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Catalepsy Induced by Manidipine, a Calcium Channel Blocker, in Mice

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Abstract

Manidipine, a calcium channel blocker, is a piperazine derivative similar to flunarizine or cinnarizine, which are known to induce parkinsonism. Since it has been reported that manidipine can worsen parkinsonian symptoms in a patient with Parkinson's disease, we have evaluated catalepsy in manidipine-treated mice and compared this with flunarizine-and haloperidol-induced catalepsy.

The minimum dose at which manidipine induced catalepsy was 200 times higher than that of haloperidol whereas for flunarizine, the minimum dose was 50 times higher than that for haloperidol. Manidipine, flunarizine and haloperidol occupied both dopamine D_1 and D_2 receptors, and D_2-receptor occupancy was higher than D_1-receptor occupancy.

These results suggest that the blockade of dopamine D_1 and D_2 receptors by drugs and the drug-induced catalepsy are related to the structure (piperazinyl substituent) of the drugs.

Flunarizine and cinnarizine are calcium channel blockers which are used in the treatment of cerebral blood flow disturbances. Chouza et al (1986) reported parkinsonism, tardive dyskinesia, akathisia, and depression in patients treated with flunarizine; Marti Masso et al (1985) reported cinnarizine-induced parkinsonism. Over the last few years, several similar clinical cases of flunarizine- and/or cinnarizine-induced extrapyramidal disorders have been reported (Laporte & Capella 1986; Meyboom et al 1986; Micheli et al 1987; Kuzuhara et al 1989).

Manidipine is also a calcium channel blocker with a piperazinyl group which is used in the treatment of hypertension. Although there has been no report of manidipine-induced parkinsonism, worsening of parkinsonian sympsoms has recently been reported after manidipine treatment (Nakashima et al 1992). These findings suggest the possibility that manidipine could induce parkinsonism. Therefore we investigated catalepsy in mice, which serves as an experimental animal model of extrapyramidal side effects.

Although the mechanism of drug-induced parkinsonism has not been fully clarified, it is accepted that parkinsonian symptoms are mainly caused by binding of the drugs to dopamine receptors at striatum and their antagonistic action at the dopaminergic system. Based on these concepts, we also measured in-vivo dopamine D_1- and D_2-receptor occupancy of the three drugs.

Materials and Methods

Animals

Male ddY mice, weighing 25–30 g, were purchased from Nippon Bio-supp, Center.

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Drugs

The following drugs were obtained as gifts from the respective companies: manidipine hydrochloride (Takeda Chemical Industries Ltd); flunarizine hydrochloride (Kyowa Hakko Kogyo Co. Ltd); haloperidol (Dainippon Pharmaceutical Co.). [^{3}H] SCH23390 (specific activity, 71·1 Ci mmol^{-1}), [^{3}H] raclopride (specific activity, 79·5 Ci mmol^{-1}), SOLVABLE and ATOMLIGHT were purchased from NEN Research Products. Other chemicals were obtained from commercial sources.

Manidipine hydrochloride was dissolved in ethanol: polyethyleneglycol 400 (PEG-400) = 1 : 1 at 50°C and diluted with PEG-400: saline = 1 : 1. Flunarizine hydrochloride and haloperidol were dissolved in 1·5% and 0·3% tartaric acid, respectively, and diluted with saline. Scopolamine was dissolved in saline. All of the unlabeled drugs were injected in a volume of 10 mL kg^{-1}.

Measurement of catalepsy

Manidipine (10, 20 mg kg^{-1}) was injected subcutaneously, flunarizine (2·5, 5, 10, 20, 30 mg kg^{-1}) and haloperidol (0·05, 0·1, 0·25, 0·5, 1 mg kg^{-1}) were injected intraperitoneally. Control animals were administered subcutaneously or intraperitoneally with the respective solvent. At different times (0·5, 1·5, 3, 4·5, 6 and 7·5 h) after administration of the drugs, catalepsy was assessed by the bar method; the front paws were gently placed on a horizontal metal bar with 2 mm diameter suspended 4 cm above and the length of time the mouse maintains this abnormal posture was measured (Fujiwara 1992).

