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Relative Importance of the Dopaminergic System in Haloperidol-Catalepsy and the Anticataleptic Effect of Antidepressants and Methamphetamine in Rats

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AL-KHATIB, Iz. M. H., M. FUJIWARA AND S. UEKI. *Relative importance of the dopaminergic system in haloperidol-catalepsy and the anticataleptic effect of antidepressants and methamphetamine in rats.* PHARMACOL BIOCHEM BEHAV **33**(1) 93–97, 1989.—The mechanisms involved in haloperidol (HPD)-catalepsy in rat and the effect of antidepressants and methamphetamine (MA) were studied. HPD-catalepsy, as measured by high bar test, lasted for 6–8 min. MA, imipramine (IMP), nomifensine (NOM) and mianserin (MIAN) reduced the duration of catalepsy on IP injection. Electrolytic lesion of the caudate-putamen (CP) and nucleus accumbens (ACC) extensively reduced HPD-catalepsy. Microinjection of MA and NOM into ACC had a similar effect. In the medial amygdala and CP, only MA displayed anticataleptic activity. Zimelidine did not reduce the duration of catalepsy. These results suggest that dopaminergic systems play a key role in mediating HPD-catalepsy and the anticataleptic activity of MA and NOM.

Rat Haloperidol Catalepsy Antidepressants Methamphetamine

CATALEPSY in the rat is an experimentally-induced akinesia characterized by an organized tonic reaction in which a stable static equilibrium prevails and the rat displays an exaggerated bracing reaction with typical posture, i.e., broad based support and kyphotic spine (6). Haloperidol (HPD) blocks dopamine (DA) receptors (20). It is a cataleptogenic neuroleptic agent (19, 22, 26) and elicits extrapyramidal side effects (15) that parallel its cataleptogenic effect. DA agonists antagonize HPD-catalepsy (12). Moreover, various treatments which indirectly influence the dopaminergic function, also modify HPD-catalepsy. These treatments include histamine histamine$_1$ blockers, γ-aminobutyric acid (24) and ablation of frontal cortex (25). Cholinergic and serotonergic interactions also play a role in this regard (12). Bilateral injection of HPD into caudate-putamen (CP), globus pallidus and nucleus accumbens (ACC) reportedly induce catalepsy accompanied by ptosis in rat (4,9). Lesions of CP and ACC reduce HPD-catalepsy (5,23).

This study was performed to determine the effects on HPD-catalepsy of various drugs of different mechanisms of action, at least as the dopaminergic function is concerned. Of the antidepressant drugs tested, only nomifensine exerts prominent agonistic effect on DA receptors (8). NOM is a potent DA-reuptake blocker (11) and its dihydroxy metabolite directly stimulates DA receptors (17). Imipramine (IMP) exerts only a poor effect on DA uptake (10). Mianserin (MIAN) exerts no significant effect on the amine reuptake but it blocks the presynaptic α_2-receptors (1), serotonin$_2$ and histamine$_1$ receptors (16). However, IMP and MIAN potently inhibit the binding of HPD in rats' striatal membranes (8). Zimelidine (ZIM) also has no dopaminergic property, but it blocks serotonin reuptake (18).The study also delineated the brain regions involved in the exhibition of HPD-catalepsy and the effects of drugs with anticataleptic activity.

METHOD

Animals

Male Wistar rats (Kuroda Experimental Animals, Kumamoto,

Japan) weighing 180–200 g were housed, 5 per Plexiglas cage(42 × 26 × 15 cm), for a week under standardized conditions of temperature (22 ± 1°C), relative humidity (55 ± 5%) and controlled lighting (lights on 07.00–19.00) with free access to food and water.

Catalepsy Test

Catalepsy was measured using the high bar test. Rats were grasped gently and the front paws placed over a bar of 4 mm diameter located 12 cm above the plate surface. The hind paws were braced to 2 pillars which were 14 cm apart and supported the bar. The rat was then gently released. The animal was timed until either at least one front paw touched the plate surface or the hind feet raised from the plate surface to climb upon the bar or descent down of the rat. The catalepsy test was carried out in a sound-proof room and inside a wood box consisting of a 5 cm thick walls and with 20 × 20 × 30 cm inner space. The box had a Plexiglas front door. The intensity of catalepsy was measured as a function of time (min) the rat remained in the cataleptic state.

Brain Lesioning

Bilateral electrical lesions of CP and ACC were performed under 40 mg/kg IP sodium poentobarbital anesthesia. A direct current of 3 mA for 20 sec was applied through stainless steel electrodes 0.4 mm in diameter, insulated except for 0.5 mm at the tip. The electrodes were implanted according to König and Klippel (14). The stereotaxic coordinates were: rostral (r)CP: frontal from lambda (F) = 9.0, lateral from the mid sagittal line (L) = 2.3, horizontal from the surface of skull (H) = 5.5; caudal (c)CP: F = 7.0, L = 3.0, H = 5.6. For rACC: F = 9.6, L = 1.1, H = 7.1; cACC: F = 8.6, L = 1.5, H = 7.5. Because of the large volume of CP and ACC, lesions were produced simultaneously in the rostral and caudal parts of either region.

