Pharmacology Biochemistry & Behavior, Vol. 34, pp. 367–374. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Serotonergic Innervation of the Rat Caudate Following a Neonatal 6-Hydroxydopamine Lesion: An Anatomical, Biochemical and Pharmacological Study[1]

A. C. TOWLE,* H. E. CRISWELL,† E. H. MAYNARD,‡ J. M. LAUDER,§
T. H. JOH,¶ R. A. MUELLER# AND G. R. BREESE**[2]

*Departments of *§Anatomy, #Anesthesiology, #**Pharmacology and †‡**Psychiatry*
*and the *†‡**Biological Sciences Research Center*
University of North Carolina School of Medicine, Chapel Hill, NC 27599
and ¶Laboratory of Neurobiology, Cornell University Medical College, New York, NY 10021

Received 30 January 1989

TOWLE, A. C., H. E. CRISWELL, E. H. MAYNARD, J. M. LAUDER, T. H. JOH, R. A. MUELLER AND G. R. BREESE. *Serotonergic innervation of the rat caudate following a neonatal 6-hydroxydopamine lesion: An anatomical, biochemical and pharmacological study*. PHARMACOL BIOCHEM BEHAV **34**(2) 367–374, 1989.—6-Hydroxydopamine (6-OHDA) treatment of neonatal rats resulted in a dose-related loss of striatal dopamine (DA). These reductions corresponded closely with the loss of tyrosine hydroxylase-containing terminals at this brain site. Striatal serotonin (5-HT) concentration increased only after DA was maximally depleted by the highest dose of 6-OHDA. Quantitative immunohistochemistry revealed that the increased 5-HT content after neonatal 6-OHDA lesioning was due to a proliferation of 5-HT nerve terminals. The density of immunoreactive 5-HT-containing terminals appeared to increase more than did the 5-HT content. The present study examined whether 5-HT hyperinnervation was playing a role in behavioral responses induced by D_1-DA agonists and antagonists in neonatally lesioned rats, because reports have suggested that these drugs may interact with 5-HT receptors. However, SCH-23390, the D_1-DA antagonist (0.3 mg/kg), did not alter behavioral responses to 5-HTP and SKF-38393 (3 mg/kg), a D_1-DA agonist did not produce any signs of activating 5-HT receptors in 5,7-DHT-lesioned rats. These data indicate that these compounds affecting D_1-DA receptors do not have a significant effect on 5-HT function at doses which have maximal effects on D_1-DA receptor function. Pretreatment with the 5-HT antagonist methysergide did not produce a change in apomorphine-induced locomotion and did not antagonize the self-mutilation or the other behaviors produced by L-DOPA or SKF-38393 in neonatally lesioned rats, suggesting that 5-HT hyperinnervation is not responsible for these drug-induced changes in neonatal 6-OHDA-lesioned rats.

6-Hydroxydopamine Dopamine Serotonin Caudate nucleus Serotonin hyperinnervation
Development SKF-38393 SCH-23390 D_1-agonist D_2-antagonist Serotonin syndrome

STACHOWIAK *et al.* (25) showed that treatment of neonatal rats with 6-hydroxydopamine (6-OHDA) leads to an increase in the serotonin content of the caudate nucleus. This observation has been confirmed (4,18). Similar treatment of adult rats with 6-OHDA does not result in any apparent change in serotonin content, although dopamine is completely depleted in the caudate by either neonatal or adult 6-OHDA administration (4). Breese *et al.* (4, 5, 7) have recently demonstrated that the age of 6-OHDA administration profoundly alters the behavioral responses to subsequent L-DOPA administration or to drugs having D_1- or D_2-dopamine receptor agonist and antagonist properties. Rats treated neonatally and tested as adults respond to L-DOPA with the appearance of a variety of behaviors including self-biting of the forepaws, that do not occur in rats treated with 6-OHDA as adults

[1]Supported by USPHS Grants HD-03110, MH-36294, NS-21345 and HD-23042.
[2]Requests for reprints should be addressed to Dr. George R. Breese, 226 Biological Sciences Research Center, CB #7250, University of North Carolina School of Medicine, Chapel Hill, NC 27599.

and tested under identical conditions (4). Evidence indicates that this effect is due to an action on D_1-dopamine receptors (5). In contrast, the behavioral response to D_2-dopamine agonists is less in neonatally lesioned rats than in adult 6-OHDA-lesioned rats (5,13). Furthermore, the immobility to dopamine antagonists observed in control rats and adult-lesioned rats is not seen in neonatally lesioned rats (11,14). Thus, the neuronal adaptations that ocur following neonatal 6-OHDA treatment are different from those seen after adult treatment and result in different behavioral responses to dopamine agonists.

There have been only limited investigations of the serotonin hyperinnervation of rats treated neonatally with 6-OHDA (1, 4, 18, 25). To examine the possible mechanism underlying the regulation of serotonin terminal growth after lesioning dopaminergic neurons, a graded reduction of brain dopamine was produced to see what effect this treatment would have on the changes seen when dopamine content is altered neonatally with 6-OHDA. Because binding studies have suggested that SCH-23390 influences S_2- and S_{1c}- serotonin receptors (2, 3, 15, 22), studies also were performed to determine if this action could contribute to the effects of SCH-23390 at doses which maximally antagonize D_1-dopamine receptor function. Further, there have been few reports to assess whether the increase in serotonin innervation could be contributing to the behavioral responses produced by dopamine agonist administration in the neonatal 6-OHDA-lesioned rats. This possibility was also investigated.

METHOD

Animals

Spraque-Dawley rats (Charles River Laboratories, Wilmington, MA) were treated with 25, 50 or 100 μg of 6-OHDA (intracisternal injection; Regis Chemical Co., Chicago, IL) on postnatal day 5 (9). Adult rats (150–200 g) were treated with 6-OHDA hydrobromide (200 μg free base, IC) 30 min after pargyline (50 mg/kg, IP) followed one week later by a second identical 6-OHDA treatment (4). Neonatal rats 5 days of age were treated with pargyline (50 mg/kg) plus 5,7-dihydroxytryptamine (5,7-DHT; 50 μg) as previously described to reduce brain serotonin (8,27).

