini, 1984). In particular, abnormal involuntary movements of the mouth with or without tongue protrusion, called tardive dyskinesia, are a severe side effect of long-term neuroleptic administration (Tarsy and Baldessarini, 1984).

Orofacial dyskinesia can be elicited by the local administration of drugs into several distinct brain areas, including the subthalamic nucleus (Parry et al., 1994; Eberle-Wang et al., 1996; Mehta et al., 1999). The subthalamic nucleus is a small collection of glutamatergic neurons located above the cerebral peduncle rostrally to the substantia nigra. Based on a better understanding of the anatomical and functional circuitry of the basal ganglia, this region is now considered an integral part of the basal ganglia (Parent and Hazrati, 1995; Chesselet and Delfs, 1996). Clinical and experimental data indicate that lesions of the subthalamic nucleus lead to irrepressible abnormal movements (Whittier, 1947; Hamada and DeLong, 1992). Thus, decreased firing activity in the

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0091-3057/01/$ – see front matter
PII: S0091-3057(00)00476-7

subthalamic nucleus may be responsible for a variety of hyperkinetic movement disorders (Crossman et al., 1988; Albin et al., 1989).

We have recently shown that local administration of the 5-hydroxytryptamine (5-HT)$_{2C/1B}$ agonist 1-(*m*-chlorophenyl)piperazine (*m*-CPP) into the subthalamic nucleus of awake rats induces a dose-dependent increase in orofacial movements, including vacuous chewing, jaw tremor, gaping and tongue protrusion (Eberle-Wang et al., 1996). Pharmacological characterization of the effect indicates that it is mediated by stimulation of 5-HT$_{2C}$ receptors (Eberle-Wang et al., 1996). The mRNA encoding 5-HT$_{2C}$ receptors is present in the subthalamic nucleus (Mengod et al., 1990; Eberle-Wang et al., 1997), suggesting that these receptors are expressed by intrinsic subthalamic neurons. Further supporting a role for the subthalamic nucleus in oral dyskinesia induced by peripherally administered 5-HT$_{2C}$ agonists, local administration of 5-HT$_{2C}$ antagonists into the subthalamic nucleus markedly reduced orofacial dyskinesia induced by intraperitoneal administration of *m*-CPP (Eberle-Wang et al., 1996). These data strongly suggest that excess stimulation of 5-HT$_{2C}$ receptors in the subthalamic nucleus could play a role in hyperkinetic movement disorders. However, it remains unclear whether these receptors are stimulated by endogenous serotonin under normal conditions.

To investigate this question, we took advantage of the observation that denervation supersensitivity, resulting in an enhanced response to agonist stimulation, often develops when a receptor is no longer exposed to its endogenous ligand. Such phenomenon has been shown to occur for some behavioral and biochemical responses elicited by 5-HT$_{2C}$ agonists (Conn et al., 1987; Berendsen et al., 1991; Rocha et al., 1993; Sawynok and Reid, 1994), but it is unknown whether it occurs for behaviors mediated by stimulation of subthalamic 5-HT$_{2C}$ receptors such as oral dyskinesia. In the present study, serotonergic projections to the forebrain were lesioned by intraventricular injections of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), and the loss of serotonergic terminals was assessed by ligand-binding autoradiography for ^{3}H-cyanoimipramine, a ligand for serotonin uptake sites. Lesioned and vehicle-injected rats were administered *m*-CPP (ip) prior to and at various times after surgery, and the occurrence of orofacial dyskinesia was recorded following each drug injection. Tissue sections of the subthalamic nucleus were processed for 5-HT$_{2C}$ ligand-binding autoradiography to determine whether changes in behavioral responses were correlated with alterations in 5-HT$_{2C}$ binding sites.

2. Experimental procedures

2.1. Surgery

All procedures were performed following an experimental protocol approved by an institutional review committee for the use of animal subjects at the University of Pennsylvania and UCLA. Prior to surgery, male Sprague–Dawley rats (300–400 g; Charles River, $n=34$) were housed in groups of three and maintained on a 12:12 h light–dark schedule with food pellets and water ad libitum. Under anesthesia with Equithesin (0.003 ml/mg ip prepared following a protocol from Jensen-Saltbury Laboratories, Kansas City, MO), the rats received bilateral infusions of either the neurotoxin 5,7-DHT (200 μg/20 μl) or its vehicle (0.1% ascorbic acid) into the lateral ventricles (100 μg/10 μl on each side over 1.5 min; coordinates: 1.4 mm posterior from bregma, 1.8 mm lateral to midline, and 1.3 mm dorsal to the surface of the cortex). Thirty minutes prior to surgery, all rats were pretreated with desipramine (25 mg/kg ip) to prevent uptake of 5,7-DHT into noradrenergic neurons.

