ROLE OF BRAIN MONOAMINE SYSTEMS IN THE JUMPING BEHAVIOR INDUCED IN RATS BY THE COMBINATION OF HARMINE AND APOMORPHINE

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Abstract—The role of brain monoamine systems in the jumping behavior induced by the combination of harmine and apomorphine was studied by using a MT pick-up to assess jumping behavior in rats. The jumping induced by the combination of harmine 10 mg/kg and apomorphine 2 mg/kg was enhanced by pretreatment with p-chlorophenylalanine, methysergide and by treatment with clonidine, while, it was reduced by pretreatment with 5-hydroxytryptophan, haloperidol, perphenazine, \( \alpha \)-methyl-p-tyrosine, chlorpromazine, phenoxycamphetamine, phentolamine, atropine and pilocarpine. The combination of harmaline or harmane and apomorphine also induced jumping with the aid of p-chlorophenylalanine. The combination of benserazide, L-DOPA and harmine induced jumping similar to that induced by harmine and apomorphine. Despite pretreatment with p-chlorophenylalanine the combination of apomorphine and benzylhydrazine, iproniazid, tranylcypromine or pargyline failed to induce jumping. These results suggest that this jumping behavior is induced not by the monoamine oxidase inhibitory effect of harmine but probably by the specific central action of harmine and on condition that the dopaminergic system is activated. The activation of this system appears to be essential, the noradrenergic system plays a facilitatory role, the serotonergic system an inhibitory role and the cholinergic system probably a specific role.

In the harmala alkaloids, harmine in particular is a tremorogenic substance (1–5). Harmine also has potent monoamine oxidase (MAO) inhibitory effects (3, 6) and hallucinogenic properties (7). Apomorphine has the ability to stimulate the medullary CTZ and to produce excitation and depression of CNS (8) and has been used experimentally as a dopaminergic agonist.

The combination of these drugs induces violent jumping accompanied by vocalization and irritation and this hyperactive state differs from the usual stereotyped behavior (gnawing, biting, licking) induced by apomorphine (9). Although there is a report (10) indicating that the jumping behavior may be associated with the MAO inhibitory effect of harmine, evidence for the mechanism of induction of the jumping behavior is scanty.

We thus attempted to determine whether or not the MAO inhibitory effect of harmine plays a major role in jumping behavior and also to elucidate the role of brain monoamine systems in this behavior.

MATERIALS AND METHODS
Animals: Male Wistar rats, weighing 120±20 g and in groups of 5 were placed in
the observation cage (41 x 25 x 15 cm) at least 30 min before injections of drugs to allow for adaptation to the new environment. The well-lighted-room was maintained at a temperature of 20±2°C.

Measurement of jumping: Fine vibration of the cage, (which was rested on Animex), induced by jumping movements of the rats could be detected by MT pick-up attached under the center of the cage. Vibration detected was amplified and continuously recorded using an inkwriting-oscilloscope. Locomotor activity was also simultaneously recorded.

Assessment of jumping: The recorded data were scored for quantitative and objective analysis by the scoring system shown in Fig. 1, that is, data were scored at 2 min intervals and averaged every 10 min.

Drugs and methods of administration: Harmine hydrochloride, harmaline hydrochloride, harmane hydrochloride, apomorphine hydrochloride, benserazide hydrochloride, clonidine hydrochloride, atropine sulfate, pilocarpine hydrochloride, benzylhydrazine hydrochloride, iproniazid phosphate, tranylcypromine hydrochloride and pargyline hydrochloride were dissolved in distilled water. Methysergide hydromaleate and phenoxybenzamine hydrochloride were dissolved in weak acid solution by heating. Haloperidol, perphenazine, chlorpromazine hydrochloride, phentolamine mesylate and propranolol hydrochloride were the usual retail products. DL-p-chlorophenylalanine (PCPA), 5-hydroxy-DL-tryptophane (5-HTP), L-DOPA and DL-α-methyl-p-tyrosine (α-MT) were prepared as an aqueous suspension in 2% carboxymethylcellulose.

