PREDICTION OF DRUG-INDUCED CATALEPSY BASED ON DOPAMINE D_1, D_2, AND MUSCARINIC ACETYLCHOLINE RECEPTOR OCCUPANCIES

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ABSTRACT:

It is known that catalepsy serves as an experimental animal model of parkinsonism. In this study, the relationship between *in vivo* dopamine D_1 and D_2 receptor occupancies and catalepsy was investigated to predict the intensity of catalepsy induced by drugs that bind to D_1 and D_2 receptors nonselectively. ^{3}H-SCH23390 and ^{3}H-raclopride were used for the labeling of D_1 and D_2 receptors, respectively. The ternary complex model consisting of agonist or antagonist, receptor, and transducer was developed, and the dynamic parameters were determined. After coadministration of SCH23390 and nemonapride, catalepsy was stronger than sum of the values predicted by single administration of each drug, and it was intensified synergistically. This finding suggested the existence of interaction between D_1 and D_2 receptors, and the necessity for constructing the model including this interaction. To examine the validity of this model, catalepsy and *in vivo* dopamine receptor occupancy were measured after administration of drugs that induce or have a possibility to induce parkinsonism (haloperidol, flunarizine, manidipine, oxatomide, hydroxyzine, meclizine, and homochlorcyclizine). All of the tested drugs blocked both dopamine D_1 and D_2 receptors. Intensity of catalepsy was predicted with this dynamic model and was compared with the observed values.

In contrast with haloperidol, flunarizine, manidipine, and oxatomide (which induced catalepsy), hydroxyzine, meclizine, and ho-
mochlorcyclizine failed to induce catalepsy.

Intensities of catalepsy predicted with this dynamic model considering the interaction between D_1 and D_2 receptors overestimated the observed values, suggesting that these drugs have catalepsy-reducing properties as well. Because muscarinic acetylcholine (mACh) receptor antagonists inhibit the induction of catalepsy, the anticholinergic activities of the drugs were investigated. After SCH23390, nemonapride and scopolamine were administered simultaneously; catalepsy and *in vivo* mACh receptor occupancy were measured to evaluate quantitatively the anticholinergic activity. Relationship between mACh receptor occupancy and change in catalepsy was used as the measure of catalepsy-reducing effects of the drugs.

Measurement of *in vivo* mACh receptor occupancy revealed a significant blockade of mACh receptor by all of the tested drugs except for haloperidol. The predicted values of catalepsy, when corrected for the mACh receptor-related reduction, approached the observed values. This finding indicates the possibility that mACh receptor antagonism of drugs may contribute to the reduction of catalepsy. In conclusion, the dynamic model considering D_1, D_2, and mACh receptor occupancies and synergism between D_1 and D_2 receptors may be useful for quantitative prediction of drug-induced catalepsy.

Catalepsy, a behavioral immobility, is associated with varying degrees of enhanced muscular rigidity (1). The state is induced in animals that have been given certain drugs, particularly dopaminergic blockers such as neuroleptics, which are known to induce parkinsonism in clinical practice. In this study, catalepsy was investigated in mice as an experimental animal model of extrapyramidal side effects (2).

Flunarizine and cinnarizine are calcium channel blockers with a piperazinyl group, which are used in the treatment of cerebral blood flow disturbances. Chouza *et al.* (3) first reported parkinsonism, tardive dyskinesia, akathisia, and depression in patients treated with flunarizine; Marti Masso *et al.* (4) reported cinnarizine-induced parkinsonism. Over the last decade, several similar clinical cases of flunarizine- and/or cinnarizine-induced extrapyramidal disorders have been reported (5–8).

Manidipine is also a calcium channel blocker with a piperazinyl

group, which is used in the treatment of hypertension. There have been reports of worsening of parkinsonian symptoms after manidipine treatment (9) and manidipine-induced parkinsonism (10). Oxatomide is a histamine H_1 antagonist with a piperazinyl group, which is used in the treatment of allergies. Although there has been no report of oxatomide-induced parkinsonism, extrapyramidal symptoms have been reported after oxatomide treatment (11, 12).

Because all of these drugs have a piperazinyl group, a bis-phenyl methyl group or its analogs and an alkyl group (fig. 1), it is proposed that these groups are related with the ability of inducing parkinsonism (8, 13–15). In the present study, we focused on drugs with similar chemical structure (hydroxyzine, meclizine, and homochlorcyclizine), which are clinically used in Japan.

Although the mechanism of drug-induced parkinsonism has not been fully clarified, it is accepted that those symptoms are mainly caused by binding of the drugs to dopamine receptors at the striatum and their antagonistic action at the dopaminergic system (16). Both dopamine D_1 and D_2 receptor antagonists induce catalepsy in rats (17, 18). Moreover, it has been reported that the incidence of catalepsy is increased synergistically by the combination of D_1 and D_2 receptor antagonists (18, 19). Based on these concepts, a dynamic model has
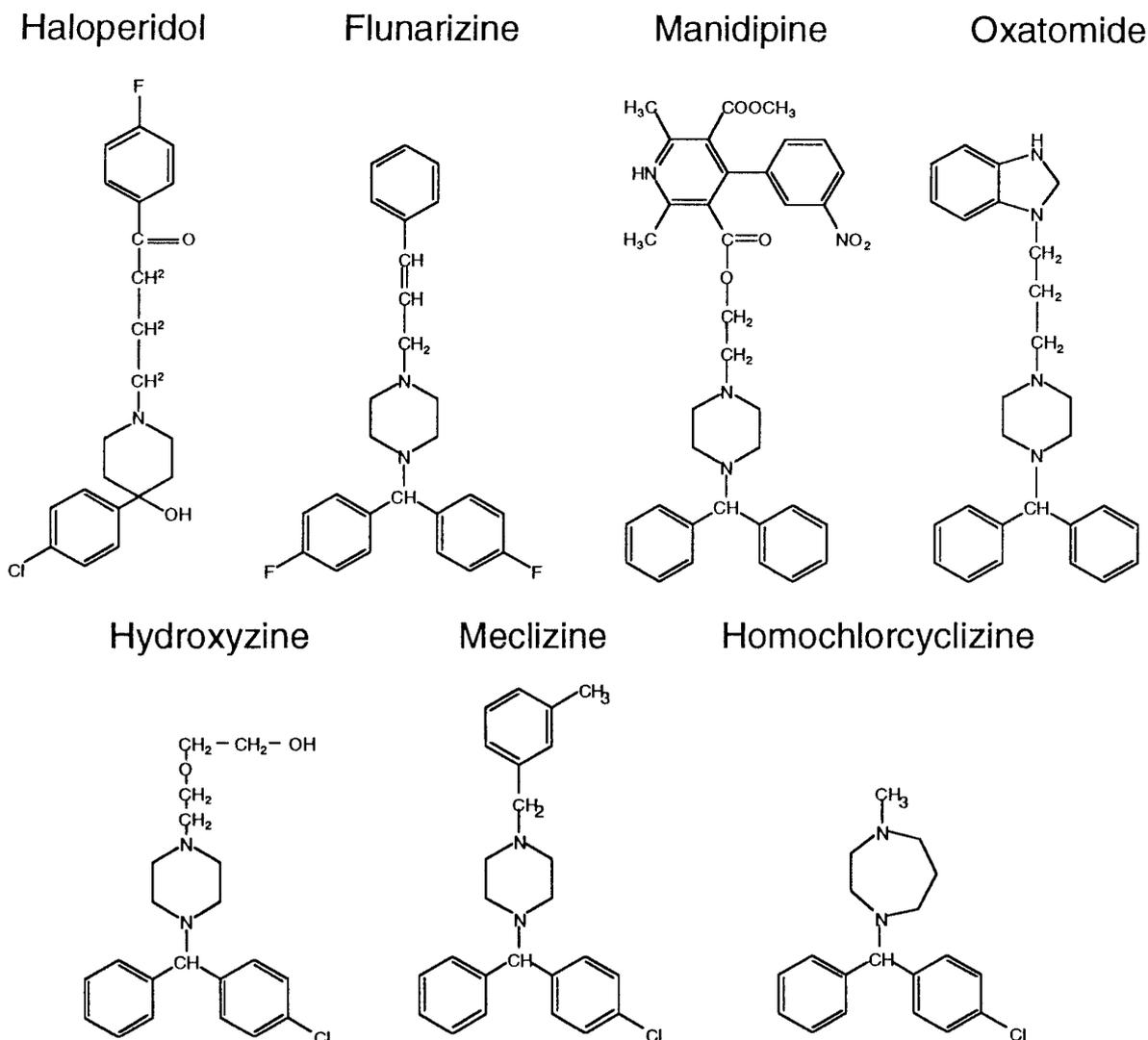
FIG. 1. *The chemical structures of drugs.*