2.2. Behavioral testing

Following a 60-min habituation, rats were observed individually in circular plastic chambers (12 in. diameter, 18 in. height) for a total of 60 min. Rats were injected with the 5-HT$_{2C/1B}$ agonist *m*-CPP (1.0 mg/kg ip) or saline vehicle 5 min prior to the onset of behavioral observations. The number of oral bouts per 60 min was quantified by an observer blind to the drug and surgical treatment of the animal. An oral bout was defined as any combination of continuous oral movements including vacuous chewing, jaw tremor, and tongue darting distinct from feeding, grooming, or exploration (Parry et al., 1994; Eberle Wang et al., 1996; Mehta et al., 2000). A clear cessation of oral activity was required to consider the oral bout fully terminated. The frequency of oral bouts was measured over the 60-min observation period. The duration of oral bouts was not simultaneously measured. One group of rats was tested for *m*-CPP-induced oral dyskinesia 2 days prior and 4 days postsurgery, and sacrificed on the following day. The baseline frequencies of orofacial movements were assessed following vehicle injection 7 days before surgery. An additional group of rats was treated similarly and tested 2 days prior to surgery and 4, 8, 12, 16, and 20 days after surgery with the same dose of *m*-CPP. The baseline frequencies of orofacial movements were assessed following vehicle injection 6 days before and again 22 days after surgery. Rats were sacrificed following the behavioral test on Day 22. To verify the lesion of serotonergic neurons behaviorally, all rats were rated for the presence of hyperreactive behaviors, which have been previously described as an effect of 5,7-DHT lesions (Soderpalm and Svensson, 1999). These behaviors included vocalizations, defensive orienting, explosive motor behavior and tail wagging, and were measured 3 (both groups) and 22 days (second group) following surgery.

2.3. Autoradiography for 5-HT$_{2C}$ receptors

Rats were sacrificed by decapitation, the brain removed, frozen on dry ice, and sectioned into 10-μm-thick coronal