PCPA, 5-HTP, L-DOPA and α-MT were administered by the i.p. route and all other agents by the s.c. route.

Injection time of each agent is shown in Figures, and only atropine and pilocarpine were administered 30 min before the injections of harmine and apomorphine.

RESULTS

Abnormal behaviors induced by the combination of harmine and apomorphine

The combination of harmine 10 mg/kg and apomorphine 2 mg/kg induced not only violent jumping but also vocalization, reflexive movement of hind limbs, irritation and rearing in the rats. The jumping occurred as early as 3 min after the simultaneous administration of both drugs, showed maximal state within 5 to 10 min and persisted for about
The magnitude of this jumping was divided into 5 grades as shown in Fig. 1.

Relation between jumping activity and the doses of harmine or apomorphine

On condition that the dose of apomorphine was 2 mg/kg, jumping activity was dose-dependently potentiated by increasing the dose of harmine from 1 to 10 mg/kg. Harmine at a higher dose of 20 or 50 mg/kg inversely reduced jumping activity. On the other hand, when the dose of apomorphine was increased from 0.1 to 10 mg/kg, when the dose of harmine was 10 mg/kg, jumping was not observed with a dose of 0.1 mg/kg but was dose-dependently potentiated from 0.2 to 2 mg/kg. Apomorphine at a higher dose of 5 or 10 mg/kg inversely reduced jumping activity, especially the intensity of jumping. As shown in Fig. 2, the combination of harmine 10 mg/kg and apomorphine 2 mg/kg induced the most violent jumping.

Effects of some agents causing modification of serotonergic system on the jumping induced by the combination of harmine and apomorphine

1) Effect of pretreatment with PCPA at high doses of harmine: In the group not pretreated with PCPA, the combination of harmine 10, 20, or 50 mg/kg and apomorphine 2 mg/kg induced jumping in an inverse order of the doses described above, while, in the group pretreated with PCPA, jumping activity was dose-dependently enhanced, accordingly the difference in the intensity and duration of jumping between the groups PCPA treated and non-treated was increased in proportion to the increase of a dose of harmine (Fig. 3). The total
scores given from each curve are presented in the inserted graph. The total score of PCPA-treated group was also dose-dependently increased inversely with that of the non-treated group. Pretreatment with PCPA did not modify significantly the jumping induced by the combination of harmine 10 mg/kg and

Fig. 3. Effect of pretreatment with PCPA on the jumping induced by the combination of apomorphine and a high dose of harmine. PCPA 150 mg/kg was administered daily x 3, and the effects were assessed on the fourth day. Significant difference from control by t-test: *p<0.05, **p<0.01.
apomorphine 5 or 10 mg/kg.

2) Effects of some agents on the jumping induced by the combination of harmine 10 mg/kg and apomorphine 2 mg/kg (Fig. 4): Pretreatment with methysergide 10 mg/kg enhanced the intensity and frequency of the jumping for about 30 min in the early stage.

Although pretreatment with 5-HTP 100 mg/kg failed to modify significantly both the onset and intensity of the jumping, 5-HTP at higher doses of 200 and 300 mg/kg markedly increased the latency before the onset of jumping. Animals treated with 5-HTP displayed the serotonin receptor activation syndrome, precisely side-to-side head weaving, forepaw padding and splaying hind limbs.

3) Effect of pretreatment with PCPA at low doses of harmine: As pretreatment with PCPA or methysergide did not present such a marked enhancement on the jumping induced by harmine 10 mg/kg and apomorphine 2 mg/kg, the effect of basal serotonergic activity on the jumping was not determined, and such must be checked after giving low doses of harmine which should have weaker MAO inhibitory effects than that of higher doses. As shown in Fig. 5, Pretreatment with PCPA on the jumping after low doses of harmine showed no marked difference from controls.

