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BRIEF COMMUNICATION

Bromocriptine Promotes Recovery of Self-Stimulation in 6-Hydroxydopamine-Lesioned Rats

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CAREY, R. J. *Bromocriptine promotes recovery of self-stimulation in 6-hydroxydopamine-lesioned rats.* PHARMACOL BIOCHEM BEHAV **18**(2) 273–276, 1983.—Rats with stable self-stimulation response rate-current intensity functions were subjected to bilateral 6-hydroxydopamine injections into the substantia nigra. The effect of several drug treatments were evaluated on the 6-hydroxydopamine lesion self-stimulation deficit. d-Amphetamine (1.0, 2.0 and 3.0 mg/kg) and scopolamine (0.25, 0.5 and 1.0 mg/kg) had little or no effect on self-stimulation, but bromocriptine (2.0 and 4.0 mg/kg) produced a nearly complete recovery of self-stimulation performance. In contrast, scopolamine increased locomotor activity; whereas, bromocriptine did not increase activity. These results point up the efficacy of bromocriptine in reversing self-stimulation deficits induced by a dopamine deficiency, and indicate that self-stimulation may be a more useful behavior than locomotor activity for evaluating drugs which might alleviate Parkinsonism.

Self-stimulation 6-Hydroxydopamine Bromocriptine Amphetamine Scopolamine

EVER since the pioneering studies of Hornykiewicz [4] it has become clear that Parkinson's Disease is characterized by a depletion of dopamine in the striatum and degeneration of the substantia nigra. With the subsequent development of L-Dopa therapy [3] the functional link between dopamine and motoric capability became firmly established. Animal studies have added to this relationship between dopamine and movement control through the demonstration of akinesia and rigidity in animals sustaining the 6-hydroxydopamine lesions of dopamine neurons in the substantia nigra [12].

A recent development in the pharmacological management of Parkinson's Disease has involved the use of the ergot alkaloid bromocriptine [11]. The present study was undertaken in an attempt to assess the functional efficacy of bromocriptine in reversing deficits in motoric function in animals subjected 6-hydroxydopamine lesions of the substantia nigra. Two types of motoric function were evaluated. One was simply the spontaneous locomotor activity of rats and the other was the more goal-directed behavior of self-stimulation. As a comparison, two other pharmacological agents which can facilitate self-stimulation and locomotor activity were used; namely, amphetamine and scopolamine [1, 2, 10]. d-Amphetamine, as an indirect acting dopamine agonist, serves to evaluate the functional effectiveness of the dopamine lesion, and scopolamine represents a completely different pharmacological treatment modality (i.e., anticholinergic) which can also ameliorate Parkinsonian symptoms.

METHOD

Surgery

Rats were implanted with bipolar platinum electrodes (Plastic Products Co., Roanoke, VA) insulated except for the cross-sectional area at the cut end. Surgery was aseptic and performed with the rats under deep equithesin anesthesia (0.3 ml/100 g). Using a Kopf-stereotaxic instrument, the electrodes were placed in the brain and bonded to a roughened dry skull with cranioplastic cement. The electrodes were aimed at the lateral hypothalamus using the following stereotaxic coordinates: 1.2 mm posterior to bregma, 1.5 mm lateral to the midline and 8.0 mm ventral to the dura mater. The incisor bar was set at 3.2 mm above the interaural line. Following surgery, each rat was maintained on oral tetracycline for 1 week.

Apparatus

Self-stimulation testing was conducted in three conven-

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tional operant chambers situated within sound attenuating enclosures (B.R.S./L.V.E. No. 1417). Each chamber contained a response lever (Ralph Gerbrands Co., No. G6312) centered in a chamber wall 3.5 cm above the grid floor. A 28 V DC miniature lamp (#304) illuminated each chamber and white noise was broadcast whenever stimulation was available. Relay circuits with timers and digital counters programmed reinforcement, recorded bar presses and controlled session duration.

A Grass Brief-Pulse Biphasic Stimulator, Model BBS1, was the source for the brain stimulation reinforcement. The stimulation consisted of pairs of biphasic rectangular pulses of 0.1 msec separated by 0.1 msec interval between positive and negative pulses. The frequency of stimulation was 100 pulses/sec and the train duration 0.2 sec. Current intensity was monitored continuously on each of three occiloscopes (Textronix No. 502A) from a voltage drop across a 1 K ohm resistor in series with the animal. The rat was connected to the stimulator through a mercury-swivel commutator mounted above each chamber.

