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Effects of amphetamine and 6-hydroxydopamine lesions on reserpine-induced oral dyskinesia

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Abstract

The present study examined whether reserpine-induced oral dyskinesia is mediated by release of residual endogenous dopamine. Amphetamine produced a dose-dependent change in reserpine-induced oral dyskinesia in which the response was exacerbated by 0.6 mg/kg amphetamine and inhibited by 1 mg/kg. The latter dose also produced stereotypy that may have interfered with expression of reserpine-induced oral dyskinesia. Nigrostriatal 6-hydroxydopamine lesions attenuated expression of reserpine-induced oral dyskinesia. These lesions did not reduce locomotor activity, however, indicating that the attenuation of reserpine-induced oral dyskinesia was not due to a general depressant effect of the lesions on motor behavior. These results suggest that increasing dopamine release by administration of amphetamine exacerbates reserpine-induced oral dyskinesia, whereas decreasing the amount of releasable dopamine in the striatum by 6-hydroxydopamine lesions attenuates reserpine-induced oral dyskinsia. These findings may have implications for understanding tardive dyskinesia and L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia.

Keywords: Reserpine; Oral dyskinesia; Tongue protrusion; Dopamine; 6-Hydroxydopamine; Amphetamine; Tardive dyskinesia; Nigrostriatal; Caudate-putamen; (Rat)

1. Introduction

Tardive dyskinesia is defined as a motor disorder involving orofacial dyskinesia that develops as a result of long-term administration of neuroleptics (Baldessarini et al., 1980; Gerlach and Casey, 1988). We have previously reported that rats develop spontaneous oral dyskinesia following administration of reserpine, and have suggested that this response may offer a new animal model of tardive dyskinesia (Neisewander et al., 1991a,b, 1994). Although reserpine is not classified as a neuroleptic, it has been used as an antipsychotic agent and has been associated with the development of tardive dyskinesia (Shonecker, 1957; Uhrbrand and Faurbye, 1960). Reserpine-induced oral dyskinesia in rats is characterized by twitching of the facial musculature, jaw movements and tongue protrusions, similar to some of the symptoms of tardive dyskinesia. The response persists for at least 60 days following termination of reserpine treatment (Neisewander et al., 1991b), similar to the persistence of tardive dyskinesia in humans following termination of neuroleptic treatment (Gardos and Cole, 1983; Klawans et al., 1984; Smith and Baldessarini, 1980). The rate of development of the dyskinesia is dose-dependent. At high doses (1 mg/kg) the response appears within 3 days, whereas at low doses (0.05 mg/kg) the response is not evident until approximately 6–8 weeks of treatment (Neisewander et al., 1994). The delayed development at low doses is similar to the protracted development of tardive dyskinesia in humans (Gerlach and Casey, 1988). Lastly, the response is dose dependently blocked by the dopamine D_2 receptor antagonist spiroperidol (Neisewander et al., 1991a), consistent with reports indicating that the symptoms of tardive dyskinesia are alleviated by dopamine receptor antagonists (Baldessarini et al., 1980; Jeste and Wyatt, 1982; Kazamatsuri et al., 1972; Klawans, 1973).

The finding that reserpine-induced oral dyskinesia is reversed by administration of a dopamine D_2 receptor antagonist suggests that residual endogenous dopamine may be involved in this response. Furthermore, chronic administration of either reserpine or neuroleptics results in

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altered dopaminergic functions, including increased behavioral sensitivity to direct dopamine receptor agonists (Arnt, 1985; Hong et al., 1987; Quinton and Halliwell, 1963; Tarsy and Baldessarini, 1974), increased number of dopamine receptors (Burt et al., 1977; Joyce, 1991; Neisewander et al., 1991a,b), and increased dopamine release and turnover (Callaway et al., 1989; Shannak and Hornykiewicz, 1980; See, 1991, 1993; See et al., 1992). Reserpine-treated animals exhibit an increase in firing rate of nigrostriatal dopamine neurons (German et al., 1981), as well as measurable levels of extracellular dopamine in the striatum that, in some cases, is actually increased relative to controls (Arbuthnott et al., 1991; Bean et al., 1989; Butcher et al., 1988; Cadoni et al., 1995; Callaway et al., 1989; Florin et al., 1995). Furthermore, reserpine-treated animals exhibit an increase in dopamine release following an amphetamine challenge that, in some cases, is greater relative to controls (Cadoni et al., 1995; Dluzen and Kratko, 1992).

In the present study, two experiments were conducted to test the hypothesis that reserpine-induced oral dyskinesia is mediated by residual endogenous dopamine. In the first experiment, we examined whether reserpine-induced oral dyskinesia is exacerbated by amphetamine-induced release of dopamine from the reserpine-resistant pool. In the second experiment, we examined whether reserpine-induced oral dyskinesia is attenuated by 6-hydroxydopamine lesions of nigrostriatal dopamine neurons that deplete the residual dopamine pool.

2. Materials and methods

2.1. Animals and reserpine administration

Male Sprague-Dawley rats (Charles River) weighing 250–300 g were housed in a climate-controlled animal colony with a 12 h light/dark cycle. They were acclimated to handling for 5–7 days prior to the start of an experiment. Animals were randomly assigned to treatment groups that received either vehicle or 1.0 mg/kg reserpine subcutaneously every other day for 5–9 days depending on the experiment. Reserpine (Sigma, St. Louis, MO, USA) was dissolved in glacial acetic acid, then diluted to the correct concentration with distilled water. Vehicle consisted of the same amount of acetic acid and water as in the reserpine solution. Both solutions were injected at a volume of 1 ml/kg. Some of the rats that received reserpine experienced severe weight loss initially and were given 6–8 ml of a liquid diet consisting of 200 ml tap water, 100 ml sweetened condensed milk, 1 package of chocolate flavored instant breakfast mix, and 22.5 ml Kaopectate via intubation for the first 1–7 days. The reserpine-treated rats were also given access to ground Purina rat chow moistened with the liquid diet throughout the experiment in order to prevent dehydration and weight loss. We have not observed any relationship between consumption of the liquid diet and expression of oral dyskinesia. For instance, animals continue to exhibit reserpine-induced oral dyskinesia at a maximal level for 20 days following termination of both reserpine treatment and access to the liquid diet (Neisewander et al., 1991b).