been constructed in the present study that accounts for this synergistic effects using selective D_1 and D_2 receptor antagonists. We quantitatively estimated the intensity of catalepsy induced by dopamine receptor antagonists that bind to D_1 and D_2 receptors nonselectively.

Although drug-induced parkinsonism and catalepsy are both inhibited by anticholinergic agents (17, 20, 21), it is possible that catalepsy is hardly induced in the case of drugs that have anticholinergic activity as well. Therefore, *in vivo* mACh[1] receptor occupancies of the drugs were measured, and the predicted intensity of catalepsy based on D_1 and D_2 receptor occupancies was modified by mACh receptor occupancy.

Methods

Animals. Male ddY mice, weighing 25–30 g, were purchased from the Nippon Bio-Supp Center (Tokyo, Japan).

Drugs. The following drugs were obtained as gifts from the respective companies: haloperidol (Dainippon Pharmaceutical Co., Osaka, Japan); flunarizine hydrochloride and oxatomide (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan); manidipine hydrochloride (Takeda Chemical Industries, Ltd., Osaka,

Japan); hydroxyzine and meclizine (Pfizer Pharmaceuticals, Inc., Tokyo, Japan); homochlorcyclizine (Eisai Co., Ltd., Tokyo, Japan); and nemonapride (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan). Scopolamine hydrobromide monohydrate and atropine sulfate monohydrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and R-(+)-SCH23390 hydrochloride from Research Biochemicals, Inc. ^{3}H-SCH23390 (specific activity: 71.1 Ci/mmol), ^{3}H-raclopride (specific activity: 79.5 Ci/mmol), ^{3}H-QNB (specific activity: 52.3 Ci/mmol), Solvable and Atomlight were purchased from NEN Research Products (Boston, MA). Other chemicals were obtained from commercial sources.

In the *in vivo* study, haloperidol and nemonapride were dissolved in 0.3% tartaric acid and diluted with saline. Flunarizine hydrochloride was dissolved in 1.5% tartaric acid and diluted with saline. Hydroxyzine, homochlorcyclizine, scopolamine hydrobromide monohydrate, atropine sulfate monohydrate, and R-(+)-SCH23390 hydrochloride were dissolved in saline. Manidipine hydrochloride was dissolved in ethanol:PEG-400 = 1:1 at 50°C and diluted with PEG-400:saline = 1:1. Oxatomide and meclizine were used as aqueous suspensions containing 0.3% carboxymethylcellulose sodium. All of the unlabeled drugs were injected in a volume of 10 ml/kg.

In the *in vitro* study, flunarizine hydrochloride, hydroxyzine, and homochlorcyclizine were dissolved in distilled water. Manidipine hydrochloride, haloperidol, and meclizine were dissolved in 10% methanol, 0.3% tartaric acid, and 10% dimethylsulfoxide, respectively.

Measurement of Catalepsy. Nemonapride (0.01–1 mg/kg), haloperidol

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[1] Abbreviations used are: mACh, muscarinic acetylcholine; QNB, quinuclizinyl benzilate; PEG-400, polyethyleneglycol 400; IC_{50}, inhibitory concentration of 50%.
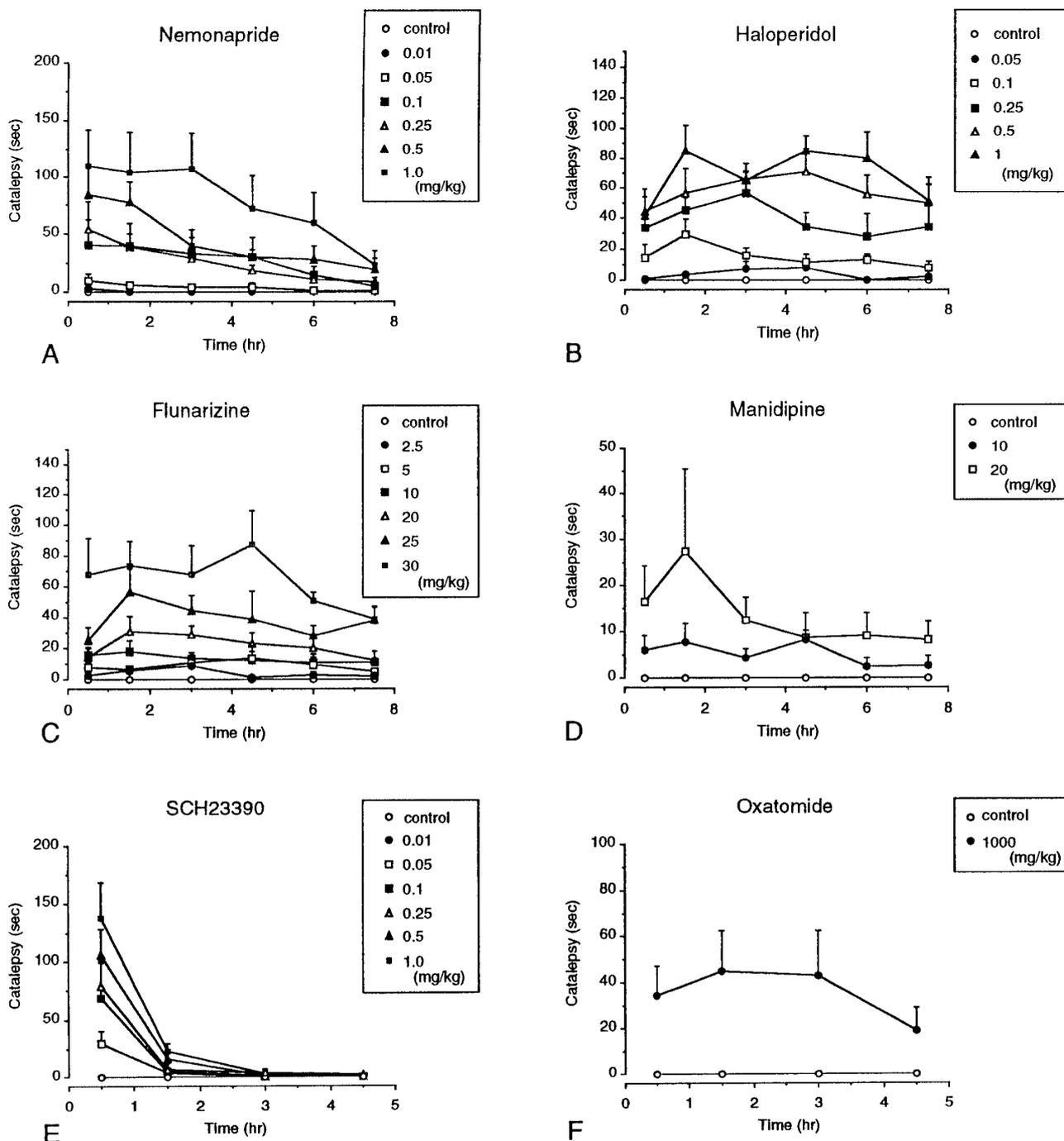
FIG. 2. *Time course of catalepsy induced by six drugs.*