Biochemistry

Animals were decapitated and the brain was rapidly removed. The striatum was dissected and frozen at −70°C until assayed. Dopamine and serotonin were determined in the same sample by reverse phase HPLC and electrochemical detection as described by Kilts *et al.* (16).

Immunocytochemistry

The immunocytochemistry procedures for localizing tyrosine hydroxylase (26) and serotonin (27) have been described previously. Briefly, the animals were perfused with a saline prewash, 150 ml of 4% paraformaldehyde, 70 mM sodium phosphate pH 7.0 and finally 250 ml of 4% paraformaldehyde in 100 mM sodium borate, pH 10.5. The tissue was prepared for sectioning by routine paraffin embedding in Paraplast Plus.

Ten micron sections were deparaffinized and rehydrated into phosphate buffered saline (PBS) and then treated with trypsin [(1.2 mg/ml PBS), Boehringer Mannheim] for 10 minutes. After rinsing the sections with PBS, the primary antiserum (antityrosine hydroxylase, 1:1000; antiserotonin, 1:2000) was applied for 48 hr at 4°C. The avidin-biotin (ABC) method was used to localize the immunoreactivity by utilizing a bridge of biotinylated goat anti-rabbit IgG (1:500)-avidin-biotinylated peroxydase (Vector Lab, Burlingame, CA) (26). Diaminobenzidine and H_2O_2 were used as the peroxidase substrate. Sections were counterstained with toluidine blue and mounted with permount. To quantitate the immunocytochemical results, the number of immunoreactive terminals which intersected the border of a 0.25 mm^2 box were counted. This method provides a relative quantitative assessment of terminal density which is useful for comparisons between samples. Ten micron coronal sections through the caudate nucleus were used for quantification. At least 4 sections from 3 animals were used for each treatment group.

TABLE 1

DOPAMINE CONTENT AND NEURAL DENSITY OF TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVITY IN CAUDATE FOLLOWING NEONATAL 6-OHDA TREATMENT[a]

Treatment	Dopamine Content (ng/mg Tissue)	Percent of Control	TH Terminal Density (Intersections/mm)	Percent of Control
Saline	138.0 ± 19	100	237.6 ± 7	100
6-OHDA				
100 μg	2.5 ± 2.5*	1.8	5.7 ± 1.4*	2.4
50 μg	27.7 ± 6.2*	20.0	55.2 ± 3.9*	23.2
25 μg	36.8 ± 18.9*	26.5	73.2 ± 4.8*	30.8

[a]Animals were treated with 6-OHDA at 5 days of age as described in the Method section. Data are presented as the mean ± SEM. N = 6 for dopamine content; N = 4 for TH terminal density.

*$p<0.05$ when compared to saline.

Behavioral Assessments

For behavioral observations, rats were placed in clear plastic cages (23 × 44 cm) for 60 min before administration of L-DOPA (100 mg/kg) or 5-hydroxytryptophan (5-HTP; 45 mg/kg) (1 hr after 50 mg/kg of RO4-4602, a decarboxylase inhibitor) (4). Other animals were habituated to the behavioral chambers for 60 min and were given SKF-38393 (3 mg/kg) (5). Once L-DOPA, 5-HTP, or SKF-38393 were administered, observations were made for 1 min of every 10 min for 2 hr. Each 1-min period was divided into four 15-sec periods and behaviors observed in each of these periods were scored for occurrence (4). Behavior is reported as a percentage of the total possible (i.e., 4 for presence in each of the four 15-sec periods times 12 observations; 48 = 100%). Behaviors recorded following L-DOPA and SKF-38393 administration to control and 6-OHDA-lesioned rats included sniffing, rearing, grooming, head nodding, locomotion, repeated movements of the paws away from the snout (taffy pulling), paw treading, licking, jumping, digging, eating woodchips, and self-biting. If the skin was broken by the self-biting (SMB), the rats were given SCH-23390 (0.5 mg/kg) to prevent this behavior (5). Behaviors recorded following 5-HTP and SKF-38393 administration to 5,7-DHT-lesioned rats included straub tail, hind-limb abduction, rigidity, tremors, salivation, lateral head weaving and forepaw treading (10).

Activity was measured in circular activity cages as previously described. Rats were habituated to the chamber for 60 min before receiving apomorphine (7).

Drugs

The 6-hydroxydopamine hydrobromide (6-OHDA), and 5,7-dihydroxytryptamine (5,7-DHT) (Sigma Chemical Co., St. Louis,