Effect of scopolamine on catalepsy

To confirm that catalepsy induced by manidipine, flunarizine and haloperidol is not caused by peripheral action, we investigated the effect of scopolamine which is used in the treatment of Parkinson's disease (Morelli & Chiara 1985).

Manidipine (20 mg kg^{-1}) and scopolamine (5 mg kg^{-1})

were simultaneously administered subcutaneously to mice and catalepsy was measured as above. For flunarizine (30 mg kg^{-1}) and haloperidol (1 mg kg^{-1}), catalepsy was measured at 60 min after intraperitoneal injection of each drug and scopolamine (10 mg kg^{-1}) was administered subcutaneously. Subsequently, catalepsy was measured every hour for 3 h.

Measurement of in-vivo dopamine and D_1- and D_2-receptor occupancy

Manidipine, flunarizine, haloperidol or vehicle was administered to mice under the same conditions as given previously for measurement of catalepsy. At 25 or 85 min after administration of the drugs, D_1-specific antagonist [^{3}H] SCH23390 (3 μCi per mouse) or D_2-specific antagonist [^{3}H] raclopride (3 μCi per mouse) was injected intravenously. At 10 min post injection, mice were decapitated and striatum and cerebellum were dissected on a glass plate. Each sample was weighed in a vial, and 1 mL of SOLVABLE added and incubated at 50°C until it became clear solution. After 0·2 mL of 30% H_2O_2 was added, the vial was left at room temperature (21°C) for 60 min and 10 mL scintillation fluid, ATOMLIGHT was added. The radioactivities were measured in a liquid scintillation counter (LSC-3100, Aloka), and receptor occupancy was calculated as follows:

$$\phi(\%) = \left(1 - \frac{A - 1}{B - 1}\right) \times 100 \qquad (1)$$

where A is the radioactivity ratio (striatum/cerebellum) in the presence of drugs and B is the radioactivity ratio (striatum/cerebellum) in the absence of drugs. The cerebellum was utilized as D_1- and D_2-receptor free regions to estimate nonspecific binding of ligands.

Results

Induction of catalepsy

Fig. 1A, B and C show the time course of catalepsy induced by manidipine (10, 20 mg kg^{-1}), flunarizine (2·5–30 mg kg^{-1}), haloperidol (0·05–1 mg kg^{-1}) or vehicle. Manidipine-induced catalepsy was weaker than catalepsy induced by flunarizine or haloperidol. The degree of catalepsy induced by flunarizine (30 mg kg^{-1}) was comparable with that induced by haloperidol (1 mg kg^{-1}), whereas greater than 20 mg kg^{-1} of manidipine was similar to 0·1 mg kg^{-1} of haloperidol in its ability to induce catalepsy.

The degree of catalepsy induced by the drugs showed dose-dependency (Fig. 1D). In any cases, catalepsy was not observed in the mice treated with vehicle.

Effect of scopolamine on catalepsy

Fig. 2 shows the effects of scopolamine on the catalepsy observed after administration of manidipine (Fig. 2A), flunarizine or haloperidol (Fig. 2B). Scopolamine inhibited catalepsy induced by any of the tested drugs.

