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Blockade of MK-801 Induced Ipsiversive Turning in 6-OHDA Lesioned Rats by α_1-Adrenoceptor Antagonists

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MARTIN, G E AND N. L. PAPP. *Blockade of MK-801 induced ipsiversive turning in 6-OHDA lesioned rats by α_1-adrenoceptor antagonists* PHARMACOL BIOCHEM BEHAV **20**(6) 893–897, 1984.— Previously the ipsiversive turning response elicited by MK-801 in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra has been shown to be reduced by the α_1-receptor antagonist, prazosin. In these experiments the effects of additional α_1-adrenoceptor antagonists were examined to verify the involvement of α_1-adrenoceptors in the elucidation of the ipsiversive turning response elicited by MK–801 Both aceperone and azapetine did significantly reduce the ipsiversive turning evoked by MK-801. In contrast, neither agent produced a statistically significant reduction in the contraversive turning evoked by the direct acting dopamine agonist, apomorphine. In addition, aceperone also produced a weak but dose-related inhibition of amphetamine-induced ipsiversive rotation, whereas azapetine partially reduced amphetamine-induced turning in a non-dose related manner. These data suggest α_1-adrenoceptors may be partially involved in the ipsiversive turning response caused by MK-801 and to a lesser extent by amphetamine. This theory was further supported by the finding that reduction of endogenous norepinephrine levels, via administration of the dopamine-β-hydroxylase inhibitor FLA-63, markedly reduced the turning evoked by MK-801 and to a lesser degree that produced by amphetamine.

MK-801 Amphetamine α_1-Adrenoceptors Aceperone Azapetine

MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine] is a unique pharmacological agent insofar as it simultaneously possesses potent anticonvulsant [3] anxiolytic [4] and sympathomimetic properties [5]. The sympathomimetic property of this compound is manifest in the turning elicited from the rat with a unilateral lesion of the substantia nigra created by 6-hydroxydopamine (6-OHDA). MK-801 produces turning ipsiversive to the lesion similar to the direction of turning evoked by amphetamine in this model. Amphetamine is thought to produce the turning indirectly via the release of endogenous dopamine [15]. The ipsiversive direction of the MK-801-induced turning response, therefore, suggests that MK-801 releases endogenous stores of dopamine [15]. Indeed, the turning elicited by MK-801 is blocked by either dopamine receptor antagonist haloperidol or clozapine and is severely attenuated in rats in which endogenous catecholamines have been depleted with reserpine or α-methylparatyrosine [4]. More intriguing perhaps is the finding that the α_1 receptor antagonist, prazosin, can also attenuate MK-801-induced turning in a dose-related fashion [4]. Furthermore, although more potent *in vivo* in eliciting turning, MK-801, added to a push-pull medium perfusing the striatum, does not evoke the release of ^{3}H-dopamine newly synthesized from ^{3}H-tyrosine as do amphetamine and amfonelic acid, two agents which produce ipsiversive turning in this rat model [12]. The present paper further examines the possible role of adrenergic receptors in the ipsiversive turning evoked by MK-801 and amphetamine by testing the action of two additional α_1-adrenoceptor antagonists with different chemical structures, aceperone [2] and azapetine [14] on the turning response evoked by MK-801. Aceperone is an α_1-antagonist that is a butyrophenone derivative with very weak antidopaminergic blocking activity [9,10]. Azapetine, on the other hand, is an α_1-antagonist that acts primarily at postsynaptic sites [14]. The effect of depletion of endogenous norepinephrine with a dopamine-β-hydroxylase inhibitor, FLA-63 [5], on MK-801-induced turning was also examined.

