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Behavioral Quantification of Striatal Dopaminergic Supersensitivity After Bilateral 6-Hydroxydopamine Lesions in the Mouse

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MANDEL, R. J., R. E. WILCOX AND P. K. RANDALL. *Behavioral quantification of striatal dopaminergic supersensitivity after bilateral 6-hydroxydopamine lesions in the mouse.* PHARMACOL BIOCHEM BEHAV **41**(2) 343-347, 1992. — Quantitative studies using dopamine (DA) agonist-induced rotational behavior after denervation have found that the behavioral sensitivity is much greater than would be predicted on the basis of striatal DA receptor upregulation alone. The sensitivity to DA agonists after chronic treatment with neuroleptics, which elicits striatal receptor alterations equal to denervation, displays increases more consistent with alterations in striatal receptor density. Since the behavioral paradigms used to assess agonist supersensitivity after denervation are different than that for chronic neuroleptic treatment (rotational vs. stereotypic behavior), we measured the behavioral supersensitivity after bilateral denervation using stereotypic behavior. The increase in sensitivity to apomorphine after bilateral nigrostriatal 6-hydroxydopamine lesions was consistent with the increases measured previously with rotational behavior. These data suggest that the quantitative difference observed in behavioral supersensitivity resulting from the different preparations lies with the biological consequences of denervation rather than with the behavioral paradigm.

Dopamine Dopamine receptors 6-Hydroxydopamine Supersensitivity D_1 receptors D_2 receptors Stereotypic behavior

INCREASES in response to dopamine (DA) agonists following denervation or chronic treatment with DA antagonists are generally agreed to reflect postsynaptic supersensitization in the striatum. Consistent with this hypothesis is the increase in binding sites for dopaminergic ligands in striatal tissue following such treatment (23). Several investigators have reported large increases of behavioral sensitivity to apomorphine after unilateral 6-hydroxydopamine (6-OHDA) lesions in the rat and mouse (12,14,16). For instance, Marshall and Ungerstedt (14) reported a 10- to 40-fold increase in response to apomorphine, a direct DA agonist, after unilateral nigrostriatal 6-OHDA lesions in the rat. Using a similar preparation in the mouse, Mandel and Randall (12) found a 31.5-fold increase in sensitivity (shift to the left of the dose-response curve). Explanations for mild increases in DA binding sites (on the order of 30-40%) supporting great shifts in behavioral agonist sensitivity have been proposed (23) but are generally inadequate.

Much of the apparent increase in agonist sensitivity after unilateral 6-OHDA lesion as measured by rotational behavior is due to the additional depletion of DA in the nucleus accumbens (8). Lesions that deplete only striatal DA with no additional effect on DA levels in nucleus accumbens yield only a five-fold increase in apomorphine sensitivity. These data for a specific depletion of the caudate nucleus agree well with one other report of a five-fold increase in apomorphine sensitivity after unilateral 6-OHDA lesion (22). Rats with depletions of both accumbens and caudate increased their sensitivity to apomorphine on the order of 20-fold.

Randall (19) recently described the use of "null" pharmacological models in behavioral experiments. While these models have been used with simple in vitro systems for many years, the more recent development of generally available nonlinear curve-fitting procedures (15,26) has made their application to behavioral data more practical. The expected consequences of increases in receptor number on agonist response can be calculated using a modification of the classical null model designed to estimate agonist affinity via comparing dose-response curves obtained before and after occlusion of a portion of the receptor sites with an irreversible antagonist. The shift in an agonist dose-response curve measured after receptor upregulation should be a linear function of agonist efficacy.

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If behavior is treated as the direct tissue response to agonist stimulation, then, under this assumption, the increase in agonist sensitivity due solely to increased receptor number can be calculated using the modified null model technique. With an agonist with high efficacy, an increase of 30% in striatal receptor density would be predicted to produce a nearly parallel shift to the left of the dose-response curve on the order of two-fold. Obviously, a model based on assumptions intended for in vitro systems when applied to an in vivo system seems simplistic since many biological variables can intervene in the intact animal (e.g., the impact of the additional DA depletion of the nucleus accumbens described above). However, most investigators still discuss DA behavioral sensitivity in terms of increased striatal DA receptor density.