The incidence of catalepsy was assessed at 0.5, 1.5, 3, 4.5, 6, and 7.5 hr for (*A*); nemonapride (*B*); haloperidol (*C*); flunarizine and (*D*); manidipine or 0.5, 1.5, 3, and 4.5 hr for (*E*); SCH23390 and (*F*); oxatomide after administration of drugs. Data are means ± SE (*N* = 7–10).

(0.05–1 mg/kg), flunarizine (2.5–30 mg/kg), and meclizine (300 mg/kg) were injected intraperitoneally; SCH23390 (0.01–1 mg/kg), manidipine (10–20 mg/kg), hydroxyzine (100 mg/kg), and homochlorcyclizine (200 mg/kg) were injected subcutaneously; oxatomide (1,000 mg/kg) was given orally. Control animals were administered with the respective solvent under the same condition as previously described. At 0.5, 1.5, 3, 4.5, 6, and 7.5 hr after administration of drugs, except for SCH23390 and nemonapride (0.5, 1.5, 3, and 4.5 hr), 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 (22).

Effect of Scopolamine on Catalepsy. In an attempt to confirm that catalepsy induced by drugs is not caused by peripheral action, we investigated the effect of scopolamine, which is used in the treatment of Parkinson's disease (17, 20). SCH23390 (0.5 mg/kg) or manidipine (20 mg/kg) and scopolamine (10 mg/kg) were simultaneously administered subcutaneously to mice, and catalepsy was measured as previously described. For nemonapride (0.5 mg/kg), flunarizine (30 mg/kg), haloperidol (1 mg/kg), and oxatomide (1,000 mg/kg), catalepsy was measured at 60 min after injection of each drug under the same condition as in *Measurement of Catalepsy* and then scopolamine (10 mg/kg) was administered subcutaneously. Subsequently, catalepsy was measured every hour for 3 hr.
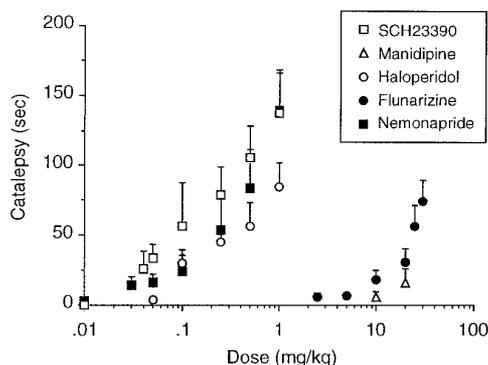
FIG. 3. *Dose-dependent induction of catalepsy.*

($\square$) SCH23390-; ($\triangle$) manidipine-induced catalepsy 30 min after subcutaneous administration; ($\bigcirc$) haloperidol-; ($\bullet$) flunarizine-; and ($\blacksquare$) nemonapride-induced catalepsy 90 min after intraperitoneal administration. Data are means $\pm$ SE ($N = 7-10$).

Measurement of *In Vivo* Dopamine D_1, D_2, and mACh Receptor Occupancies. Each drug or vehicle was administered to mice under the same condition as in *Measurement of Catalepsy*. At 25 or 85 min after administration of the drugs, D_1-selective antagonist ³H-SCH23390 (3 μCi/body), D_2-selective antagonist ³H-raclopride (3 μCi/body), or a mACh-specific antagonist ³H-QNB (3 μCi/body) was injected intravenously (23). At 10 min postinjection, mice were decapitated, and striatum and cerebellum were dissected on a glass plate. Each sample was weighed in a vial, added with 1 ml of Solvable, and incubated at 50°C until it became a clear solution. After 0.2 ml of 30% H_2O_2 was added, the vial was left at room temperature for 60 min and 10 ml of Atomlight was added. The radioactivities were measured in a liquid scintillation counter (LSC-3100, Aloka).

Dopamine and mACh receptor occupancies were calculated according to eqs. 1 and 2, respectively:

$$\Phi(\%) = \left(1 - \frac{A-1}{B-1}\right) \times 100, \tag{1}$$

where A and B are the radioactivity ratios (striatum/cerebellum) in the presence and absence of drugs, respectively. The cerebellum was used as a dopamine receptor-free region to estimate nonspecific binding of ligands.

$$\Phi(\%) = \left(1 - \frac{A-C}{B-C}\right) \times 100, \tag{2}$$

where A and B are the radioactivities in the striatum in the presence and absence of drugs, respectively, and C is the nonspecific binding that was determined by subcutaneous administration of atropine (50 mg/kg) at 25 min before administration of ³H-QNB.

***In Vitro* Dopamine D_1, D_2, and mACh Receptor Binding Studies.** Homogenates of striatal tissue from mice were prepared in 100 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with a Teflon-on-glass tissue homogenizer. Homogenates were centrifuged (20,000*g* for 10 min at 4°C) twice with intermediate resuspension in ice-cold 50 mM Tris-HCl buffer (pH 7.4). The final pellets were resuspended in 200 and 300 volumes (w/v) of the buffer for dopamine and mACh receptors, respectively (24).

Aliquots of the membrane preparations were incubated with each drug and 0.3 nM ³H-SCH23390 (for D_1 receptor binding) or 1 nM ³H-raclopride (for D_2 receptor binding) for 15 min at 37°C in 50 mM Tris-HCl buffer (pH 7.4) containing (in millimolar): NaCl, 120; KCl, 5; CaCl₂, 2; and MgCl₂, 1. For mACh receptor binding, aliquots of the membrane preparations were incubated with each drug and 0.2 nM ³H-QNB for 30 min at 37°C in 50 mM Tris-HCl buffer (pH 7.4). The final tissue concentrations were 1 mg of original wet weight tissue per 1 ml for D_1 and D_2 receptor bindings and 2 mg/3 ml for mACh receptor binding.
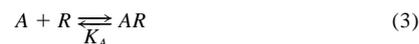
Incubation was terminated by rapid pouring of the contents of the tubes over Whatman GF/C glass fiber filters under vacuum. Filters were rinsed twice with 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and placed in glass

scintillation vials; 8 ml of Atomlight was then added. Vials were thoroughly shaken and counted for tritium at 40% average efficiency.