METHOD

Animals

Anesthetized female Sprague-Dawley (Blue Spruce Farms, Altamont, NY) rats weighing 140–170 g were placed in a stereotaxic frame for microinjection of 6-hydroxydopamine hydrobromide (6-OHDA) dissolved in 0.9% saline containing 0.1 mg/ml of ascorbic acid. Using coordinates of 3.0 mm anterior, 2.0 mm lateral, and −3.0 mm dorsoventral (derived from the atlas of Konig and Klippel [11]), a 28-ga stainless steel cannula for the microinjection was lowered just rostral to the right substantia nigra. Four μl containing 8 μg (base) of 6-OHDA were then injected over a 4-min period using a Sage syringe pump. One and two weeks following surgery, each rat was given 1.0 mg/kg IP of apomorphine and its turning was measured in an automated rotometer [8] for two consecutive 30-min intervals. Any

animal which did not turn in a direction contralateral to the lesion at least 90 turns/30 min was discarded. In subsequent experiments, no animal in the colony was used more than once a week

Drug Pretreatment

A 50 μg/kg dose given PO was selected as the test dose of MK-801 since it produced robust turning in previous studies. A crossover design was used to study the putative antagonists of MK-801-induced turning. As pretreatment, one-half of the rats in a given study were administered the vehicle (0.5% Methocel or 0.9% saline) and the other half the test compound (SC) one hour before MK-801 was administered PO. Complete turns were then recorded in rotometers for consecutive 30-min epochs. The number of 30-min epochs examined differed with the drug used. Since the number of turns elicited by MK-801 in the initial half-hour after dosing was quite low, turns during this initial half-hour period were not recorded in all tests. After an interval of at least seven days, the counterbalanced design was completed by switching the pretreatments. Any vehicle pretreated animal which failed to turn ≥45 turns/30 min during any 30-min epoch was eliminated from the statistical analyses. Hence, some of the studies were not completely balanced; viz., there was not an equal number of vehicle and drug pretreated rats for both halves of the experiment. Whether or not a given pretreatment produced a significant fall in the number of turns produced by MK-801 was determined using a simple crossover analysis, $p<0.05$ [12]. Where possible the dose required to produce a 50% inhibition of turning was determined using log probit paper [13]. A similar experiment was conducted using amphetamine (1 mg/kg IP) in place of MK-801.

The effect of FLA-63 on MK-801-induced turning was also examined using a counterbalanced design. FLA-63, 25 mg/kg SC, was given three hours before either amphetamine (1.0 mg/kg IP) or MK-801 (50 μg/kg PO). The mean number of turns for the 30 to 90 minute period after dosing with MK-801 was compared between pretreatment regimens using a simple crossover analysis.

As a control for a possible non-specific ataxic effect of the α_1-receptor blocking drugs, azapetine (6–24 mg/kg SC) and aceperone (3–24 mg/kg SC) were given to rats which were placed on a rotorod revolving at 6 revolutions/minute. A rat was considered ataxic if it failed in three attempts to remain on the rod for 15 consecutive seconds.

For purposes of comparison the effects of aceperone and azapetine on turning elicited by the direct acting dopamine agonist apomorphine (1 mg/kg IP) were also ascertained. The design and statistical analyses were similar to those just described. The doses of amphetamine and apomorphine were selected from previous studies as doses which would be comparable to the dose of MK-801 in the number of turns elicited.

Drugs

All dose levels are given in the results section and are expressed as the free base. MK-801 was synthesized in the Department of Medicinal Chemistry by Dr. Paul Anderson. The following compounds were obtained from the indicated sources as gifts: aceperone (Janssen Pharmaceuticals, New Brunswick, NJ); azapetine (Hoffmann-LaRoche, Nutley, NJ), FLA-63 (Astra Pharmaceuticals, Sodertalje, Sweden); amfonelic acid (Sterling Winthrop, Rensselaer, NY); methylphenidate (CIBA, Summit, NJ). Amphetamine sulfate was made at Merck and apomorphine was purchased from Sigma (St. Louis, MO).