2.2. Behavioral testing

On each test day, rats were placed into a clear Plexiglas cage (44 × 24 × 20 cm high) that had a metal bar floor and a perforated metal lid. To quantify the occurrence of oral dyskinesia, the incidence of tongue protrusions was recorded continuously for 30 min. Tongue protrusions were operationally defined as a visible extension of the tongue outside of the mouth, and individual tongue protrusions during a bout of oral dyskinsia were each preceded by visible retraction of the tongue. A mirror was placed behind the back wall of the cage to enable observation of tongue protrusions when the animal was faced away from the observer. Each cage was also equipped with two sets of photocells and light sources mounted onto a rack outside of the cage such that the emitted photobeams were located 32 cm apart and 4 cm above the floor. A computer-automated relay system recorded the number of times the animals broke the two photobeams consecutively by moving from one end of the cage to the other. The latter measure is referred to as crosses.

2.3. Experiment 1: effects of amphetamine on reserpine-induced behaviors

Rats were injected subcutaneously with either vehicle ($n = 39$) or reserpine (1 mg/kg; $n = 38$) every other day for 9 days. On day 10, the rats in each treatment condition were randomly assigned to groups that were injected intraperitoneally with either 0 ($n = 8–11$), 0.1 ($n = 6–7$), 0.3 ($n = 8–9$), 0.6 ($n = 7$), or 1.0 ($n = 6$) mg/kg amphetamine (Sigma, St. Louis, MO, USA). Amphetamine was dissolved in sterile saline and injected at a volume of 1 ml/kg. Immediately after the injection, the animals were placed into the test cages for a 30-min period, and tongue protrusions and locomotor activity were measured as described previously. Reserpine treatment produces stereotypic walking in some rats that is characterized by abnormal 'robotic-like' movement. Stereotypic walking also appears purposeless since the animals typically engage in the behavior continuously, walking from one end of the cage to the other, and do not exhibit other exploratory-like behaviors such as sniffing or rearing. Animals engaged in stereotypic walking also display ptosis, hunched posture, and tongue protrusions. Amphetamine produces stereotypic sniffing, chewing and licking directed at the floor, walls, and ceiling that also appears purposeless and repetitive. The presence and intensity of these behaviors were measured every 2.5 min based on a 10-s observation period.

Intensity was rated on a scale of 1–3 depending on the percentage of time the animal was engaged in the behavior as follows: 1 = < 25%, 2 = 25–75%, and 3 = > 75%. The sum of these ratings yielded an overall score for the behavior. The behaviors were recorded by an observer who was unaware of the animals' amphetamine dose.

2.4. Experiment 2: effects of nigrostriatal 6-hydroxydopamine lesions on reserpine-induced behaviors

The rats were deprived of food 24 h prior to surgery. They were anesthetized with pentobarbital (50 mg/kg, i.p.) in combination with atropine (10 mg/kg, i.p.). They were then placed into a stereotaxic instrument with their head positioned with Bregma and Lambda horizontal. They were injected with desmethylimipramine (25 mg/kg, i.p.), and 15–30 min later they received bilateral infusions of either vehicle (0.1% ascorbate, $n = 27$) or 6-hydroxydopamine (5 μg/4 μl/side, $n = 37$) into the substantia nigra using the following coordinates: −5.0 mm AP and ±2.0 mm ML with respect to Bregma, and −7.4 DV with respect to the skull. The cannulae (30 gauge stainless steel) were connected to gastight Hamilton syringes by PE-20 tubing and the syringes were placed into a pump (Harvard Apparatus) that delivered the infusion over a 195-s period. A minute air bubble was made in the tubing so that its movement could be measured in order to verify that the proper amount of fluid had been infused. Following a 10–14 day recovery period, half of each group was injected with vehicle and half was injected with reserpine (1 mg/kg, s.c.) every other day for six days. Twenty-four hours after their last injection, the animals were placed into the test cage, and tongue protrusions and locomotor activity were recorded for 30 min as described previously. Within 2 h of behavioral testing, the rats were decapitated. The brains were rapidly removed, placed on an ice-cold plate, and cut coronally at the caudal border of the olfactory tubercle. The striata were dissected from the anterior portion of the brains. The tissue samples were weighed, frozen on dry ice, stored at −70°C, and then later assayed for monoamine and metabolite levels. This experiment includes two replications due to the loss of tissue from the first experiment as a result of a power failure to our freezer. The tissue from the second experiment was prepared for the assay by homogenizing the samples in 0.05 N perchloric acid containing the internal standard dihydroxybenzylamine (n per group is indicated in the results). Homogenates were centrifuged at 1500 × g for 4 min and the filtered supernatant was assayed using high performance liquid chromatography with electrochemical detection as described by Robinson et al. (1987).

2.5. Data analysis

Tongue protrusions, crosses, and neurochemical measures were analyzed using analyses of variance (ANOVA) and Newman-Keuls tests were performed for pairwise comparisons. For Experiment 1, reserpine pretreatment (i.e., 0 vs. 1 mg/kg) and dose of amphetamine were between subjects factors. For Experiment 2, surgical treatment (i.e., vehicle vs. 6-hydroxydopamine) and reserpine treatment (i.e., 0 vs. 1 mg/kg) were between subjects factors. The behavior from the two replications of Experiment 2 were analyzed separately. The same findings were statistically significant in each separate experiment, and therefore, the data have been combined for presentation of the results. Stereotypy ratings from Experiment 1 were analyzed using nonparametric Kruskall-Wallis ANOVAs and Mann-Whitney U-test were used for pairwise comparisons.