Induction of jumping by the combination of harmine analogs and apomorphine

We observed whether jumping behavior would be induced by the combination of harmaline or harmane at various doses and apomorphine 2 mg/kg. Jumping lasting about 10 min was observed only in case of harmaline 50 mg/kg. Although this drug in a dose of 20 mg/kg and harmane in a dose of 50 mg/kg failed to induce jumping, such behaviors as vocalization, reflexive movement of hind limbs and irritation were observed.

In the animals pretreated with PCPA, harmaline at a dose of 10 mg/kg induced jumping to the same extent as that of harmine 10 mg/kg. Harmaline in a dose of 10 mg/kg failed to induce jumping, but a higher dose of 50 mg/kg induced not only jumping lasting 30 min in the early stage but also tonic and clonic convulsion within 15 min after injection and most of the rats died. These results are summarized in Table 1.

Jumping inducitivity by the combination of harmine and dopamine agonist

Whether or not jumping could be induced

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Fig. 4. Effects of some agents which modify the serotonergic system with regard to the jumping induced by the combination of harmine 10 mg/kg and apomorphine 2 mg/kg. Methysergide and 5-HTP were administered 10 min and 30 min, respectively, before the administration of harmine and apomorphine. Significant difference from control by t-test: *p<0.05, **p<0.01.
by the use of L-DOPA in place of apomor-
phine was then observed.

Benseragide 5 mg/kg, L-DOPA 300 mg/
kg and harmine 10 mg/kg, given according
to the administration schedule described in
Fig. 6, induced a form of jumping which was
similar to that induced by the combination of
harmine and apomorphine, and lasted for

Fig. 5. Effect of pretreatment with PCPA on the jumping induced by the combination of
apomorphine and a low dose of harmine. PCPA 150 mg/kg was administered daily x 3,
and the effects were assessed on the fourth day. Significant difference from control
by t-test: *p<0.05.
Effects of agents causing modification of catecholaminergic system on the jumping induced by the combination of harmine 10 mg/kg and apomorphine 2 mg/kg

Since an induced excitation of the dopaminergic system seems to be essential for the induction of jumping, agents which reduce the dopaminergic functions should depress the jumping.

Pretreatment with haloperidol 0.01 mg/kg or perphenazine 0.02 mg/kg had a moderate inhibitory effect, and haloperidol at doses from 0.02 to 2 mg/kg or perphenazine at doses from 0.05 to 0.5 mg/kg abolished the jumping behavior. Pretreatment with α-MT which interferes with catecholamine synthesis, or chlorpromazine 0.1 mg/kg, markedly reduced both the intensity and duration of the jumping. Pretreatment with phentolamine 20 mg/kg or phenoxybenzamine 10 mg/kg, α-adrenergic blocking agents, increased the latency before onset of the jumping and markedly reduced both the intensity and duration of the jumping. Treatment with clonidine 0.1 mg/kg, a direct stimulant for α-adrenergic receptor, enhanced the intensity of the jumping (Fig. 7). Pretreatment with propranolol 20 mg/kg, a β-adrenergic blocking agent, did not significantly modify the jumping (not shown in Fig.).

Effects of agents causing modification of cholinergic system on the jumping induced by the combination of harmine 10 mg/kg
and apomorphine 2 mg/kg (not presented in Fig.)

Pretreatment with atropine 10 mg/kg, an anticholinergic agent, did not significantly modify the jumping, however, there was a tendency to reduce both the intensity and duration. Pilocarpine 5 mg/kg, a cholinomimetic agent, markedly reduced both the intensity and duration of the jumping.

**Induction of jumping by the individual administration of harmine or apomorphine and by various combinations of related drugs**

As shown in Table 2, jumping was never induced by harmine alone at doses ranging from 0.1 to 50 mg/kg or by apomorphine alone at doses ranging from 0.1 to 20 mg/kg. The combination of harmine and dopamine agonists induced jumping as described above, while the combination of harmine 10 mg/kg or apomorphine 2 mg/kg and clonidine 0.1 mg/kg or PCPA did not induce such behavior. The combination of apomorphine 2 mg/kg and an other MAO inhibitor, benzylhydrazine 20 mg/kg, iproniazid 100 mg/kg, tranylcypromine 15 mg/kg or pargyline 75 mg/kg did not induce jumping, although each MAO inhibitor was simultaneously administered with apomorphine at
a dose known to produce maximal MAO inhibition. Pretreatment with PCPA to rats given apomorphine and a MAO inhibitor markedly enhanced locomotor activity but did not induce jumping. Apomorphine in a dose of 20 mg/kg and the combination of apomorphine 2 mg/kg and clonidine 0.1 mg/kg also induced irritation and marked enhancement of locomotor activity, but not the violent jumping behavior.