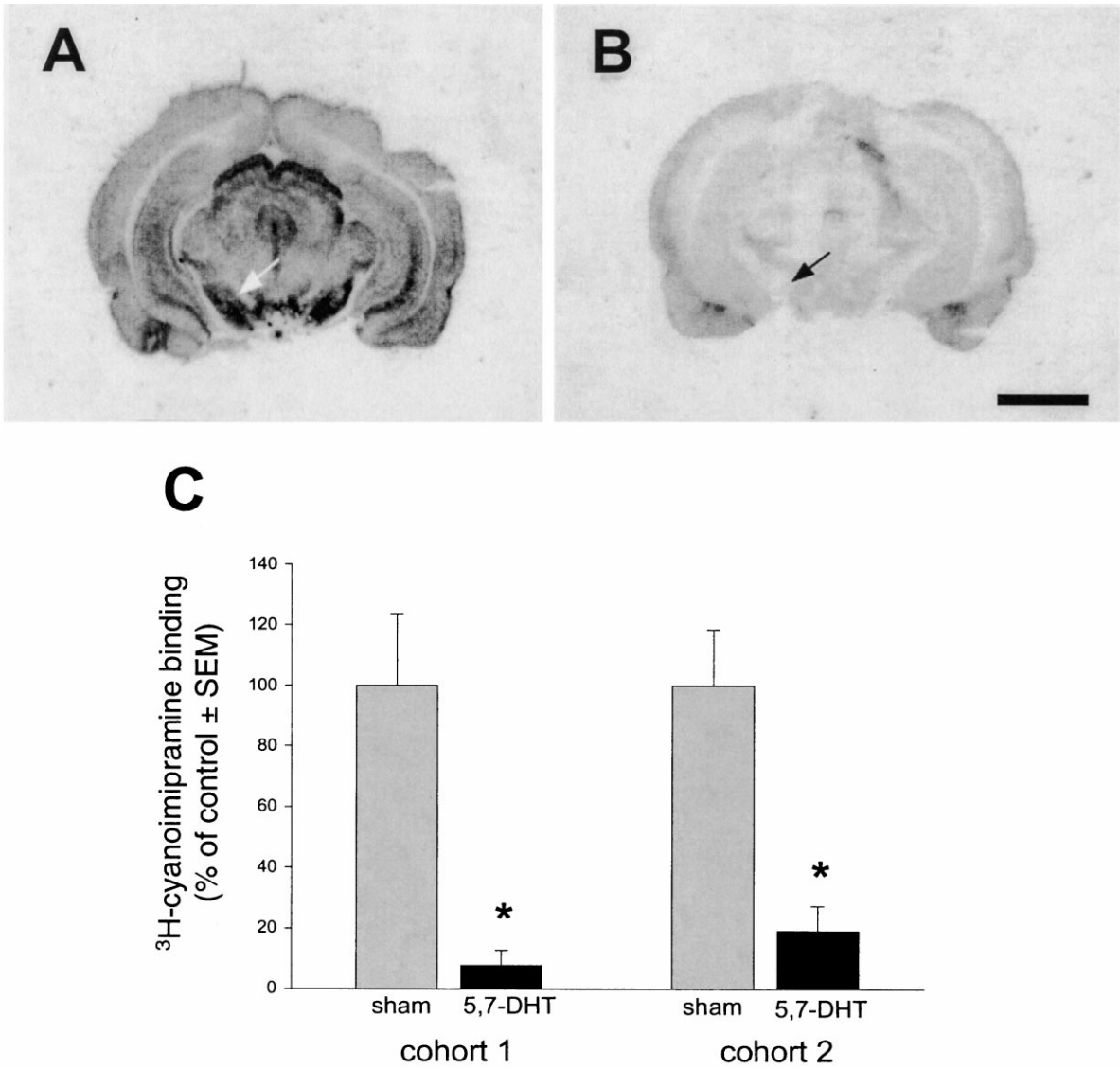

Fig. 1. Representative autoradiograms of total ^{3}H-cyanoimipramine binding for serotonergic uptake sites on (A) sham-operated and (B) 5,7-DHT-lesioned tissue sections. Arrows indicate the substantia nigra, a structure richly innervated by serotonergic nerve terminals from the dorsal raphe nucleus like the subthalamic nucleus (Lavoie and Parent, 1990). Scale bar = 4 mm. (C) The effect of 5,7-DHT-lesions on ^{3}H-cyanoimipramine binding in the substantia nigra in both cohorts of rats. Data are expressed as percent of control ± S.E.M. * $P < .05$, compared to data in sham-operated rats, according to Student's unpaired *t* test. Statistics performed on absolute values.

sections at the level of the substantia nigra and subthalamic nucleus. The sections were processed for 5-HT$_{2C}$ receptor binding autoradiography according to the protocol of Rocha et al. (1993). A single dose of ligand was used because the small size of the subthalamic nucleus precluded a full Scatchard analysis. Briefly, the sections were washed (1 × 3 min) in Tris–HCl buffer pH 7.5 (170 mM) containing 100 μM spiperone (to prevent binding to 5-HT$_{2A}$ receptors) at room temperature. The sections were then incubated for 2 h at room temperature in the same Tris–HCl buffer containing either 3.0 nM ^{3}H-mesulergine (total binding) or an excess of cold displacer 1.0 μM mianserin (nonspecific binding). Following 2 × 10 min washes in Tris–HCl buffer at 4°C and a dip in ice-cold dH$_2$O, the slides were desiccated overnight and exposed to ^{3}H-sensitive Hyperfilm for 5–6 weeks at room temperature.

2.4. ^{3}H-cyanoimipramine binding

The brains were removed and cut as described in the previous section. Tissue sections at the level of the substantia nigra, which is innervated by the dorsal raphe like the subthalamic nucleus (Lavoie and Parent, 1990), were used to conserve sections at the level of the subthalamic nucleus. The sections were processed for 5-HT uptake binding autoradiography according to the protocol of Tejani-Butt and Labow (1994). Briefly, the sections were incubated for 24 h at 4°C in Tris–HCl buffer pH 7.4 (50 mM) containing

either 0.5 nM ^{3}H-cyanoimipramine (total binding) or an excess of cold displacer 5.0 μM sertraline (nonspecific binding). Following a 60-min postincubation in ice-cold buffer and a dip in ice-cold dH_2O, the slides were desiccated overnight and exposed to ^{3}H-sensitive Hyperfilm for 5–6 weeks at room temperature.