3. Results

3.1. Experiment 1: effects of amphetamine on reserpine-induced behaviors

Amphetamine produced an increase in reserpine-induced tongue protrusions at a dose of 0.6 mg/kg and a decrease at a dose of 1.0 mg/kg (see Fig. 1a). The overall ANOVA indicated a significant interaction between reser-

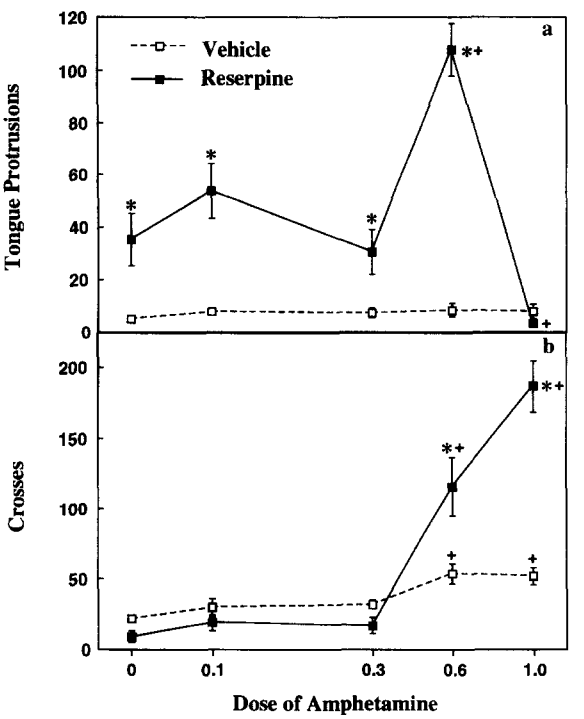

Fig. 1. Effects of amphetamine on tongue protrusions (panel a) and locomotor activity (panel b) in animals pretreated with vehicle or reserpine every other day for 9 days. Values represent the means ± S.E.M. Asterisks (*) represent a significant difference between the reserpine-treated group and respective vehicle-pretreated group challenged with a given dose of amphetamine, $P < 0.01$, Newman-Keuls test. Plus sign (+) represents a significant difference between groups challenged with amphetamine and the group challenged with 0 mg/kg amphetamine within respective vehicle or reserpine pretreatment conditions, $P < 0.05$, Newman-Keuls test.

pine and amphetamine treatments, [$F(4,67) = 14.4$, $P < 0.0001$]. Subsequent pairwise comparisons indicated that reserpine-treated animals exhibited more tongue protrusions relative to their respective vehicle-treated control group at each dose of amphetamine tested (Newman-Keuls test, $P < 0.05$), except the dose of 1 mg/kg. In addition, reserpine-treated animals tested at the dose of 0.6 mg/kg amphetamine exhibited more tongue protrusions relative to reserpine-treated controls tested at the dose of 0 mg/kg amphetamine (Newman-Keuls test, $P < 0.05$), whereas reserpine-treated animals tested at the dose of 1.0 mg/kg amphetamine exhibited fewer tongue protrusions relative to reserpine-treated controls (Newman-Keuls test, $P < 0.05$). Amphetamine did not alter tongue protrusions in vehicle-treated animals at any dose.

Both of the doses of amphetamine that altered reserpine-induced tongue protrusions also produced an increase in locomotor activity that was more robust in reserpine-treated animals relative to vehicle-treated animals (see Fig. 1b). The overall ANOVA indicated a significant interaction between reserpine and amphetamine treatments, [$F(4,67) = 25.9$, $P < 0.0001$]. Subsequent pairwise comparisons indicated that animals receiving 0.6–1.0 mg/kg amphetamine exhibited more crosses relative to their respective control group receiving 0 mg/kg (Newman-Keuls test, $P < 0.05$). In addition, reserpine-treated animals tested at these doses exhibited more crosses relative to their respective vehicle-treated control groups (Newman-Keuls test, $P < 0.05$).

The effects of reserpine and/or amphetamine on stereotypic behaviors are summarized in Table 1. Amphetamine increased stereotypic walking in reserpine-treated animals, but not in vehicle-treated animals. The Kruskall-Wallis ANOVA indicated a significant group effect [$H = 42.1$, $P < 0.001$]. Reserpine-treated animals receiving 0.1 and 0.6 mg/kg amphetamine exhibited an increase in stereotypic walking relative to both respective vehicle-treated controls and reserpine-treated animals receiving 0 mg/kg amphetamine (Mann-Whitney U-tests, $P < 0.05$). Amphetamine increased sniffing behavior and this effect was evident at a lower dose of amphetamine in animals treated with reserpine. The Kruskall-Wallis ANOVA indicated a significant group effect [$H = 47.7$, $P < 0.001$]. Subsequent pairwise comparisons indicated that in vehicle-treated animals, sniffing was decreased in animals receiving 0.1 mg/kg amphetamine, and increased in animals receiving 1.0 mg/kg amphetamine, relative to controls receiving 0 mg/kg amphetamine (Mann-Whitney U-tests, $P < 0.05$). In reserpine-treated animals, sniffing was increased in animals receiving 0.6–1.0 mg/kg amphetamine relative to controls receiving 0 mg/kg amphetamine (Mann-Whitney U-tests, $P < 0.05$). Baseline sniffing was decreased by reserpine treatment alone since animals receiving reserpine alone exhibited less sniffing relative to animals receiving vehicle treatment alone (Mann-Whitney U-tests, $P < 0.05$). Amphetamine did not produce a significant amount of oral stereotypy in any of the treatment groups [$H = 14.1$, $P < 0.12$], although there was a trend for an increase in oral stereotypy in reserpine-treated animals receiving 1.0 mg/kg amphetamine.

