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Evaluation of Risperidone in the Neonatal 6-Hydroxydopamine Model of Lesch-Nyhan Syndrome

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ALLEN, S. M., J. N. FREEMAN AND W. M. DAVIS. *Evaluation of risperidone in the neonatal 6-hydroxydopamine model of Lesch-Nyhan syndrome.* PHARMACOL BIOCHEM BEHAV **59**(2) 327–330, 1998.—Rats were treated as neonates with 6-hydroxydopamine (6-OHDA) 100 mg free base in 10 μl intracisternally. Upon maturation, animals were injected with L-dopa and placed in photocell cages for monitoring of locomotion, stereotypies, and self-mutilation. Pretreatment with either risperidone or SCH-23390 significantly reduced locomotion and stereotypies. SCH-23390 eliminated L-dopa induced self-mutilation in all subjects, while risperidone eliminated self-mutilation in all but one subject. © 1998 Elsevier Science Inc.

Risperidone 6-Hydroxydopamine SCH-23390 Serotonin Dopamine Self-mutilation

RISPERIDONE (R 64 766, 3-{2-[4-(6-flouro-1,2-benzisoxazol-3-yl)-1-pyperidinl] ethyl}-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]-pyrimidon-4-one) is a new atypical neuroleptic with affinities for both dopaminergic and serotonergic receptors (14,16,20,21). These affinities are high for D_2, 5-HT_2A, 5-HT_2C, 5-HT_3, 5-HT_6, and 5-HT_7 receptors. The ability of this agent to bind with both dopaminergic and serotonergic receptors produces both unique therapeutic results and a much lower incidence of extrapyramidal side effects as compared to classical neuroleptics. Specifically, risperidone has shown clinical efficacy in treatment of both positive and negative symptoms of schizophrenia (7), as well as a reduction of the self-mutilation in a Lesch-Nyhan patient (1).

Lesch-Nyhan syndrome is an X-linked disease characterized by a lack of the purine salvage enzyme hypoxanthine guanine phosphoribosyltransferase (HPRT). Individuals with this disorder exhibit several severely abnormal behaviors, the most debilitating symptom by far being the tendency to self-mutilate (18). Individuals with this malady tend to bite themselves on the fingers, hands, legs, orofacial area, or buccal mucosa. Other manifestations include dystonia, involuntary spastic movements, and usually some degree of mental retardation. Lloyd et al. (12) discovered severely depleted levels of dopamine in the caudate and putamen, and increased levels of serotonin in the putamen from postmortem biochemical analysis of three Lesch-Nyhan patients. The amounts of GABA, glutamate, and acetylcholine were not significantly different from controls in any brain region. Dopamine depletion in dopaminergic pathways has since been confirmed in vivo for Lesch-Nyhan subjects compared to controls using positron emission tomography (9,24). Currently, there is an animal model for this syndrome consisting of the neonatally 6-hydroxydopamine-lesioned (6-OHDA) rat (2). In this model, rats are administered 6-OHDA intracisternally at a neonatal age. This technique produces a permanent reduction in dopaminergic neurons and a hyperproliferation of serotonergic nerve terminals in the caudate and putamen (17,22). Dopamine content is reduced by as much as 98%, while serotonin content is increased approximately 50%. This severe depletion of dopamine produces a supersensitivity to dopamine in the remaining neurons in the striatum. The exact mechanism of this increased sensitivity is unknown, but may be related to an enhancement of second-messenger systems (11), or a vastly increased synthesis and release of dopamine by the surviving

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terminals (17). Whatever the mechanism, the cause of this change in sensitivity is probably the alteration of the functional relationship of dopamine and serotonin in the area.