TABLE 1

ACTION OF ACEPERONE ON THE IPSIVERSIVE TURNING ELICITED BY MK-801† (50 μg/kg PO) IN FEMALE RATS WITH A UNILATERAL 6-OHDA LESION IN THE SUBSTANTIA NIGRA

Pretreatment (mg/kg SC, −1 hr)	n	Mean Turns ±SEM*	% Inhibition‡
Aceperone (24)	7	14 ± 6§	96
Vehicle	7	275 ± 89	
Aceperone (6)	10	175 ± 33§	58
Vehicle	10	412 ± 65	
Aceperone (0.6)	10	295 ± 65§	52
Vehicle	10	619 ± 94	
Aceperone (0.3)	11	101 ± 24§	54
Vehicle	11	221 ± 27	
Aceperone (0.075)	9	304 ± 110§	46
Vehicle	9	559 ± 124	

*Turns ipsilateral to the lesioned substantia nigra for the 90 minutes after treatment

†% inhibition = 100 [(mean vehicle group − mean treatment group)/mean vehicle group]

‡Significantly less than vehicle pretreated control rats, $p<0.05$ simple crossover analysis $ID_{50}\approx 250$ μg/kg SC

RESULTS

Both α_1-adrenoceptor antagonists, aceperone and azapetine, significantly reduced the ipsilateral turning evoked by 50 μg/kg of MK-801 in a dose-related fashion (Tables 1 and 2). The dose-response curve charting blockade of MK-801-induced turning vs. the dose of aceperone given was quite steep. Using log probit paper, a dose of 250 μg/kg was estimated to be the dose required to reduce MK-801-induced turning by 50% (ID_{50}). Since the dose-response curve was so steep, it precluded determination of 95% confidence limits. The dose-response curve for azapetine was also quite steep, preventing a precise determination of the dose required to inhibit turning by 50%. However, a dose of 8 mg/kg was determined as an approximate ID_{50} value using log probit paper. When given prior to MK-801 aceperone produced statistically significant falls in turning in a dose range of 0.075 to 24 mg/kg (Table 1). As suggested by their respective ID_{50} values, azapetine was less potent than aceperone, producing statistically significant diminutions in MK-801-induced turning following the 12 and 18 mg/kg doses, but failing to do so when given in the dose of 6 mg/kg (Table 2).

In contrast to the marked inhibition of MK-801-evoked turning by aceperone, this α_1-antagonist given at the top dose of 24 mg/kg produced only a 44% reduction of the turning produced by amphetamine (Table 3). Nonetheless, aceperone, administered in doses of 24 and 6 mg/kg, did produce statistically significant reductions in the ipsiversive turning elicited by amphetamine (1.0 mg/kg IP). Although no dose of aceperone produced a 50% or greater reduction in turning elicited by amphetamine, aceperone did reduce turn-

TABLE 2

ACTION OF AZAPETINE ON THE IPSIVERSIVE TURNING ELICITED BY MK-801 (50 μg/kg PO) IN FEMALE RAT WITH A UNILATERAL 6-OHDA LESION IN THE SUBSTANTIA NIGRA

Pretreatment (mg/kg SC, −1 hr)	n	Mean Turns ±SEM*	% Inhibition†
Azapetine (18)	9	96 ± 22‡	89
Vehicle	9	612 ± 65	
Azapetine (12)	8	161 ± 93‡	62
Vehicle	8	421 ± 84	
Azapetine (6)	9	221 ± 47	38
Vehicle	9	360 ± 55	

*For the period 30 to 90 minutes post dosing
†Determined as in Table 1
‡Significantly less than vehicle pretreated control rats, $p<0.05$ simple crossover analysis $ID_{50}\approx 8$ mg/kg SC

TABLE 3

ACTION OF ACEPERONE ON THE IPSIVERSIVE TURNING ELICITED BY AMPHETAMINE (1.0 mg/kg IP) IN THE FEMALE RAT WITH A UNILATERAL 6-OHDA LESION IN THE SUBSTANTIA NIGRA

Pretreatment (mg/kg SC, −1 hr)	n	Mean Turns ±SEM*	% Inhibition†
Aceperone (24)	10	301 ± 59‡	44
Vehicle	10	533 ± 100	
Aceperone (6)	9	687 ± 155‡	23
Vehicle	9	897 ± 133	
Aceperone (0.6)	10	729 ± 135	10
Vehicle	10	809 ± 102	

*For the period 0 to 90 minutes post dosing
†Determined as in Table 1.
‡Significantly less than vehicle pretreated control rats, $p<0.05$ simple crossover analysis