Clearly, the increases in agonist sensitivity reported above, measured using rotational behavior, are far greater than the two-fold shift predicted by the null model. However, one experiment (18) using apomorphine-induced stereotypic behavior before and after chronic DA receptor blockade with haloperidol found increased agonist sensitivity that agreed well with theoretical expectation. The two-fold shift has also been replicated in rats after chronic treatment with eticlopride, a specific D_2 antagonist (10). Conformation to the simplified pharmacological prediction may not be too surprising in this case since stereotypic behavior is generally agreed to be generated solely by striatum (17) and haloperidol specifically upregulates striatal D_2 receptors (11). Thus, DA behavioral hypersensitivity as measured by stereotypic behavior in response to chronic antagonist treatment may represent a unique and simplified in vivo pharmacological paradigm.

However, since both the stimulus for supersensitization (denervation vs. chronic neuroleptic) and the behavioral measurement differ between rotational and stereotypic behavior measured after denervation and chronic neuroleptic treatment, respectively, but the amount of striatal receptor proliferation is similar, the current experiment was conducted to determine whether the behavioral paradigm or the supersensitization stimulus accounts for the disparity between the theoretical expectations and empirical data concerning the magnitude of DA supersensitivity. Thus, the present experiment assesses the quantitative extent of supersensitization using stereotypic behavior as the agonist response after bilateral DA denervation of the striatum.

METHOD

Forty-five male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) weighing 20.8 ± 0.4 g at the time of surgery were administered bilateral 6-OHDA lesions as described previously (12,18). 6-Hydroxydopamine hydrobromide (Sigma Chemical Co., St. Louis, MO) was dissolved in a 0.2% ascorbate-0.9% saline solution at a concentration of 4 $\mu g/\mu l$ and kept cold throughout the intracerebral injection. Four μl were injected via a 30-ga cannula continuously over 20 min directly into the caudate nucleus (+0.5 mm AP, 2.4 mm ML from bregma, and −3.1 mm DV from the skull) and the cannula was allowed to remain in place for an additional 10 min to allow for passive diffusion. This identical lesion technique has previously been shown to induce both a robust behavioral supersensitivity syndrome (12) and a 30% increase in striatal DA receptor density (24). Twenty mice weighing 20.7 ± 0.3 g mice received sham lesions (bilateral injection of 0.02% ascorbate vehicle solution). An additional 15 animals of the same cohort were used as the untreated control group.

Even with extreme care (18), by 3 wk postoperatively only 14 6-OHDA-lesioned animals remained alive. In contrast, 16 mice in the vehicle group remained alive at 3 wk postsurgery.

Remaining animals were randomly assigned to blocks in an incomplete block design (6) where each animal received four of six doses of apomorphine hydrochloride (a gift of Merck, Sharp, and Dohme, Rahway, NJ). Apomorphine was dissolved in 0.09% saline and injected IP at doses 4.0, 2.0, 1.0, 0.5, 0.25, and 0.125 mg/kg. Mice were injected once a week and tested for stereotypic behavior as described below. This experimental design yields $n = 10$ for each dose; however, since only 14 mice remained in the bilateral 6-OHDA-lesioned group, for four doses of apomorphine (4.0, 1.0, 0.5, and 0.125 mg/kg) the $n = 9$.

Six mice were observed each session in enclosures of wire mesh (15 × 15 × 15 cm). After a 10-min habituation period, the appropriate dose of apomorphine was administered and ratings assigned every 6 min on the basis of a 20-s observation for a total of 1 h. Ratings were assigned according to Randall and Randall (20). Briefly, the behaviors were assigned numerical scores as follows: 0, no drug effect; 1, reduced locomotion; 2, reduced locomotion and intermittent sniffing; 3, continual nondirected sniffing; 4, upright posture (usually climbing up on the enclosure) with intermittent sniffing; 5, upright posture with continual sniffing or intermittent gnawing; 6, upright posture with extreme arching of the back and continual gnawing at the cage. In addition, animals that displayed asymmetrical behavior were rated on the severity of their rotation. Rotational behavior was rated 0 to 3, 0 indicating no rotational behavior; 1, postural asymmetry with no locomotion; 2, movement slowly around in a unidirectional circular pattern of less than two full 360° rotations; and 3, rapid head to rump rotational behavior for the entire 20-s observation period.

