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Prediction of Catalepsies Induced by Amiodarone, Aprindine and Procaine: Similarity in Conformation of Diethylaminoethyl Side Chain[1]

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ABSTRACT

Recently, clinical cases of parkinsonism due to antiarrhythmics drugs amiodarone and aprindine and a local anesthetic drug procaine have been reported. We performed both *in vivo* and *in vitro* experiments to quantitatively predict the intensity of catalepsy by these drugs and haloperidol in mice. Haloperidol showed the most potent relative intensity of catalepsy, followed by aprindine, metoclopramide, tiapride, amiodarone and procaine, in that order. *In vivo* dopamine D_1 and D_2 receptor occupancies of the six drugs to the striatum were observed. *In vitro* binding affinity (K_i) of these drugs to the D_1 and D_2 receptors in the striatum synaptic membrane was within the range of 60 nM to 706 μM, 0.5 nM to 75 μM and 860 nM to 115 μM, respectively. A good correlation between the relative intensity of drug-induced catalepsy and the K_i values for the dopamine D_1 and D_2 receptors was obtained ($r = .911$ and $r = .896$, respectively; $P < .05$). The partial tertiary structure of the tested drugs was well superimposed on that of haloperidol. In conclusion, these drug-induced catalepsies were due to the blockade of the D_1 and D_2 receptors, which was related to the analogous tertiary structures (diethylaminoethyl side chain).

It is generally known that antipsychotic drugs, such as phenothiazine derivatives and butyrophenone derivatives, induce the parkinsonism as a serious side effect in clinical practice. It is very important to predict the intensity of the drug-induced parkinsonism because its progress is rapid and the patients' quality of life become worse. Besides antipsychotic drugs, flunarizine and cinnarizine, used in the treatment of cerebral blood flow disturbances, induce parkinsonism (Chouza *et al*., 1986; Kuzuhara *et al*., 1989; Negrotti and Calzetti, 1997). Recently, it has been reported that amiodarone (fig. 1) and aprindine, antiarrhythmic drugs, and procaine, a local anesthetic, induced parkinsonism (Dotti and Federico, 1995; Itou *et al*., 1996, Marti Masso *et al*., 1993; Gjerris, 1971). The structures of amiodarone, aprindine and procaine possess a highly similar in diethylaminoethyl side chain like metoclopramide (fig. 1) and tiapride, which belong to the benzamide derivatives and selectively block dopamine D_2 receptors. It was suggested that these structures were involved in the induction of drug-induced parkinsonism (Itou *et al*., 1996). It is likely that the drug-induced parkinsonism is mainly due to the blocking of dopamine receptors in the striatum by administrated drugs, although the detailed mechanism of drug-induced parkinsonism is unclear (Gershanik, 1994).

In this study, we quantitatively estimated the occurrence of catalepsy induced by amiodarone, aprindine and procaine as an index of the behavioral pharmacological effect in mice (Sanberg *et al*., 1988; Haraguchi *et al*., 1997, 1998). Moreover, it has been reported that the drug-induced parkinsonism and catalepsy are related to the specific binding to the dopamine D_1 and D_2 receptors (Wanibuchi and Usuda, 1990) and mACh receptor (Ushijima *et al*., 1997) in the brain. In this study, the *in vivo* specific binding affinity and *in vitro* occupancies to the dopamine D_1, D_2 and mACh receptors were estimated to predict the intensity of catalepsy induced by drugs. Ogawa *et al*. (1990) analyzed the tertiary structures of various drugs using computer graphics and compared the structure of haloperidol and pimozide, typical dopamine receptor blockers, and flunarizine. In this study, we compared the tertiary structures of amiodarone, aprindine, procaine, metoclopramide and tiapride with that of haloperidol according to the method of Ogawa *et al*. and found a similar side chain in these six drugs.

Materials and Methods

Animals. Male ddY mice, 5 weeks old, weighing 25 to 30 g, were purchased from Seac Yoshitomi Co. (Fukuoka, Japan).

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ABBREVIATIONS: mACh, musucarinic acetylcholine; SCH23390, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; [^{3}H]QNB, [^{3}H]L-quinuclizinyl benzilate; 5-HT, 5-hydroxytryptamine; GABA, γ-aminobutyric acid.

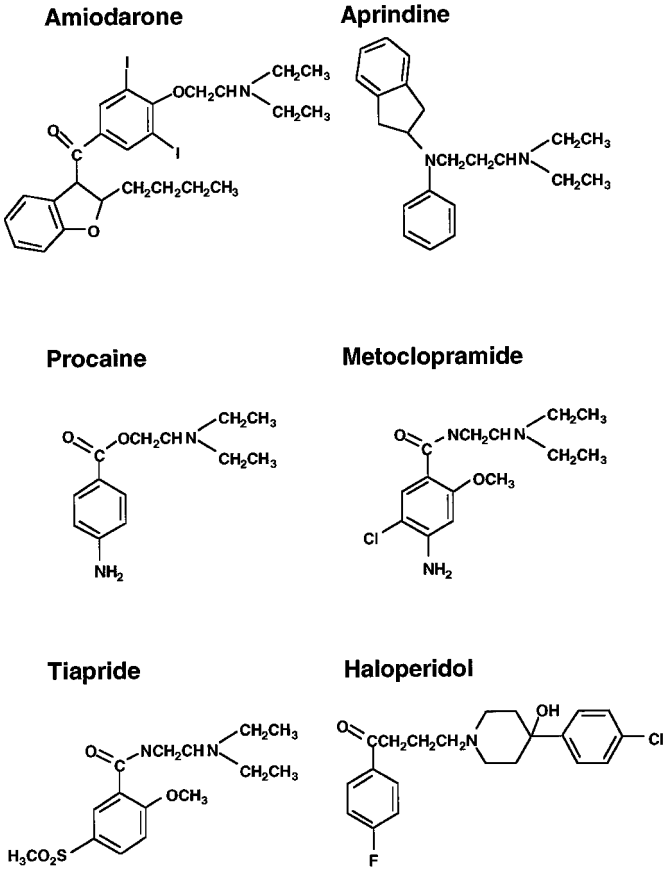

Fig. 1. The chemical structures of test drugs.