3.2. Experiment 2: effects of nigrostriatal 6-hydroxydopamine lesions on reserpine-induced behavioral and neurochemical changes

6-Hydroxydopamine lesions of the nigrostriatal pathway did not alter tongue protrusions or locomotion in vehicle-treated animals, but attenuated tongue protrusions and increased locomotion in reserpine-treated animals (see Fig. 2). The overall ANOVA indicated a significant interaction between surgical and reserpine treatments both for tongue protrusions, [$F(1,60) = 13.1$, $P < 0.001$] and locomotor activity, [$F(1,60) = 5.8$, $P < 0.05$]. Subsequent pairwise comparisons indicated that in both cases reserpine-treated animals given sham lesions were significantly different from all other groups ($P < 0.05$, Newman-Keuls tests). Vehicle-treated groups did not differ from each other on either behavioral measure. The incidence of tongue protrusions in reserpine-treated animals given 6-hydroxydopamine lesions did not differ from the vehicle-treated animals given 6-hydroxydopamine lesions, but was significantly greater relative to vehicle-treated animals given sham lesions ($P < 0.05$, Newman-Keuls test). These findings indicate that reserpine-induced tongue protrusions

Table 1
Stereotypic behaviors following amphetamine administration in animals pretreated with vehicle or reserpine every other day for 9 days [1]

Dose (mg/kg) of amphetamine	Walking		Sniffing		Oral	
	Vehicle	Reserpine	Vehicle	Reserpine	Vechicle	Reserpine
0	0.64 ± 0.64	0.13 ± 0.13	12.6 ± 2.4	3.0 ± 1.5 [b]	0.27 ± 0.27	0 ± 0
0.1	1.12 ± 0.83	6.29 ± 1.70 [a,b]	5.2 ± 2.1 [a]	3.7 ± 1.9	0.83 ± 0.40	0 ± 0
0.3	0.12 ± 0.12	1.56 ± 0.85	21.5 ± 3.5	8.6 ± 3.2 [b]	0.38 ± 0.26	0 ± 0
0.6	2.00 ± 0.58	14.70 ± 3.21 [a,b]	19.0 ± 2.9	14.1 ± 2.1 [a]	0.43 ± 0.30	0.57 ± 0.43
1.0	0.17 ± 0.17	0.50 ± 0.50	34.3 ± 1.4 [a]	26.5 ± 3.2 [a]	0 ±0	2.67 ± 2.12

[1] Values represent mean overall scores (±S.E.M.) computed by summating time-sampled ratings as described in Methods. [a] Represents a significant difference from respective control group receiving 0 mg/kg amphetamine, $P < 0.05$, Mann-Whitney U-test. [b] Represents a significant difference from respective vehicle-treated control group, $P < 0.05$, Mann-Whitney U-test.

Table 2
Levels of dopamine and dopamine metabolites (ng/mg tissue ± S.E.M.) and ratios of metabolite to dopamine from striata of rats given sham or 6-OHDA lesions and treated with either vehicle or reserpine every other day for 6 days

Drug treatment	Surgical treatment						
	Sham lesion			6-OHDA lesion			
	Vehicle ($n = 6$)	Reserpine ($n = 9$)	% change	Vehicle ($n = 9$)	% change	Reserpine ($n = 8$)	% change
DA	17.28 ± 1.54 [b]	0.65 ± 0.09	96	5.36 ± 1.16 [b]	69	0.45 ± 0.10	98
DOPAC	2.42 ± 0.09 [b]	1.27 ± 0.16	48	1.16 ± 0.14	52	0.56 ± 0.17 [b]	77
DOPAC/DA	0.14 ± 0.01	2.73 ± 0.75 [b]	1850	0.37 ± 0.12	164	1.28 ± 0.23 [a]	814
HVA	1.31 ± 0.15 [b]	0.78 ± 0.13	40	0.75 ± 0.07	43	0.53 ± 0.18	60
HVA/DA	0.08 ± 0.01 [b]	1.17 ± 0.15	1362	0.26 ± 0.10 [b]	225	1.12 ± 0.14	1300

[a] Represents a significant difference from sham-lesioned/vehicle-treated group, $P < 0.01$, Newman-Keuls test. [b] Represents a significant difference from all groups, $P < 0.05$, Newman-Keuls test.

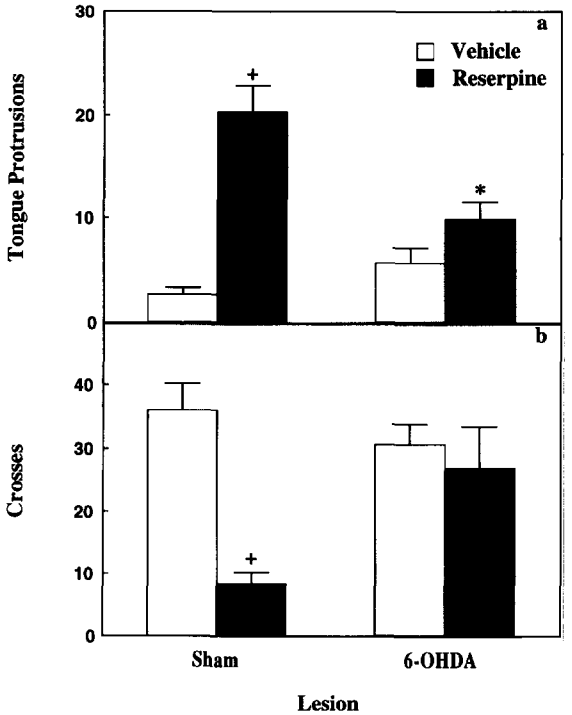

Fig. 2. Effects of 6-hydroxydopamine lesions and/or reserpine treatment on tongue protrusions (panel a) and locomotor activity (panel b). Values represent the means ± S.E.M. Asterisks (*) represent a significant difference from control group (i.e., vehicle/vehicle), $P < 0.05$, Newman-Keuls test. Plus sign (+) represents a significant difference from all other groups, $P < 0.01$, Newman-Keuls test.

were attenuated by the 6-hydroxydopamine lesions. Locomotor activity in reserpine-treated animals given 6-hydroxydopamine lesions was significantly greater relative to animals receiving reserpine alone, but did not differ from either of the vehicle-treated groups. These findings indicate that the lesion increased locomotor activity in reserpine-treated animals.

