Pharmacology Biochemistry & Behavior, Vol. 19, pp. 231–234, 1983. © Ankho International Inc. Printed in the U.S.A.

An *In-Vivo* Method for Testing Drugs That Influence Striatal Dopaminergic Functions

Y. K. FUNG

Department of Pharmacology, College of Pharmacy, Ohio Northern University, Ada, OH 45810

AND

R. D. SCHWARZ[1]

Department of Pharmacology, College of Medicine, The Ohio State University, Columbus, OH 43210

Received 31 January 1983

FUNG, Y. K. AND R. D. SCHWARZ. *An* in-vivo *method for testing drugs that influence striatal dopaminergic functions.* PHARMACOL BIOCHEM BEHAV **19**(2) 231–234, 1983.—A procedure is described for evaluating the effects of drugs on dopaminergic function in the striatum of mice. Mice, anesthetized with chloral hydrate are injected with 6-hydroxydopamine into one striatum to destroy dopamine nerve terminals at this site. Several days later, the mice are anesthetized with halothane, and test drugs or saline are injected into the intact striatum either before or after the systemic administration of amphetamine. The effect of these drugs on amphetamine-induced circling behavior is evaluated. Using this model, we have found that pilocarpine and muscimol can inhibit amphetamine-induced circling and their effects are blocked by the systemic administration of scopolamine and picrotoxin, respectively. In addition, ethyleneglycol-bis-(β-amino-ethyl ether) N,N'-tetraacetic acid (EGTA), a calcium chelating agent, inhibited amphetamine-induced circling behavior and this effect is prevented by adding calcium to the EGTA solution. Finally, the intrastriatal administration of prostaglandin E_2 but not its metabolite, 13,14-dihydro-15-keto-prostaglandin E_2 inhibited amphetamine-induced circling suggesting a selective effect of the active prostaglandin. These results suggest that this procedure could be used for evaluating both the mechanism of action of drugs in the striatum as well as screening drugs for their therapeutic potential.

Intrastriatal injection Dopamine Circling behavior

AFTER destruction of the striatal dopaminergic pathway on one side of the brain with 6-hydroxydopamine (6-OHDA), rodents will circle in response to drugs that release dopamine (DA) from presynaptic nerve terminals or that act directly on DA receptors [10, 11, 13]. Thus, in response to the administration of amphetamine which releases DA from the presynaptic nerve terminals in the intact striatum, animals will rotate toward the lesioned side (ipsilateral rotation). In contrast, a direct-acting DA agonist, such as apomorphine, will produce rotation in the opposite direction, i.e., away from the lesioned side (contralateral rotation). This is probably caused by a direct effect of apomorphine on supersensitive postsynaptic DA receptors that were denervated by 6-OHDA administration [9,11]. This circling model is used extensively to assess striatal dopaminergic function *in-vivo*.

Dopaminergic function in the striatum is altered by a variety of agents [6]. The neuronal circuitry of the striatum is known to contain dopaminergic, cholinergic and GABAergic neurons [1, 4, 5] with dopaminergic function being influenced by agents that interact with these neurotransmitter systems [6]. In addition, endogenous substances such as prostaglandins may also modify dopaminergic function. The present study presents a simple technique which might prove valuable to localize the action of agents that affect striatal function. This is achieved by the direct intrastriatal injection of various agents into the intact striatum of mice previously lesioned in one striatum with 6-OHDA.

METHOD

Animals

Male Swiss-Webster mice (Laboratory Supply, IN) weighing between 23–30 g were allowed free access to food (Purina Laboratory Chow) and water and were housed in plastic cages (5/cage) in a room maintained at 23±1°C with an automatic 12-hr light-dark cycle. The animals were housed for at least 2 days before surgery to allow them to become acclimated to the animal facilities.

Drugs

EGTA was dissolved in 0.1 M N-2-hydroxyethyl-

[1]Requests for reprints should be addressed to R. D. Schwarz at his present address: Department of Pharmacology, Warner Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48106.

piperazine-N'2 ethanesulfonic acid buffer adjusted to pH 7 with sodium hydroxide. 6-Hydroxydopamine hydrobromide (6-OHDA HBr) and d-amphetamine sulfate were purchased from Sigma Chemical Company. For other intrastriatal injections, the drugs were dissolved in saline and the pH adjusted to 7.