Nonspecific binding was determined in the presence of 100 nM SCH23390, 1 μM nemonapride, and 1 μM atropine for D_1, D_2, and mACh receptor bindings, respectively.

IC_{50} values for the displacement of ³H-SCH23390, ³H-raclopride, and ³H-QNB were determined by log-probit analysis of data from inhibition experiments. K_i values were calculated according to the equation: $K_i = IC_{50}/(1 + L/K_d)$ with L the concentration and K_d the dissociation constant of the ³H-ligand obtained from Scatchard analysis of saturation experiment data.

Quantitative Analysis of Relationship between Dopamine D_1 and D_2 Receptor Occupancies and Catalepsy. Relationship between D_1 and D_2 receptor occupancies and catalepsy after coadministration of SCH23390 (0–0.5 mg/kg) and nemonapride (0–0.5 mg/kg) was analyzed with ternary complex model (25–27). In this model, dopamine (agonist) interacts with dopamine receptor and imagined GTP binding protein (transducer). The agonist-receptor-transducer complex elicits pharmacological responses. Catalepsy, one of the effects of antagonists, would be induced by inhibition of the formation of this ternary complex. The model can be represented as follows:

$$A + R \underset{K_A}{\rightleftarrows} AR \tag{3}$$

$$AR + T \underset{K_{AR}}{\rightleftarrows} ART \tag{4}$$

$$B + R \underset{K_B}{\rightleftarrows} BR, \tag{5}$$

where A, B, R, and T represent agonist, antagonist, receptor, and transducer, respectively; K_A, K_{AR}, and K_B represent the dissociation constants for the AR, ART, and BR complexes, respectively. According to this model, the total concentrations of receptor (R_0) and transducer (T_0) can be expressed as follows:

$$R_0 = [R] + [AR] + [BR] + [ART] \tag{6}$$

$$T_0 = [T] + [ART]. \tag{7}$$

The effect of agonist (E) is assumed to be proportional to the concentration of the ART complex:

$$E = a[ART], \tag{8}$$

where a is a constant.

Receptor occupancy of the antagonist can be expressed by the following equation:

$$\Phi(\%) = \frac{[BR]}{R_0} \times 100. \tag{9}$$

The effects of an antagonist (F) can be expressed as follows:

$$F = E_{-B} - E_{+B}, \tag{10}$$

where E_{-B} and E_{+B} represent the effects of agonist in the absence and presence of antagonist, respectively.

The Dynamic Model for the Interaction between D_1 and D_2 Receptors. Because catalepsy (G) induced by coadministration of SCH23390 and nemonapride was increased synergistically, it was hypothesized that G can be expressed as sum of mutual addition and multiplication of catalepsies induced by D_1 and D_2 receptor blockades (F_1 and F_2, respectively).

$$G = \alpha F_1 + \beta F_2 + \gamma F_1^x \cdot F_2^y, \tag{11}$$

where α and β represent the contribution of each receptor in the additional effects, x and y represent the contribution of each receptor in the synergistic effects, and γ represents the contribution of synergism.

Quantitative Analysis of the Effect of mACh Receptor Blockade on Change in Catalepsy. The relationship between mACh receptor occupancy
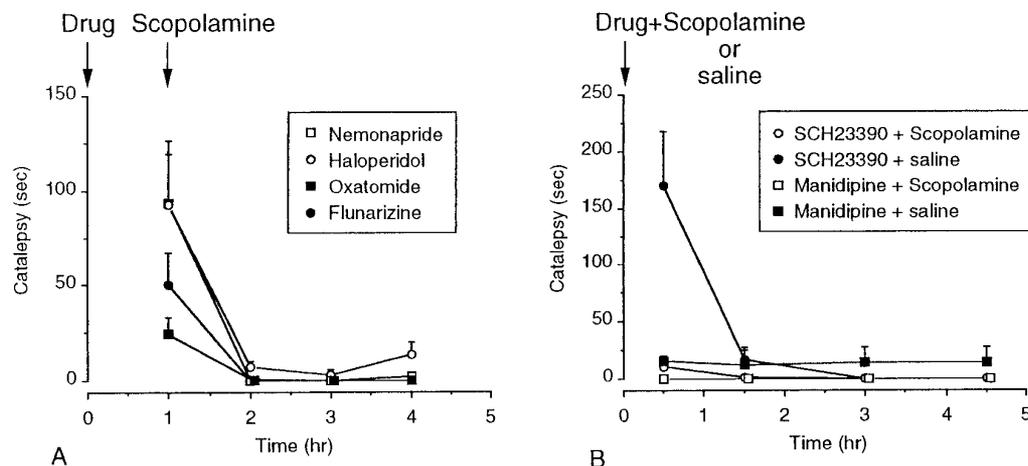
FIG. 4. *Effect of scopolamine on drug-induced catalepsy.*

(A) □, Nemonapride (0.5 mg/kg ip); ○, haloperidol (1 mg/kg ip); ■, oxatomide (1,000 mg/kg po); and ●, flunarizine (30 mg/kg ip) were administered 60 min before subcutaneous injection of scopolamine (10 mg/kg). (B) ○, SCH23390 (0.5 mg/kg sc) and scopolamine (10 mg/kg sc); ●, SCH23390 (1 mg/kg sc) and saline; □, manidipine (20 mg/kg sc) and scopolamine (10 mg/kg sc); and ■, manidipine (20 mg/kg sc) and saline were co-administered. Data are means ± SE ($N = 5$–7).

and catalepsy at 30 min after simultaneous administration of SCH23390 (0.04 mg/kg), nemonapride (0.03 mg/kg), and scopolamine (0–1 mg/kg) was analyzed by linear regression analysis.

Results

Time Course of Drug-Induced Catalepsy. Time courses of catalepsy induced by nemonapride, haloperidol, flunarizine, SCH23390, manidipine, and oxatomide are shown in fig. 2 (*A–F*). All of these drugs induced catalepsy in a dose-dependent manner. Catalepsy was observed for several hours, except for SCH23390. In any case, catalepsy was not observed in the mice treated with vehicle.

Dose-Dependency of Drug-Induced Catalepsy. Catalepsy induced by SCH23390, nemonapride, haloperidol, flunarizine, and manidipine showed dose-dependency (fig. 3). The minimum doses at which flunarizine and manidipine induced catalepsy were ~100 times higher than those for SCH23390, nemonapride, and haloperidol.

Effect of Scopolamine on Catalepsy. Scopolamine reduced catalepsy induced by any tested drugs to the baseline level (fig. 4).

Dose-Dependency of *In Vivo* D_1 and D_2 Receptor Occupancies. Relationships between dose and dopamine receptor occupancy of SCH23390, nemonapride, haloperidol, flunarizine, and manidipine are shown in fig. 5 (*A* and *B*). Both D_1 and D_2 receptor occupancies increased in a dose-dependent manner. The binding characteristic of haloperidol to D_2 receptor was similar to that of nemonapride. All of the tested drugs, except for SCH23390, showed higher affinity for D_2 receptor than for D_1 receptor.