Microinjection Into Brain Regions

Injections were made in unanesthetized, manually restrained rats. Motor-driven microsyringes (Scientific Glass Engineering, PTY, Australia) were connected by a polyethylene tube (PE-10) to injection cannulae of 0.35 mm outer diameter, directed via stainless steel guide cannulae of 0.7 mm outer diameter and 15 mm length. The guide cannulae were implanted bilaterally in the following regions: medial amygdala (AME): F = 5.7, L = 3.3, H = 8.0. cCP: F = 7.0, L = 3.0, H = 5.1. cACC: F = 8.6, L = 1.5, H = 7.0. Lateral septum (LS): F = 8.7, L = 0.5, H = 4.5. Injection cannulae were designed so that they protruded 0.5 mm beyond the tips of guide cannulae to end at the desired brain regions. The cannulae were cemented to screws fixed in the skull using dental cement, and when not in use were occluded with stainless steel stylets.

Experimental Paradigm

Prior to the catalepsy experiments, rats were tested in three trials for catalepsy. Generally, rats did not retain the cataleptic posture and soon left the bar, except in a few cases (20/280) where some rats grasped the bar for about 5 sec. In such cases the duration of catalepsy was corrected for. Each rat received only one drug (besides HPD) to avoid any residual drug effect. In IP injection, saline or one of the test drugs was injected 30 min before HPD 2 mg/kg and catalepsy was tested 30 min and 1 hr after HPD. For each drug, injections were started with the lowest dose and proceeded to a higher dose with an interval of 5 days. One week was allowed for the animals to recover after either lesioning or implantation of the cannulae. In the microinjection experiments into ACC or CP, 5 rats/group were injected bilaterally with one drug or saline into the specific brain regions 30 min after HPD. Catalepsy was measured 5 min and 30 min later. Each rat received two injections with an interval of 5 days between the first and second injections. Five brain-lesioned rats received the first injection of either saline or one drug and after 5 days they received a second injection. Catalepsy was tested as mentioned for IP injection.

Verification of Lesion and Injection Sites

At the end of each experiment and under deep ether anesthesia, brains were perfused with 10% formalin through the carotid artery, removed and fixed with formalin for a week. Frozen sections (80 μm) were sliced and stained with cresyl violet. The sites and extent of microinjection and lesion were verified histologically. Data from rats with aberrant lesion sites (only 5/35 in ACC) or injection sites (3/33 in AME, 4/34 in ACC and 5/35 in LS) were excluded.

Drugs

Haloperidol, HPD (seranase ampules) and methamphetamine HCl, MA (philopon powder) were obtained from Dainippon. Antidepressants used were: nomifensine maleate, NOM (Hoechst), imipramine HCl, IMP (Sigma), mianserin HCl, MIAN (Organon) and zimelidine HCl, ZIM (Fujisawa). HPD was diluted with distilled water. Vehicles for other drugs were 0.5% carboxymethylcellulose (CMC) for NOM and distilled water for MA, IMP, MIAN and ZIM. In case of IP injections, the volumes of injections were adjusted at 1 ml/kg. For microinjection into brain regions, drugs were dissolved in distilled water and rendered isotonic by adding proper amount of NaCl and infused in 2 μl volume containing 10 μg drug (except MA 3 μg/2 μl) at the rate of 1 μl/2 min. The injection cannulae were left in place an additional 2 min before replacing the stylets.

Statistical Analysis

Effect on HPD-catalepsy refers to significant one at each point. Statistical significances were evaluated using Mann-Whitney U-test.

RESULTS

Effect of IP Injection of Tested Drugs on HPD-Catalepsy (Table 1)

HPD produced a cataleptic state that lasted for 6–8 min accompanied by ptosis, wide base with head reclined on fore limb. Rats squealed upon handling.

MA reduced the catalepsy only for 30 min after HPD. IMP reduced catalepsy only at 10 mg/kg and for 30 min. Of the atypical antidepressants studied, NOM produced the most prominent reduction of catalepsy and the significant effect of NOM 10 mg/kg was even more potent and lasted longer than MA 3 mg/kg. MIAN also reduced catalepsy following doses >10 mg/kg. At such doses MIAN sedated the rats and potentiated HPD-ptosis. ZIM did not reduce catalepsy. At 5 and 10 mg/kg ZIM showed a tendency to potentiate catalepsy 1 hr after HPD.