Table 2 illustrates the effect of 6-hydroxydopamine lesions and/or reserpine treatment on levels of striatal dopamine, dopamine metabolites, and the ratio of metabolite to dopamine. The ANOVAs indicated significant differences among the groups for each measure ($P < 0.001$). 6-Hydroxydopamine lesions alone significantly depleted dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) to 69%, 52%, and 43% of sham/vehicle control levels, respectively (Newman-Keuls test, $P < 0.05$). Reserpine treatment alone significantly depleted dopamine, DOPAC, and HVA to 96%, 48%, and 40% of sham/vehicle control levels, respectively (Newman-Keuls test, $P < 0.05$). The combined treatment with 6-hydroxydopamine and reserpine significantly potentiated the decrease in DOPAC levels produced by reserpine treatment alone (i.e., 77% vs. 48% of sham/vehicle control levels, respectively; Newman-Keuls test, $P < 0.05$). The combined treatment of 6-hydroxydopamine and reserpine also produced greater depletion of dopamine (98%) and HVA (60%) than reserpine treatment alone, although these differences were not significant. However, a planned t-test comparison of dopamine levels in these groups indicated a trend toward greater depletion in the combined treatment group [$t(14) = 1.43$, $P < 0.09$]. 6-Hydroxydopamine lesions alone produced a nonsignificant increase in DOPAC/dopamine ratios (164%) and a significant increase in HVA/dopamine ratios (225%) relative to sham/vehicle controls (Newman-Keuls test, $P < 0.05$). Reserpine treatment alone produced a significant increase in DOPAC/dopamine (1850% relative to sham/vehicle controls) and HVA/dopamine (1362% relative to sham/vehicle controls) ratios relative to both sham/vehicle and 6-hydroxydopamine/vehicle groups (Newman-Keuls test, $P < 0.05$). The combined treatment of 6-hydroxydopamine and reserpine attenuated significantly the increase in DOPAC/dopamine ratios produced by reserpine treatment alone (814% vs. 1850% relative to sham/vehicle controls, respectively; Newman-Keuls test, $P < 0.05$).

4. Discussion

The results from both experiments are consistent with our hypothesis that reserpine-induced tongue protrusions are mediated, at least in part, by residual endogenous dopamine. The results from Experiment 1 indicate that amphetamine exacerbates expression of reserpine-induced tongue protrusions. Amphetamine produced an inverted U-shaped dose-dependent change in reserpine-induced tongue protrusions, with an increase observed at a dose of 0.6 mg/kg, and decrease observed at a dose of 1.0 mg/kg. Amphetamine also produced an increase in locomotor activity at doses of 0.6 and 1.0 mg/kg that was more robust in reserpine-treated animals relative to vehicle-treated controls. The nature of the locomotor activity observed at the dose of 0.6 mg/kg appeared to differ depending on the animals' pretreatment. Reserpine-pretreated animals were predominantly engaged in stereotypic walking characterized by 'robotic-like' movement without sniffing, whereas vehicle-pretreated animals were engaged in low intensity stereotypy in which they exhibited simultaneous head-down sniffing and rapid locomotion. At the dose of 1.0 mg/kg amphetamine, the locomotion was of the latter nature in both groups. Stereotypic walking also occurs spontaneously in reserpine-treated animals, however, it is idiosynchratic since it is not reliably observed in all animals, nor is it reliably observed within an animal across test days during the course of chronic reserpine treatment (unpublished observation). Thus, it is difficult to examine the mechanism of this behavior. However, the results from the present study suggest that amphetamine may increase the probability of observing this behavior in reserpine-treated animals since a significant increase in this behavior was observed at doses of 0.1 and 0.6 mg/kg amphetamine.

Collectively, the results from Experiment 1 indicate that the dose of 0.6 mg/kg amphetamine increases reserpine-induced stereotypic walking and tongue protrusions, whereas the dose of 1.0 mg/kg amphetamine produces stereotypic sniffing and locomotion that may interfere with expression of reserpine-induced tongue protrusions. Amphetamine causes release of dopamine from a reserpine-resistant extravesicular pool (Arnold et al., 1977; Fisher and Cho, 1979; Langer and Arbilla, 1984; Niddam et al., 1985; Parker and Cubeddu, 1986; Raiteri et al., 1979; Sulzer et al., 1993). This pharmacologic action is thought to mediate amphetamine-induced locomotion and stereotypy (Costall and Naylor, 1977; Creese and Iversen, 1974; Moore, 1978; Scheel-Krüger, 1971), although other neurotransmitters may also play a role (Cadoni et al., 1995; Callaway et al., 1989; Florin et al., 1995). The finding that amphetamine-induced locomotion was enhanced in reserpine-pretreated animals relative to vehicle-pretreated animals is consistent with previous research demonstrating behavioral supersensitivity to amphetamine (Hong et al., 1987; Quinton and Halliwell, 1963; Scheel-Krüger, 1971; Smith, 1963; Stolk and Rech, 1967; Van Rossum et al., 1962), as well as direct dopamine receptor agonists (Arnt, 1985; LaHoste and Marshall, 1992; Neisewander et al., 1991a; Ross et al., 1988; Starr et al., 1987) in reserpine-pretreated animals. The latter findings suggest that enhanced sensitivity of postsynaptic dopamine receptors may be involved in the behavioral supersensitivity. This enhanced sensitivity of postsynaptic dopamine receptors may also be involved in reserpine-induced tongue protrusions. Consistent with this idea, reserpine-induced tongue protrusions are reversed by the dopamine D_2 receptor antagonist spiroperidol (Neisewander et al., 1991a). Amphetamine also causes release of norepinephrine, however, this effect is dependent on vesicular stores since it is absent in reserpine-treated animals (Florin et al., 1994, 1995; Kuczenski and Segal, 1992). Therefore, it is unlikely that the increase in reserpine-induced tongue protrusions by amphetamine is mediated by norepinephrine.