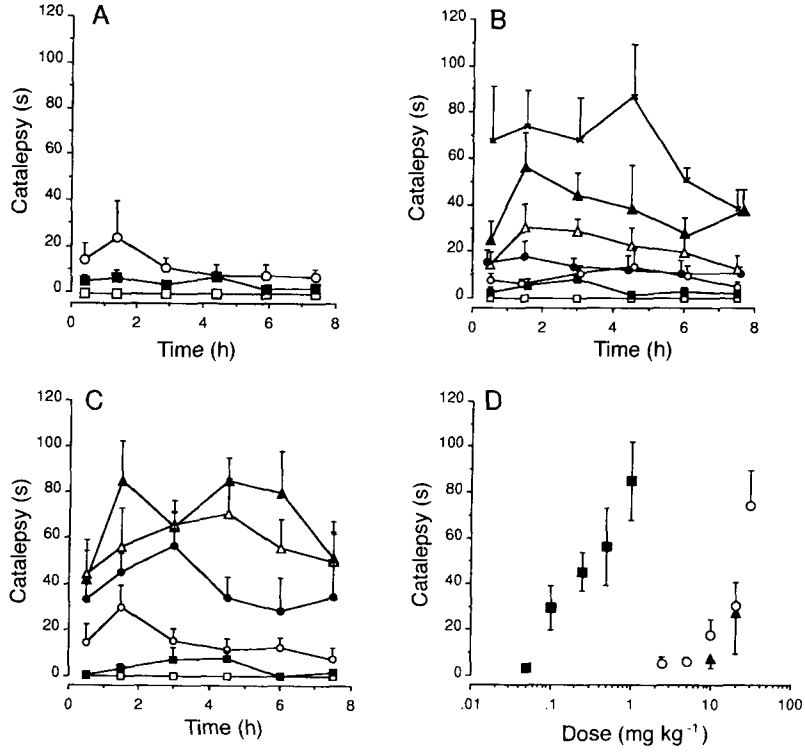

FIG. 1. Time course of catalepsy induced by manidipine (A), flunarizine (B) and haloperidol (C). Dose-dependent induction of catalepsy (D). The incidence of catalepsy was assessed at 0·5, 1·5, 3, 4·5, 6 and 7·5 h after subcutaneous administration of manidipine (n = 5) and intraperitoneal administration of flunarizine (n = 8–10) and haloperidol (n = 8–10). A: □, control; ■, 10; ○, 20 mg kg^{-1} manidipine, B: □, control; ■, 2·5, ○, 5; ●, 10; △, 20; ▲, 25 and ×, 30 mg kg^{-1} flunarizine, C: □, control; ■, 0·05; ○, 0·1; ●, 0·25, △, 0·5 and ▲, 1·0 mg kg^{-1} haloperidol. D: ▲, manidipine; ○, flunarizine; ■, haloperidol. Catalepsy was assessed at 1·5 h after administration of each drug. Data are means ± s.e.

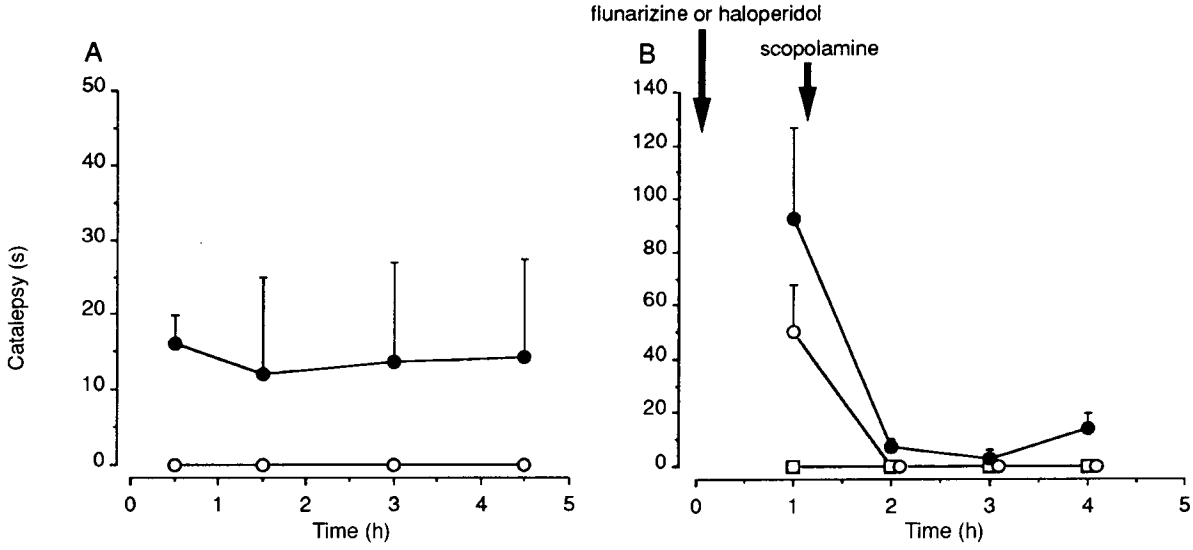

FIG. 2. A. Effect of scopolamine on catalepsy induced by manidipine. ○; manidipine (20 mg kg^{-1} s.c.) + scopolamine (5 mg kg^{-1} s.c.). ●; manidipine (20 mg kg^{-1} s.c.) + saline (s.c.). Data are means ± s.e. (n = 5). B. Effect of scopolamine on catalepsy induced by flunarizine or haloperidol. Flunarizine (30 mg kg^{-1}, ○), haloperidol (1 mg kg^{-1}, ●) or vehicle (□) was administered intraperitoneally 60 min before subcutaneous injection of scopolamine (10 mg kg^{-1}). Data are means ± s.e. (n = 5).