2.5. Drugs

The neurotoxin 5,7-DHT (Sigma, St. Louis, MO) was prepared in 0.1% ascorbic acid and kept covered with aluminum foil on ice due to its sensitivity to light, air, and heat. Ascorbic acid was prepared in 0.9% saline. The noradrenaline uptake inhibitor desipramine, the 5-$HT_{2C/1B}$ receptor agonist *m*-CPP, the 5-$HT_{1A/2A}$ antagonist spiperone, and the 5-$HT_{2C/2A}$ antagonist mianserin were purchased from Sigma. ^{3}H-mesulergine and ^{3}H-cyanoimipramine were purchased from NEN Dupont (Boston, MA).

2.6. Data analysis

2.6.1. Behavioral studies

For each study, the total number of oral bouts was calculated for a 60-min observation period for each rat, and the data were analyzed with a repeated-measures analysis of variance (ANOVA) followed by a Dunnett's test or Student–Newman–Keuls test using Statview 512+ Interactive Statistics and Graphics Package (SIS Institute, Cary, NC) with $P<.05$ considered significant. Data are reported as means ± S.E.M.

2.6.2. Autoradiography

Optical density readings for areas of interest were measured with the NIH Image program (version 1.62) and converted to nanocuries per milligram of protein using a standard curve based on tritium-containing plastic standards. Measurements were made on both sides of the brain and then averaged for each rat. Specific binding of ^{3}H-mesulergine was determined by subtracting the value for nonspecific binding obtained in the presence of mianserin from total binding in each region of interest. Labeling density for each brain region was compared between treatment groups using a two-tailed Student's *t* test. The threshold for significance was $P<.05$. All statistics were performed on absolute values.

3. Results

3.1. Effects of 5,7-DHT on serotonin uptake sites

To verify the efficacy of the lesion, sections at the level of the substantia nigra were processed for ^{3}H-cyanoimipramine binding, a ligand of serotonergic uptake sites. All rats injected intraventricularly with 5,7-DHT had a widespread and profound loss of ^{3}H-cyanoimipramine binding (Fig. 1). Optical density levels measured over the substantia nigra decreased by 92% in the first cohort of rats (sacrificed 5 days after lesion) and by 81% in the second cohort (sacrificed 22 days after lesion (Fig. 1C). Further confirming the lesion of serotonergic inputs to the forebrain, the lesioned rats displayed a qualitative increase in hyperreactive behaviors elicited by physical interaction with the investigator, which has been previously described as an effect of 5,7-DHT lesions (Soderpalm and Svensson, 1999). These included an increase in vocal response upon pick-up, defensive orienting, explosive motor behavior and tail wagging on Day 3 postsurgery (data not shown). The rats displayed normal behavior when observed in their cages.

3.2. Effects of 5,7-DHT on m-CPP-induced orofacial dyskinesia

In the first group of rats, baseline levels of oral movement (oral bouts) following injection saline vehicle (ip) were measured 7 days before lesioning and were found to be 8.2 ± 2.0 ($n=6$) oral bouts per 60 min for the sham-operated group and 9.0 ± 2.0 ($n=7$) for the lesioned group. The dose of *m*-CPP (1.0 mg/kg ip) used in this study was chosen based on a dose–response curve previously established in our laboratory (Eberle-Wang et al., 1996), showing a half maximum increase in orofacial dyskinesia with this dose. The presurgery response to *m*-CPP (54.5 ± 4.3 oral bouts per 60 min for the sham-operated controls, and 44.1 ± 3.9 for the lesioned rats) was significantly greater than the baseline response for both groups (according to Dunnett's test, $P<.05$) and similar to our previously published value of 51.5 ± 6.2 ($n=6$) (Eberle-Wang et al., 1996). Four days after surgery, administration of *m*-CPP (ip)