6-OHDA–lesioned animals do not demonstrate any spontaneous functional abnormality upon reaching adulthood. However, injection of dopaminergic agonists such as L-dihydroxyphenylalanine (L-dopa), apomorphine, or SKF-38393, causes a variety of behaviors which resemble the clinical manifestations of Lesch-Nyhan syndrome. Among the abnormal behaviors exhibited by these animals are stereotypies, increased motility and self-biting. These behaviors are blocked by the specific D_1 antagonist SCH-23390, and have therefore been attributed to selective activation of the striatal D_1 neuronal system (3,4). This assumption is further supported by a lack of antagonism of these behaviors by D_2 blockers such as haloperidol (2,5).

Criswell used clozapine, another atypical neuroleptic, to antagonize self-mutilation in neonatally 6-OHDA–lesioned rats (8). The self-mutilation was attenuated in a dose-dependent fashion, with complete elimination at the highest dose. The present study examined the ability of risperidone to antagonize the self-mutilation induced in the neonatally 6-OHDA–lesioned rat. These data will be compared to the antagonistic qualities of the selective D_1 receptor antagonist SCH-23390.

METHOD

Twelve pregnant Sprague–Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). Upon arrival, the animals were individually housed in solid-bottom cages with paper bedding. All animals were housed in rooms with a constant temperature of 23–25°C and a 12-h lighting schedule. Upon birth, pups were pooled and randomly divided into two groups and treated with either 6-OHDA (100 μg free base in 10 μl) or 10 μl saline into the cisterna cerebellomedularis at 5 days of age. Afterwards, the pups were divided by group, raised by the dams, and weaned at 30 days of age.

Neonatally lesioned pups were primed at 40 days of age by administering four injections of SKF-38393 (3 mg/kg IP) weekly (5). Beginning at 65 days of age, all animals were injected with carbidopa (25 mg/kg, IP) 30 min prior to L-dopa, 100 mg/kg, IP) to induce the abnormal behaviors associated with this model. Testing was conducted in clear plastic chambers (42 × 42 × 30 cm) located in Digiscan activity monitors (Omnitech Electronics) during 60-min sessions. Animals were placed in the activity chamber for a 30-min acclimation period following L-dopa injection. Both activity counts and gross stereotypies were automatically recorded by the activity monitoring equipment. Activity counts were defined as number of photobeam interruptions. Gross stereotypies were defined as a break in the same beam or set of beams repeatedly. Incidence of self-mutilation was recorded by human observation, and was defined by the presence of blood on the animal. Animals exhibiting self-mutilation were administered SCH-23390 (1.0 mg/kg, IP) and topical lidocaine immediately following the session to prevent significant tissue damage and provide relief of pain. No severe lesions due to self-mutilation were observed during testing. Immediate injection of SCH-23390 was not conducted in this study to maximize observation of L-dopa induced stereotypical behaviors. This evaluation was necessary to establish the full expression of behaviors induced in this model and the full antagonistic effect of risperidone.

Following initial testing procedures, the neonatally lesioned animals were used to evaluate the abilities of SCH-23390 (1.0 mg/kg, IP), and risperidone (0.5 mg/kg, IP) to block the L-dopa induced behaviors in this model. Animals were randomly divided into two groups to receive either risperidone or SCH-23390 pretreatment during the first session. Activity counts, gross stereotypies, and self-mutilation were recorded under antagonist pretreatment as described above. All methodology involving any intrusive treatment in the subjects was approved by the Institutional Animal Care and Use Committee prior to the study.

Differences in means between control and treatment groups were assessed by student's *t*-test for both activity count and gross stereotypies. The presence or absence of self-mutilation was recorded for each animal at the end of the session. Repeated measures ANOVAs were used to compare means between baseline, risperidone, and SCH-23390 conditions for the lesioned animals both for activity counts and number of gross stereotypies. The incidence of self-mutilation under each condition for the treatment group was analyzed with a χ^2 test.

RESULTS

Administration of 100 mg/kg L-dopa produced self-mutilation in 57.7% of all neonatally 6-OHDA–lesioned animals. No self-mutilation was observed in the control group. Activity counts for control and lesioned animals differed significantly after administration of L-dopa, $t(30) = 5.309$, $p < 0.001$, with much higher rates observed in the 6-OHDA–treated group. Gross stereotypies also differed significantly between groups in response to L-dopa, $t(30) = 6.613$, $p < 0.001$, with much higher rates demonstrated by the lesioned animals.