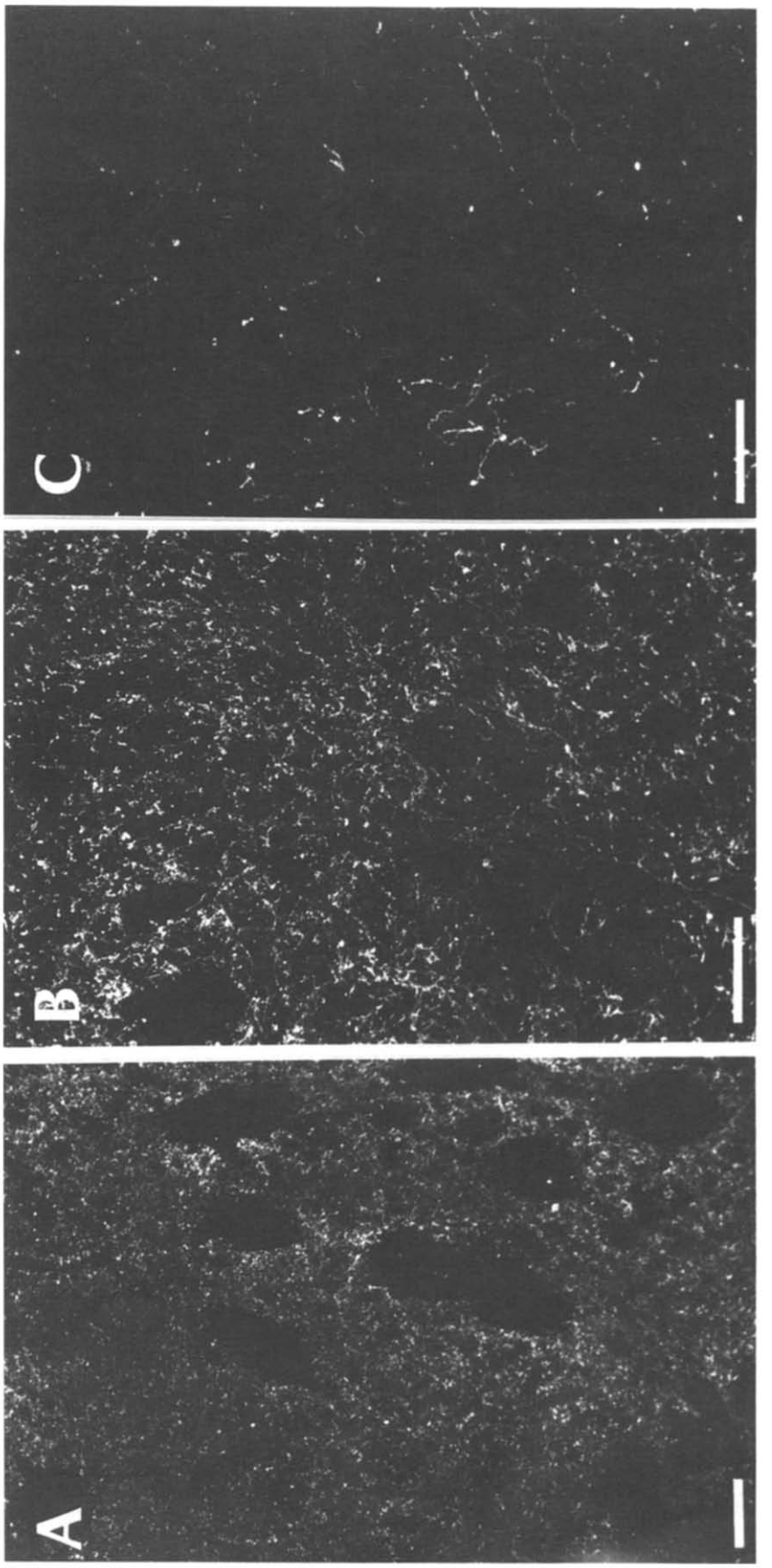

FIG. 1. Tyrosine hydroxylase immunoreactive terminals in rat caudate nucleus. (A) Saline treatment (control). (B) Treatment with 50 μg 6-OHDA. (C) Treatment with 100 μg 6-OHDA. All animals were treated on day 5 and sacrificed when 60 days of age. Bar = 100 μM.

TABLE 2

SEROTONIN CONTENT AND IMMUNOREACTIVITY IN CAUDATE FOLLOWING NEONATAL 6-OHDA TREATMENT [a]

Treatment	Serotonin Content (ng/mg Tissue)	Percent of Control	Serotonin Fiber Density (Intersections/mm^2)	Percent of Control
Saline	6.2 ± 0.6	100	18.3 ± 2.3	100
6-OHDA				
100 μg	9.1 ± 0.2*	147	45.3 ± 1.4†	248
50 μg	7.5 ± 0.5	120	26.4 ± 1.3*	144
25 μg	8.2 ± 0.9	132	24.6 ± 1.4*	134

[a]Animals were treated with 6-OHDA at 5 days of age as described in the Method section. Data are expressed as the mean ± SEM. N = 6 for serotonin content; N = 4 for serotonin neural density.

*$p<0.05$ when compared to saline; †$p<0.01$ when compared to saline.

TABLE 3

EFFECT OF METHYSERGIDE ON ACTIVITY RESPONSE TO APOMORPHINE ADMINISTRATION TO NEONATAL 6-OHDA-LESIONED RATS[a]

Treatment	Dose (mg/kg)	Control (Counts/120 min)	Neonatal 6-OHDA (Counts/120 min)
Saline	—	648 ± 79	558 ± 130
Methysergide (Methy)	5.0	772 ± 172	1600 ± 172*
Apomorphine (APO)	1.0	3307 ± 303*	5107 ± 673*[b]
Methy + APO	5.0 + 1.0	4279 ± 379*	6684 ± 1302*

[a]Rats were treated with 6-OHDA when 3 days of age as described in the Method section. Responses to D_1-dopamine agonists were primed as described by Breese *et al.* before being tested with apomorphine. Methysergide (5 mg/kg, IP) or saline was given 30 min prior to apomorphine (1 mg/kg) administration. There are at least 6 rats in each group. Control refers to unlesioned rats; neonatal 6-OHDA refers to rats that received 100 μg 6-OHDA when 5 days of age.

[b]Responses in control and adult 6-OHDA-lesioned rats were 3980 ± 933 and 13,514 ± 840 counts/120 min, respectively ($p<0.01$), demonstrating the greater response observed after adult treatment compared to that observed in the neonate 6-OHDA-lesioned rats (see above APO response in neonatal 6-OHDA-lesioned rats).

*$p<0.05$ when compared to saline.

MO) were dissolved in saline containing 0.5% ascorbic acid and were administered intracisternally. SKF-38393 (Research Biochemicals Inc., Wayland, MA) and SCH-23390 (Schering Pharmaceutical Company, Bloomfield, NJ) were dissolved in water and administered. IP L-DOPA (Sigma) was suspended in carboxymethylcellulose (0.5%). The RO4-4602 (Hoffmann-La Roche, Nutley, NJ) was dissolved in saline.

Statistics

For behavioral studies, data for each behavior were analyzed by a one factor, unweighted means ANOVA. For each ANOVA that yielded a significant F ratio, a Newman-Keul's test was used to make comparisons. For biochemical and anatomical investigations, differences among group means were tested for statistical significance with a one-way ANOVA for the main effect and the least Significant Differences Test was used for multiple comparisons of means.

