PHARMACOLOGICAL CHARACTERISTICS OF ABNORMAL BEHAVIOR INDUCED BY HARMINE WITH SPECIAL REFERENCE TO TREMOR IN MICE

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Harmine, a hallucinogen with potent monoamine oxidase inhibitory properties, induced abnormal behavior, including tremor, scratching, head twitch and cage biting, in the mouse. A dose-dependent tremor was produced by all routes of administration of harmine. Although oxotremorine tremor was markedly suppressed by atropine, harmine tremor was unaffected by cholinergic drugs, remarkably inhibited by dopaminergic drugs, antidepressants and diazepam, mildly diminished by *p*-chlorophenylalanine, markedly augmented by 5-hydroxytryptophan and mildly increased by α -methyl-*p*-tyrosine. These findings suggest that a catecholaminergic (particularly dopaminergic) and serotonergic system imbalance plays an important role in the manifestation of harmine tremor. In view of these characteristics, harmine tremor may be useful as an effective experimental model for the evaluation of anti-parkinsonism drugs, along with oxotremorine tremor because of the different mechanism of occurrence. In addition, harmine tremor appears to be useful in characterizing the properties of antidepressant drugs.

Keywords—harmine tremor; oxotremorine tremor; brain amines; antidepressants; mice

INTRODUCTION

Harmine produces tremor in monkey, dog, guinea pig, rat and mouse. Similar effects have been observed in man and harmine tremor has been linked to an abatement of striatal dopaminergic function resembling that occurring in Parkinson's diseases.¹⁾ Harmine tremor in animals reportedly involves serotonergic^{2,3)} GABAergic⁴⁾ and noradrenergic mechanisms⁵⁾ in addition to dopaminergic mechanisms. The present work was designed to systematically investigate the variety, type and course of harmine-induced abnormal behavior and the relationship with dosage and other factors. To clarify the mechanism of harmine tremor, the influence of drugs capable of

altering the metabolism and activity of brain amines was concurrently studied. To clearly characterize the properties of harmine-induced abnormal behavior, particularly tremor, comparative studies were carried out with oxotremorine tremor.

MATERIALS AND METHODS

Animals—Male dd mice weighing 20–28g, obtained from Kuroda Animal Farms (Kumamoto) were used. The animals were maintained on a 12-h light-dark schedule (lights on 7:00 a.m.)

Procedure—The mice were housed in groups of two in wire mesh cages (26 × 12.5 × 16.5 cm). Behavior was observed for 60 min commencing

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ing immediately after harmine or oxotremorine administration. Tremor of at least 2 min in duration was considered to be positive. Scratching and head twitch were regarded as positive for animals showing an incidence of at least 30 and 10 times, respectively.

To investigate the effects of various drugs on harmine- and oxotremorine-induced abnormal behavior, particularly tremor, harmine and oxotremorine were administered 5 min and 10 min, respectively, before the peak effect time of the test drug. The peak time of the drugs was estimated by preliminary experiments measuring either the effect on the tremor or behavioral toxicity.

The experiment was performed in a room maintained at a constant temperature of $23 \pm 1^\circ\text{C}$. ED50 values were calculated by the method of Litchfield-Wilcoxon.⁶⁾ Statistical analysis was done using Fischer's exact probability test (one-tailed).⁷⁾