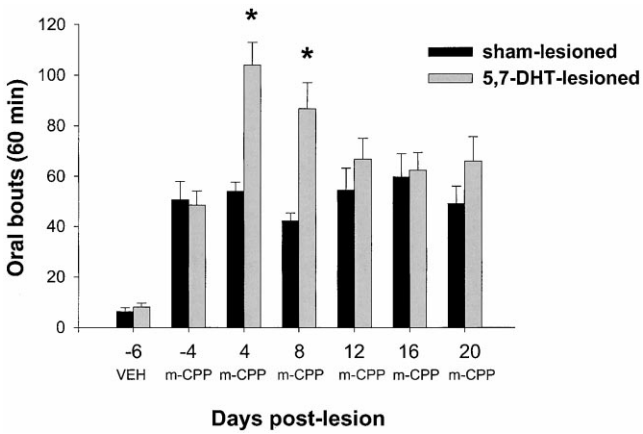

Fig. 2. Time course of *m*-CPP-induced oral dyskinesia in rats with 5,7-DHT lesions. Lesion of serotonergic neurons by 5,7-DHT transiently increased the behavioral response to peripheral administration of *m*-CPP (1.0 mg/kg ip) in rats. Data are expressed as means ± S.E.M. of total oral bouts per 60 min ($n=7$ for sham and $n=8$ for lesion). * $P<0.05$, compared to corresponding sham-operated data, according to Student–Newman–Keuls test following a repeated-measures ANOVA.

elicited a 54% increase in oral bouts in the lesioned rats (82.6 ± 12.2 oral bouts per 60 min) compared to the sham-lesioned rats (54.2 ± 4.4) (according to Student–Newman–Keuls test, $P < .05$). Furthermore, the behavior of the lesioned group was significantly increased compared to the effect of the same drug injection prior to surgery (according to Dunnett's test, $P < .05$) [repeated-measures ANOVA, $F(1,11) = 9.9$, $P < .01$].

A second group of rats was used to determine the time course of the enhanced behavioral response to *m*-CPP injections after lesioning with 5,7-DHT. Baseline levels of oral bouts following injection of saline vehicle (ip) were measured 6 days before lesioning and a presurgery response to *m*-CPP was measured 4 days before surgery ($P < .05$, compared to corresponding baseline responses, according to Dunnett's test). Following surgery, the sham-operated rats received injections of *m*-CPP on the same days as the lesioned animals (every 4 days) to verify the absence of sensitization or desensitization to *m*-CPP with this administration schedule. As shown in Fig. 2, the sham-operated rats showed a consistently stable response to *m*-CPP over the 20-day time course. In contrast, statistical analysis with a repeated measure ANOVA revealed a significant effect of time on *m*-CPP-induced oral dyskinesia following 5,7-DHT lesions [$F(4,52) = 3.5$, $P < .05$]. Administration of *m*-CPP (ip) 4 days after surgery elicited a 93% increase in oral bouts in 5,7-DHT-lesioned rats compared to sham-lesioned controls (Fig. 2). An increased response was still observed 8 days postsurgery (105% above sham-lesioned controls). By 12 days postsurgery, the response to *m*-CPP in the lesioned rats had returned to control levels and remained at control levels until the end of the experiment (20 days postlesion). To verify the stability of the baseline level of oral bouts, another baseline measurement following vehicle injection was measured 22 days after surgery and was found to be 9.7 ± 2.2 ($n = 7$) oral bouts per 60 min for the sham-lesioned group and 13.0 ± 1.4 ($n = 8$) for the lesioned group.

In addition to the increase in oral dyskinesia that was present in all lesioned rats, some of the lesioned animals showed signs of seizure after *m*-CPP administration (Table 1). In the two groups used in this study, 10 out of the 15 lesioned rats displayed this behavior after the first postsurgery administration of *m*-CPP (Day 4). For three of these rats (one-fifth of the lesioned rats), the symptoms were severe, including extreme rigidity in the forelimbs and hindlimbs, sporadic forelimb spasms (a "boxing"-like movement), poor balance, and periods of what seemed to be total body paralysis. The severity of the symptoms was greatest immediately following injection. During periods of paralysis, which lasted only a few minutes, the rats did not display oral dyskinesia. Since the apparent suppression of *m*-CPP-induced oral dyskinesia occurred during a very short period of the total observation time, these rats were included in the study. The other seven rats showed milder symptoms with a lesser extent of rigidity and the absence of paralysis. In the second group of rats used for the time course study (Fig. 2), two of the eight lesioned rats showed mild seizures after the second postsurgery administration of *m*-CPP (Day 8). These episodic behaviors did not interfere with the measurement of oral dyskinesia and could not be confounded with the oral behavior. By Day 12, none of the rats displayed abnormal behaviors. These behaviors were never observed after administration of *m*-CPP in sham-operated rats.