Data were analyzed using analysis of variance (ANOVA) (6) and nonlinear curve-fitting techniques of De Lean et al. (7) and Randall and Randall (20). The latter technique is an extension of quantal techniques for ordered categories (i.e., ratings). The stereotypic rating system can be viewed as a series of ordered quantal responses where the probability of meeting or exceeding thresholds for the different categories define quantal dose-response to curves for each rating level. All curves for all groups are fit simultaneously using a minimum χ^2 criterion. This method allows one to determine if all behavioral categories (ratings) shift in a homogeneous manner. In the Randall (18) experiment, all individual curves shifted in an approximately equal manner, indicating that all behavioral components of apomorphine-induced stereotypes were affected similarly by the receptor supersensitization (i.e., representing a parallel shift in the dose-response curve for an underlying *continuous* response variable). In addition, the equal shift of all the curves in Randall's experiment (18) indicates that the inherent ceiling effect concomitant with rating data (i.e., no animal can receive a rating higher than 6) does not affect interpretations of the data when two dose-response curves are compared, that is, it is the between-curve comparison of estimated ED_{50}'s that is relevant, not characteristics of individual curves.

RESULTS

Total stereotype ratings increased with dose in all three groups [bilateral 6-OHDA, $F(5,40) = 4.25$; sham, $F(5,40) = 5.2$; untreated controls, $F(5,40) = 4.94$; $p < 0.01$, for each]. ED_{50} for total stereotype ratings of the sham lesion group did not differ from that of the untreated control group, $F(13,9) = 1.13$; $p > 0.05$, and therefore the data from these two control groups were combined in all further comparisons. In addition, logistic slopes and maximum total rating parameters were equal among all three dose-response curves, $F(11,9)$

= 0.4 and $F(14,4) = 2.96$, respectively; $p > 0.05$ for both. The bilaterally lesioned mice were more sensitive to apomorphine (ED_{50} = 0.75 mg) than the combined control groups [ED_{50} = 1.2 mg/kg; $F(14,9) = 5.2$; $p < 0.01$]. The best-fitting logistic dose-response curves are presented in Fig. 1A.

Six of the 14 lesioned mice exhibited some rotational behavior. The dose-response to apomorphine of these animals for both total stereotypic ratings and rotational behavior are shown in Fig. 1B.

Dose-response curves for the individual rating levels of lesioned and control animals are presented in Fig. 2 and the dose ratios of control vs. lesioned mice ED_{50}'s are shown in the inset to Fig. 2. Increased sensitivity to apomorphine was restricted to lower rated behaviors (ratings 1-3) that shifted between four- and five-fold to the left, while highly rated behaviors did not shift to any appreciable extent.