More direct support for our hypothesis was obtained in Experiment 2, which demonstrated that expression of reserpine-induced tongue protrusions is attenuated by lesions of nigrostriatal dopamine neurons. Reserpine alone produced a 96% depletion of dopamine and 48% and 40% depletions of DOPAC and HVA, respectively. 6-Hydroxydopamine lesions in combination with reserpine produced further depletion of dopamine to 98% and of DOPAC and HVA to 77% and 60%, respectively. Only the further reduction of DOPAC produced by the combined treatments relative to reserpine treatment alone reached significance. However, the lack of a significant difference in dopamine levels between these treatment groups is likely due to limitations in the sensitivity of the tissue assay for detecting changes in the small residual pool of dopamine. Indeed, studies have revealed discrepancies between measures of tissue and dialysate levels of dopamine following either reserpine or 6-hydroxydopamine administration, and the latter measure reflects synaptic levels (Callaway et al., 1989; Castañeda et al., 1990). Thus, the significant reduction of DOPAC and the nonsignificant trend toward reduction of dopamine produced by 6-hydroxydopamine in reserpine-treated animals likely reflect a decrease in the releasable and synaptic pools of dopamine. We further suggest that this 6-hydroxydopamine-induced reduction of residual dopamine attenuated expression of reserpine-induced tongue protrusions.

Surprisingly, the results from Experiment 2 also indicate that 6-hydroxydopamine lesions increased locomotor activity in reserpine-treated animals. One explanation for this finding is that more of the animals receiving both 6-hydroxydopamine and reserpine exhibited stereotypic walking than animals treated with reserpine alone. Although we did not include time-sampled ratings of stereotypic walking in this experiment, we did note whether or not animals exhibited this behavior during the test session. In animals treated with reserpine alone, 36% exhibited stereotypic walking, whereas 58% of the animals treated with both reserpine and 6-hydroxydopamine exhibited this behavior. This finding suggests that stereotypic walking is not dopamine-mediated, and may actually be inhibited by dopamine. It is possible that this behavior involves changes in norepinephrine or serotonin neurotransmission, since reserpine also depletes vesicular stores of these transmitters. Alternatively, stressors in general, including an amphetamine challenge or 6-hydroxydopamine lesion, may increase the incidence of stereotypic walking. Consistent with this idea, 6-hydroxydopamine-lesioned animals exhibit behavioral activation in response to stressors that is not blocked by dopamine receptor antagonists (Keefe et al., 1989). In any case, the finding that locomotor activity in animals receiving both 6-hydroxydopamine and reserpine did not differ from vehicle-treated controls suggests that the attenuation of reserpine-induced tongue protrusions by the 6-hydroxydopamine lesion was not due to a general depressant effect of the lesion.

In conclusion, the findings that reserpine-induced tongue protrusions are exacerbated by amphetamine and attenuated by nigrostriatal 6-hydroxydopamine lesions support the hypothesis that this behavior is mediated, at least in part, by residual endogenous dopamine. However, an increase in dopamine release alone is not sufficient to produce oral dyskinesia, since normal animals treated with amphetamine do not exhibit this behavior. Dopamine depletion may cause changes in postsynaptic receptors resulting in altered responses to residual endogenous dopamine, including oral dyskinesia. Consistent with this idea, oral dyskinesia develops in Parkinson's disease patients following repletion of endogenous dopamine by L-3,4-dihydroxyphenylalanine (L-DOPA) treatment. A change in dopamine receptor sensitivity has also been suggested as a mechanism of neuroleptic-induced oral dyskinesia, an animal model of tardive dyskinesia (Klawans, 1973; Baldessarini

et al., 1980; Tarsy, 1983). More recently, however, changes in neurotransmitter systems efferent to nigrostriatal dopamine neurons, such as γ-amino-butyric acid and acetylcholine, have been implicated in neuroleptic-induced oral dyskinesia (Fibiger and Lloyd, 1984; Gunne and Häggström, 1983; Gunne et al., 1984; Jenner and Marsden, 1988; Scheel-Krüger and Arnt, 1985). Thus, it is possible that abnormal activity in neurotransmitter systems efferent to the nigrostriatal dopamine pathway is initiated by residual endogenous dopamine in reserpine-treated animals, resulting in oral dyskinesia.

We have previously demonstrated that reserpine-induced oral dyskinesia is decreased by the dopamine D_2 receptor antagonist spiroperidol. This finding suggests that reserpine-induced oral dyskinesia is mediated, at least in part, by endogenous dopamine stimulating D_2-like dopamine receptors. Future research is needed to determine whether stimulation of D_1-like dopamine receptors also plays a role in reserpine-induced oral dyskinesia, as has been demonstrated for neuroleptic-induced oral dyskinesia (Ellison et al., 1988; Lublin and Gerlach, 1988; Lublin et al., 1992; Peacock et al., 1990; Stoessl et al., 1989). Elucidating the mechanism of reserpine-induced oral dyskinesia may have important implications for understanding tardive dyskinesia and L-DOPA-induced dyskinesia, and for developing safe and effective treatments for these disorders.

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References

Arbuthnott, G.W., I.S. Fairbrother and S.P. Butcher, 1991, Dopamine release and metabolism in the rat striatum: an analysis by 'in vivo' brain microdialysis, Pharmacol. Ther. 48, 281.

Arnold, E.B., P.B. Molinoff and C.O. Rutledge, 1977, The release of endogenous norepinephrine and dopamine from cerebral cortex by amphetamine, J. Pharmacol. Exp. Ther. 202, 544.

Arnt, J., 1985, Behavioral stimulation is induced by separate D-1 and D-2 receptor sites in reserpine-pretreated but not in normal rats, Eur. J. Pharmacol. 113, 79.

Baldessarini, R.J., J.O. Cole, J.M. Davis, G. Simpson, D. Tarsy, G. Gardos and S.H. Preskorn, 1980, Tardive dyskinesia: summary of a task force report of the American Psychiatric Association, Am. J. Psychiatry 137, 1163.

Bean, A.J., M.J. During, A.Y. Deutch and R.H. Roth, 1989, Effects of dopamine depletion on striatal neurotensin: biochemical and immunohistochemical studies, J. Neurosci. 9, 4430.