Drugs—The drugs used in the experiment were harmine HCl (Sigma), oxotremorine (Aldrich), L-5-hydroxytryptophan (5-HTP) (powder, Sigma), *p*-chloroamphetamine HCl (PCA) (powder, Sigma), *p*-chlorophenylalanine methylester HCl (PCPA) (powder, Sigma), Ro 4-4602 (powder, Roche), α -methyl-*p*-tyrosine methylester HCl (α -MT) (powder, Sigma), L-3, 4-dihydroxy-dopamine (L-DOPA) (powder, Kyowa Hakko), apomorphine HCl (powder, Takeda), amantadine (powder, Fujisawa), physostigmine (powder, Sigma), atropine H₂SO₄ (powder, Tanabe), biperiden HCl (powder, Dainippon), diphenhydramine HCl (powder, Kyowa Hakko), aminooxyacetic acid (AOAA) (powder, Sigma), diazepam (powder, Roche), haloperidol HCl (Serenase injection, Dainippon) imipramine HCl (powder, Fujisawa), desipramine HCl (powder, CIBA-GEIGY), clomipramine HCl (powder, CIBA-GEIGY), amitriptyline HCl (powder, CIBA-GEIGY) lofepramine (powder, Daiichi) and maprotiline (powder, CIBA-GEIGY). L-DOPA, PCPA, 5-HTP, lofepramine, maprotiline and AOAA were suspended in 0.5% carboxymethylcellulose (CMC). All other drugs

were dissolved in distilled water. All drugs were administered *i.p.* unless specified otherwise.

RESULTS

Harmine given *s.c.* in doses ranging from 0.1 mg/kg to 2.0 mg/kg resulted in a marked scratching and head twitch (Fig. 1). A dose of 5 mg/kg *s.c.* conversely evoked a reduction in scratching and head twitch, and tremor was manifested (Fig. 1). The incidence of tremor increased with increases in the dose of harmine. Moreover, doses of harmine eliciting tremor also induced aggressive behavior, such as biting a rod thrust in front of the mouse's nose, biting the wire mesh cage and biting between cagemates, in about 30% of the mice.

The incidence of scratching and head twitch was low following *p.o.*, *i.p.*, *i.v.* and *i.v.c.* administration of harmine. However, tremor occurred in a dose-dependent fashion, regardless of the route of administration. The ED50 for tremorogenic effect of harmine was 10.7 (5.7–19.4) mg/kg *s.c.*, 10.8 (7.7–15.2) mg/kg *i.p.*, 3.8 (2.7–5.4) mg/kg *i.v.*, 29.3 (20.0–41.0) mg/kg *p.o.* and 11.7 (4.3–31.6) $\mu\text{g/kg}$ by the *i.v.c.* route.

Oxotremorine in doses of 0.3, 0.6, 0.8 and 1.5 mg/kg *i.p.*, also induced tremor in 3, 5, 7 and 10 among groups of 10 mice, respectively. The tremorogenic ED50 was 0.49 (0.33–0.72) mg/kg *i.p.* Oxotremorine consistently produced tremor with all routes of administration, but unlike harmine, abnormal behavior such as scratching, head twitch and cage biting were not apparent.

Effects of 5-HTP on tremor and head twitch induced by 2 and 10 mg/kg *i.p.* of harmine are summarized in Table I. Although tremor was not manifested and head twitch occurred only in a few of 10 mice following the administration of 5-HTP alone, combined administration of harmine and 5-HTP elicited a dose-dependent increase in tremor as well as in head twitch. In addition, the duration of tremor induced by harmine 10 mg/kg *i.p.* alone was about 10 min, but tremor persisted for about 50 min after the combined treatment with 5-HTP. This tremor-potentiating effect of 5-HTP became even more remarkable by the concomitant use of Ro 4-4602, a peripheral

FIG. 1. *Abnormal Behavior induced by Harmine in Mice (n= 10)*TABLE I. *Effect of 5-HTP on Harmine-induced Tremor and Head Twitch in Mice*

Treatment drugs mg/kg <i>i.p.</i>		Saline		Harmine 2 mg/kg		Harmine 10 mg/kg	
		TR	HT	TR	HT	TR	HT
Saline				0/10	0/10	3/10	1/10
5-HTP	10	—	—	—	—	4/10	6/10*
(60 min)	20	—	—	0/10	7/10**	7/10	8/10**
	50	0/10	0/10	2/10	8/10**	8/10*	8/10**
	100	0/10	3/10	5/10*	10/10**	10/10**	10/10**
	200	0/10	1/10	7/10**	10/10**	—	—
Ro 4-4602							
(10 mg/kg <i>i.p.</i>)	2	—	—	—	—	2/10	1/10
(90 min)	5	—	—	—	—	6/10	4/10
+	10	0/10	0/10	0/10	0/10	8/10*	6/10*
5-HTP	20	0/10	0/10	1/10	6/10**	10/10**	8/10**
(60 min)	50	0/10	1/10	5/10*	10/10**	—	—
	100	0/10	3/10	8/10**	10/10**	—	—