Relationship between Receptor Occupancies of D_1- and D_2-Selective Antagonists and Catalepsy. Relationships between receptor occupancy and catalepsy induced by administration of SCH23390 (0.01–1 mg/kg) or nemonapride (0.01–1 mg/kg) are shown in fig. 6 (*A* and *B*). The ternary complex model described herein was used for curve fitting. A nonlinear relationship was observed for all drugs in which catalepsy greatly increased by dose escalation.

Synergism between D_1- and D_2-Selective Antagonists in Inducing Catalepsy. Figure 7 shows cataleptic responses after injection of SCH23390 (0.05 mg/kg), nemonapride (0.1 mg/kg), or a combination of the two drugs. The intensity of catalepsy in the combination of SCH23390 and nemonapride significantly exceeded the sum of that

predicted from D_1 and D_2 receptor occupancies (fig. 6), suggesting the existence of synergism between D_1 and D_2 receptor antagonists in inducing catalepsy.

Relationship between Receptor Occupancy and Catalepsy in the Combination of D_1 and D_2 Receptor-Selective Antagonists. Relationships between receptor occupancy and catalepsy in the combination of SCH23390 (0–0.5 mg/kg) and nemonapride (0–0.5 mg/kg) are shown in fig. 8 (*A* and *B*). Catalepsy was increased synergistically at any doses, and the degree of increase was dose-dependent. The fitting curves were obtained using the ternary complex model considering synergistic interaction. Figure 9*A* illustrates the relationship between D_1 and D_2 receptor occupancies and catalepsy calculated using dynamic parameters in a three-dimensional graph. Figure 9*B* shows the sections of fig. 9*A* at which intensity of catalepsy was 100, 200, and 300 sec. Using these graphs, the intensity of catalepsy might be predicted by receptor occupancies of the drug.

***In Vivo* and *In Vitro* D_1 and D_2 Receptor Binding and Catalepsy.** Intensities of catalepsy measured at 90 min after administration of haloperidol (0.5 mg/kg) and flunarizine (10 mg/kg) and at 30 min after administration of manidipine (20 mg/kg), oxatomide (1,000 mg/kg), hydroxyzine (100 mg/kg), meclizine (300 mg/kg), and homochlorcyclizine (200 mg/kg) are listed in table 1. Catalepsy was not observed in the mice treated with hydroxyzine, meclizine, and homochlorcyclizine. *In vivo* D_1 and D_2 receptor occupancies of the drugs under the same condition as in the measurement of catalepsy are also shown in table 1, together with *in vitro* K_i values. Figure 10 (*A* and *B*) shows the inhibition curves for *in vitro* binding of D_1 or D_2 receptor-selective radioligands in the presence of the tested drugs. Both D_1 and D_2 receptors were blocked by all of these drugs both *in vivo* and *in vitro*, with the binding affinity for D_2 receptor higher than that for D_1 receptor in any cases.

Relationship between Observed Values of Catalepsy and Those Predicted by D_1 and D_2 Receptor Occupancies. Although the intensity of catalepsy predicted by the model considering interaction between D_1 and D_2 receptors significantly correlated with the observed values ($r = 0.77$, $p < 0.001$), the predicted values overestimated the observed values (fig. 11).

Relationship between mACh Receptor Occupancy and Change in Catalepsy in the Case of Combination of D_1 and D_2 Receptor-
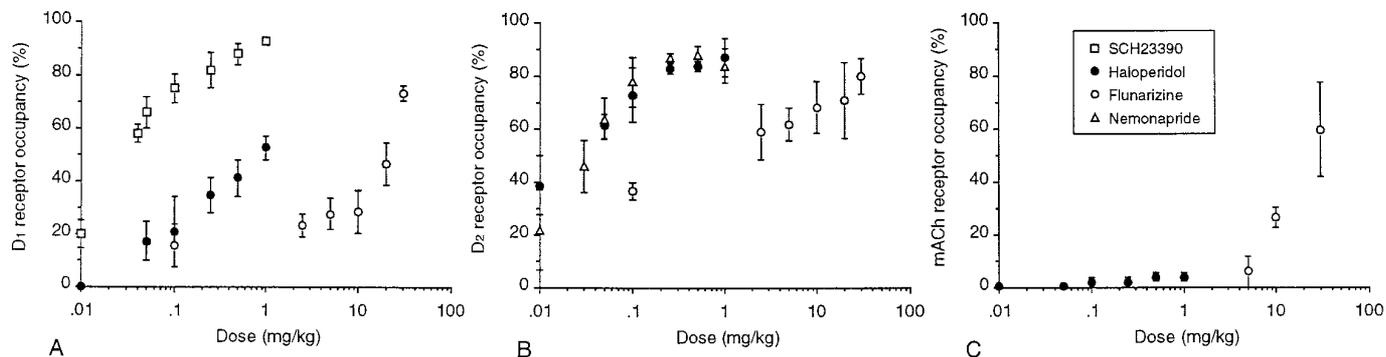
FIG. 5. *(A) Dose-dependency of D_1 receptor occupancy. (B) Dose-dependency of D_2 receptor occupancy. (C) Dose-dependency of mACh receptor occupancy.*

□, SCH23390 30 min after subcutaneous administration; ●, haloperidol; ○, flunarizine; and △, nemonapride 90 min after intraperitoneal administration. Data are means ± SE (N = 3–5).
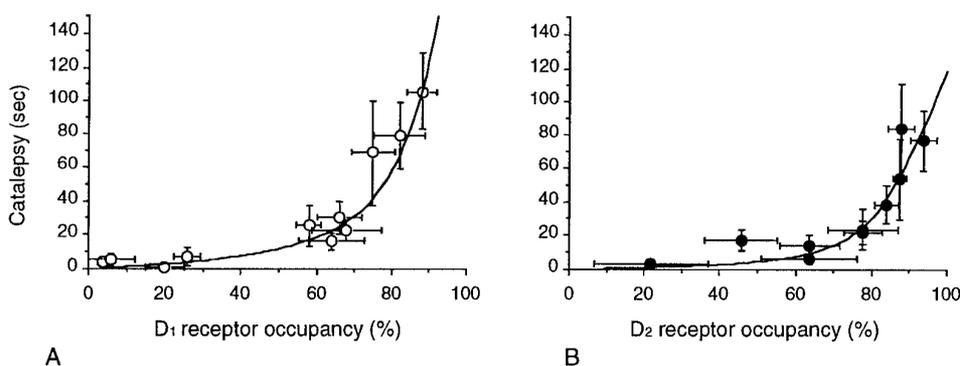

FIG. 6. *(A) Relationship between D_1 receptor occupancy and SCH23390-induced catalepsy. (B) Relationship between D_2 receptor occupancy and nemonapride-induced catalepsy.*

Curves represent best fit to the data obtained by the ternary complex model.
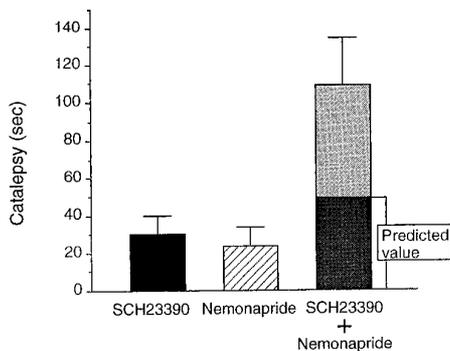

FIG. 7. *Catalepsy induced by SCH23390 (0.05 mg/kg) and nemonapride (0.1 mg/kg) and coadministration of SCH23390 (0.05 mg/kg) and nemonapride (0.1 mg/kg).*

Data are means ± SE (N = 7–8).