Neurosurgery

6-OHDA-induced lesions of the right striatum of mice. The mice were anesthetized with chloral hydrate (420 mg/kg, IP). After the incision was made in a longitudinal direction, we injected 4 microliters (μl) of chilled physiological saline, containing 16 micrograms (μg) of 6-OHDA HBr and 2.4 μg of ascorbic acid into the right striatum of the mice over a 4-minute interval, using a stereotaxic instrument (David Kopf) (incisor bar at same height as intraaural line) and a 10 μl Hamilton syringe. The solution was injected into the center of the corpus striatum which was 3.5 mm below the skull surface, 2.2 mm lateral to the midline and 5 mm anterior to the occipital suture. Employing the same anterior and lateral coordinates, we made a hole in the left side of the skull for drug injections into the left striatum to be done at a later time. The incision was closed with one wound clip.

"Free-hand" intrastriatal injections into the left intact striatum. A small piece of cotton, soaked in halothane, was placed in a 10 ml beaker, which was taped to the bottom of a 400 ml beaker. The larger beaker was covered with a petri dish. Under these conditions, the beaker was saturated with halothane within 3–4 min. A mouse was then placed inside the beaker until it was anesthesized, as indicated by a slow rate of breathing and shallow respiration. The mouse was then taken from the beaker, the wound clip removed from the incision site, and the drugs injected into the striatum. If, during the manipulation of the animal, the mouse showed signs of recovery from anesthesia, additional halothane was applied by placing a 10 ml beaker containing a cotton ball soaked with halothane near the animal's nose. If the mouse stopped breathing due to too much inhalation of halothane, it was revived by blowing air into its lungs with a 1 ml glass syringe.

The various compounds to be injected intrastriatally were dissolved in 0.9% saline and the pH of the solution adjusted to 7. Two microliters of this solution were injected into the left striatum with a 10 microliter Hamilton syringe. The syringe was fitted with a polyethylene cuff so that only the distal 3.8 mm of the needle was exposed. Under these conditions, the oriface of the needle was 3.5 mm below the skull. The "free-hand" injection was made over a period of 15 seconds, before withdrawing it from the skull. The incision was closed with a wound clip. All animals recovered from halothane anesthesia within 2–3 minutes after intrastriatal injection. The dose of drug injected intrastriatally was exposed as nmoles/g body weight.

Assessment of circling behavior. After amphetamine (4 mg/kg, IP) administration, the mice were placed in 2-liter round bottom flasks and the number of 360 degree turns to the right or left was observed and recorded at 3-minute intervals. Net ipsilateral turns toward the lesioned side were calculated by subtracting the turns to the left from the turns to the right. Different mice were used in each experiment and they were used only once.

In order to determine the success of the 6-OHDA-induced lesions, we injected mice with amphetamine (4 mg/kg, IP) 5 days after surgery and determined their circling behavior at 25 minutes after the amphetamine injection, the time of peak amphetamine effects [2]. Only those lesioned mice that circled 10 or more times in 2 minutes were selected for testing the effects produced by the intrastriatal administration of various chemicals the following day.

RESULTS

Effect of Intrastriatal Injection of Saline

To control for the effects produced by the intrastriatal administration of pilocarpine, muscimol, and EGTA, saline was injected intrastriatally 10 minutes before the systemic administration of amphetamine, and circling behavior was determined 25 minutes after amphetamine. As shown in Table 1, amphetamine produced marked circling behavior in saline-treated animals.

The effects produced by the intrastriatal administration of prostaglandins are present for only short periods of time. Consequently, prostaglandins or saline was injected intrastriatally 10 minutes after amphetamine and circling measured 20 minutes later. As reported above, the mice injected with amphetamine and saline on this time schedule also exhibited marked circling behavior (Table 1).

