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Effect of chlorpromazine on mouse ambulatory activity sensitization caused by (+)-amphetamine

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Abstract—The development of sensitization to the ambulation-increasing effect of (+)-amphetamine (2·5 mg kg^{-1}) was found to be dose-dependently inhibited when 1 or 2 mg kg^{-1} chlorpromazine was administered concomitantly, and the sensitization to (+)-amphetamine was almost completely suppressed when co-administered with 4 mg kg^{-1} chlorpromazine. Following a challenge dose of 2·5 mg kg^{-1} (+)-amphetamine, mice pretreated with (+)-amphetamine alone or with (+)-amphetamine plus 1 or 2 mg kg^{-1} chlorpromazine showed similar marked enhancement of the sensitization. However, mice that had been given (+)-amphetamine plus 4 mg kg^{-1} chlorpromazine displayed only slight enhancement of the effect compared with the activity level in saline-pretreated mice.

Many investigators (Pickens & Crowder 1967; Rushton et al 1968; Tilson & Rech 1973; Segal & Mandell 1974; Short & Shuster 1976; Kokkinides & Zacharko 1980) who have studied the effects of repeated administration of amphetamines to animals have suggested enhancement of sensitivity to the ambulation-increasing and stereotypy-producing effects of the drug. However, our studies of sensitization to the ambulation-increasing effects of amphetamines (Tadokoro & Ohashi 1975; Hayashi et al 1980; Hirabayashi & Alam 1981) have indicated that this phenomenon is strongly affected by dose and interval of the administration, as well as by environment. Neuroleptics, such as chlorpromazine and haloperidol, are known to be effective in attenuating the stimulant effects of amphetamines (Sulser & Dingell 1967; Kuczenski & Leith 1981; Ihara 1983; Kashiwara et al 1984; Kuribara & Tadokoro 1985); the purpose of these experiments was to examine the mechanism by which chlorpromazine blocks sensitization to the ambulation-increasing effects of (+)-amphetamine.

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Materials and methods

Animals. Adult male dd strain mice, 24–32 g at the beginning of the experiment, were supplied by the Institute of Experimental

Table 1. Effect of concomitant administration of chlorpromazine on the ambulation-increasing activity of (+)-amphetamine.

Treatment group	Administration					Challenge dose
	1st	2nd	3rd	4th	5th	
I	1123±51	1950±83[a]	2670±63[b]	2721±101[b]	2854±85[b]	2983±201[c]
II	303±96	707±83[a]	1220±74[a]	1110±155[a]	1303±220[a]	2657±267[c]
III	311±156	618±108	713±109	765±138[a]	1009±61[a]	2870±251[c]
IV	108±33	109±41	111±43	78±38	130±55	1007±101
V	31±17	46±21	52±18	23±15	31±13	1103±153

Group I, (+)-amphetamine alone 2·5 mg kg^{-1}. Groups II, III, IV, (+)-amphetamine plus chlorpromazine (1, 2 or 4 mg kg^{-1}, respectively). Group V saline control. Results are number of counts over a 3 h period (n=20, each). [a]$P<0·05$; [b]$P<0·01$ compared with the 1st administration; [c]$P<0·01$ compared with saline control (group V).

Animal Research, Gunma University School of Medicine, Japan. The mice were housed in groups of ten in aluminium cages (35 × 25 × 10 cm) with wooden-flake bedding, and given free access to a solid diet (MF, Oriental Yeast Co, Tokyo) and tap water except during the experiment. The animal room was illuminated by fluorescent lamps with a 12 h light–dark cycle (lights on 0600 h), and the room temperature was maintained at 23±2°C.

Measurement of ambulatory activity. The ambulatory activity of the mice was determined by the tilting cage method (AMB-M20, Ohara and Co. Ltd, Tokyo), as reported previously by Hirabayashi et al (1978). Briefly, each slight tilt of the round Plexiglass activity cage (20 cm in diameter and 18 cm height) caused by horizontal movement made by a mouse, was detected by three microswitches fixed to the cage box. Each mouse was placed in the activity cage, and ambulatory activity counts were recorded every 10 min for 30 min before, and for 180 min after, the drug administration. The measurement of ambulatory activity was usually carried out between 1000 and 1500 h.

Drugs and repetition procedures. The drugs used were chlorpromazine hydrochloride (1–4 mg kg^{-1}) administered intraperitoneally (Yoshitomi Pharmaceutical Co., Japan) and (+)-amphetamine sulphate (2·5 mg kg^{-1}) (Dainippon Pharmaceutical Co., Japan) subcutaneously. The drugs were dissolved in purified water and the volume administered was 0·1 mL per 10 g of body weight.