*: $p < 0.05$, **: $p < 0.01$.

Numerals are the incidence of tremor (TR) and head twitch (HT) (number of mice exhibiting TR and HT/ number of mice used). The peak time of each test drugs is indicated in parentheses.

aromatic L-amino acid decarboxylase inhibitor. The ED₅₀ for this effect of 5-HTP was 113.0 (68.5–186.5) mg/kg *i.p.* and was 53.5 (33.0–86.7) mg/kg *i.p.* when combined with Ro 4-4602 (10 mg/kg *i.p.*).

Effects of various drugs on tremor induced by 20 mg/kg *i.p.* of harmine and 1.5 mg/kg *i.p.* of oxotremorine are summarized in Table II.

Pretreatment with PCPA and PCA,⁹⁾ inhibitors of 5-HT synthesis, suppressed tremor as well as head twitch induced by harmine. 5-HTP, PCPA and PCA had no effect on oxotremorine-induced tremor.

α -MT, an inhibitor of catecholamine synthesis produced no change in the occurrence of tremor induced by either harmine 20 mg/kg *i.p.* or

TABLE II. *Effects of Various Drugs on Harmine and Oxotremorine Tremor in Mice*

Drug (Peak time)	Dose (mg/kg <i>i.p.</i>)	Harmine (20 mg/kg <i>i.p.</i>)	Oxotremorine (1.5 mg/kg <i>i.p.</i>)
Saline		10/10	10/10
PCPA (24 h)	200	8/10	10/10
	300	7/10	10/10
	600	5/10*	10/10
PCA (60 min)	2	8/10	10/10
	5	5/10*	10/10
	10	6/10*	10/10
α -MT (4 h)	100	10/10	9/10
	200	10/10	9/10
L-DOPA (30 min)	50	6/10*	—
	100	5/10*	10/10
	200	3/10**	10/10
Apomorphine (30 min)	2	6/10*	10/10
	5	4/10**	10/10
	10	3/10**	—
Amantadine (30 min)	1	5/10*	—
	2	5/10*	10/10
	5	4/10**	10/10
	10	3/10**	—
Haloperidol (30 min)	0.5	8/10	7/10
	1	5/10*	5/10*
	2	3/10**	5/10*
	5	—	3/10**
Physostigmine (30 min)	0.2	9/10	—
	0.5	8/10	—

(continued)			
	1	—	7/10
Atropine	2	10/10	4/10**
(30 min)	5	10/10	2/10**
	10	7/10	0/10**
	5	7/10	9/10
Biperiden	10	9/10	5/10*
(60 min)	20	10/10	1/10**
	10	9/10	5/10*
Diphenhydramine	20	9/10	5/10*
(60 min)	50	9/10	4/10**
	25	9/10	10/10
AOAA	50	8/10	10/10
(6 h)	100	6/10*	10/10
	10	7/10	10/10
Diazepam	20	4/10**	10/10
(30 min)	50	3/10**	9/10
	10	8/10	9/10
Imipramine	20	9/10	7/10
(30 min)	50	8/10	3/10**
	10	8/10	10/10
Desipramine	20	6/10*	10/10
(30 min)	50	5/10*	9/10
	100	3/10**	—
	10	8/10	9/10
Clomipramine	20	7/10	8/10
(30 min)	50	7/10	5/10*
	2	6/10*	6/10*
Amitriptyline	5	5/10*	3/10**
(30 min)	10	3/10**	1/10**
	20	0/10**	—
	10	6/10*	10/10
Lofepramine	20	6/10*	10/10
(30 min)	40	4/10**	10/10
	5	7/10	10/10
Maprotiline	10	6/10*	10/10
(30 min)	20	4/10**	10/10

*: $p < 0.05$, **: $p < 0.01$.

