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Biphasic Locomotor Response to Intra-Accumbens Dopamine in a Nonhuman Primate

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JONES, D. L., S. L. BERG, R. L. DORRIS AND R. E. DILL. *Biphasic locomotor response to intra-accumbens dopamine in a nonhuman primate.* PHARMAC. BIOCHEM. BEHAV. 15(2) 243–246, 1981.—Locomotor activity of ten squirrel monkeys, *Saimiri sciureus*, was evaluated by means of a photocell activity cage following intracranial application of dopamine (DA). A biphasic response consisting of an initial quiet period followed by increased locomotor activity was seen following intra-accumbens DA, 12.5–100 μg bilaterally. Both the length of the quiet phase and intensity of locomotor activity were positively related to DA dose. Intra-caudate DA (50 μg) was significantly less effective in producing locomotor effects. The specificity of the DA response was substantiated by dose-related inhibition with both systemic (0.1 or 0.05 mg/kg) and intra-accumbens (2–10 μg) administration of the DA antagonist haloperidol. Additionally, the intra-accumbens application of haloperidol was found to be ineffective in inducing catalepsy, a state readily produced by systemically administered haloperidol.

Nucleus accumbens Squirrel monkey Locomotor activity Dopamine Catalepsy

ALTHOUGH there has been increased interest in the psychogenic and motor function of the nucleus accumbens, a mesolimbic structure, quantitative studies of the role of the nucleus accumbens in primate activity have yet to be reported [1, 7, 11, 14]. In the first primate study of the effects of dopamine (DA) on the nucleus accumbens, we reported from subjective observations [4] that intra-accumbens (IA) injection of DA (100 μg) produced increased locomotor activity and several other behavioral changes in the squirrel monkey. We report here a dose-response study using a photocell cage to quantify locomotor activity. The unusual biphasic response of this primate species to IA DA seen in the first study was confirmed.

METHOD

Five adolescent and five mature male squirrel monkeys *(Saimiri sciureus)* were prepared for intracranial injection by bilateral cannulation in the nucleus accumbens septi and the head of the caudate nucleus with the respective coordinates: A +15, L ±2.75, V +2.3 and A +13, L ±2.0, V +8.3 mm [6]. Placement of the cannulae was identified radiographically in all animals and verified histologically in six of the ten as four have not yet been terminated. With the exception of one pair of cannulae located in the tuberculum olfactorium, all cannulae were determined to be correctly positioned. Details of the cannulation procedure and techniques used for intracranial drug injection have been previously described [3]. All intracranial infusions were made bilaterally. Dopamine hydrochloride for intracranial injection was made up fresh daily in sterile physiologic saline in concentrations such that the injection volume did not exceed 2 μl. Haloperidol injections were Haldol commercial injectable solution (McNeil). Injection of the drug solution was followed by injection of 2 μl of sterile saline, so that the total volume injected intracranially on each side was 4 μl. The rate of injection was approximately 2 μl/min.

A photocell activity cage was used for the quantification of locomotor activity. The cage (30×28×60 cm) was constructed with front and sides of clear plastic, opaque back and top, and wire mesh floor. A 3/8 in. diameter bar perch was located horizontally in the center of the cage, 20 cm above the cage floor. Four photocell detectors and light sources were mounted externally on the sides of the cage. The photocell assemblies were mounted in two parallel rows, the beams oriented horizontally 15 and 40 cm above the cage floor, and 10 and 20 cm from the front of the cage. Thus, the interior of the cage was divided into quadrants, each centered around a photocell beam.

During the experimental sessions, the photocell cage was located in an isolated observation room equipped with a one-way glass window and cable ports. All instrumentation and equipment was located in the adjacent laboratory. Ventilation and masking noise were provided by a small fan mounted atop the cage. All sessions were monitored on closed-circuit television, and videotape records made of selected portions of the experiments. Written records of subjective observation during each test session were also kept.

The interruptions of the light beams were counted and stored in one-min blocks by a microcomputer in the adjacent laboratory. The raw data were expressed as the number of beam interruptions (counts) per unit time. From the raw data, the following scores were then derived: (1) maximum rate per minute, (2) latency to onset of effect, and (3) latency to maximum effect. By comparing the saline control condition and the drug treatment condition, each animal served as his own control for determining the latency scores. The latency to onset was defined as the time from drug injection until the average count per min significantly exceeded that of the corresponding period of the saline control, and remained so for at least 20 min. The absolute maximum effect was the highest count recorded during any one-min interval during the test session. The latency to maximum effect was simply the time from drug injection to the occurrence of the maximum count per min. For comparison across treatment conditions, the mean and standard error of the scores for each measure were calculated, and Tukey's HSD [8] used for comparison among means.