RESULTS

Dopamine Content and Tyrosine Hydroxylase Immunohistochemistry in the Caudate of Neonatal and Adult 6-OHDA-Lesioned Rats

Dopamine content has been demonstrated to be dramatically reduced following intracisternal administration of 100 μg of 6-OHDA to neonatal rats (4,9). In this study, the consequence of giving different doses of 6-OHDA to neonatal rats was examined. As expected, neonatal treatment with 100 μg 6-OHDA resulted in a nearly complete loss (98% reduction) of dopamine in the striatum (Table 1). Rats treated with lower doses of 6-OHDA (50 and 25 μg) had significantly less dopamine depletion (Table 1).

Immunohistochemical analysis of tyrosine hydroxylase (TH-IR) in caudate was also performed in these animals. The qualitative results of this work are illustrated in Fig. 1. The TH-IR innervation of the caudate was dramatically reduced by neonatal treatment with 100 μg of 6-OHDA. Treatment of neonatal rats with lesser amounts of 6-OHDA (25 or 50 μg) resulted in correspondingly smaller decreases in the extent of TH-IR terminal density throughout the caudate (Fig. 1C). The only brain region containing TH neurons that was not affected markedly by administration of 100 μg of 6-OHDA to neonatal rats was the hypothalamus (data not shown).

In order to quantify the degree of catecholamine-containing fiber loss in the striatum, the terminal density of tyrosine hydroxylase-containing neurons in the caudate was measured in control and 6-OHDA-lesioned rats (Table 1). Neonatal 6-OHDA (100 μg) treatment reduced the TH-IR terminal density in the caudate by more than 97%. This is a conservative estimate because the TH-IR terminal density was so high in normal rats that it was difficult to accurately count all intersecting terminals. Rats treated with 25 or 50 μg 6-HODA retained significantly more TH terminals than rats treated with 100 μg 6-OHDA, yet the terminal densities were still greatly reduced when compared to saline-treated rats (Table 1). Thus, the results of this morphological quantification of catecholamine terminal density correlate well with the loss of dopamine content observed after the 6-OHDA treatment (Table 1). These results indicate that a dose-dependent reduction of TH terminal density follows neonatal 6-OHDA treatment.

Effect of Various Doses of 6-OHDA on Content and Immunohistochemical Staining of Serotonin in the Striatum

Previous data have demonstrated that the striatum has increased serotonin content in the caudate whereas other brain regions with a rich dopaminergic innervation (e.g., olfactory tubercle and nucleus accumbens) do not have such an increase in serotonin content (4,25). For this reason, the present study focused on serotonin content in the striatum after 6-OHDA treatment. Our first study examined the relationship between the extent of dopamine depletion by the 6-OHDA and the corresponding rise in striatal serotonin content (Table 2). While the highest dose of 6-OHDA was associated with a 50% increase in striatal serotonin content, no significant change in serotonin level was observed after the lower doses of 6-OHDA.

The increase in serotonin content of the striatum following the 100 μg 6-OHDA treatment could be due to an increase in serotonergic terminal density, an increase in the serotonin content per terminal or to an interaction between these factors. To examine