Locomotor activity was measured using photoactivity cylinders (B.R.S./L.V.E PAC-001). These black metal activity chambers were 61 cm in diameter and 53 cm high with a metal meshwork floor. Six infrared photocells symmetrically spaced 2.5 cm above the floor detected movement. The photobeam interruptions were recorded on 2 digital counters with each counter recording from three photocells.

Three drugs were used in this experiment; 2-bromo-alpha-ergocryptine methane sulfonate; i.e., bromocriptine mesylate (Sigma Chemical Co.,), d-amphetamine HCl (K and K Chemical, Jamaica, NY), scopolamine HCl (Sigma Chemical Co.). The bromocriptine was first dissolved with a few drops of absolute ethanol and then brought up to the appropriate concentration with distilled water. The amphetamine and scopolamine were dissolved in 0.9% saline.

Procedure

After a 2-week post-implantation recovery period, the rats were trained to bar press for the 0.2 sec train of brain stimulation. After the response was well established, the rats were given daily 15 minute sessions with brain stimulation available on a continuous reinforcement schedule until reliable response rates were established. Next, rate-intensity functions were determined for each rat. Current intensity adjustments were made to generate three rates of response, a low, 5–15 responses per minute, a half-maximal (40–80) and a maximal response rate (120–150). After reliable performances were established for these 3 current intensities, the intensities were then always kept the same throughout the course of the experiment.

Phase I

After reliable rate-intensity functions were established, 8 of the rats under deep ether anesthesia were subjected to bilateral 6-hydroxydopamine lesions of the substantia nigra, and the remaining 2 rats were run through the test procedures as unoperated controls. The stereotaxic coordinates were: 4.5 mm posterior to bregma, 8.0 mm below the dural touch point and 2.0 mm lateral to the midline. At this loci, 2 μl of a 4 μg/μl of 6-OHDA HBr (calculated as the base) dissolved in 0.15 m NaCl containing 0.2 mg/ml ascorbic acid, was injected. The 6-OHDA was injected through a 30 gauge cannula at a rate of 0.5 μl/min. Thirty minutes before the 6-OHDA treatment, the rats were given an IP injection of desipramine HCl (25 mg/kg). From 2–7 weeks postoperative; all the rats were retested on self-stimulation. Five rats showed persistent deficits (overall performance of self-stimulation reduced to 50% of preoperative level) were used in this study.

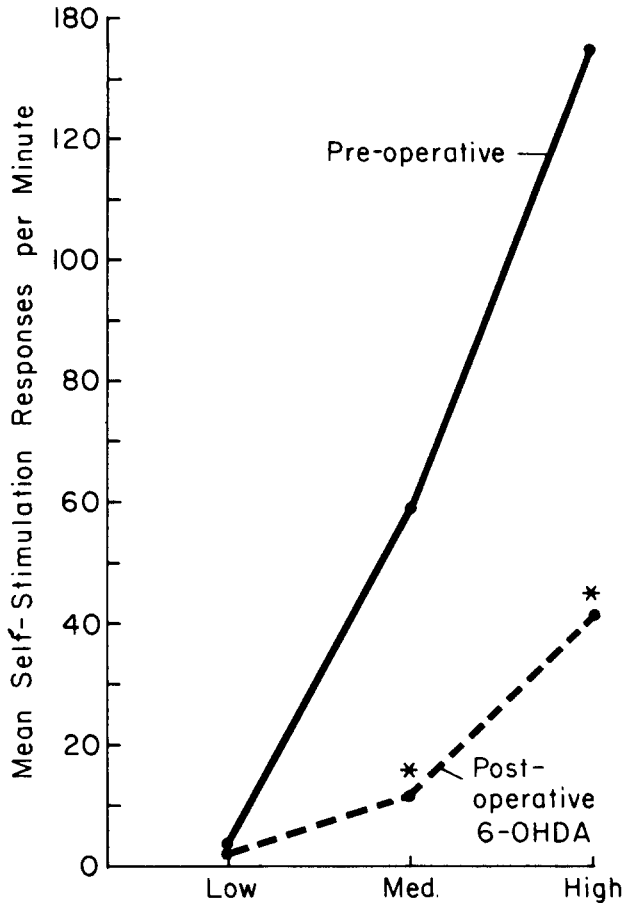

FIG. 1. Mean self-stimulation response rates before and 1 month after bilateral 6-hydroxydopamine injections into the substantia nigra. The response rates were generated under 3 current intensities, low, medium and high. *Denotes $p<0.01$, t-test for difference scores.