The criterion for ascertaining the presence of catalepsy was defined as the maintenance of an awkward position, suspended by the hind limbs beneath the horizontal bar in the photocell cage, for at least 10 min.

After a post-cannulation recovery period of at least one week, each animal was placed in the photocell cage for 6 hr on two consecutive days. The first day served to adapt the animal to the cage and the second to establish a baseline of activity. The locomotor activity was then recorded for each of six animals receiving IA injections at 10-day intervals in a counterbalanced sequence of one control (4 μl saline) and three test conditions (25, 50 and 100 μg DA). Subsequent to the initial dose-response measures and other test treatments, a dose of 12.5 μg DA IA was administered to assess responsiveness of the site as well as to obtain an additional lower dose point. Animals were routinely treated 18 hr prior to intracranial injection with the monoamine oxidase inhibitor tranylcypromine, 1 mg/kg subcutaneously (SC). Fifty μg DA injected into the head of the caudate nucleus served as a control for site specificity. The specificity of the IA DA response was tested in two ways: (1) systemic administration of the DA antagonist haloperidol (0.1 or 0.05 mg/kg SC) 30 min prior to IA DA, and (2) injection of 10 μg haloperidol IA during the maximum locomotor activity produced by 100 μg DA IA. Since systemic injection of haloperidol produced catalepsy in some animals, this response was studied further by the injection of haloperidol (2–10 μg) into both the nucleus accumbens and the caudate nucleus.

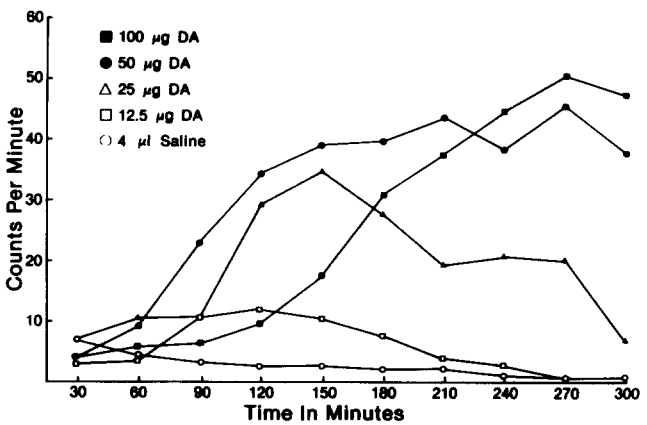

FIG. 1. Time course of locomotor activity of 6 squirrel monkeys injected bilaterally in the nucleus accumbens with four dose levels of dopamine hydrochloride or 4 μl saline. The points on each curve represent the mean of the average counts perminute of each of the animals during the preceding 30 min interval. Statistical analyses of the data are presented in Table 1.

RESULTS

The effects of IA DA on locomotor activity presented in Fig. 1 demonstrate a sustained period of inactivity preceding a period of increased locomotor activity. Latency to peak locomotor activity was positively correlated with IA DA dose, as high doses showed longer initial periods of sustained inactivity (Table 1). The latency to onset of locomotor activity (defined as the point at which activity differed significantly ($p<0.05$) from saline-injected controls) showed a trend in the same direction, but there were no significant

TABLE 1

LOCOMOTOR EFFECTS OF INTRA-CRANIAL DOPAMINE IN SIX SQUIRREL MONKEYS

	Dose of DA (bilateral) in μg							
		Accumbens						Caudate
Measure of Locomotor Activity	Saline 4 μl	12.5	25	50	100	25 + Hal	100 + Hal	50
Latency to maximum effect in min. Mean ±S.E.M.	—	116.7 ±12.9	153.3 ±21.8	212.5 ±63.0	283.3 ±42.3*	—	225.0 ±33.9	158 ±19.85
Latency to onset in min. Mean±S.E.M.	—	86.0 ±32.7	71.7 ±21.4	77.5 ±25.1	142.0 ±38.9	—	143.3 ±24.4	116 ±29.59
Maximum effect counts/ min. Mean±S.E.M.	15.1 ±3.39	23.5 ±7.5	51.3 ±5.2	93.5 ±13.6*	77.8 ±11.4†	2.06 ±1.79	78.4 ±12.5†	38.88 ±11.54

*=vs 12.5 $p<0.01$; vs 25, $p<0.05$.
†=vs 12.5 $p<0.01$.
Hal=0.05 mg/kg haloperidol SC.