Mice were divided into five groups receiving (+)-amphetamine alone (2·5 mg kg^{-1}, group I), (+)-amphetamine plus chlorpromazine (1, 2 or 4 mg kg^{-1}, groups II, III and IV, respectively) or 0·9% NaCl (saline, group V) five times at intervals of seven days. In groups II, III and IV, (+)-amphetamine was given 30 min after chlorpromazine.

The ambulatory activity of each mouse was observed for 3 h after (+)-amphetamine administration. Seven days after the final (5th) administration, all groups of mice were given a challenge dose of (+)-amphetamine (2·5 mg kg^{-1}), and the activity level of each group was again measured and compared with that of controls (group V). Pilot studies had shown that chlorpromazine at a dose of 4 mg kg^{-1} almost completely antagonized the ambulation-increasing effect of (+)-amphetamine (2·5 mg kg^{-1}).

Statistical evaluation. Differences between mean activity counts were statistically evaluated with one- or two-way analysis of variance.

Results and discussion

The results of the study are summarized in Table 1. The present results indicate that the dosage of concurrently-administered chlorpromazine influences the extent to which it induces inhibition of sensitization to the ambulation-increasing effect of (+)-amphetamine; concomitant administration of 1 or 2 mg kg^{-1} chlorpromazine was dose-dependently effective in inhibiting the development of sensitization to (+)-amphetamine, whereas 4 mg kg^{-1} chlorpromazine not only suppressed the ambulation-increasing effect of (+)-amphetamine but also prevented the development of sensitization to (+)-amphetamine. Similar observations after the combined administration of methamphetamine plus haloperidol have been reported by Kashiwara et al (1984) and Kuribara & Tadokoro (1985).

However, after a challenge dose of (+)-amphetamine, mice pretreated with (+)-amphetamine plus 1 or 2 mg kg^{-1} chlorpromazine, showed marked enhancement of the sensitization, similar to that seen in mice treated with (+)-amphetamine alone. Carlsson & Lindquist (1963), Andén et al (1964) and Nybäck (1971) have suggested that repeated administration of small doses of chlorpromazine accelerates synthesis and turnover of dopamine by blocking central dopamine receptors. Sulser & Dingell (1967) also suggested that potentiation by chlorpromazine of the stimulant effects of (+)-amphetamine is observed only after the administration of lower doses of the neuroleptic. These phenomena would account for the marked enhancement of the sensitization to the challenge dose of (+)-amphetamine observed.

In contrast, following the challenge dose of (+)-amphetamine, mice pretreated with (+)-amphetamine plus 4 mg kg^{-1} chlorpromazine showed no enhancement of the effect. However, this level of sensitization is apparently not due to accumulation of chlorpromazine at higher doses.

Amphetamines cause marked behavioural effects through release of both brain dopamine and noradrenaline, and through inhibition of the reuptake of both catecholamines (Robinson & Becker 1986). However, chlorpromazine blocks receptors for several neurotransmitters (Worms et al 1983). Hayashi et al (1987) of our laboratory reported that when methamphetamine (which has somewhat stronger CNS-stimulation action than does (+)-amphetamine (Hirabayashi et al 1978)) was repeatedly administered to rats in activity cages, the number of brain catecholamine receptor binding sites and catecholamine concentrations were decreased, and catecholamine metabolite concentrations were increased, while no change in either number of binding sites or catecholamine turnover was detected when the drug was repeatedly administered to rats in a narrow cage. These results suggest that there is a correlation between enhancement of the ambulation-increasing effects of methamphetamine and neurochemical changes in cerebral catecholaminergic neurons, and moreover that rats cannot be sensitized when their ambulation is impeded, even under the drug effect.

Although previous reports (Segal & Mandell 1974; Short & Shuster 1976) failed to demonstrate sensitization associated with the conditioning effect of (+)-amphetamine, we have previously

confirmed this conditioning effect of (+)-amphetamine (Tadokoro & Ohashi 1975; Hayashi et al 1980) and methamphetamine (Hirabayashi & Alam 1981). Other investigators (Pickens & Crowder 1967; Tilson & Rech 1973; Schreiber et al 1976; Schiff 1982; Krank & Bennett 1987) have also pointed out the important role of distinctive environment in the conditioned drug effects, but have demonstrated no activity-cage dimensional factor. We have suggested (Hirabayashi et al 1991) that, in accordance with the all-or-none law, the development of sensitization to methamphetamine in mice is controlled by the size of the activity cage, and that to be effective, a round cage must be more than 15 cm in diameter.

Although further investigations are required to elucidate the mechanism of no marked potentiation of the stimulant effect of (+)-amphetamine by pretreatment with chlorpromazine at high dose, this finding may be explained as follows: the concurrently administered chlorpromazine at high dose exerts its blockade effect on the priming effect of (+)-amphetamine, which induces a marked reaction the next time the mice are exposed to the drug in the distinctive environment.

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