Phase 2

Pharmacological testing was conducted between weeks 5 through 7 postoperative. On each of 3 successive weeks, the 6-OHDA treated rats were tested with 1 of the 3 drugs. For each drug the same format was used. The first day of testing was a non-drug test and then each successive day was a drug test with the drug dosage administered in ascending order. In week 5, 3 doses of d-amphetamine were used; 1.0, 2.0 and 3.0 mg/kg; in week 6, 2 doses of bromocriptine, 2.0 and 4.0 mg/kg; and in the seventh week, 3 doses of scopolamine, 0.25, 0.5 and 1.0 mg/kg. For the scopolamine and amphetamine tests the rats were injected IP 10 minutes before being placed in the photobeam activity chambers for 15 minutes. Five minutes after completion of the activity testing, the rats were tested for self-stimulation of each of 3 current intensity levels. Self-stimulation was tested for 10 minutes at each current intensity with 5 minute non-

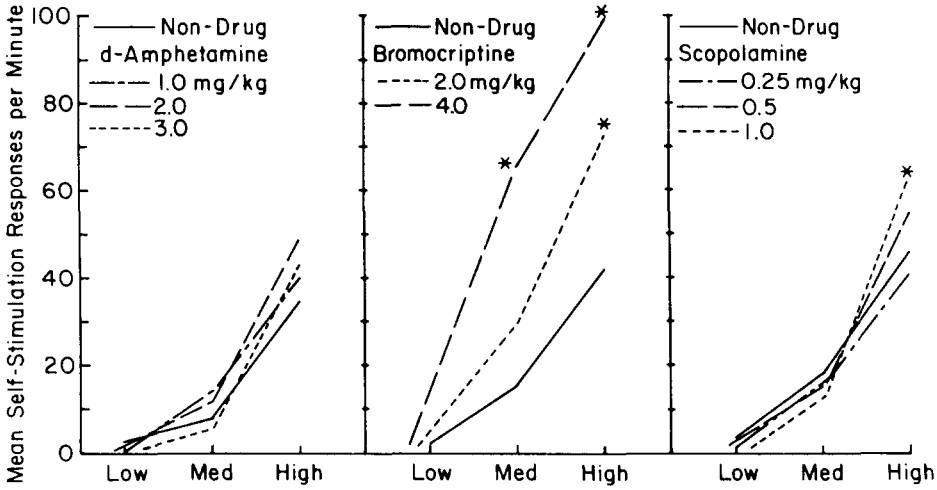

FIG. 2. Mean self-stimulation response rates obtained for 3 current intensities (low, medium, high) under non-drug and drug conditions. All rats had bilateral 6-hydroxydopamine lesions of the substantia nigra. *Denotes $p<0.05$ for t-test for difference scores between non-drug and drug conditions.

stimulation intervals separating changes in current intensity. The current intensity was increased in ascending order. The same testing format was followed for bromocriptine except the delay between drug injection and the start of testing was 2 hours. Bromocriptine can have initial inhibitory effects (up to 1 hour) but then has dopamine agonist effects lasting for several hours [5,7].

Biochemical Assay

At least 1 week after completion of the testing, the rats (under ether anesthesia) had their electrodes removed stereotaxically, then were decapitated and their brains removed rapidly over ice. Each brain was cut coronally immediately anterior to the optic chiasm. This anterior brain section was then dissected to remove striatal and limbic (olfactory lobe and accumbens nucleus) tissue samples. These tissue samples were assayed for dopamine (DA) and norepinephrine (NE) using high performance liquid chromatography with electrochemical detection (LCEC) [3]. The DA and NE peak heights were ratioed to the dihydroxybenzylamine (DHBA) internal standard peak height and the concentrations calculated knowing the relative response of standards, the amount of DHBA added and the brain sample weight. The caudal section of the brain was used for histological verification of electrode and cannula placements.

RESULTS

Figure 1 shows the marked decrease in self-stimulation produced by the bilateral 6-OHDA injections into the substantia nigra. Although the 6-OHDA treatment markedly reduced self-stimulation at half-maximal and maximal response rates, the rats still remained sensitive to increases in current intensity but were deficient in their response performance at the two highest current intensities.

In Fig. 2, the pharmacological modification of the self-stimulation in the 6-OHDA treated rats is presented. Amphetamine had no statistically reliable effect on self-stimulation and an overall similar result was apparent for scopolamine, although there was a statistically significant elevation in self-stimulation at the highest dose level of scopolamine, but only at the highest current intensity. Bromocriptine, however, produced large and statistically significant increases in self-stimulation at both dose levels. An important consideration in these findings is that the baseline non-drug self-stimulation performance remained unchanged across the test intervals. The effects of the drug treatments on locomotor activity, however, were quite different.