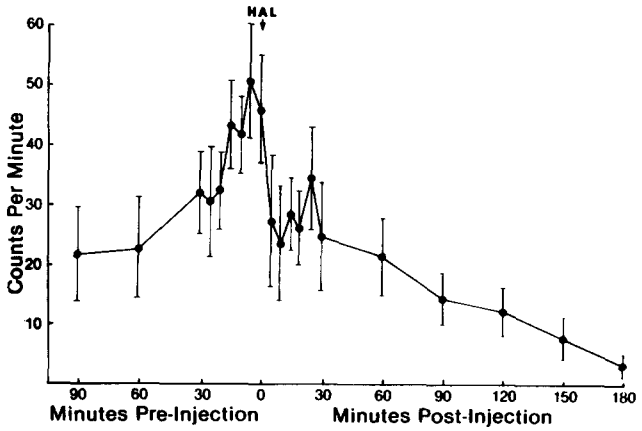

FIG. 2. Six squirrel monkeys were injected bilaterally in the nucleus accumbens with 100 μg dopamine HCl. During the early phase of the resulting increase in locomotor activity, 10 μg haloperidol were injected bilaterally into the same site (indicated by arrow). A significant decrease ($p<0.05$) in locomotor activity was seen within 5 min.

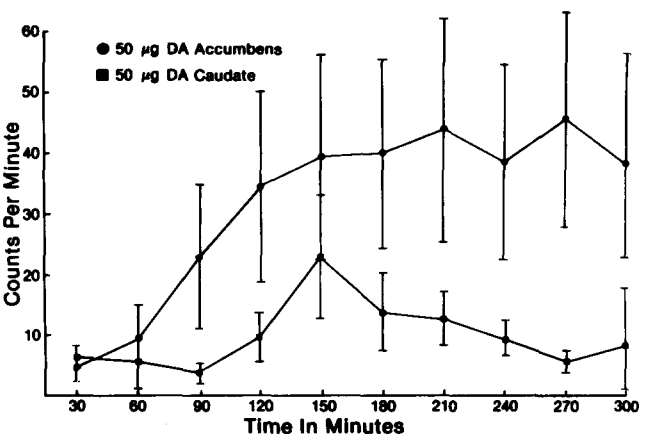

FIG. 3. Comparison of the time course of locomotor activity produced by the intracranial injection of 50 μg dopamine HCl into the nucleus accumbens and the caudate nucleus in 6 squirrel monkeys. The points on each curve represent the mean of the average counts per minute of each of the animals during the preceding 30 min interval.

differences between dose levels. The maximum locomotor response to IA DA was dose-related, showing a significant difference ($p<0.01$) between the highest and lowest doses. Statistical analyses of these data are presented in Table 1.

Haloperidol, 0.1 mg/kg, SC, completely blocked (mean < 1.0 counts/min) the effects of 25 μg DA IA, while haloperidol 0.05 mg/kg, SC blocked the effects of 25 μg DA IA, but not 100 μg (Table 1). The increased activity following IA DA was rapidly reversed by the IA injection of haloperidol, 2–3 hr following the initial DA treatment, at the peak of locomotor excitation. Locomotor activity following injection of haloperidol (mean = 28.9 ± 11.4 counts/min) decreased significantly ($p<0.01$) from pre-injection levels (mean = 47.3 ± 8.8 counts/min). This decrease occurred within five min following haloperidol injection, and was followed by a continued decline in activity throughout the remainder of the session (Fig. 2).

A significant increase in locomotor activity occurred following the injection of 50 μg DA into the head of the caudate nucleus. However, the absolute maximum effect for the group (N=6, mean = 38.88 ± 11.54 counts/min) was significantly ($p<0.01$) less than that produced by the IA injection of the same amount of DA (mean = 93.5 ± 13.6 counts/min). This relative difference in locomotor effects is presented graphically in Fig. 3. The latency to onset of increased locomotor activity following intra-caudate injection (mean = 116.0 ± 29.59 min) was also longer than that following IA injection of DA (mean = 77.5 ± 25.1 min).