Table 1
Induction of seizure behavior by *m*-CPP 4 days after 5,7-DHT lesion

	Number of animals that displayed seizure behavior		
	No seizures	Mild seizures	Severe seizures
Sham-operated	13	0	0
5,7-DHT-lesioned	5	7	3

Table 2
Effect of 5,7-DHT lesion on 5-HT_{2C} receptor density in the subthalamic nucleus and other brain regions

	5-HT_{2C} receptor binding (nCi/mg)	
	Sham-lesioned ($n = 6$)	5,7-DHT-lesioned ($n = 6$)
Subthalamic nucleus	29.9 ± 1.4	30.1 ± 0.8
Choroid plexus	338.0 ± 7.8	321.1 ± 7.7
Cingulate cortex	10.4 ± 1.2	13.5 ± 1.9
Piriform cortex	23.8 ± 2.3	23.6 ± 2.1

The data represent the means ± S.E.M. of 5-HT_{2C} receptor binding (nCi/mg tissue).

3.3. Effects of 5,7-DHT lesions on 5-HT_{2C} binding sites

To correlate the robust, but transient, increase in *m*-CPP-induced motor behaviors observed after 5,7-DHT lesioning to eventual changes in 5-HT_{2C} receptor density in the subthalamic nucleus, ligand-binding autoradiography was performed on tissue from rats of the first cohort sacrificed 5 days after lesioning, i.e., at a time when increased behavioral effects were observed. Analysis of the 5-HT_{2C} autoradiograms did not reveal statistically significant differences between the control and lesioned groups in the subthalamic nucleus (Table 2). Other regions of the brain with relatively high 5-HT_{2C} densities were measured as well to confirm any changes due to serotonergic denervation. No significant differences in receptor density between the control and lesioned groups were found in these brain areas either.

4. Discussion

Lesions of serotonergic afferents markedly increased the behavioral response to a submaximal dose of peripherally administered *m*-CPP. The most reliable response was an increase in orofacial dyskinesia, a motor response also induced in sham-operated rats by *m*-CPP. This observation is compatible with previous evidence of denervation supersensitivity of other 5-HT_{2C} receptor-mediated behaviors,

such as penile erection and conditioned place aversion, after serotonergic denervation in rats (Berendsen et al., 1991; Rocha et al., 1993). Furthermore, biochemical responses induced by 5-HT_{2C} receptor stimulation in the choroid plexus, a brain area particularly rich in these receptors (Mengod et al., 1990), are increased after lesions of serotonergic neurons (Conn et al., 1987).

In general, denervation supersensitivity has been interpreted as a compensatory response to a decrease in the amount of endogenous agonist to a receptor. Lesions performed with a protocol similar to that used in the present study have shown a profound decrease in serotonin content in rat forebrain (Rocha et al., 1993). The autoradiographic method used in this study to assess serotonin depletion is very sensitive and has the advantage to provide information on the topographic extent of the neurotransmitter loss. In the present study, injection of 5,7-DHT resulted in a widespread and profound decrease in serotonin uptake sites. The number of serotonergic uptake sites measured by autoradiography of ^{3}H-cyanoimipramine binding correlates with the loss of serotonergic innervation (Hensler et al., 1991; Tejani-Butt and Labow, 1994). The observation of a similar magnitude and extent of uptake site loss in animals killed 5 and 22 days after injection of 5,7-DHT indicates that the loss of serotonergic terminals is rapid enough to account for the early onset of the increase in behavioral response, and that the return to normal levels of behaviors is not due to recovery of serotonergic innervation.