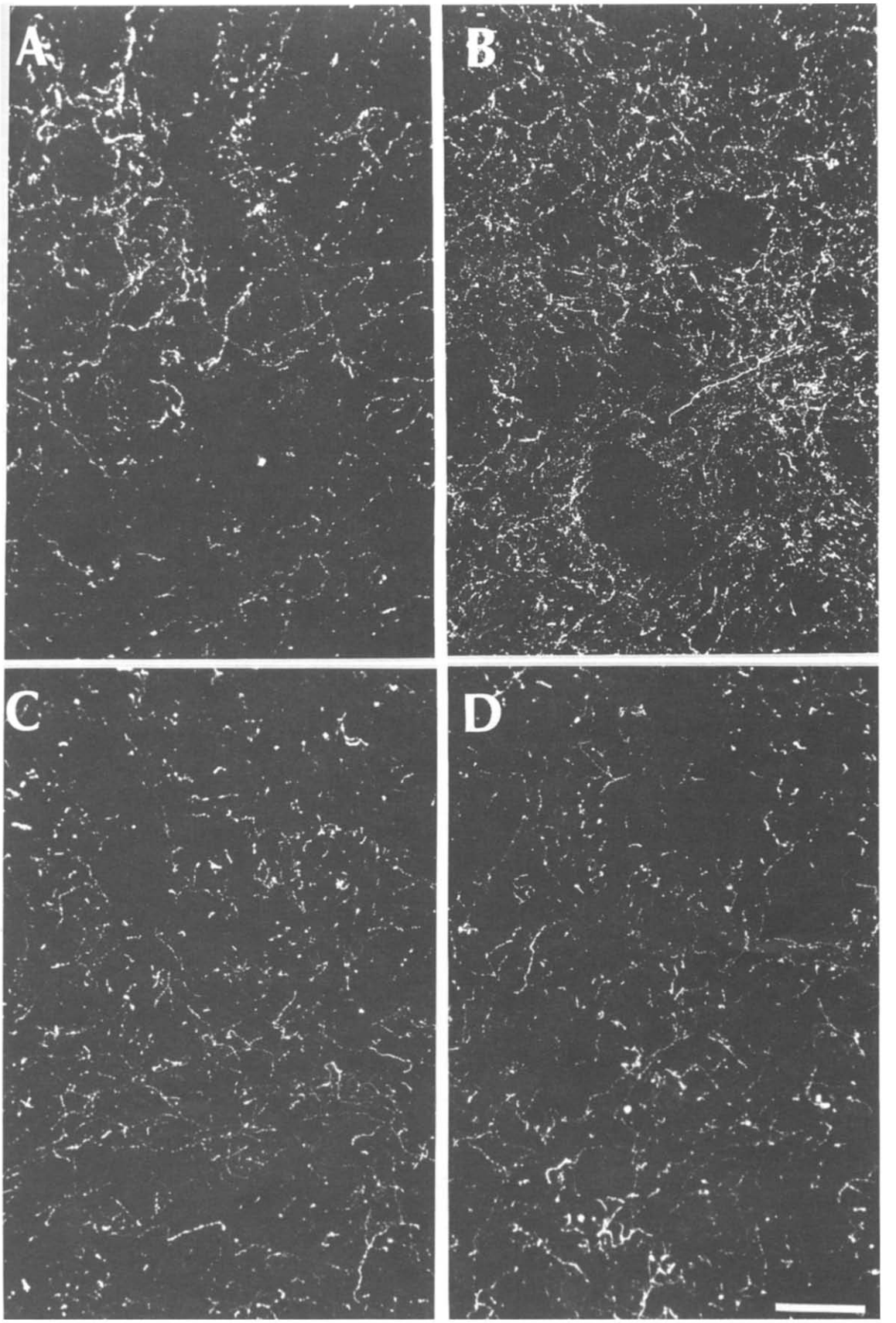

FIG. 2. Serotonin immunoreactive nerve terminals in rat caudate nucleus. (A) Saline treatment (control). (B) Treatment with 100 μg 6-OHDA on day 5 of age. (C) Treatment with 50 μg 6-OHDA on day 5 of age. (D) Treatment with 100 μg 6-OHDA on day 45 of age. All animals were killed 2 months after the various treatments and sacrificed when 60 days of age. Bar = 100 μM.

TABLE 4

BEHAVIORAL SCORES FOR L-DOPA AND SKF-38393 IN NEONATAL 6-OHDA-LESIONED RATS AFTER METHYSERGIDE PRETREATMENT[a]

Behaviors	Saline Neonates	SKF-38393 Saline	SKF-38393 Methysergide	L-DOPA Saline	L-DOPA Methysergide
Self-biting	1 ± 1	2 ± 1	0 ± 0	28 ± 4	18 ± 8
Taffy Pulling	0 ± 0	19 ± 9	9 ± 5	11 ± 4	20 ± 11
Paw Licking	0 ± 0	28 ± 7	14 ± 7	9 ± 2	9 ± 3
Sniffing	65 ± 11	83 ± 6	97 ± 1	23 ± 6	76 ± 7*
Rearing	37 ± 10	56 ± 14	91 ± 3*	38 ± 6	30 ± 7
Paw Treading	3 ± 1	46 ± 13	80 ± 6	19 ± 10	22 ± 6
Locomotion	19 ± 6	40 ± 17	47 ± 10	49 ± 9	30 ± 9
Digging	1 ± 1	6 ± 3	30 ± 8*	12 ± 2	6 ± 3

[a]SKF-38393 (3.0 mg/kg, IP) and L-DOPA (100 mg/kg, IP) were administered to neonate 6-OHDA-lesioned rats as described in the Method section. Methysergide (5.0 mg/kg) was administered 30 min prior to drug treatment. Values presented for drug treatment are all significantly different from saline treatment in neonatal 6-OHDA-lesioned rats. There are 7–10 rats in each of the groups.

*$p<0.05$ when compared to saline administration.

these possibilities, we performed qualitative and quantitative immunocytochemistry to define serotonin terminal density (Fig. 2). Quantitative analyses of these data documented that the density of serotonin-containing terminals in the caudate was increased in rats neonatally treated with 100 μg 6-OHDA (Table 2). The increase measured by this approach demonstrated that the number of fibers increased by 1.5 times in the lesioned rats compared to controls. It should be noted that this measure of serotonin-containing neurons resulted in a greater increase than that reflected by the measure of serotonin content in this brain region (Table 1).

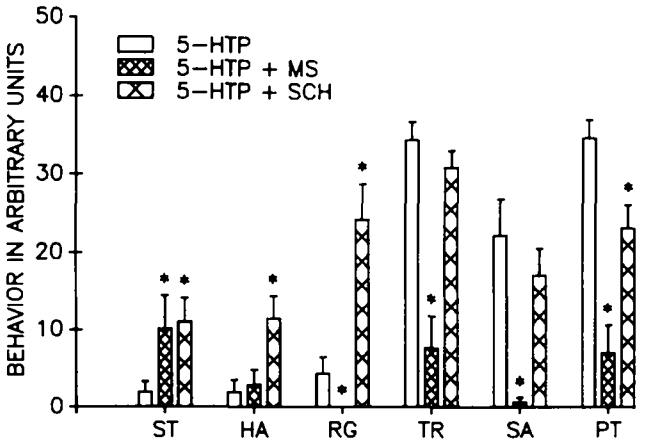

FIG. 3. Effects of SCH-23390 or methysergide on behavioral scores produced by 5-HTP administration to 5,7-DHT-treated rats. Behavioral scores are the mean ± SEM of the percent time an animal displays behavior over a 60 min period. Dose of 5-HTP as 45 mg/kg. There are 7 rats in each group. ST = straub tail; HA = hind-limb abduction; RG = rigidity; TR = tremor; SA = salivation; PT = paw tread. *Indicates that the behavioral score is significantly different from 5-HTP alone.

TABLE 5

EFFECT OF METHYSERGIDE AND SCH-23390 ON THE INCIDENCE OF SMB INDUCED BY L-DOPA IN NEONATE 6-OHDA-LESIONED RATS[a]

Treatment	Dose mg/kg	L-DOPA-Induced Incidence of SMB No./Total	%
Saline	0	7/7	100%
Methysergide	5.0	7/8	88%
SCH-23390	0.3	0/7*	0

[a]All rats were treated with RO4-4602 (50 mg/kg) 60 min before injection of L-DOPA (100 mg/kg). All rats used in this investigation had previously shown SMB when given this treatment. Methysergide was administered 30 min before L-DOPA. SMB = self-mutilatory behavior.

*$p<0.05$ when compared to saline + L-DOPA.