TABLE 4

ACTION OF AZAPETINE ON THE IPSIVERSIVE TURNING ELICITED BY d-AMPHETAMINE (1.0 mg/kg IP) IN THE FEMALE RAT WITH A UNILATERAL 6-OHDA LESION IN THE SUBSTANTIA NIGRA

Pretreatment (mg/kg SC, −1 hr)	n	Mean Turns ±SEM*	% Inhibition†
Azapetine (24)	9	317 ± 54‡	33
Vehicle	9	473 ± 54	
Azapetine (18)	9	291 ± 55‡	30
Vehicle	9	414 ± 58	
Azapetine (12)	9	374 ± 55‡	37
Vehicle	9	594 ± 77	
Azapetine (6)	10	279 ± 61	35
Vehicle	10	426 ± 62	

*For the 90 minute period after treatment
†Determined as in Table 1
‡Significantly less than vehicle pretreated control rats, $p<0.05$ simple crossover analysis

TABLE 5

ACTION OF ALPHA-ADRENERGIC ANTAGONISTS ON ROTOROD PERFORMANCE IN THE FEMALE RAT ANIMALS WERE TESTED ONE HOUR AFTER DRUG ADMINISTRATION

Drug (mg/kg SC)		n	No. Ataxic*/ No. Dosed	% Ataxic
Prazosin	(3)	8	2/8	25
	(1)	8	1/8	12.5
	(0.33)	8	2/8	25
	(0.11)	8	0/8	0
Azapetine	(24)	8	1/8	12.5
	(18)	8	1/8	12.5
	(12)	8	1/8	12.5
	(6)	8	0/8	0
Aceperone	(24)	10	2/10	20
	(12)	10	5/10	50
	(6)	10	2/10	20
	(3)	10	2/10	20
Vehicle	(−)	18	4/18	22

*Ataxic = a rat unable to maintain its balance on a rotorod turning at 6 rpm for 15 sec when given three chances to remain on the rod

ing in a dose-related manner. Azapetine, on the other hand, produced a 30 to 37% reduction in amphetamine-evoked turning across the entire dose range tested (6–24 mg/kg, Table 4). The reductions in amphetamine-evoked turning were statistically significant following azapetine in doses of 24, 18 or 12 mg/kg SC. No dose of azapetine produced a reduction in amphetamine-induced turning as great as 50% of the control level.

Neither azapetine nor aceperone produced a dose-related decrement in the ability of rats to maintain their balance on a rotorod. Both exerted some action as shown in Table 5. Although aceperone caused half of 10 animals to fall from the rod after the 12 mg/kg dose, only 2 of 10 were unable to maintain their balance after the 24 mg/kg dose. Hence, neither agent caused a significant dose-related number of rats to fall from the rotorod relative to the vehicle-treated control rats. The observed reduction in amphetamine and MK-801-induced turning, therefore, was probably not due to a non-specific action of either agent.

The dopamine-β-hydroxylase inhibitor FLA-63 (25 mg/kg SC, −3 hours) also produced a significant reduction in the ipsilateral turning response elicited in 6-OHDA-lesioned rats by either MK-801 or d-amphetamine (Table 6). The magnitude of the inhibition, however, was three times greater for MK-801 (72%) than for amphetamine (24%).

The contraversive turning produced by the direct acting

TABLE 6

MEAN IPSILATERAL TURNS ELICITED FROM THE FEMALE SPRAGUE-DAWLEY RAT WITH A UNILATERAL 6-OHDA LESION IN THE SUBSTANTIA NIGRA FOLLOWING THE ADMINISTRATION OF d-AMPHETAMINE OR MK-801 DATA ARE PRESENTED FOR TURNS EVOKED FOLLOWING PRETREATMENT WITH EITHER FLA-63 OR A VEHICLE SOLUTION

Pretreatment (mg/kg SC −3 hr)	n	Mean Turns (±SEM) for the 30–90 Minute Interval Post Treatment: Treatment	Mean Turns	% Reduction
FLA-63 (25)	9	d-amphetamine	484 ± 44	24
Vehicle		1 0 mg/kg IP	639 ± 59	
FLA-63 (25)	9	MK-801	75 ± 40	72
Vehicle		0 05 mg/kg PO	273 ± 63	