Effect of Brain Lesioning on Catalepsy and the Activity of Drugs

Separate lesions of CP and ACC reduced catalepsy (Fig. 1). However, a lesion of ACC resulted in even more reduction, a

TABLE 1

EFFECT OF ANTIDEPRESSANTS AND METHAMPHETAMINE ON HALOPERIDOL (HPD)-CATALEPSY

Treatment	Dose mg/kg IP	Duration of Catalepsy (min) 30 min	60 min
Saline	—	6.9 ± 1.5	6.8 ± 1.2
Methamphetamine	0.5	5.8 ± 1.1	6.2 ± 1.2
	1	4.2 ± 0.9*	5.6 ± 1.4
	3	3.8 ± 0.8*	6.6 ± 1.8
Saline	—	7.6 ± 1.8	6.4 ± 1.8
Imipramine	1	6.9 ± 1.2	6.8 ± 1.6
	5	7.4 ± 1.6	8.8 ± 1.7
	10	4.4 ± 0.9*	5.7 ± 1.2
Saline	—	7.6 ± 1.4	8.2 ± 1.3
Nomifensine	0.5	7.9 ± 1.5	7.9 ± 1.4
	2	4.2 ± 0.6*	4.3 ± 0.4†
	5	3.3 ± 0.8†	5.0 ± 1.1*
	10	1.2 ± 0.2‡	1.9 ± 0.5‡
Saline	—	7.7 ± 1.7	7.7 ± 1.3
Mianserin	2	6.5 ± 1.5	5.0 ± 0.9
	10	4.3 ± 0.8*	4.1 ± 0.5*
	20	4.8 ± 0.4*	4.1 ± 1.0*
Saline	—	7.3 ± 1.1	8.2 ± 0.8
Zimelidine	5	7.6 ± 1.1	11.6 ± 3.2
	10	6.2 ± 2.4	9.7 ± 2.2
	20	7.1 ± 0.8	7.0 ± 1.3

Catalepsy was tested 30 min after HPD (2 mg/kg IP). The test drugs were administered IP 30 min prior to HPD. Values are mean ± S.E. of ten rats. Statistically significant differences vs. saline group (HPD preceded by saline): *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

result significant on comparison with the CP-lesioned group ($p<0.05$). On the other hand, none of the drugs altered catalepsy in CP- and ACC-lesioned rats (data not shown).

Effect of Microinjection of Tested Drugs on Catalepsy

Table 2 shows that only MA reduced the duration of catalepsy on injection into AME and CP.

MA and NOM reduced catalepsy on injection into ACC. However, MA displayed a more significant effect.

None of the drugs injected into LS exerted any effect on catalepsy. IMP, MIAN and ZIM injected into all the aforementioned regions did not interfere with the catalepsy.

DISCUSSION

In this study, the effect on HPD-catalepsy of some atypical antidepressants compared to MA and IMP besides the brain region(s) and effect of lesion of ACC and CP were investigated. Although some naive rats grasped the bar for 5 seconds, they showed exploratory movements and grasped, in addition, the pillars with fore paws. This result was different from HPD-treated rats. HPD-catalepsy results from a blockade of dopamine receptors especially in CP and ACC. It is expected that drugs that potentiate dopaminergic function (whether directly or indirectly) consequently antagonize or reduce the catalepsy. Accordingly, MA and NOM (potent dopaminergics) produced the most prominent reduction in the intensity of catalepsy. However, MA, especially at 3

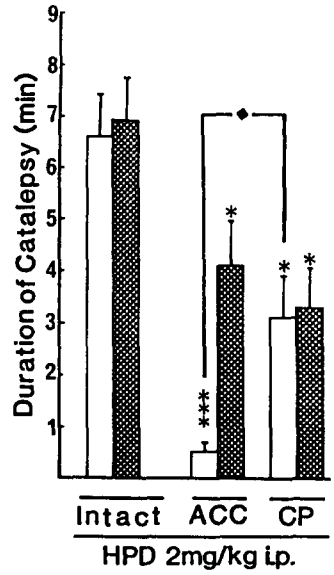

FIG. 1. Effect of lesions of caudate-putamen (CP) and nucleus accumbens (ACC) on haloperidol (HPD)-catalepsy. Catalepsy was measured 30 min (empty columns) and 60 min (hatched columns) after HPD. Values are mean ± S.E. of two experiments each included five rats received two injections, five days apart. Vertical lines are the upper parts of S.E. Statistically significant differences (Mann-Whitney U-test): *$p<0.05$, ***$p<0.001$ vs. intact. ◆$p<0.05$ ACC lesion vs. CP lesion.