Selective Antagonists and Scopolamine. As shown in fig. 12, catalepsy induced by coadministration of SCH23390 and nemonapride was reduced by scopolamine, depending on mACh receptor occupancy.

In Vivo **and** *In Vitro* **mACh Receptor Binding.** *In vivo* mACh receptor occupancies of the tested drugs are shown in table 1 and fig. 5C. Although haloperidol and manidipine hardly blocked mACh receptor, flunarizine at high dosage and the other drugs were more

active in blocking mACh receptor. Figure 10C shows the inhibition curves for *in vitro* binding of mACh receptor-specific radioligand in the presence of the tested drugs. *In vitro* K_i values are listed in table 1. The K_i values of the tested drugs were high except for homochlorcyclizine (102 nM).

Relationship between Observed Values of Catalepsy and Those Predicted Considering mACh Receptor Occupancy. As shown in fig. 13, predicted values of catalepsy corrected by considering the contribution to mACh receptor occupancy approached the observed values, showing the larger correlation coefficient (r = 0.86, $p <$ 0.001) compared with that in fig. 11.

Discussion

The objective of the present study was to estimate quantitatively the intensity of catalepsy induced by dopamine receptor antagonists based on the relationship between *in vivo* D_1 and D_2 receptor occupancies and intensity of catalepsy in mice.

It is known that haloperidol and other neuroleptics induce catalepsy in a dose-dependent manner (22, 28). The present findings indicate that catalepsy induced by SCH23390, nemonapride, flunarizine, and manidipine also shows dose-dependency (fig. 3).

In an attempt to confirm that the observed catalepsy is not caused by peripheral mechanism, anticholinergic agent scopolamine, which is transported into the 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 and that it is mediated by cholinergic
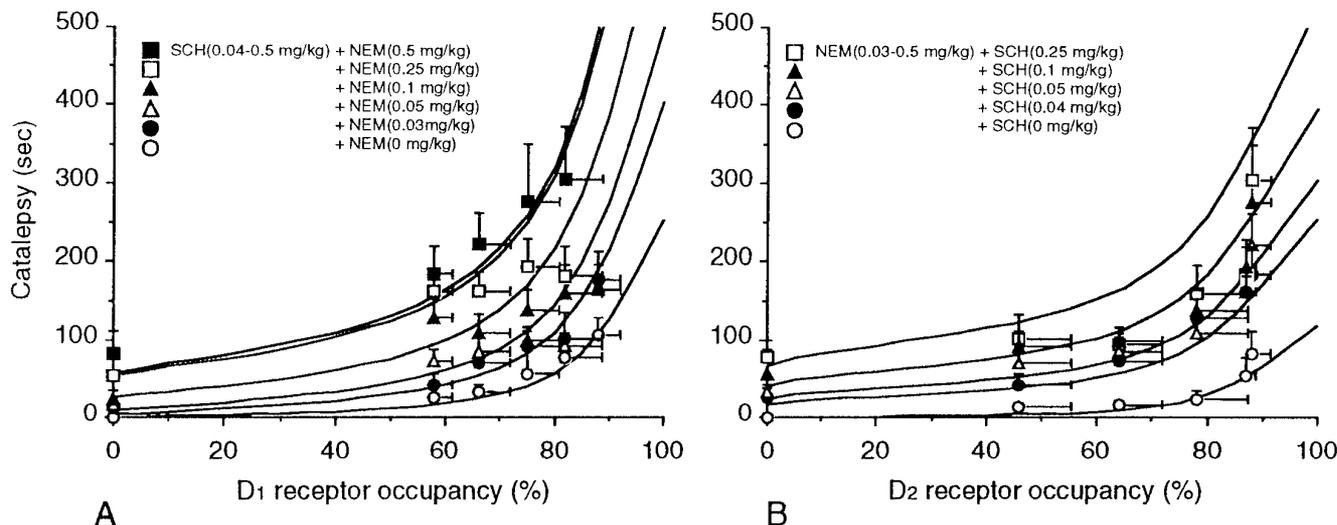
FIG. 8. *Relationship between D_1 (A) or D_2 (B) receptor occupancy and catalepsy induced by coadministration of SCH23390 (0.04–0.5 mg/kg) and nemonapride (0.03–0.5 mg/kg).*

Data are means $\pm$ SE (receptor occupancy: $N = 3$–5; catalepsy: $N = 7$–8). *Curves* represent best fit to the data obtained by the ternary complex model.
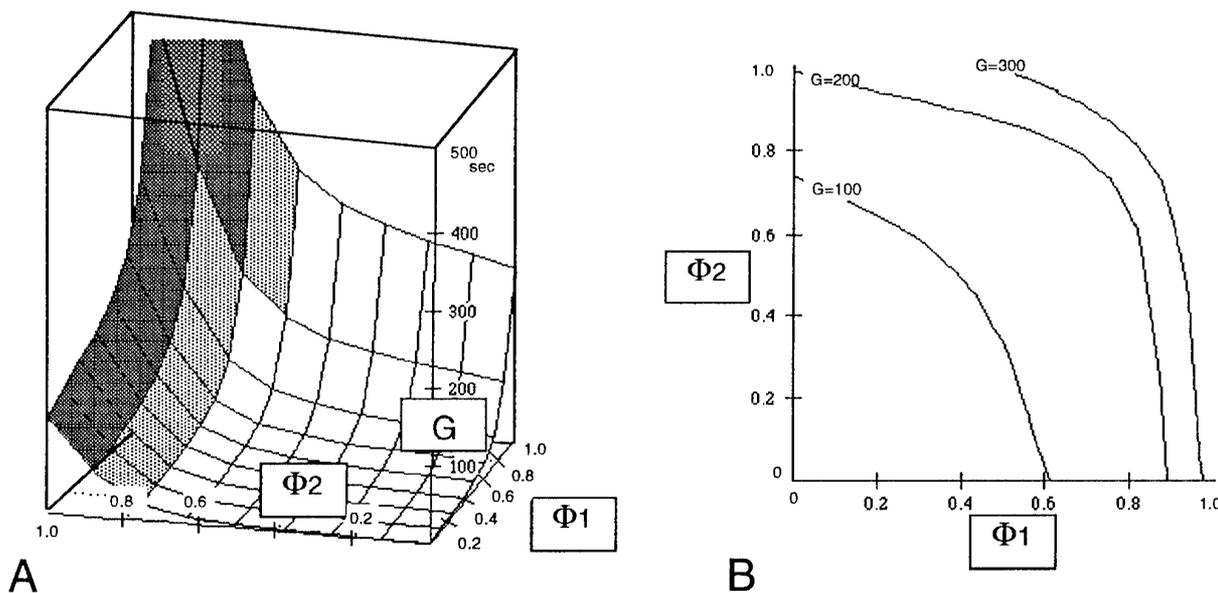

FIG. 9. *(A) Relationship between D_1 and D_2 receptor occupancies (Φ_1 and Φ_2) and catalepsy (G). (B) Sections of (A) at G = 100, 200, and 300 sec.*

stimulation *via* muscarinic receptor. Catalepsy, although weaker than that for haloperidol, was observed in the mice treated with flunarizine, manidipine, and oxatomide. Oxatomide-induced catalepsy was observed at considerably high dose of 1,000 mg/kg. On the other hand, catalepsy was not induced by hydroxyzine, meclizine, and homochlorcyclizine at the doses used in this study (table 1).