The serotonin terminal density was found to be increased significantly after either 25 or 50 μg 6-OHDA, but this change was considerably less than observed at the highest dose of 6-OHDA (Table 1; Fig. 2 and Table 2). The density of serotonin-containing terminals after neonatal 6-OHDA lesions was fairly uniform throughout the caudate, which is in marked contrast to the rostral-caudal density gradient in control rats (25,27). It was also noted that 100 μg of 6-OHDA administration to a rat 45 days of age caused no elevation in serotonin content (Fig. 2).

Effect of Methysergide on Behavioral Responses to Apomorphine, SKF-38393, and L-DOPA

In order to explore whether the serotonin hyperinnervation in the neonatally treated rats could be contributing to the unique behavioral responses of these animals to dopamine agonists, rats were treated with a serotonin antagonist, methysergide, prior to administering apomorphine, SKF-38393, or L-DOPA. In Table 3, apomorphine is shown to produce an attenuated response in neonatally 6-OHDA-lesioned rats when compared to adult 6-OHDA-lesioned rats (value in table legend), confirming our earlier results (4). The reduced response to apomorphine in the neonatal 6-OHDA-lesioned rats was not altered by methysergide, but methysergide itself seemed to enhance activity level in the neonatal 6-OHDA-lesioned rats.

The effect of methysergide against the behavioral responses observed following administration of SKF-38393 and L-DOPA is shown in Table 4. SKF and L-DOPA produced the behavioral responses usually observed in neonatal 6-OHDA-lesioned rats (5). Concomitant administration of methysergide with SKF-38393 altered only two behaviors; the increase in rearing and the paw treading induced by SKF-38393. Pretreatment with methysergide had no significant effect on most behaviors induced by L-DOPA in neonatal 6-OHDA-lesioned rats except sniffing (Table 4). Furthermore, methysergide did not alter significantly the frequency of self-mutilation displayed by the neonatal 6-OHDA-lesioned rats to L-DOPA (Table 5).

Effect of SKF-38393 and SCH-23390 on 5-HT Receptors

Several publications have implied that SCH-23390 could interact with 5-HT receptors in vitro (2, 3, 12, 15, 22). Because of these observations, we explored whether this action of SCH-23390 on serotonergic receptors was apparent at doses of SCH-23390 used to antagonize the action of D_1-dopamine agonists. This was accomplished by testing the action of SCH-23390 against 5-HTP

in 5,7-DHT-lesioned rats. As shown in Fig. 3, SCH-23390 (0.3 mg/kg), a dose having maximal effectiveness against D_1-dopamine agonist responses, reduced only the paw treading response to 5-HTP. However, hindlimb abduction, straub tail and rigidity following 5-HTP were enhanced by SCH-23390. The increased rigidity could be attributed to the neuroleptic effect produced by SCH-23390 alone (data not shown). Some interaction of this property with the behavior produced by 5-HTP could account for the increased incidence of the other behaviors. Methysergide, primarily a S_2-serotonin antagonist (3), was included as a positive control and this drug antagonized the prominent behaviors observed after 5-HTP administration. Thus, these data suggest that SCH-23390 is not having a prominent action on serotonergic receptor function.

In addition to SCH-23390, the effects of SKF-38393 in 5,7-DHT-lesioned rats were examined to assess whether any serotonin agonist action could be demonstrated. Administration of 3 mg/kg of SKF-38393 to the 5,7-DHT-treated rats produced no significant alteration in behavioral responses compared to saline (data not shown), which contrasts to the change observed following administration of 5-HTP (Fig. 3). Thus, this D_1-dopamine agonist also does not appear to interact with serotonin receptors in any significant way.

DISCUSSION

Data presented in this investigation confirm the original observation by Stachowiak *et al.* (25) that a neonatal 6-OHDA treatment which reduces dopamine content more than 90 percent increases serotonin content in striatum. This change is not apparent in rats given 6-OHDA as adults (4,25), indicating that the phenomenon of serotonin hyperinnervation in the striatum following 6-OHDA treatment is age dependent. Quantitative immunohistochemical evaluation of serotonin-containing neurons in the caudate provided a clear indication that the increased serotonin content in the caudate following neonatal 6-OHDA lesioning of dopaminergic neurons can be attributed to an increase in terminal density, rather than to other factors. This finding is consistent with the report by Berger *et al.* (1) who reported a 300% increase in serotonin fiber density. However, it is not clear why there is such a large increase in serotonin-containing fibers (150%) with only a 50% increase in serotonin-content in the caudate.

One possible explanation for the approximately 50% increase in serotonin concentration and the increase in fiber density could be the shrinkage of the caudate that results from the neonatal-6-OHDA-lesion. However, an argument against this view is that the size of the caudate is reduced by only 20–25% in the lesioned rats whereas fiber density of serotonin-containing neurons is increased by 150%. In addition, caudate shrinkage of a similar magnitude was seen in some rats treated with lower doses of 6-OHDA (25 and 50 μg), yet serotonin concentration was not altered in these animals. However, at these lower doses of 6-OHDA there was an increase in fiber density of serotonin-containing neurons in the neonatally lesioned rats suggesting that such shrinkage within the caudate might account for the small, but significant increase in fiber density in these animals. Another explanation for the serotonin hyperinnervation in the caudate unrelated to any physical change in the caudate could be that dopamine and serotonin-containing nerve terminals compete during development for termination sites in this brain region. This explanation proposes that the presence of dopaminergic terminals may limit the extent of the innervation by serotonergic neurons. Another possible reason for the hyperinnervation could be that the destruction of dopaminergic neurons during development results in the release of growth-promoting factors in response to the removal of dopaminergic terminals in the striatum. If this should be the case, the growth factor(s) released in response to the removal of dopaminergic terminals in the striatum likely would be relevant to the growth of both dopaminergic and serotonergic neurons.