Pretreatment with either SCH-23390 or risperidone was successful in antagonizing self-mutilation in the 6-OHDA–treated animals as shown in Table 1 ($\chi^2 = 28.067$, $p < 0.001$). Both risperidone and SCH-23390 pretreatments significantly reduced the high frequency of activity counts observed in response to L-dopa in the 6-OHDA–treated group, $F(2, 50) = 29.042$, $p < 0.001$. Figure 1 represents these data. Risperidone and SCH-23390 pretreatments also reversed the high rates of stereotypies observed in lesioned animals after administration of L-dopa, $F(2, 50) = 48.717$, $p < 0.001$, as shown in Fig. 2.

DISCUSSION

The present study confirms the clinical efficacy of risperidone in treating self-mutilation in Lesch-Nyhan syndrome.

TABLE 1

OCCURENCE OF SELF-MUTILATION IN RESPONSE TO L-DOPA

Treatment	Occurence of Self-Mutilation in Response to 100 mg/kg L-Dopa	
Control	0/26	0.0%
6-OHDA	15/26	57.7%*
6-OHDA + SCH 23390	0/26	0.0%†
6-OHDA + Risperidone	1/26	3.8%†

All animals received carbidopa (25 mg/kg) 30 min prior to L-dopa (100 mg/kg) administration. 6-OHDA–treated animals were tested first with L-dopa only, and L-dopa with both risperidone (1.0 mg/kg), and SCH-23390 (0.5 mg/kg) pretreatment. Both risperidone and SCH-23390 were administered at the same time as carbidopa.

*$p < 0.001$ when compared to control animals.

†$p < 0.001$ when compared to L-dopa treatment alone.

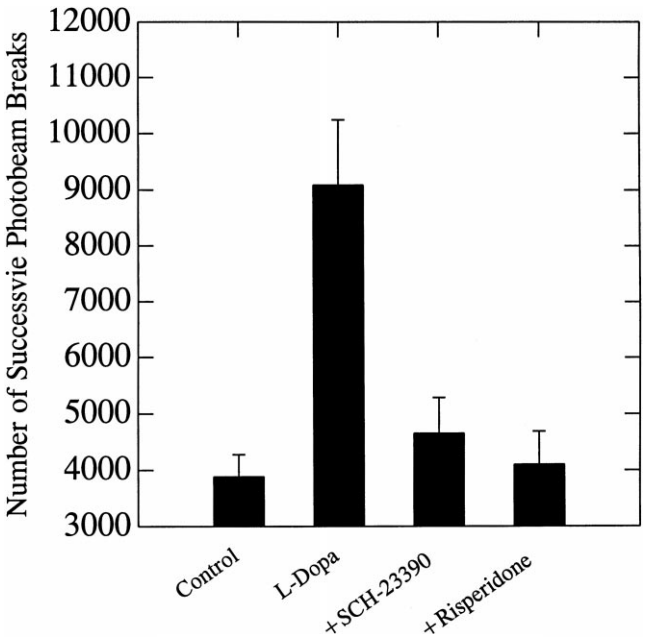

FIG. 1. Effects of pretreatment with SCH-23390 and risperidone on activity counts in neonatally lesioned animals compared to baseline and control responses to Ldopa (100 mg/kg, IP). Each bar represents the mean for each treatment lever ± the SEM.