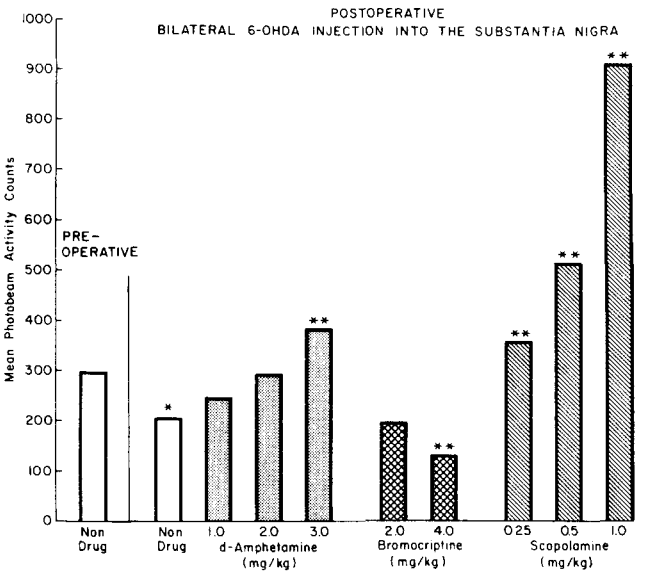

FIG. 3. Mean photobeam activity counts pre- and postoperative. All drug tests are postoperative. *$p<0.05$, t-test for difference scores between non-drug pre- and postoperative activity. **Denotes $p<0.05$, t-test for difference scores between postoperative non-drug versus drug activity.

As can be seen in Fig. 3, amphetamine produced a small increase in activity and only at the highest dose level, 3.0 mg/kg. Scopolamine, however, produced a large dose dependent increase in activity. Bromocriptine, however, had no effect on activity at 2.0 mg/kg and produced a small but a significant decrease at 4.0 mg/kg.

TABLE 1

MEAN LIMBIC AND STRIATAL DOPAMINE (DA) AND NOREPINEPHRINE (NE) LEVELS (μg/g)

	Striatal		Limbic	
	(NE)	(DA)	(NE)	(DA)
6-OHDA Treated	0.25	0.51*	0.78	0.925*
Unoperated	0.36	8.91	0.98	2.51

*Denotes $p<0.01$, t-test for difference scores.

Table 1 presents the striatal and limbic dopamine and norepinephrine levels in the animals sustaining 6-OHDA lesions. As is apparent from Table 1, the 6-OHDA injections produced severe losses in both striatal and limbic dopamine. Microscopic examination of histologically prepared brain sections showed that the electrode tips were located in the medial forebrain bundle area between the internal capsule and fornix at the level of the ventral medial nucleus pars medialis. The tips of the cannula tracts were situated in the medial region of the pars reticulata at the level of the interpeduncular nucleus.

DISCUSSION

The functional reversal by bromocriptine of a self-stimulation deficit induced by 6-OHDA lesions of dopamine neurons is in keeping with an increasing body of clinical data supportive of the efficacy of bromocriptine in alleviating Parkinsonian symptomatology. This demonstration offers further support for a motoric dysfunction interpretation of self-stimulation deficits produced by pharmacological or lesion-produced deficits in dopamine neurotransmission [4,8]. An unexpected facet of the present study was that pharmacological manipulations in animals with lesions of dopamine neurons were not uniform in their effect on self-stimulation and locomotor activity. In fact, bromocriptine tended to reduce locomotor activity and markedly enhance self-stimulation; whereas, scopolamine had little or no effect on self-stimulation, but increased locomotor activity several fold. This latter effect by scopolamine on locomotor activity in dopamine depleted rats has also been reported by Schallert *et al.* [9]. These authors have also pointed out that this exaggerated activity is characterized by strong perseveration and is quite abnormal. In the present study, this hyperactivity seemingly could not become goal-directed so that self-stimulation performance was only slightly affected. Another consideration is the use of photobeam interruptions as a measure of locomotor activity. That is, increases in locomotion can produce increases in photobeam interruptions; whereas, more restricted activity changes such as increased oral activity (e.g., biting and gnawing) might not be detected but yet be associated with a decrease in photobeam counts. Perhaps, such a change in the topography of activity accounts for the apparent decrease in activity associated with bromocriptine. Photobeam activity, therefore, does not appear to be a useful measure to assess the functional effectiveness of a pharmacological treatment in restoring motoric capability. Self-stimulation, on the other hand, which requires instrumental performance linked to reinforcement would appear well suited to such an assessment since the restoration of functional capacity would be expressed by a behavioral response of strong functional significance to the animal.

ACKNOWLEDGEMENTS

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