Whatever the explanation may be for the increased concentration of serotonin in the caudate, the serotonin hyperinnervation appears to be all or none, since rats with partial destruction of dopaminergic neurons showed at best only a small alteration in serotonergic innervation of the caudate. It should also be pointed out that the change described in the caudate after neonatal 6-OHDA treatment appears to be unique to the striatum, since other brain regions with both dopaminergic and serotonin innervation (e.g., nucleus accumbens and olfactory tubercle) do not exhibit an increase in serotonin content after neonatal lesioning of dopaminergic neurons (4). Thus, for as yet unexplained reasons, serotonergic hyperinnervation occurs only after a dramatic loss of dopaminergic innervation in the neonatal caudate.

As indicated by the introductory remarks, SCH-23390 has been demonstrated to interact with serotonin receptors (3, 12, 15, 19, 22). Except for the work by Bishoff *et al.* (2), there has not been an evaluation of the in vivo effects of SCH-23390 on serotonin-mediated behaviors. In the present study, this evaluation was performed by determining whether 0.3 mg/kg of SCH-23390 would modify responses to 5-HTP in rats made supersensitive by treating them with 5,7-DHT. At this dose of SCH-23390, which has a maximal effect on D_1-dopamine receptor mediated functions, no significant inhibition of the pharmacological actions of 5-HTP was observed. Because of the structural similarity of SKF-38393 to SCH-23390, we also explored whether the D_1-dopamine agonist (SKF-38393) might produce an agonist action in the 5,7-DHT-lesioned rats, reasoning that any action on serotonin receptors would be observed in rats supersensitive to serotonin agonists. No change in behavior was observed after SKF-38393 administration to the 5,7-DHT-treated rats. Thus, it can be assumed that the action of these compounds in 6-OHDA-lesioned rats at the doses used by our laboratory is due to effects on D_1-dopamine receptors and not to effects on serotonergic receptors.

Another purpose of this study was to evaluate whether the elevated serotonin content in the caudate of neonatal 6-OHDA-lesioned rats might be contributing to their unique behavioral responses to dopamine agonists. The strategy taken to evaluate the potential influences of the serotonergic hyperinnervation was to pretreat rats with methysergide to antagonize primarily 5-HT_2 receptors prior to administering agonists influencing dopamine receptors. Methysergide did not alter the response to apomorphine (i.e., primarily a D_2-dopamine agonist response) or the SMB and behavioral responses observed after L-DOPA in rats treated neonatally with 6-OHDA. Since the dose of methysergide used reduced the majority of behaviors observed after 5-HTP administration, it seems unlikely that the serotonergic hyperinnervation is contributing in a major way to the behavioral responses observed after dopamine agonist administration. If the recent observation that 5-HT_2 antagonists interact with the 5-HT_{1C} receptor in the choriod plexus (23) applies to other brain sites, then the action of SCH-23390 on the 5HT_{1C} receptor subtype (22) could not account for its effects against dopamine agonists, because methysergide administration did not alter behaviors after L-DOPA but SCH-23390 did. Nevertheless, drugs antagonistic for other subtypes of serotonin receptors will need to be tested further before one can be absolutely certain of this conclusion.

While no support for an involvement of serotonergic hyperinnervation in neonatally lesioned rats for the reduced response to apomorphine or behavioral responses to L-DOPA were apparent, methysergide altered selectively some behaviors produced by SKF-38393. Previous work has demonstrated that reduction of serotonin function can elevate activity produced by d-amphet-

amine (6, 17, 21, 24). Segal (24) has attributed such an alteration after reducing serotonin (6, 17, 21) to a change in behavioral pattern noting a decrease in stereotyped behavior and enhanced motor activity and rearing after such treatment. In the present study, methysergide enhanced rearing and digging in wood chips induced by SKF-38393 without altering locomotion and other behaviors. Why similar changes in these behaviors were not observed after methysergide administration to rats that received L-DOPA is unknown. One could speculate that the degree to which D_1- or D_2-dopamine receptors are activated might explain this difference between L-DOPA and SKF-38393. Regardless, such results with SKF-38393 would seem consistent with the view that altered serotonergic function (6) can influence dopamine function. The fact that such changes were specific for selected behaviors should be emphasized, in agreement with the conclusion by Segal (24).

In summary, the present work confirms that neonatal 6-OHDA treatment results in an increase in the number of serotonin-containing fibers in the caudate. In spite of this documentation, we were unable to associate this change in serotonin content with behavioral responses characteristic of neonatal 6-OHDA-lesioned rats after dopamine agonists. Furthermore, we could obtain no evidence that SCH-23390 or SKF-38393 at doses producing maximal effects on D_1-dopamine receptors was influencing serotonin receptor function in any major way. Thus, additional work is required to define whether there is a physiological consequence of this increase in striatal serotonin when neonatal dopaminergic neurons are destroyed.

ACKNOWLEDGEMENTS

The authors are grateful for the skillful typing of this manuscript by Ms. Doris Lee and for the determination of monoamines by Ms. Marcine Garrison and Ms. Edna Edwards.