*Significantly less than vehicle pretreated control group, $p<0$ 05 simple cross-over analysis

TABLE 7

ACTION OF ACEPERONE OR AZAPETINE ON CONTRALATERAL TURNING INDUCED IN FEMALE RATS WITH A UNILATERAL 6-OHDA LESION IN THE SUBSTANTIA NIGRA BY APOMORPHINE (1 mg/kg IP)

Pretreatment (mg/kg SC −1 hr)	n	Mean Turns/ 30 Min + SEM: +30 Min	+60 Min	% Inhibition
Azapetine (24)	10	114 ± 20	131 ± 24	25
Vehicle*	10	161 ± 20	166 ± 26	
Aceperone (6)	9	176 ± 16	154 ± 22	2
Vehicle*	9	184 ± 23	154 ± 27	

*No significant difference at any time interval, $p<0$ 05, simple crossover analysis

dopamine agonist apomorphine was not significantly altered by aceperone (6 mg/kg SC, Table 7). Although azapetine did cause a 25% inhibition of apomorphine-induced turning, the reduction was not statistically signficant.

DISCUSSION

Employing two structurally distinct α_1-receptor antagonists, the present experiments verify the observation [4] that α_1-receptor blockade does indeed lead to a reduction in the number of turns elicited by MK-801 in the 6-OHDA-lesioned rat Although these experiments do further implicate adrenergic mechanisms in MK-801-induced turning they do not delineate whether this agent exerts a direct or indirect α-adrenergic effect One might surmise, however, that MK-801 exerts an indirect action on adrenergic receptors since MK-801 has previously been shown to have a low affinity for adrenergic receptors in receptor binding studies [5] and depletion of endogenous norepinephrine also reduces the turning evoked by MK-801.

The apparent lack of effect of either α_1-antagonist in blocking the contralateral turning evoked by the direct dopamine agonist, apomorphine, suggests that neither agent directly blocks dopamine receptor activation. Hence, each must exert its effect via adrenolytic activity. This finding is especially important for aceperone since it also possesses very weak antidopaminergic activity [9,10]. The results from the rotorod test show that a drug-induced lack of motor coordination was not responsible for the reduction in turning produced by the α_1-adrenergic receptor antagonists

The important role of endogenous norepinephrine in the ipsiversive turning is further delineated in the experiments in which selective depletion of norepinephrine markedly diminished MK-801-induced turning (Table 6).

The α_1-adrenergic receptors utilized in this study also partially blocked the ipsilaterally-directed turning elicited by amphetamine. Amphetamine is known to release endogenous dopamine as well as norepinephrine [1] from nerve terminals. The blockade of amphetamine-induced turning, however, was not as great as the blockade of the MK-801-induced turning, suggesting that the adrenergic releasing effects of amphetamine may be less important to the evocation of ipsilateral turning than in the case of MK-801. It is interesting to note that when perfused locally within the striatum in equimolar concentrations, amphetamine does evoke the efflux of ^{3}H-dopamine newly synthesized from ^{3}H-tyrosine, whereas MK-801 does not [12]. Perhaps this is due to the fact that if MK-801 does elicit dopamine release when given IP, it

does not exert this effect directly within the striatum. Alternatively, MK-801 may preferentially evoke norepinephrine release which may then modulate turning either directly at adrenergic sites somewhere in the brain or via a noradrenergic receptor modulating dopamine release. The α_1-adrenergic antagonist prazosin has previously been shown to reduce not only the turning elicited by MK-801, but also MK-801-induced increases in locomotor activity [4] and its anticonvulsant activity [3]. Since the turning elicited by MK-801 is ipsilateral to the lesion and it can be abolished by dopamine receptor antagonists, it seems to occur via release of endogenous dopamine [15], MK-801 unlike amphetamine does not elicit a full blown stereotypic response which is thought to be mediated by dopamine receptor activation [7].

So MK-801 shares some common properties with amphetamine, yet it retains a unique profile of action Although the precise site of action is unknown, as well as the specific mechanisms, it is clear that some of the effects of MK-801 are mediated via α_1-receptors. Further studies will have to be done to determine the precise site and mechanism of action of MK-801.

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