Effect of the Intrastriatal Injection of Various Compounds on Amphetamine-Induced Circling

The intrastriatal administration of pilocarpine, a muscarinic receptor agonist, produced a marked inhibition of amphetamine-induced circling (Table 1). In order to determine whether this effect of pilocarpine was mediated by the activation of cholinergic muscarinic receptors, scopolamine was administered (5 mg/kg, IP) 15 minutes before pilocarpine. Scopolamine, when injected alone, produced a small increase in amphetamine-induced circling, but it was not statistically significant. However, scopolamine completely reversed the inhibition of amphetamine-induced circling produced by pilocarpine.

Similarly, muscimol, a GABA receptor agonist, also markedly inhibited amphetamine-induced circling, and this inhibition was blocked by pretreatment with picrotoxin (2 mg/kg, IP), a GABA antagonist.

Prostaglandins have been postulated to be neuromodulatory substances in the brain, and are found in the striatum [14]. Table 1 shows that prostaglandin E_2 (PGE_2) when injected intrastriatally, significantly inhibited amphetamine-induced circling at a dose of 0.1 nmole/g body weight of mouse. In contrast, the major PGE_2 metabolite, 13,14-dihydro-15-keto-PGE_2, failed to inhibit amphetamine-induced circling at the same dose.

Amphetamine has been shown to stimulate DA synthesis *in-vitro* and *in-vivo* by a calcium dependent mechanism [3, 7, 8, 12]. We have previously studied the importance of calcium in the behavioral effects of amphetamine by injecting EGTA into the intact striatum of mice previously injected with 6-OHDA in the other striatum, and then determining the effects of amphetamine [2]. Table 1 shows data that confirms our previous observations that the intrastriatal administration of EGTA inhibits amphetamine-induced circling, and that this effect is prevented by adding calcium to the EGTA solution.

DISCUSSION

This procedure provides a simple, rapid, and inexpensive means of examining the effects of various compounds on

TABLE 1

EFFECT OF THE INTRASTRIATAL INJECTION OF VARIOUS DRUGS ON AMPHETAMINE-INDUCED CIRCLING

Experiment No.	Treatment	n	No. of turns in 3 min
1	Saline, IS	7	30.2 ± 1.4
	Pilocarpine, IS (0.7 nmoles/g)	4	9.0 ± 3.0*
	Scopolamine, IP (5 mg/kg)	4	42.5 ± 4.5
	Scopolamine, IP—pilocarpine I.S.	4	29.3 ± 1.0
2	Vehicle, IS	4	33.4 ± 6.7
	EGTA, IS (2 nmole/g)	4	7.5 ± 1.5*
	$CaCl_2$, IS (5 nmole/g)	4	30.0 ± 1.7
	EGTA + $CaCl_2$	6	25.0 ± 2.0
3	Saline, IS	4	35.5 ± 2.9
	Muscimol, IS (0.004 nmoles/g)	4	5.0 ± 2.0*
	Picrotoxin, IP (2 mg/kg)	4	29.5 ± 6.9
	Picrotoxin + muscimol	4	29.0 ± 1.6
4	Saline, IS	4	33.3 ± 2.1
	Prostaglandin E_2, IS (0.1 nmole/g)	4	7.5 ± 2.6*
	13,14-Dihydro-15-keto prostaglandin E_2, IS (0.1 nmole/g)	4	29.7 ± 1.8

In Experiments 1, 2 and 3 either pilocarpine, muscimol, EGTA, or saline was injected intrastriatally (IS) 10 minutes before amphetamine (4 mg/kg, IP) and circling determined 25 min later. Scopolamine and picrotoxin were injected, IP, 15 and 10 minutes before either pilocarpine or muscimol, respectively. In Experiment 4, prostaglandin E_2 or its metabolite was injected IS, 10 min after amphetamine (4 mg/kg, IP) administration.

Each value is the mean ± S.E.M.

*$p<0.05$ Compared to controls, (Dunnett's test).

striatal mechanisms. Mice are initially anesthetized with chloral hydrate and a hole is made on each side of the skull directly above the striatum. 6-OHDA is then injected into one striatum. At a time subsequent to the degeneration of dopamine nerve terminals, mice are anesthesized with halothane and test drugs or saline are injected into the intact striatum either before or after amphetamine administration and their effect on amphetamine-induced circling behavior is evaluated.