REFERENCES

1. Berger, T. W.; Kaul, S.; Stricker, E. M.; Zigmond, M. J. Hyperinnervation of the striatum by dorsal raphe afferents after dopamine-depleting brain lesions in neonatal rats. Brain Res. 336:354–358; 1985.
2. Bishoff, S.; Heinrich, M.; Krauss, J.; Sills, M. A.; Williams, M.; Vassout, A. Interaction of the D_1 receptor antagonist SCH-23390 with the central 5-HT system: radioligand binding studies, measurements of biochemical parameters and effects on L-5-HTP syndrome. J. Recept. Res. 8:107–120; 1988.
3. Bischoff, S.; Heinrich, M.; Sonntag, J. M.; Krauss, J. The D-1 dopamine receptor antagonist SCH-23390 also interacts potently with brain serotonin ($5\text{-}HT_2$) receptors. Eur. J. Pharmacol. 129:367–370; 1986.
4. Breese, G. R.; Baumeister, A. A.; McCown, T. J.; Emerick, S. G.; Frye, G. D.; Crotty, K.; Mueller, R. A. Behavioral differences between neonatal and adult 6-hydroxydopamine-treated rats to dopamine agonists: Relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. J. Pharmacol. Exp. Ther. 231:343–354; 1984.
5. Breese, G. R.; Baumeister, A. A.; Napier, C. T.; Frye, G. D.; Mueller, R. A. Evidence that D-1 dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. J. Pharmacol. Exp. Ther. 235:287–295; 1985.
6. Breese, G. R.; Cooper, B. R.; Mueller, R. A. Evidence for involvement of 5-hydroxytryptamine in the actions of amphetamine. Br. J. Pharmacol. 52:307–314; 1974.
7. Breese, G. R.; Duncan, G. E.; Napier, T. C.; Bondy, S. C.; Iorio, L. C.; Mueller, R. A. 6-Hydroxydopamine treatments enhance behavioral responses to intracerebral microinjection of D_1- and D_2-dopamine agonists into nucleus accumbens and striatum without changing dopamine antagonist binding. J. Pharmacol. Exp. Ther. 240:167–176; 1987.
8. Breese, G. R.; Cooper, B. R. Behavioral and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered to adult and developing rats. Brain Res. 98:517–527; 1975.
9. Breese, G. R.; Traylor, T. D. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. Br. J. Pharmacol. 44:210–222; 1972.
10. Breese, G. R.; Vogel, R. A.; Kuhn, C. M.; Mailman, R. B.; Mueller, R. A.; Schanberg, S. M. Behavioral and prolactin responses to 5-hydroxytryptophan in rats treated during development with 5,7-dihydroxytryptamine. Brain Res. 155:263–275; 1978.
11. Bruno, J. P.; Stricker, E. M.; Zigmond, M. J. Rats given dopamine-depleting brain lesions as neonates are subsensitive to dopaminergic antagonists as adults. Behav. Neurosci. 99:771–775; 1985.
12. Christensen, A. V.; Arnt, J.; Hyttel, J.; Larsen, J. J.; Svendsen, O. Pharmacological effects of a specific dopamine D-1 antagonist SCH-23390 in comparison with neuroleptics. Life Sci. 34:1529–1540; 1984.
13. Criswell, H. E.; Mueller, R. A.; Breese, G. R. Assessment of purine-dopamine interactions in 6-hydroxydopamine-lesioned rats: evidence for pre- and postsynaptic influences by adenosine. J. Pharmacol. Exp. Ther. 244:493–500; 1987.
14. Duncan, G. E.; Criswell, H. E.; McCown, T. J.; Paul, I. A.; Mueller, R. A.; Breese, G. R. Behavioral and neurochemical responses to haloperidol and SCH-23390 in rats treated neonatally or as adults with 6-hydroxydopamine. J. Pharmacol. Exp. Ther. 243:1027–1034; 1987.
15. Hicks, P. E.; Schoemaker, H.; Langer, S. Z. 5-HT receptor antagonist properties of SCH-23390 in vascular smooth muscle and brain. Eur. J. Pharmacol. 105:339–342; 1984.
16. Kilts, C. D.; Breese, G. R.; Mailman, R. B. Simultaneous quantification of dopamine, 5-hydroxytryptamine and four metabolically related compounds by means of reverse phase HPLC with electrochemical detection. J. Chromatogr. Biol. Med. Appl. 225:347–357; 1981.
17. Lucki, I.; Harvey, J. A. Increased sensitivity to *d*- and *l*-amphetamine action after midbrain raphe lesions as measured by locomotor activity. Neuropharmacology 18:243–249; 1979.
18. Luthman, J.; Bolioli, B.; Tustsumi, T.; Verhofstad, A.; Jonsson, G. Sprouting of striatal serotonin nerve terminals following selective lesions of nigro-striatal dopamine neurons in neonatal rat. Brain Res. Bull. 19:269–274; 1987.
19. McQuade, R. D.; Ford, D.; Duffy, R. A.; Chipken, R. E.; Iorio, L. C.; Barnett, A. Serotonergic component of SCH-23390: in vitro and in vivo binding analysis. Life Sci. 43:1861–1869; 1988.
20. Markstein, R.; Hoyer, D.; Engel, G. $5\text{-}HT_{1A}$-receptors mediate stimulation of adenylate cyclase in rat hippocampus. Naunyn Schmiedebergs Arch. Pharmacol. 333:335–341; 1986.
21. Neill, D. B.; Grant, L. D.; Grossman, S. P. Selective potentiation of locomotor effects of amphetamine by midbrain raphe lesions. Physiol. Behav. 9:655–657; 1972.
22. Nicklaus, K. J.; McGonigle, P.; Molinoff, P. B. [^{3}H] SCH-23390 labels both dopamine-1 and $5\text{-hydroxytryptamine}_{1C}$ receptors in the choroid plexis. J. Pharmacol. Exp. Ther. 247:343–348; 1988.
23. Sanders-Bush, E.; Breeding, M. Putative selective 5-HT-2 antagonists block serotonin $5\text{-HT-}1_C$ receptors in the choroid plexis. J. Pharmacol. Exp. Ther. 247:169–173; 1988.
24. Segal, D. S. Differential effects of para-chlorophenylalanine on amphetamine-induced locomotion and stereotype. Brain Res. 116: 267–276; 1976.
25. Stachowiak, M. K.; Bruno, J. P.; Snyder, A. M.; Stricker, E. M.; Zigmond, M. J. Apparent sprouting of striatal serotonergic terminals after dopamine-depleting brain lesions in neonatal rats. Brain Res. 291:164–167; 1984.
26. Towle, A. C.; Joh, T. H.; Lauder, J. M. Optimization of tyrosine hydroxylase immunocytochemistry in paraffin section using pretreatment with proteolytic enzymes. J. Histochem. Cytochem. 32:766–770; 1984.
27. Towle, A. C.; Breese, G. R.; Mueller, R. A.; Coyle, S.; Lauder, J. M. Early postnatal administration of 5,7 DHT: Effects on serotonergic neurons and terminals. Brain Res. 310:67–75; 1984.