In-vivo dopamine D_1- and D_2-receptor occupancy

Table 1 represents in-vivo dopamine D_1- and D_2-receptor occupancy of manidipine (20 mg kg^{-1}, 30 min after s.c.), flunarizine (10 mg kg^{-1}; 90 min after i.p.) and haloperidol (0·25 mg kg^{-1}, 90 min after i.p.). These drugs occupied both of the receptor subtypes and D_2-receptor occupancy was higher than D_1-receptor occupancy.

Discussion

Catalepsy, although relatively mild, was observed in the mice treated with manidipine (Fig. 1). The minimum dose at which manidipine induced catalepsy was 200 times higher than that for haloperidol. In the case of flunarizine, the minimum dose of inducing catalepsy was 50 times higher than that for haloperidol, but when the dose of flunarizine was increased to 30 mg kg^{-1}, the intensity of catalepsy was comparable to that induced by 1 mg kg^{-1} haloperidol. It is known that haloperidol and other neuroleptics induce catalepsy in a dose-dependent manner (Fujiwara 1992; Ossowska et al 1990). The present findings indicate that catalepsy induced by manidipine and flunarizine also shows dose-dependency (Fig. 1D).

Table 1. In-vivo dopamine D_1- and D_2-receptor occupancy of the tested drugs.

Drug	In-vivo receptor occupancy (%)	
	D_1	D_2
Manidipine (20 mg kg^{-1})	24·3 ± 5·5	38·0 ± 9·9
Flunarizine (10 mg kg^{-1})	28·7 ± 8·1	68·3 ± 9·8
Haloperidol (0·25 mg kg^{-1})	26·7 ± 16·3	73·0 ± 17·3

Drugs were administered intraperitoneally 85 min before tracer injection except for manidipine, which was administered subcutaneously 25 min before. Data are means ± s.e. (n = 4–5).

When the dose of manidipine was increased to over 20 mg kg^{-1}, mice were depressed and the degree of catalepsy was not further intensified. Since the dose at which manidipine induced catalepsy was higher than that for haloperidol and flunarizine, it is possible that catalepsy was hidden by peripheral effects including hypotension. To confirm that the observed catalepsy is not caused by a peripheral mechanism, the anticholinergic agent, scopolamine, which is transported into brain in-vivo, was administered subcutaneously. In the presence of scopolamine, the catalepsy induced by the tested drugs was remarkably reduced, indicating that catalepsy reflects the effect in the central nervous system.

It is suggested that the main cause of drug-induced parkinsonism is binding of drugs to dopamine receptors and blockade of function of dopaminergic neurons. Therefore, it seems likely that the drugs with high affinity for dopamine receptors have potential for inducing catalepsy. The reported values of K_i for D_1 and D_2 receptors are 532 ± 39 and 112 ± 9 nM (Ambrosio & Stefanini 1991), respectively, for flunarizine and 76 and 2·6 nM (Andersen 1988), respectively, for haloperidol, which shows that the affinities of these drugs for the D_2 receptor are higher than those for the D_1 receptor. Our in-vivo results in this study are in agreement with these results in-vitro (Table 1).

The chemical structure of manidipine is partly similar to that of flunarizine and cinnarizine, which are known to induce catalepsy; all having a piperazinyl group. Since manidipine occupied both dopamine D_1 and D_2 receptors at the same dose as inducing catalepsy, it was suggested that the blockade of dopamine receptors and the ability of inducing catalepsy are related to the structure of the drugs. We suggest that for drugs which possess a piperazinyl group or similar structure, even if parkinsonism has not previously been reported, drug-induced parkinsonism might occur. The incidence may be increased in the case of

elderly patients or those who use antipsychotics concurrently. Using this method, therefore, screening of drugs with a potential risk of inducing parkinsonism might be necessary.

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