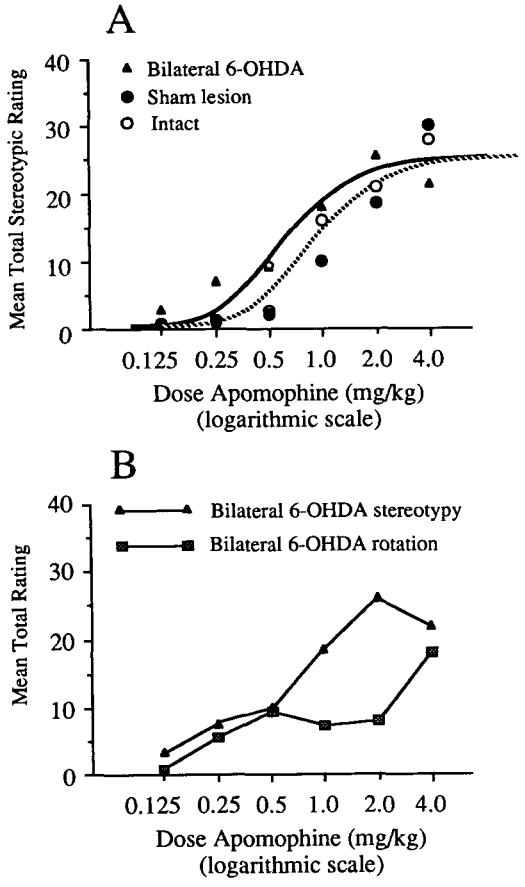

FIG. 1. (A). Presentation of individual data points and estimated smooth dose-response curves from the mean of the stereotypic rating data summed over 1 h. ▲ represents the data from the mice with bilateral 6-OHDA lesions, ● represents the data from sham-lesioned mice, and ○ represents the data from untreated mice. Data from the sham-lesioned mice and the intact mice were combined to calculate the apomorphine dose-response curve (broken curve). The solid, smooth curve is the estimated dose-response curve from the bilateral 6-OHDA-lesioned mice. The difference between the ED_{50} of both curves is approximately two-fold. (B). Presentation of the rotational response of the six bilaterally lesioned mice that displayed consistent rotational behavior in response to apomorphine. Rotational behavior (■) is plotted against the mean total stereotypic rating (▲) for the bilaterally lesioned mice, which is identical to the data presented in panel A. These data indicate that the increase in rotational behavior that occurred at the higher doses of apomorphine may have interfered with the expression of highly rated stereotypic behaviors since highly rated stereotypic behaviors only appear at high doses of apomorphine. The abscissa is scaled based on $\log_2$.

DISCUSSION

The dose-response curve for total ratings over the 1-h test period showed a shift to the left of 1.6-fold, is in good agreement with theoretical expectation and with the supersensitization occurring in chronic haloperidol treatment (18). However, if the increase in sensitivity represents a parallel shift in the overall apomorphine dose-response curve all individual component curves should have shifted to the same extent. This was not the case. The curves for behaviors occurring at lower doses shifted to a much greater extent, nearly five-fold, while the highest component (a rating of 5) in fact shifted slightly to the right. Since, in general, the expected shift is a linear function of the agonist dose, the present data are not consistent with a simple increase in receptor density, even if apomorphine is acting as a partial agonist.

One plausible explanation of the decreasing shift at higher doses is that estimation of supersensitivity was contaminated by competing behaviors occurring in the lesioned animals that were not present in controls. A number of lesioned mice showed asymmetric behavior, probably resulting from unequal depletion in the two hemispheres. Rotational behavior (Fig. 1B) rose sharply at the higher doses of apomorphine, whereas the mean total stereotypic rating decreased at the highest dose (4.0 mg/kg). Therefore, the quantal dose-response curves for the lower-rated behaviors (ratings 1-3) are better estimates of the shift in lesion-induced agonist sensitivity because rotational behavior did not appear at the doses of apomorphine most likely to induce the low-rated behaviors.

The increase in sensitivity of low-rated behaviors (four- to five-fold) agrees well with the behavioral supersensitivity with that for rotational behavior using the specific striatal depletion group in the Hartgraves et al. (8) experiment. The behavioral supersensitivity observed in this experiment is greater than that observed by Randall (18) for stereotypic behavior after chronic haloperidol. Therefore, the reported differences between haloperidol- and 6-OHDA-induced supersensitization probably results from differences between chronic antagonist treatment and denervation, rather than differences in the behavioral response employed for quantification.

Since both 6-OHDA lesions and chronic haloperidol are thought to produce relatively specific D_2 as opposed to D_1 upregulation (1,5,11), the major difference between the chronic neuroleptic regime and the 6-OHDA denervation is that the DA neurons (and endogenous DA) are lost in the latter. It is now well accepted that the synergistic interaction of D_1 and D_2 receptors as revealed by specific DA agonist interactions is altered as a result of DA depletion with 6-OHDA or by pharmacological means (DA synthesis inhibition and/or reserpine treatment) (2-4,9,13,21). Moreover, three pieces of evidence indicate that lesion-induced striatal D_2 receptor proliferation is unrelated to the manifestation of behavioral hypersensitivity to DA agonists. First, rats rotate contralaterally in response to apomorphine within 4 days after unilateral 6-OHDA lesion, earlier than striatal D_2 receptor number can increase (25). Second, unilaterally 6-OHDA-lesioned mice exposed to chronic infusions of the D_2 agonist, quinpirole, which results in normal levels of striatal D_2 receptors on the lesioned side, retain their rotational response to acute injections of quinpirole (27). Finally, unilaterally 6-OHDA-lesioned rats treated chronically with the D_2 antago-