Numerals are the incidence of tremor. The peak time of each test drugs is indicated in parentheses.

oxotremorine 1.5 mg/kg (Table II), but the incidence of tremor induced by harmine 10 mg/kg *i.p.* increased from 3/10 to 7/10 and 10/10 after pretreatment with 100 and 200 mg/kg *i.p.* of α -MT. Tremor induced by 0.3 mg/kg *i.p.* of oxotremorine was not intensified by pretreatment with α -MT. In the 200 mg/kg α -MT group, 4 of 10 mice died after oxotremorine administration.

L-DOPA, a catecholamine precursor, apomorphine, a dopamine receptor agonist, amantadine, a dopamine releaser, significantly inhibited harmine tremor. Combined treatment with harmine and L-DOPA 200 mg/kg resulted in aggressive behaviors such as rod-attack and cage-biting in 6 of 10 mice, in addition to salivation and hyperactivity. With harmine administration, death occurred in 2 and 5 mice in the 100 mg/kg and 200 mg/kg L-DOPA groups, respectively. L-DOPA, apomorphine and amantadine did not inhibit tremor induced by 1.5 mg/kg *i.p.* of oxotremorine (Table II), and death followed oxotremorine administration in 2 mice each in the 5 mg/kg apomorphine and 5 mg/kg amantadine groups. Harmine given concomitantly with either apomorphine or amantadine did not result in salivation, aggressive behavior, scratching, head twitch or death. On the other hand, haloperidol significantly inhibited harmine and oxotremorine tremor only at doses larger than 1 mg/kg *i.p.*

Physostigmine and atropine had no significant effect on tremor induced by 10 and 20 mg/kg harmine. On the contrary, inhibition of oxotremorine-induced tremor by atropine was dose-dependent, and a tremor induced by 0.3 mg/kg of oxotremorine increased to 6 in the group of 10 mice given 0.5 mg/kg of physostigmine. Biperiden and diphenhydramine did not affect the harmine-induced tremor but did significantly suppress the oxotremorine-induced tremor. This action may be attributed to the anticholinergic effects of these drugs.

AOAA, a GABA transaminase inhibitor, tended to inhibit and diazepam, which potentiates the action of GABA,¹⁰⁾ significantly suppressed harmine tremor, yet these compounds had no effects on the oxotremorine-induced

tremor.

Among the antidepressants tested, desipramine, amitriptyline, lofepramine and maprotiline significantly inhibited harmine tremor, but imipramine and clomipramine did not. Tremor induced by 10 mg/kg *i.p.* of harmine was not augmented by imipramine and clomipramine either. On the other hand, oxotremorine tremor was significantly inhibited by imipramine, amitriptyline and clomipramine, while it was unaffected by other antidepressants tested.

DISCUSSION

In the present study, lower doses of harmine produced scratching and head twitch, whereas higher doses caused tremor and aggressive behavior. Oxotremorine produced tremor concurrently with lacrimation and salivation. However, influence of oxotremorine differed from that of harmine: Scratching, head twitch, biting or other signs of aggressive behavior were not observed with oxotremorine.