The reported values of K_i for D_1 and D_2 receptor in rats are 532 $\pm$ 39 nM and 112 $\pm$ 9 nM (29), respectively, for flunarizine and 76 and 2.6 nM (30), respectively, for haloperidol. Our findings in this study in mice are in agreement with these results. Manidipine and oxatomide, which induced catalepsy, also blocked both D_1 and D_2 receptors *in vitro* and *in vivo* [table 1, fig. 10 (*A* and *B*)].

The chemical structures of manidipine, oxatomide, hydroxyzine, meclizine, and homochlorcyclizine are partly similar to that of flunarizine and cinnarizine (which are known to induce catalepsy), having piperazinyl or the bis phenyl methyl group in common (fig. 1). Although catalepsy was not induced by hydroxyzine, meclizine, and

homochlorcyclizine, both D_1 and D_2 receptors were blocked *in vitro* and *in vivo* by these drugs [table 1, fig. 10 (*A* and *B*)], suggesting that the ability of blocking dopamine receptors is profoundly related with the structures of the drugs.

Both D_1 and D_2 receptor occupancies were increased in a dose-dependent manner [fig. 5 (*A* and *B*)]. Relationship between D_1 and D_2 receptor occupancies and catalepsy could be analyzed by the ternary complex model [fig. 6 (*A* and *B*)].

To simulate catalepsy induced by drugs that bind to D_1 and D_2 receptors nonselectively, both D_1 and D_2 receptors were blocked by coadministration of SCH23390 and nemonapride. Catalepsy was increased synergistically, compared with the case of selective blockade of each receptor (fig. 7). There have been some reports of synergistic interaction between D_1 and D_2 antagonists in inducing catalepsy (18, 19). A number of reports, using selective agonists, have suggested interaction between D_1 and D_2 receptors both *in vivo* and *in vitro*. In a rat model of Parkinson's disease, concurrent administration of the

TABLE 1

Catalepsy, in vivo receptor occupancy, and in vitro K_i values of the tested drugs for D_1, D_2, and mACh receptor

	Dose	Catalepsy	Receptor Occupancy (%)			K_i (nM)		
			D_1	D_2	mACh	D_1	D_2	mACh
	mg/kg	*sec*						
Haloperidol	0.5	56.1 ± 16.9	41.3 ± 6.8	84.0 ± 1.9	0	74	1.1	2,333
Flunarizine	10	17.9 ± 6.8	28.7 ± 8.1	68.3 ± 9.7	26.7 ± 3.9	905	71	3,513
Manidipine	20	16.4 ± 8.9	24.3 ± 5.5	38.0 ± 9.9	14.3 ± 6.5	124	117	>10,000
Oxatomide	1,000	34.4 ± 12.5	8.0 ± 6.9	54.4 ± 8.9	35.7 ± 10.8	>10,000	25	>10,000
Hydroxyzine	100	0	58.7 ± 7.9	75.1 ± 6.0	48.8 ± 10.6	>10,000	378	>10,000
Meclizine	300	0	12.9 ± 5.8	36.9 ± 1.1	40.8 ± 11.0	>10,000	109	>10,000
Homochlorcyclizine	200	0	36.8 ± 7.0	72.8 ± 14.4	87.3 ± 5.7	1,835	157	102

[3]H-SCH23390, ^{3}H-raclopride, and ^{3}H-QNB were used for labeling of dopamine D_1, D_2, and mACh receptor, respectively, both *in vivo* and *in vitro*. Data are means ± SE (receptor occupancy: $N = 3–5$; catalepsy: $N = 7–8$).

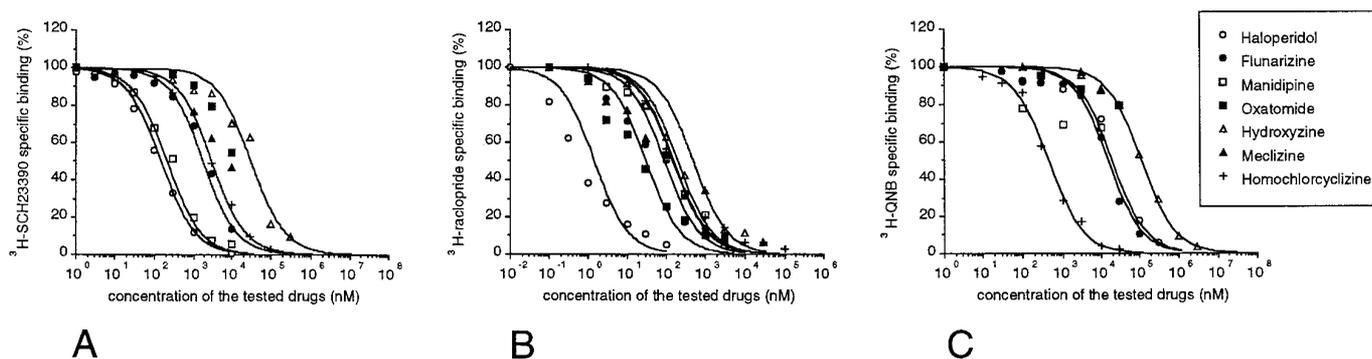A   B   C

FIG. 10. *Inhibition curves for binding of ^{3}H-SCH23390 (A), ^{3}H-raclopride (B), and ^{3}H-QNB (C) to mouse striatal membranes in the presence of haloperidol (○), flunarizine (●), manidipine (□), oxatomide (■), hydroxyzine (△), meclizine (▲), and homochlorcyclizine (+).*

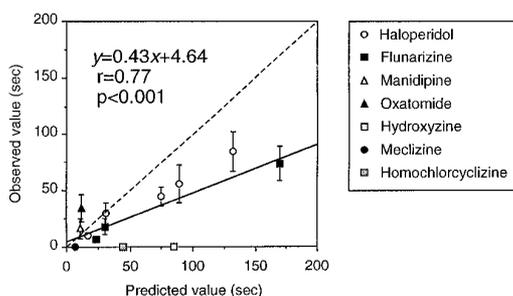Each *point* is the mean of three determinations performed in duplicate.

FIG. 11. *Relationship between observed values of catalepsy and those predicted by D_1 and D_2 receptor occupancies.*

Dashed line is the line of identity, and the *solid line* represents best fit to the data obtained by linear regression analysis. Each drug was administered to mice under the same condition as in *Methods*.

FIG. 12. *Relationship between mACh receptor occupancy of scopolamine and change in catalepsy induced by coadministration of SCH23390 (0.04 mg/kg) and nemonapride (0.03 mg/kg).*

Data are means ± SE (receptor occupancy: $N = 3–5$; catalepsy: $N = 5–7$). *Solid line* represents best fit to the data obtained by linear regression analysis.