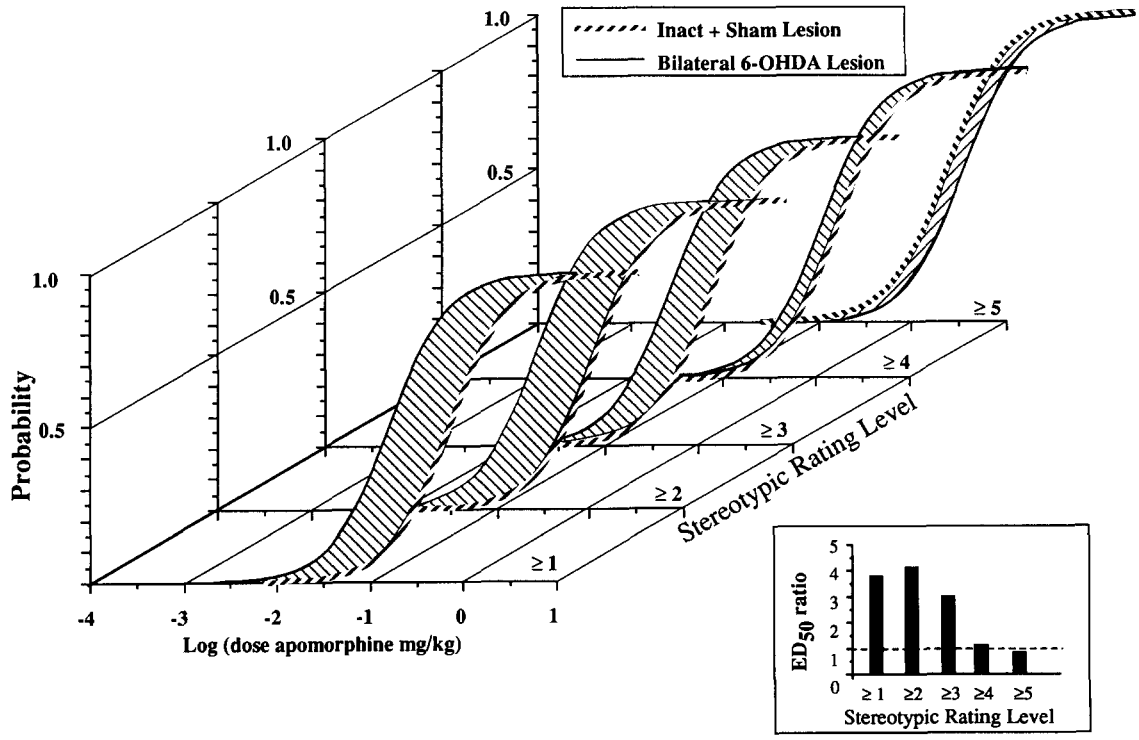

FIG. 2. Presentation of quantal dose-response curves calculated from the percentage of mice displaying a particular rating score or greater at a given dose of apomorphine. The estimated smooth dose-response curves from the bilaterally lesioned mice are represented by solid curves, while the dose-response curves from combined control group are represented by broken curves. The area between the dose-response curves is hatched to highlight the magnitude of the difference between them. The inset presents the actual agonist sensitivity difference between the dose-response curves for each individual stereotypic rating level illustrated by the ratio of the ED_{50}'s of the two curves. The ED_{50} ratio is calculated by dividing the ED_{50} calculated from each control curve by the ED_{50} calculated from the appropriate dose-response curve of lesioned mice, and the bars in the inset are directly proportional in height to the distance between the curves in the main graph.

nist, eticlopride, which also equalizes the striatal D_2 binding levels in both hemispheres by increasing D_2 receptors in the intact striatum, continue to rotate in response to quinpirole (9). Apomorphine is an agonist at both D_1 and D_2 receptors and the altered relationship between the two DA receptor subtypes in DA-depleted vs. nondepleted preparations may account for the magnitude of difference in agonist sensitivity between the two preparations (four- to five-fold) reported here rather than the mild proliferation of striatal D_2 receptors.

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