4.1. Role of the subthalamic nucleus in m-CPP-induced oral dyskinesia

The subthalamic nucleus contains both 5-HT_{2C} binding sites and the mRNA encoding the 5-HT_{2C} receptor (Mengod et al., 1990; Eberle-Wang et al., 1997), suggesting that the receptor is, at least in part, present on intrinsic neurons of the subthalamic nucleus. Previous studies of this laboratory have shown that local administration of *m*-CPP into the subthalamic nucleus induces oral dyskinesia identical to that elicited by peripheral administration of this drug (Eberle-Wang et al., 1996). Furthermore, the effect of peripheral *m*-CPP administration is markedly reduced by local administration of 5-HT_{2C} antagonists into the subthalamic nucleus (Eberle-Wang et al., 1996). This strongly suggests that 5-HT_{2C} receptors of the subthalamic nucleus play a major role in the oral dyskinesia induced by peripheral *m*-CPP administration, as used in the present study. We were unable to test the effect of local administration of *m*-CPP into the subthalamic nucleus of lesioned rats because the robust hyperreactivity induced by 5,7-DHT lesions would interfere with our method of microinjection in conscious rats. Therefore, we cannot eliminate the possibility that other brain regions participate in *m*-CPP-induced oral dyskinesia. A candidate region is the ventrolateral striatum, which contains 5-HT_{2C} receptors (Mengod et al., 1990) and is a site of induction of oral dyskinesia by many pharmacological agents (Delfs and Kelley, 1990; Yeghiayan and Kelley, 1995). However, bilateral local administration of *m*-CPP into the striatum failed to elicit abnormal oral movements in rats (Yeghiayan and Kelley, 1995). Therefore, it is likely that the increased oral dyskinesia observed after peripheral injections of *m*-CPP in lesioned rats is due, at least in part, to altered sensitivity to 5-HT_{2C} receptor stimulation in the subthalamic nucleus.

In a subset of lesioned rats, *m*-CPP induced seizure-like behaviors that were never observed in sham-lesioned animals. Since the lesion of serotonergic neurons performed in this study led to denervation of several brain regions known to play a role in similar seizure-like behaviors such as the hippocampus (Jefferys, 1998), it is possible that increased responses to the stimulation of 5-$HT_{2C\text{ or }1B}$ receptors in those regions mediated these behaviors.

4.2. Molecular mechanisms underlying the increased behavioral response

Measurements of ^{3}H-mesulergine binding under conditions that specifically label the 5-HT_{2C} receptor (Rocha et al., 1993) failed to detect changes in 5-HT_{2C} binding sites 5 days after 5,7-DHT injection, i.e., when the behavioral response was markedly increased. The use of a single dose analysis may have prevented the detection of significant changes in binding. However, a widespread increase in ^{3}H-mesulergine binding 30 days after a similar injection of 5,7-DHT was shown by Rocha et al. (1993) also using a single dose binding assay. Similarly, in a separate study (Eberle-Wang et al., unpublished observation), we found a small but significant increase in 5-HT_{2C} binding sites in the subthalamic nucleus of rats 10 days after a similar lesion. These data taken together indicate that increases in 5-HT_{2C} receptor density after serotonergic denervation are delayed and follow rather than precede the increase in behavioral response. A similar dissociation between behavioral supersensitivity and a lack of change in receptor density has been well documented for 5-HT_{1A} receptors (Hensler et al., 1991).

It is possible that the increased behavioral response observed in our study is related to supersensitivity in the transduction pathway activated by the stimulation of the 5-HT_{2C} receptors. This receptor subtype is a member of the 5-HT_2 receptor family of serotonergic receptors. These G-protein-coupled receptors activate phospolipase C, a major intracellular second messenger system (Backstrom et al., 1999). Indeed, 5,7-DHT-lesions have been shown to induce an enhancement in this transduction pathway following 5-HT_{2C} receptor stimulation in the choroid plexus (Conn et al., 1987). The very small size of the subthalamic nucleus in rats, however, precluded biochemical studies to directly test this hypothesis in our model.

4.3. Functional implications

Despite a widespread and dense innervation of all parts of the basal ganglia by serotonergic nerve terminals (Lavoie

and Parent, 1990), the role of serotonin in motor control is still poorly understood. The present data further support the hypothesis that neurons of the subthalamic nucleus that express 5-HT_{2C} receptors are normally under the control of serotonergic afferents. How serotonergic inputs to the suthalamic nucleus contribute to its normal functioning remains unknown, but the data suggest that excess stimulation of 5-HT_{2C} receptors in this region may lead to hyperkinetic movement disorders that resemble tardive dyskinesia in humans. This may be of particular importance in conditions of serotonergic depletion when an acute administration of a 5-HT_{2C} agonist could induce motor side effects. However, the short duration of the enhanced response suggests that such side effects, if observed, may only be transient. These data further highlight the need to consider potential changes in serotonergic neurotransmission in the pathophysiology of movement disorders.

Acknowledgments

We gratefully thank Dr. Philippe De Deurwaerdere for helpful discussions of the data. This work was supported by PHS grant MH-44894.

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