**DISCUSSION**

In the present experiment, the role of brain monoamine systems in the jumping behavior induced by the combination of harmine and apomorphine was studied in hopes of elucidating mechanisms involved with modification of central monoamine systems, as induced by certain drugs. At first, the doses of harmine and apomorphine, which induce the severest jumping, were determined.

As can be seen in Fig. 2, the jumping was dose-dependently induced in proportion to increase in the dose of harmine and which ranged from 1–10 mg/kg, but the induction of jumping was reduced inversely with higher doses of harmine. The same pattern was seen in the experiment when harmine 10 mg/kg and various doses of apomorphine were given concomitantly. Because jumping was not induced in cases of the combination of apomorphine 2 mg/kg and a low dose of harmine and the combination of harmine 10 mg/kg and a low dose of apomorphine, it would appear that an optimal dose of the both drugs is essential to induce jumping.

The question why the induction of jumping was reduced with high doses of harmine was investigated using PCPA. This drug decreases brain serotonin content by inhibiting the enzyme which synthesizes this neurotransmitter (11). Pretreatment with PCPA enhanced the jumping induced by the combination of apomorphine 2 mg/kg and various doses of harmine, especially in case of higher doses of harmine. This finding indicates that the pretreatment with PCPA

### Table 2. Jumping inductivity by the individual administration of harmine or apomorphine and by various combinations of related compounds

| Harmine only (1~50 mg/kg) | ~ |
|--------------------------|---|
| Apomorphine only (0.1 mg/kg~20 mg/kg) | ~* |
| Harmine (10 mg/kg) + Apomorphine (2 mg/kg) | +* |
| Harmine (10 mg/kg) + L-DOPA (300 mg/kg) + Benserazide (5 mg/kg) | +* |
| Harmine (10 mg/kg) + Clonidine (0.1 mg/kg) | ~ |
| Apomorphine (2 mg/kg) + Clonidine (0.1 mg/kg) | ~* |
| Harmine (10 mg/kg) + PCPA (150 mg/kg×3) | ~ |
| Apomorphine (2 mg/kg) + PCPA (150 mg/kg×3) | ~ |
| Apomorphine (2 mg/kg) + Benzylhydrazine (20 mg/kg) | ~ |
| Apomorphine (2 mg/kg) + L-Deprenyl (100 mg/kg) | ~ |
| Apomorphine (2 mg/kg) + Tranylcypromine (15 mg/kg) | ~ |
| Apomorphine (2 mg/kg) + Pargyline (75 mg/kg) | ~ |
| Apomorphine (2 mg/kg) + Benzylhydrazine (20 mg/kg) + PCPA (150 mg/kg×3) | ~ |
| Apomorphine (2 mg/kg) + L-Deprenyl (100 mg/kg) + PCPA (150 mg/kg×3) | ~ |
| Apomorphine (2 mg/kg) + Tranylcypromine (15 mg/kg) + PCPA (150 mg/kg×3) | ~ |
| Apomorphine (2 mg/kg) + Pargyline (75 mg/kg) + PCPA (150 mg/kg×3) | ~ |

*: jumping induced, ~: not induced, *: irritation induced. Apomorphine and benzylhydrazine, L-Deprenyl, tranylcypromine or pargyline were simultaneously administered. Other drugs were administered at the time shown in other Figs.
restores the induction of true jumping by eliminating the effects of the serotonergic system and that the serotonergic system activated by the MAO inhibitory effect of harmine depresses jumping, particularly at higher doses of harmine. However, the finding that the effects of pretreatment with PCPA on the jumping after low doses of harmine showed no marked enhancement indicates that basal serotonergic activity may not have so marked an inhibitory effect on the jumping.