This technique offers advantages over conventional procedures for injecting drugs directly into discrete brain sites. The procedure uses mice which are less expensive than rats. In addition, it alleviates the necessity to implant cannulas, which can be time-consuming and laborious. Our method does not involve extensive surgery or manipulation, and many animals can be tested in a short period of time. This can be particularly valuable in behavioral experiments where large numbers of animals may be required because of the high degree of variability in the behavioral response.

Our procedure does require the use of an anesthetic during the injection of the test drugs into the striatum. We have chosen to use halothane since in mice it can produce anesthesia with rapid recovery from anesthesia occurring within 2–3 minutes after withdrawal. Since a hole has been previously drilled in the skull for the injection of the test drugs into the striatum, the administration of these drugs can be done rapidly, usually within 2 minutes. Only on occasion has it been necessary to use additional halothane to maintain anesthesia during intrastriatal injection. We have tried using other anesthetics such as methoxyfluorane and ether, but recovery from these anesthetics was much slower than with halothane, the mice exhibiting an ataxic gait for 40–60 minutes after withdrawal.

In summary, this procedure provides a method for assessing the effect of drugs on dopaminergic function in the striatum. The procedure is inexpensive and simple enough to allow a large number of animals to be studied in a short period of time.

ACKNOWLEDGEMENTS

We would like to acknowledge the secretarial work of Anna Mae Shadley.

REFERENCES

1. Butcher, S. B. and L. L. Butcher. Origin and modulation of acetylcholine activity in the neostriatum. *Brain Res* **71:** 167–171, 1974.

2. Fung, Y. K. and N. J. Uretsky. The importance of calcium in amphetamine-induced turning behavior in mice with unilateral nigro-striatal lesions. *Neuropharmacology* **19:** 550–560, 1980.

3. Fung, Y. K. and N. J. Uretsky. The importance of calcium in amphetamine-stimulated synthesis of dopamine in mouse striata, *in vivo*. *J Pharmacol Exp Ther* **223:** 477–482, 1982.
4. McGeer, P. L. and E. G. McGeer. Evidence for glutamic acid decarboxylase containing interneurons in the neostriatum. *Brain Res* **91:** 331–335, 1975.
5. McGeer, P. L., E. G. McGeer, H. C. Fibiger and U. Wickson. Neostriatal choline acetylase and acetylcholinesterase following selective brain lesions. *Brain Res* **35:** 308–314, 1971.
6. Pycock, C. J. Turning behavior in animals. *Neuroscience* **5:** 461–514, 1980.
7. Roberts, M. and R. L. Patrick. Amphetamine and phenethylamine-induced alterations in dopamine synthesis regulation in rat brain striatal synaptosomes. *J Pharmacol Exp Ther* **209:** 104–110, 1979.
8. Schwartz, R. D., N. J. Uretsky and J. R. Bianchine. The relationship between the stimulation of dopamine synthesis and release produced by amphetamine and high potassium in striatal slices. *J Neurochem* **35:** 1120–1127, 1980.
9. Thornburg, J. E. and K. E. Moore. Supersensitivity to dopamine agonists following unilateral 6-hydroxydopamine-induced striatal lesions in mice. *J Pharmacol Exp Ther* **192:** 42–49, 1975.
10. Ungerstedt, U. 6-Hydroxydopamine-induced degeneration of central monoamine neurons. *Eur J Pharmacol* **5:** 107–110, 1968.
11. Ungerstedt, U. Striatal dopamine release after amphetamine on nerve degeneration revealed by rotational behavior. *Acta Physiol Scand* Suppl **367:** 49–68, 1971.
12. Uretsky, N. J. and S. R. Snodgrass. Studies on the mechanism of stimulation of dopamine synthesis by amphetamine in striatal slices. *J Pharmacol Exp Ther* **202:** 565–580, 1977.
13. Von Voigtlander, P. F. and K. E. Moore. Turning behavior of mice with unilateral 6-hydroxydopamine lesions in the striatum: Effects of apomorphine, L-DOPA, amantadine, amphetamine and other psychomotor stimulants. *Neuropharmacology* **12:** 451–462, 1973.
14. Wolfe, L. S. Possible roles of prostaglandins in the nervous system. *Adv Neurochem* **1:** 1–49, 1975.