Burt, D.R., I. Creese and S.H. Snyder, 1977, Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain, Science 196, 326.

Butcher, S.P., I.S. Fairbrother, J.S. Kelly and G.W. Arbuthnott, 1988, Amphetamine-induced dopamine release in the rat striatum: an in vivo microdialysis study, J. Neurochem. 50, 346.

Cadoni, C., A. Pinna, G. Russi, S. Consolo and G. Di Chiara, 1995, Role of vesicular dopamine in the in vivo stimulation of striatal dopamine transmission by amphetamine: evidence from microdialysis and fos immunohistochemistry, Neuroscience 65, 1027.

Callaway, C.W., R. Kuczenski and D.S. Segal, 1989, Reserpine enhances amphetamine stereotypies without increasing amphetamine-induced changes in striatal dialysate dopamine, Brain Res. 505, 83.

Castañeda, E., I.Q. Whishaw and T.E. Robinson, 1990, Changes in striatal dopamine neurotransmission assessed with microdialysis following recovery from a bilateral 6-OHDA lesion: variation as a function of lesion size, J. Neurosci. 10, 1847.

Costall, B. and R.J. Naylor, 1977, Mesolimbic and extrapyramidal sites for the mediation of stereotyped behavior patterns and hyperactivity by amphetamine and apomorphine in the rat, in: Cocaine and Other Stimulants, ed. E.H. Ellinwood and M.M. Kilbey (Plenum Press, New York) p. 47.

Creese, I. and S.D. Iversen, 1974, The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat, Psychopharmacologia 39, 345.

Dluzen, D.E. and F.T. Kratko, Jr., 1992, L-DOPA infusion mode differentially affects corpus striatal dopamine efflux in the presence of reserpine, J. Neural Transm. 89, 197.

Ellison, G., P. Johansson, E. Levin, R. See and L. Gunne, 1988, Chronic neuroleptics alter the effects of the D1 agonist SK and F 38393 and the D2 agonist LY171555 on oral movements in rats, Psychopharmacology 96, 253.

Fibiger, H.C. and Lloyd, K.G., 1984, Neurobiological substrates of tardive dyskinesia: the GABA hypothesis, Trends Neurosci. 7, 462.

Fisher, J.F. and A.K. Cho, 1979, Chemical release of dopamine from striatal homogenates: evidence for an exchange-diffusion model, J. Pharmacol. Exp. Ther. 208, 203.

Florin, S.M., R. Kuczenski and D.S. Segal, 1995, Effects of reserpine on extracellular caudate dopamine and hippocampus norepinephrine responses to amphetamine and cocaine: mechanistic and behavioral consideration, J. Pharmacol. Exp. Ther. 274, 231.

Florin, S.M., R. Kuczenski and D.S. Segal, 1994, Regional extracellular norepinephrine responses to amphetamine and cocaine and effects of clonidine pretreatment, Brain Res. 654, 53.

Gardos, G. and J.O. Cole, 1983, The prognosis of tardive dyskinesia, J. Clin. Psychiatry 44, 177.

Gerlach, J. and D.E. Casey, 1988, Tardive dyskinesia, Acta Psychiatry Scand. 77, 369.

German, D.C., B.A. McMillen, M.K. Sanghera, S.I. Saffer and P.A. Shore, 1981, Effect of severe dopamine depletion on dopamine neuronal impulse flow and on tyrosine hydroxylase regulation, Brain Res. Bull. 6, 131.

Gunne, L.M. and J.-E. Häggström 1983, Reduction of nigral glutamic acid decarboxylase in rats with neuroleptic-induced oral dyskinesia, Psychopharmacology 81, 191.

Gunne L.M., J.-E. Häggström and B. Sjöquist, 1984, Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis, Nature 309, 347.

Hong, M., P. Jenner and C.D. Marsden, 1987, Comparison of the acute actions of amine-depleting drugs and dopamine receptor antagonists on dopamine function in the brain in rats, Neuropharmacology 26, 237.

Jenner, P. and C.D. Marsden, 1988, Adaptive changes in brain dopamine function as a result of neuroleptic treatment, in: Advances in Neurology, Vol. 49: Facial Dyskinesias, eds. J. Jankovic and E. Tolosa (Raven Press, New York) p. 417.

Jeste, D.V. and R.J. Wyatt, 1982, Therapeutic strategies against tardive dyskinesia, Arch. Gen. Psychiatry 39, 803.

Joyce, J.N., 1991, Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine, Exp. Neurol. 113, 277.

Kazamatsuri, H., C.P. Chien and J.O. Cole, 1972, Treatment of tardive dyskinesia. II. Short term efficacy of dopamine-blocking agents haloperidol and thiopropazate, Arch. Gen. Psychiatry 27, 100.

Keefe, K.A., J.D. Salamone, M.J. Zigmond and E.M. Stricker, 1989, Paradoxical kinesia in Parkinsonism is not caused by dopamine release – studies in an animal model, Arch. Neurol. 46, 1070.

Klawans, H.L., 1973, The pharmacology of tardive dyskinesias, Am. J. Psychiatry 130, 83.

Klawans, H.L., C.M. Tanner and A. Barr, 1984, The reversibility of "permanent" tardive dyskinesia, Clin. Neuropharmacol. 7, 153.

Kuczenski, R. and D.S. Segal, 1992, Regional norepinephrine response to amphetamine using dialysis: comparison to caudate dopamine, Synapse 11, 164.

LaHoste, G.J. and J.F. Marshall, 1992, Dopamine supersensitivity and D_1/D_2 synergism are unrelated to changes in striatal receptor density, Synapse 12, 14.

Langer, S.Z. and S. Arbilla, 1984, The amphetamine paradox in dopaminergic neurotransmission, Trends Pharmacol. Sci. 5, 387.

Lublin, H. and J. Gerlach, 1988, Behavioural effects of dopamine D-1 and D-2 receptor agonists in monkeys previously treated with haloperidol, Eur. J. Pharmacol. 153, 239.