TABLE 2

EFFECT OF MICROINJECTION OF ANTIDEPRESSANTS AND METHAMPHETAMINE ON HALOPERIDOL (HPD)-CATALEPSY

Drugs	AME 5 min	AME 30 min	cCP 5 min	cCP 30 min	cACC 5 min	cACC 30 min	LS 5 min	LS 30 min
Saline	7.6 ±0.9	7.8 ±0.9	6.2 ±0.7	7.2 ±0.6	8.0 ±0.9	7.8 ±1.1	7.2 ±1.1	6.6 ±0.8
Methamphetamine	4.8 ±0.8*	5.1 ±0.2*	3.2 ±0.9*	4.5 ±0.6*	0.8 ±0.1‡	2.7 ±0.2†	7.0 ±1.2	6.7 ±1.4
Imipramine	8.6 ±1.4	8.2 ±1.5	7.2 ±1.2	7.8 ±0.6	7.2 ±0.8	7.2 ±1.2	7.0 ±1.2	6.2 ±1.2
Nomifensine	8.5 ±1.2	7.2 ±0.8	7.2 ±0.8	8.2 ±1.4	1.6 ±0.2†	2.4 ±0.2†	6.2 ±0.8	6.8 ±1.2
Mianserin	6.6 ±1.0	6.1 ±0.8	6.8 ±1.2	7.4 ±1.2	5.8 ±1.2	5.2 ±0.8	7.4 ±1.2	6.1 ±1.4
Zimelidine	7.5 ±1.6	9.2 ±1.6	9.9 ±3.2	8.3 ±2.3	9.2 ±1.8	7.6 ±1.6	7.4 ±1.2	7.5 ±1.2

Drugs (10 μg/2 μl except methamphetamine 3 μg/2 μl) were injected bilaterally 30 min after HPD (2 mg/kg IP) and catalepsy was measured 5 min and 30 min later. Values are mean ± S.E. of five rats each received two injections in two experiments separated by five days. Statistically significant differences from saline: *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

mg/kg, produced exploratory movements, while in NOM-treated rats only 3/10 rats showed such a behavior. This result may be due to the difference between behavioral (anticataleptic) effects of the two dopaminergic drugs. Although MIAN and IMP potently inhibit the binding of HPD in rats' striatal membranes (8), they exert a poor effect on dopaminergic function. In this study IMP and MIAN only at doses >10 mg/kg reduced catalepsy.

ZIM has no agonistic effect on the dopaminergic system, and in this study ZIM did not reduce, but slightly potentiated catalepsy instead. This effect may be done so by deactivating the postural support mechanisms that are actually released and exaggerated under HPD (6).

These results suggest that treatments that facilitate dopaminergic function (MA and NOM) or inhibit HPD binding (IMP and MIAN) reduce HPD-catalepsy.

There is evidence that a lesion of brain dopaminergic regions antagonizes or reduces HPD-catalepsy. The present study showed that a lesion of ACC produced a greater reduction in HPD-catalepsy and abolished any further reduction by the tested drugs. This result could be due to differences in the role of CP and ACC in mediating HPD-catalepsy. CP and ACC may comprise two dopaminergic systems, viz. an excitatory and another inhibitory (2). HPD-catalepsy may result from a blockade of the stimulatory dopaminergic system (2). It is conceivable that a reduction of HPD-catalepsy in this study reflects either a direct reversal of HPD-induced blockade of the stimulatory dopaminergic system or an indirect effect on the inhibitory one.

An injection of HPD into CP and ACC reportedly induces catalepsy with shorter latency on injection into ACC (9). These results add further support to our hypothesis that a reduction of HPD-catalepsy in this study is attributable to the reversal of the HPD effect, especially by MA and NOM, in ACC. On the other hand, the present study also revealed that LS is not involved in the anticataleptic activity of the tested drugs. This result could be due to the prevalence in LS of cholinergic function (7) which may not be involved directly in HPD-catalepsy, because HPD has no direct anticholinergic activity (21) and the tested drugs lack any appreciable effect on the cholinergic system (8).

The reduction of HPD-catalepsy by IMP and MIAN with an IP injection and the absence of any effect with a microinjection into four brain regions suggest that the anticataleptic activity of MIAN and IMP could be due to an indirect effect on the dopaminergic system apart from inhibition of HPD binding. Alternatively, it may be exerted in brain regions other than those selected in this study. Moreover, the effect of MIAN could be due to its antihistaminic and/or antiserotonergic effects accompanied by extensive sedation.

Moreover, it can be observed from the results that antidepressants displayed no anticataleptic activity on injection into AME. It is possible to suggest that this result may be because HPD exerts virtually no effect on spontaneous normal activity in AME (6), or this region is not involved in mediating the anticataleptic activity of the antidepressants investigated.

Finally, the present study verified the role of CP and ACC in HPD-catalepsy. Dopaminergic drugs reversed the catalepsy more potently, an effect that was mainly exerted in ACC. A correlation between the anticataleptic effect and clinical efficacy of the antidepressants (especially NOM with antiparkinsonism activity) should be considered.

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