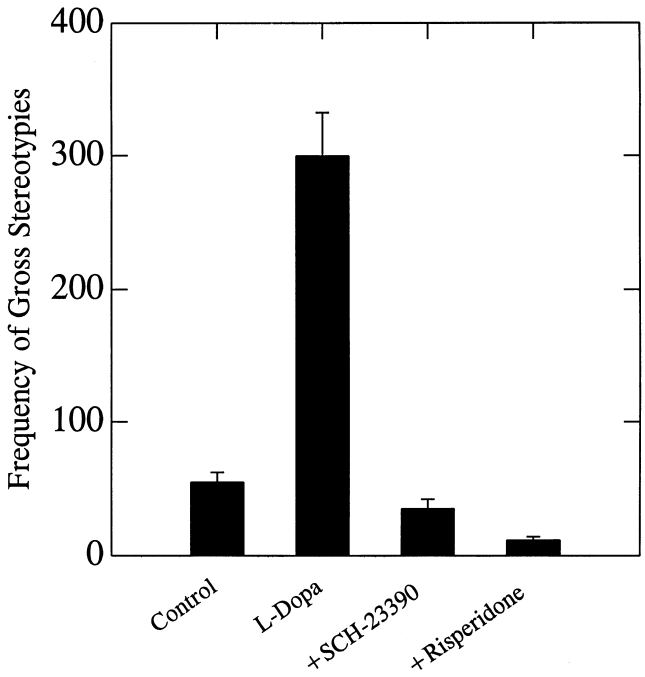

FIG. 2. Effects of pretreatment with SCH-23390 and risperidone on gross stereotypies in neonatally lesioned animals compared to baseline and control responses to L-dopa (1200 mg/kg IP). Each bar represents the mean for each treatment level ± the SEM.

Risperidone eliminated the behavior induced by L-dopa in all but one of the animals tested, and produced a similar behavioral profile to that of SCH-23390 in reduction of gross stereotypies and activity counts. These data are consistent with those presented on the effects of clozapine pretreatment in the neonatal 6-OHDA rat model (8). Clozapine blocked self-mutilation in a dose-dependent fashion, with elimination of the behavior at the highest dose. It is possible that a slightly higher dose of risperidone would have eliminated the self-mutilation in all rats treated, as in the case of clozapine. Further investigation into the dose–response relationship of risperidone upon self-mutilation in this model is warranted.

Criswell suggested that the actions of clozapine in reducing self-mutilation in the neonatal 6-OHDA rat model was due to action at the D_1 receptor (8). Although this is a plausible assumption when considering the reversal of D_1 activation by either clozapine or SCH-23390 treatment in this model, clozapine does not have a very high affinity for the D_1 receptor (13,15). However, clozapine and risperidone both have a very high affinity for the 5-HT_2c receptor. There is a considerable literature supporting a 5-HT, or specifically a 5-HT_2c, interaction with dopamine at the level of the striatum in the neonatally lesioned 6-OHDA rat (6,10,19). Also, the specific 5-HT_2c agonist m-chlorophenylpiperazine (m-CPP) induces similar behaviors as those seen with L-dopa, apomorphine, or SKF 38393 in the neonatal 6-OHDA rat. Behaviors induced by any of these drugs are blocked by pretreatment with mianserin, a serotonergic antagonist with high affinity for 5-HT_2c, although the activity induced by m-CPP is not altered by pretreatment with SCH-23390 (19). Therefore, it seems tenable to conclude that the antagonism of self-mutilation by risperidone in the neonatal 6-OHDA rat may be due to blockade of 5-HT_2c receptors, which results in a similar effect as observed with D_1 receptor blockade in the striatum.

In summary, the present study demonstrates: 1) risperidone is effective in antagonizing L-dopa induced behaviors as compared with antagonism by SCH-23390. 2) Serotonergic systems may be implicated along with dopaminergic systems in this model. 3) The neonatal 6-OHDA rat model is a useful pharmacological tool for evaluation of treatments for use in Lesch-Nyhan syndrome. Considering the effectiveness of both clozapine and risperidone in this model, evaluation of the developing class of atypical neuroleptics within this model is warranted. Therefore, this model has potential as both a viable screening tool for new treatments as well as a unique in vivo method for elucidating the neural mechanisms in this destructive malady.

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