Lublin, H., J. Gerlach and L. Peacock, 1992, Effect of D1 and D2 agonists in primates withdrawn from long-term treatment with haloperidol: the potential role of dopamine D1 receptors in dyskinesia, Clin. Neuropharmacol. 15, 448.

Moore, K., 1978, Amphetamines: biochemical and behavioral actions in animals actions in animals, in: Handbook of Psychopharmacology (New York, Plenum Press) p. 41.

Neisewander, J.L., E. Castañeda and D.A. Davis, 1994, Dose-dependent differences in the development of reserpine-induced oral dyskinesia in rats: support for a model of tardive dyskinesia, Psychopharmacology 116, 79.

Neisewander, J.L., I. Lucki and P. McGonigle, 1991a, Behavioral and neurochemical effects of chronic administration of reserpine and SKF-38393 in rats, J. Pharmacol. Exp. Ther. 257, 850.

Neisewander, J.L., I. Lucki and P. McGonigle, 1991b, Neurochemical changes associated with the persistence of spontaneous oral dyskinesia in rats following chronic reserpine treatment, Brain Res. 558, 27.

Niddam, R., S. Arbilla, B. Scatton, T. Dennis and S.Z. Langer, 1985, Amphetamine induced release of endogenous dopamine in vitro is not reduced following pretreatment with reserpine, Naunyn-Schmied. Arch. Pharmacol. 329, 123.

Parker, E.M. and L.X. Cubeddu, 1986, Effects of d-amphetamine and dopamine synthesis inhibitors on dopamine and acetylcholine neurotransmission in the striatum. I. Release in the absence of vesicular transmitter stores, J. Pharmacol. Exp. Ther. 237, 179.

Peacock, L., H. Lublin and J. Gerlach, 1990, The effects of dopamine D_1 and D_2 receptor agonists and antagonists in monkeys withdrawn from long-term neuroleptic treatment, Eur. J. Pharmacol. 186, 49.

Quinton, R.M. and G. Halliwell, 1963, Effects of α-methyl DOPA and DOPA on the amphetamine excitatory response in reserpinized rats, Nature 200, 178.

Raiteri, M., F. Cerrito, A.M. Cervoni and G. Levi, 1979, Dopamine can be released by two mechanisms differentially affected by the dopamine transport inhibitor nomifensine, J. Pharmacol. Exp. Ther. 208, 195.

Robinson, T.E., J.B. Becker, A.E. Young, H. Akil and E. Castañeda, 1987, The effects of footshock stress on regional brain dopamine metabolism and pituitary beta-endorphin release in rats previously sensitized to amphetamine, Neuropharmacology 26, 679.

Ross, S.B., D.M. Jackson, E.M. Wallis and S.R. Edwards, 1988, Enhancement by a single dose of reserpine (plus alpha methyl-p-tyrosine) of the central stimulatory effects evoked by dopamine D-1 and D-2 agonists in the mouse, Naunyn-Schmied. Arch. Pharmacol. 337, 512.

Scheel-Krüger, J., 1971, Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain, Eur. J. Pharmacol. 14, 47.

Scheel-Krüger, J. and J. Arnt, 1985, New aspects on the role of dopamine, acetylcholine, and GABA in the development of tardive dyskinesia, Psychopharmacology Suppl. 2, 46.

See, R.E., 1991, Striatal dopamine metabolism increases during long-term haloperidol administration in rats but shows tolerance in response to acute challenge with raclopride, Neurosci. Lett. 129, 265.

See, R.E., 1993, Assessment of striatal extracellular dopamine and dopamine metabolites by microdialysis in haloperidol-treated rats exhibiting oral dyskinesia, Neuropsychopharmacology 9, 101.

See, R.E., M.A. Chapman, C.E. Murray and M. Aravagiri, 1992, Regional differences in chronic neuroleptic effects on extracellular dopamine activity, Brain Res. Bull. 29, 473.

Shannak, K.S. and O. Hornykiewicz, 1980, Brain monoamines in rhesus monkey during long-term neuroleptic administration, Adv. Biochem. Psychopharmacol. 24, 315.

Shonecker, M., 1957, Ein eigentümliches Syndrom in eralen Bereich bei Megaphen Applikation, Nervenartz 28, 34.

Smith, C.B., 1963, Enhancement by reserpine and α-methyldopa of the effects of *d*-amphetamine upon the locomotor activity of mice, J. Pharmacol. Exp. Ther. 142, 343.

Smith, J.M. and R.J. Baldessarini, 1980, Changes in prevalence, severity, and recovery in tardive dyskinesia with age, Arch. Gen. Psychiatry 37, 1368.

Starr, B.S., M.S. Starr and I.C. Kilpatrick, 1987, Behavioural role of dopamine D_1 receptors in the reserpine-treated mouse, Neuroscience 22, 179.

Stoessl, A.J., C.T. Dourish and S.D. Iversen, 1989, Chronic neuroleptic-induced mouth movements in the rat: suppression by CCK and selective dopamine D1 and D2 receptor antagonists, Psychopharmacology 98, 372.

Stolk, J.M. and R.H. Rech, 1967, Enhanced stimulant effects of *d*-amphetamine on the spontaneous locomotor activity of rats treated with reserpine, J. Pharmacol. Exp. Ther. 158, 140.

Sulzer, D., N.T. Maidment and S. Rayport, 1993, Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons, J. Neurochem. 60, 527.

Tarsy, D. and R.J. Baldessarini, 1974, Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines, Neuropharmacology 13, 927.

Tarsy, D., 1983, History and definition of tardive dyskinesia, Clin. Neuropharmacol. 6, 91.

Uhrbrand, L. and A. Faurbye, 1960, Reversible and irreversible dyskinesia after treatment with perphenazine, chlorpromazine, reserpine, and electroconvulsive therapy, Psychopharmacologia 1, 408.

Van Rossum, J.M., J.B. Van der Schoot and J.A. Hurkmans, 1962, Mechanism of action of cocaine and amphetamine in the brain, Experientia 18, 229.