In attempt to elucidate the role of brain amines in the mechanism evoking harmine tremor, the effect of drugs affecting brain amine dynamics was investigated. The finding that serotonin precursor 5-HTP augmented and inhibitors of serotonin synthesis, PCPA and PCA, inhibited harmine tremor indicates that brain 5-HT plays an important role in the occurrence of tremor, as suggested by Costall *et al.*³⁾ and Kelly and Naylor.²⁾ The finding that tremor-potentiating effect of 5-HTP became even more remarkable by the concomitant administration of Ro 4-4602 which inhibits peripheral L-aromatic amino acid decarboxylase, subsequently increasing the 5-HT content in the CNS, provides additional evidence favoring this interpretation. Kim *et al.*¹¹⁾ reported that harmaline increased striatal 5-HT levels in the rat. It has been postulated that harmine also elevates 5-HT levels, because harmine, the congener of harmaline, is also a potent MAO inhibitor¹²⁾ and we have confirmed that harmaline has a marked tremorogenic action as well.

The present study also revealed that harmine

tremor was inhibited in a dose-dependent fashion by dopaminergic drugs and augmented by α -MT, an inhibitor of catecholamine synthesis. Haloperidol significantly inhibited both harmine and oxotremorine-induced tremors. In view of the data that haloperidol has virtually no anticholinergic activity and dopamine inhibits the harmine-induced tremor, the effect of haloperidol is probably not due to a dopamine receptor blocking action, but rather to a non-specific action, such as that caused by catalepsy, as purported by Kelly and Naylor.²⁾

Thus, inhibition of harmine tremor appears to be mediated by brain catecholamines, in particular, a dopaminergic mechanism. Poirier *et al.*¹³⁾ reported that harmine inhibited the formation of dopamine from L-DOPA in the striatum and suppressed dopamine release from nerve terminals, thereby inducing tremor. However, more recently obtained evidence in which L-threo-dihydroxyphenylserine, a direct precursor of noradrenaline, inhibits tremor without involving dopamine suggests that a noradrenergic mechanism may also act in inhibiting harmaline tremor.⁵⁾ Harmine tremor is thus assumed to be induced by an increase in serotonergic activity and a decrease in catecholaminergic activity, leading to an activity imbalance.

Furthermore, it is suggested that a GABAergic mechanism is also involved in the suppression of harmine-induced tremor¹⁴⁾ and cholinergic mechanisms probably do not participate.^{2, 15)}

As to the effect of antidepressants on tremor induced by harmine and oxotremorine, the present results together with the evidence that lofepramine^{16, 17)} and maprotiline^{18, 19)} which is known to have no anticholinergic activity, suggest that the higher the anticholinergic activity of antidepressants the more pronounced the inhibitory effect on oxotremorine tremor. In contrast, harmine tremor is promoted by a serotonergic mechanism and conversely inhibited by catecholaminergic mechanism. The suppression of harmine tremor by antidepressants seems, therefore, to be attributed to the inhibitory action of these drugs on the uptake mechanism of

catecholamines. Since imipramine and clomipramine are known to inhibit the uptake of serotonin rather than that of catecholamine in comparison to other antidepressants,^{20, 21)} it seems likely that the augmentative effect of serotonin and the inhibitory effect of catecholamine cancel out each other with these drugs.

From the results of our experiments, it is concluded that the serotonergic, catecholaminergic and GABAergic mechanisms are involved, but the cholinergic mechanism does not participate in harmine tremor, in contrast to oxotremorine tremor in which exclusively the cholinergic mechanism is involved.

Harmine itself is a potent MAO inhibitor.¹²⁾ This property of harmine may participate in the occurrence of tremor. However, massive doses of the MAO inhibitor pargyline or nialamide do not induce tremor or the characteristic scratching, head twitch or aggressive behavior seen after harmine administration. It is, therefore, highly unlikely that harmine induces these forms of abnormal behavior by MAO inhibition.

Oxotremorine tremor is often employed as an experimental model of parkinsonism. An imbalance in striatal serotonergic, dopaminergic and cholinergic activity has been reported in parkinsonism patients.²²⁾ Therefore, it would be extremely effective to use not only oxotremorine tremor, as an experimental model for the evaluation of antiparkinsonism drugs, but also harmine tremor due to different mechanism of occurrence. In addition, harmine tremor appears to be a useful model in further characterizing the properties of antidepressant drugs.

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