Systemic injection of 0.05 mg/kg haloperidol produced profound catalepsy in the five young (587–750 g) males, but not the five mature (963–1175 g) primates (Table 2). The cataleptic response produced by the systemic haloperidol appeared at approximately 30–40 min post-injection, at which time 25 μg DA was injected IA. The IA DA had no effect on the catalepsy, which persisted in some cases for as long as 1 hr. Although all animals were routinely pretreated

TABLE 2

RATIO* OF MONKEYS EXHIBITING CATALEPSY FOLLOWING TREATMENT WITH HALOPERIDOL (INTRACRANIAL INJECTIONS ARE BILATERAL)

	Treatment					
	Hal 0.05 mg/kg SC no pretreatment with tranylcypromine	Hal 0.05 mg/kg SC + 4 μl saline IA	Hal 0.05 mg/kg SC + 25 μg DA IA	Hal IA 10 μg	Hal IC 5 μg	Hal IC 10 μg
Young Monkeys	5/5	5/5	5/5	1/3	1/2	0/0
Mature Monkeys	0/5	0/1	0/1	0/4	0/1	0/5
Total	5/10	5/6	5/6	1/7	1/3	0/5

*Number of animals exhibiting catalepsy/number treated.
IA=intra-accumbens.
IC=intra-caudate.

with the monoamine oxidase inhibitor tranylcypromine, its omission in one group had no effect on haloperidol-induced catalepsy (Table 2).

Various routes and combinations of treatment were used to assess the role of the nucleus accumbens in the cataleptic response (Table 2). One animal showed catalepsy in response to intracranial haloperidol (Table 2) immediately following a 5 μg injection into the caudate nucleus, while a 10 μg injection into the nucleus accumbens of this animal produced catalepsy 2.5 hr post-injection.

DISCUSSION

The increase in locomotor activity produced in squirrel monkeys by the IA application of DA confirms earlier studies on rats [1, 7, 11] and our preliminary study on squirrel monkeys [4]. However, the biphasic nature of the response differs markedly from the rapid onset of increased locomotor activity in rats. A biphasic response having an initial period of depressed activity has been reported to occur in mice following treatment with DA agonists [2,5]. The biphasic response to IA DA seen in squirrel monkeys may be analogous to the quieting of children with amphetamine, which is followed hours later by an increase in activity [12].

The mechanism underlying the initial period of locomotor quiescence is unknown. In this study it is unlikely that it was a delayed response due to diffusion of the DA to an active site beyond the nucleus accumbens because: (1) latency tended to be positively related to dose, and (2) IA haloperidol rapidly reversed the DA-induced locomotor activity. Several other possible explanations for the quiescent period can be suggested. The large amount of DA injected IA may facilitate release of norepinephrine (NE) or may be converted to NE, the latter being responsible for the quiescent phase. This concept is supported by the work of Dolphin *et al.* [5] who noted that the depressed portion of the biphasic response in mice to the DA agonist bromocriptine was associated with an apparent increase in forebrain NE turnover.

Other possible mechanisms for the quiescent phase include negative feedback via presynaptic DA receptors, activation of high-threshold inhibitory post-synaptic receptors [10], or Shore's [13] theory that exogenous DA must be taken up by DA terminals, stored in granules and released in order to produce post-synaptic effects. Speculation regarding these alternatives is beyond the scope of the present report.

The weak locomotor response to intra-caudate injection of DA and its long latency to onset suggests that diffusion of DA to some other site (possibly the nucleus accumbens) had occurred. At any rate, the accumbens site was more responsive than the caudate in terms of eliciting locomotor activity.

Interestingly, the presumed inhibition of DA receptors in the nucleus accumbens by the injection of haloperidol did not readily result in catalepsy. Yet catalepsy was readily induced by systemically administered haloperidol in amounts (37–50 μg) often less than twice the total amount (20 μg) injected directly into the accumbens. Maturity of the animal seemed to play a role in the cataleptic response to systemic haloperidol since only the younger animals showed this effect. The long delay to the single response to IA haloperidol was sufficient for diffusion of the drug to other areas, and as catalepsy was readily produced by systemic injection, it seems likely that some site other than the nucleus accumbens is involved in the cataleptic response. Recent work by others [9] suggests that the corpus striatum is an important site in the mechanisms associated with neuroleptic-induced catalepsy. However, only one of eight animals injected intra-caudate with haloperidol in the present study showed catalepsy of rapid onset. Thus, it appears that increased DA activity in the nucleus accumbens is associated only with increases in locomotor activity, as inhibition of the DA receptors in this nucleus via IA haloperidol blocks the increased locomotor activity following IA DA, but fails to produce catalepsy in animals not pretreated with IA DA.

In summary, our original subjective observations on the effects of intra-accumbens application of dopamine in squirrel monkeys [4] have been supported and to a degree quantified and expanded, but much remains to be explained concerning the role of biogenic amines in the nucleus accumbens.

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