There are reports that PCPA enhances the behavior-arousing actions induced by apomorphine (12, 13). These findings would indicate that the ability virtually disappears 24 hr after the administration of PCPA and that it seems to be independent of serotonin-depleting action of PCPA. The enhancement of jumping by PCPA is probably not concerned with this phenomenon because apomorphine was injected 24 hr after the last administration of PCPA in our experiment. The reduction of jumping with high doses of apomorphine is not likely to be involved in serotonergic system.

Pretreatment with 5-HTP, which activates the serotonergic system by elevating brain 5-HT levels (14), induced serotonin receptor activation syndrome with the aid of the MAO inhibitory effects of harmine, and this syndrome may be related to the delay of the onset of jumping. Pretreatment with methysergide, which is a classical antagonist of the surmountable competitive type and its blocking effect on the serotonin receptor is achieved without evidence of stimulation (15), enhanced the intensity of the jumping. These findings are consistent with the findings seen with pretreatment with PCPA.

In the experiment in which a harmine analogue and apomorphine were given concomitantly, we found that the dose of the harmine analogue required for induction of jumping was higher than that of harmine and that the pretreatment with PCPA could induce jumping at the dose of this analogue which could not induce jumping in itself. Therefore, these analogs will also induce jumping. As harmine, harmaline and harmane are potent MAO inhibitors (6), it is suggested that the serotonergic system activated by the MAO inhibitory effect of these analogs inhibits the induction of jumping. All these findings described indicate that the serotonergic system plays an inhibitory role in inducing the jumping.

Benserazide contributes to the elevation of brain dopamine levels by inhibiting peripheral aromatic amino acid decarboxylase, when L-DOPA is administered (9). The fact that the treatment with L-DOPA and harmine in rats pretreated with benserazide, induced jumping similar to that induced by the combination of harmine and apomorphine indicates that apomorphine plays only the role of activation of the dopaminergic system and that the activation of this system is an indispensable condition to induce jumping. Therefore, it is reasonable that pretreatment with haloperidol or perphenazine would abolish the jumping at the low doses shown in Fig. 7.

The observations that the pretreatment with α-MT, chlorpromazine, phenoxybenzamine or phentolamine reduced the jumping activity, while, the treatment with clonidine enhanced the intensity, that such was not markedly affected by the pretreatment with propranolol, and that the combination of harmine and clonidine failed to induce jumping all indicate that the activation of noradrenergic system may not be an indispensable condition but may only be endowed with a facilitatory role in inducing the jumping.

As pilocarpine markedly reduced the jumping, the participation of cholinergic system requires further study.

As shown in Table 2, when both the
dopaminergic and noradrenergic systems were stimulated by apomorphine and clonidine, even when pretreatment with PCPA was given before the administration of harmine or apomorphine, jumping did not occur. Jumping was never induced by the combination of apomorphine and some MAO inhibitors and even by the additional pretreatment with PCPA, although there was a significant increase in locomotor activity. These findings indicate that the jumping is not induced only by a modification of central monoamine systems and/or by the MAO inhibitory effect of harmine.

Histological studies showed that the jumping was abolished following lesioning of the globus pallidus (10). Since the majority of the neostriatal efference is directed via the paleostriatum and lesioning of the globus pallidus must necessarily reduce neostriatal function, and it is conceivable that such a lesion more effectively reduces neostriatal activity than does lesioning of the caudate-putamen itself. Therefore, the induction of the jumping may involve the dopaminergic function in these areas, namely, the nigrostriatal pathway.

All our observations suggest that the jumping is induced not by the MAO inhibitory effect of harmine but probably by the specific central action of harmine, on conditions that the dopaminergic system is activated, that activation of this system is essential and that the noradrenergic system plays a facilitatory role, the serotonergic system plays an inhibitory role and the cholinergic system possibly a specific role in inducing the jumping.

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