D_1 agonist (SKF38393) with the D_2 agonist (quinpirole) produced a synergistic effect on active rotation (31, 32) and *c*-fos expression in the striatum (31). Pallidal cell activity was significantly potentiated by concurrent D_1 and D_2 receptor stimulation (33). In the *in vitro* study, the (Na^+, K^+)-ATPase activity of striatal neurons in the guinea pig was synergistically inhibited by the co-activation of both receptors (34). Activation of D_1 and D_2 receptors resulted in marked synergistic potentiation of arachidonic acid release when both subtypes were co-expressed in the CHO cell (35). The electrically evoked release of ^{3}H-GABA on rat cortical slices was inhibited synergistically by agonists of both subtypes (36).

These results suggest that synergistic interaction has to be taken into consideration to predict the intensity of catalepsy induced by
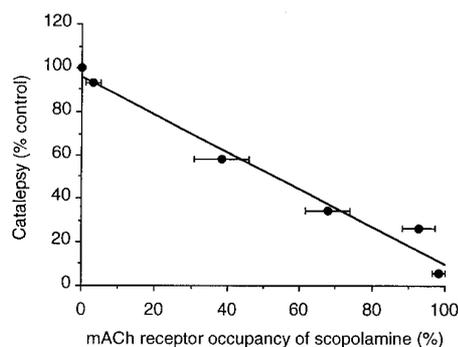
antagonists that bind to both D_1 and D_2 receptors nonselectively. Therefore, SCH23390 and nemonapride were administered simultaneously at various doses. Catalepsy was intensified synergistically at any doses, and the degree of potentiation was increased dose-dependently [fig. 8 (*A* and *B*)]. Because the mechanism of interaction between D_1 and D_2 receptors is unclear, a model was constructed that explains the relationship between D_1 and D_2 receptor occupancies, the first step in the induction of catalepsy, and the intensity of catalepsy. The model was based on the ternary complexed model, which had been shown by Yamada *et al.* (26) to be able to explain the relation-
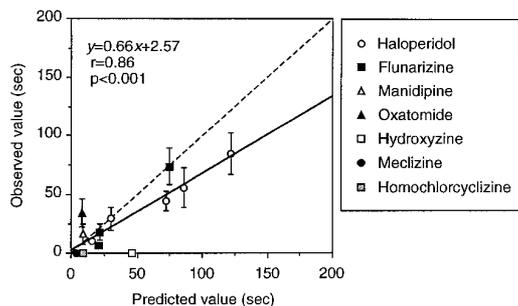
FIG. 13. *Relationship between observed values of catalepsy and those predicted by D_1, D_2, and mACh receptor occupancies.*

Each drug was administered to mice under the same condition as in *Methods*. *Dashed line* is the line of identity, and *solid line* represents best fit to the data obtained by linear regression analysis.

ship between dopamine receptor occupancy of the antagonists and their pharmacological effects.

The values of catalepsy predicted by both receptor occupancies using the model were compared with the observed values (fig. 11). The predicted values overestimated the observed values.

One of the reasons for the overestimation may be the drugs' intrinsic ability to reduce catalepsy, including anticholinergic activity. Therefore, change in catalepsy and mACh receptor occupancy was measured in combination with SCH23390, nemonapride, and scopolamine (fig. 12) to estimate the contribution of mACh receptor blockade.

mACh receptor was hardly blocked by haloperidol and manidipine *in vivo*. On the other hand, flunarizine at 30 mg/kg and the other drugs blocked mACh receptor (table 1, fig. 5C). The *in vitro* mACh receptor binding affinity of homochlorcyclizine, which failed to induce catalepsy, was relatively high, with a K_i value of ~100 nM (table 1, fig. 10C).

The predicted values of catalepsy, when corrected for the mACh receptor-related reduction, approached the observed values (fig. 13). This finding indicates that the contribution of anticholinergic activity has to be taken into account for the evaluation of catalepsy.

However, the predicted values at high doses of haloperidol and those for hydroxyzine, meclizine, and homochlorcyclizine, which failed to induce catalepsy, still do not match the observed values (fig. 13). It is known that haloperidol binds to the 5-HT$_2$ receptor, as well as to both D_1 and D_2 receptors. The reported values of K_i of haloperidol for the 5-HT$_2$ receptor measured using ^{3}H-spiperone are 95 nM in rats (37) and 36 nM in human (38). Moreover, 5-HT$_2$ receptor antagonists have been reported to reduce catalepsy (39–41). Clozapine, an atypical neuroleptic, scarcely induces extrapyramidal adverse effects, although it binds to both D_1 and D_2 receptors. This may be explained by its relatively potent antagonistic activity in central serotonergic receptor functions. The K_i value of clozapine for the 5-HT$_2$ receptor in the postmortem human brain is reported to be 1.6 nM (38). Therefore, it may be necessary that the contribution of 5-HT$_2$ receptor binding be taken into consideration for drugs such as haloperidol, which has a high affinity for 5-HT$_2$ receptor. Because the doses of haloperidol used in this study are much higher than those in clinical treatment and nemonapride also binds to the 5-HT$_2$ receptor with similar affinity as haloperidol (37), the difference between the predicted and observed values at high dosage might be caused by other factors.

Hydroxyzine, meclizine, and homochlorcyclizine are the histamine H$_1$ receptor antagonists. There have been reports that mepyramine, a H$_1$ receptor antagonist, reduced perphenazine-induced catalepsy in

rats (42) and that chlorcyclizine, another H$_1$ receptor antagonist, reduced morphine-induced catalepsy in mice (43). These findings indicate that the blockade of the H$_1$ receptor also might have some effect on catalepsy.

Drugs that possess the piperazinyl group or similar structure, even if parkinsonism has not been reported to date, might induce parkinsonism. The incidence may be increased in the elderly, patients with hepatic or renal disorder, and those who use antipsychotics concurrently. Extrapyramidal signs have been reported in a patient with severe hepatic disease, which might have been caused by an increase in plasma concentration of meclizine that failed to induce catalepsy in this study (44). Drugs that are hardly transported into the brain in normal condition might be increasingly transported into the brain in disease states. Therefore, it is necessary that binding of the drugs to dopamine receptors are investigated both *in vivo* and *in vitro*.

At first, *in vitro* K_i values are measured for a novel drug that is expected to block dopamine receptor from its chemical structure. If it binds to dopamine receptor, catalepsy and *in vivo* dopamine and mACh receptor occupancy should be measured. Catalepsy might be predicted quantitatively by the model proposed in the present study. To predict the intensity of drug-induced parkinsonism in humans, dopamine and mACh receptor occupancies have to be estimated by unbound concentration in plasma after administration of normal dose and K_i values obtained *in vitro*. Transport through the blood-brain barrier may also have to be considered in case of drugs with low lipophilicity. Intensity of catalepsy might be predicted by relationship between receptor occupancies and catalepsy in the animal model, and compared with that induced by drugs such as haloperidol or flunarizine, whose ability to induce parkinsonism is already known. The risks of drug-induced parkinsonism may thus be predicted using this method.

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