Drugs. The following drugs were kindly gifted from the respective companies: amiodarone hydrochloride (Taisho Pharmaceutical Co., Tokyo, Japan); aprindine hydrochloride (Mitsui Pharmaceutical Company, Tokyo, Japan); haloperidol and biperiden hydrochloride (Dainippon Pharmaceutical Company, Osaka, Japan); tiapride hydrochloride and metoclopramide (Fujisawa Pharmaceutical Co., Osaka, Japan); nemonapride (Yamanouchi Pharmaceutical Co., Tokyo, Japan); propantheline bromide (Monsanto, Co., Osaka, Japan). Procaine hydrochloride and Clea-sol I as a scintillation cocktail were purchased from Nakalai Tesque (Kyoto, Japan), atropine sulfate monohydrate from Wako Pure Chemical Industries (Osaka, Japan) and (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine [(*R*)-(+)-SCH23390] hydrochloride from Funakoshi Co. (Tokyo, Japan). [^{3}H]SCH23390 (specific activity, 70.3 Ci/mmol), [^{3}H]raclopride (specific activity, 79.3 Ci/mmol) and [^{3}H]L-quinuclizinyl benzilate ([^{3}H]QNB, specific activity, 49.0 Ci/mmol) were purchased from NEN Research Products (Boston, MA) and SOLVABLE from Packard. All other chemicals used in the experiments were of analytical grade.

Preparation of drug solutions. In the *in vivo* study, amiodarone hydrochloride and biperiden were dissolved in distilled water. Metoclopramide was dissolved in 1 N HCl and then neutralized with 1 N NaOH and diluted with saline. Haloperidol was dissolved in 0.3% tartaric acid and diluted with saline. Aprindine hydrochloride, procaine hydrochloride, tiapride hydrochloride, propantheline bromide and atropine sulfate were dissolved in saline. The unlabeled drugs were injected into a volume of 2.5 ml/kg for intravenous administration and a volume of 10 ml/kg for other administration, and the solvent alone was used as a control.

In the *in vitro* study, aprindine hydrochloride, procaine hydrochloride, tiapride hydrochloride and (*R*)-(+)-SCH23390 were dissolved in distilled water. Amiodarone hydrochloride was dissolved in 10% ethanol. Haloperidol, metoclopramide and nemonapride were dissolved in 0.3% tartaric acid.

Measurement of intensity of catalepsy. Measurement of catalepsy was performed according to the method of Fujiwara (1992) and Haraguchi *et al*. (1997). Amiodarone hydrochloride (10–200 mg/kg), aprindine hydrochloride (5–30 mg/kg), procaine hydrochloride (10–150 mg/kg), metoclopramide (1–25 mg/kg), tiapride hydrochloride (10–50 mg/kg) or haloperidol (0.05–0.5 mg/kg) was intraperitoneally injected. Control animals were administered with the respective solvent alone under the same conditions. Catalepsy was assessed at 0.5, 1.5, 3 and 4.5 hr after administration of the drugs by the bar method; the front paws were gently placed on a horizontal metal bar with 2 mm in diameter suspended 4 cm above, and the length of time (in sec) the mouse maintains this abnormal posture was measured. The measurement of catalepsy was performed by an observer who did not prepare the drug solutions according to the double-blind method.

Effects of central and peripheral anticholinergic drugs on catalepsy. Amiodarone hydrochloride (200 mg/kg), aprindine hydrochloride (30 mg/kg), procaine hydrochloride (150 mg/kg), metoclopramide (25 mg/kg), tiapride hydrochloride (50 mg/kg) or haloperidol (0.5 mg/kg) was intraperitoneally injected. Catalepsy was measured at 60 min after the injection of each drug under the same conditions as in the section on "Measurement of intensity of catalepsy" and then 10 mg/kg of biperiden, a central anticholinergic drug, or 2.5 mg/kg of propantheline, a peripheral anticholinergic drug, were administered subcutaneously or intravenously, respectively. After the injection of biperiden or propantheline, catalepsy was measured every hour for 3 hr.

***In vivo* dopamine D_1, D_2 and mACh receptor occupancy.** Measurement of *in vivo* receptor occupancy was performed according to the method of Haraguchi *et al*. (1997). Each drug or vehicle was administered to mice under the same conditions as in the section on "Measurement of intensity of catalepsy." At 85 min after the administration of haloperidol and at 25 min after the administration of the other drugs, D_1-selective antagonist [^{3}H]SCH23390 (2 μCi/body), D_2-selective antagonist [^{3}H]raclopride (2 μCi/body) or mACh specific antagonist [^{3}H]QNB (2 μCi/body) was injected intravenously. After 10 min, the mice were decapitated, and the striatum and cerebellum were dissected on a glass plate. Each sample was weighed in a vial, added to 1 ml of SOLVABLE and incubated at 50°C until it became a clear solution; 0.2 ml of 30% H_2O_2 was then added, and the vial was left at room temperature overnight. It was neutralized with 70 μl of 6 N HCl and 10 ml of Clea-sol I was added. The radioactivities were measured in a liquid scintillation counter (LS6500, Beckman).

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

$$\Phi_1\,(\%) = (1 - (A - 1)/(B - 1)) \times 100 \qquad (1)$$

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

$$\Phi_2\,(\%) = (1 - (A' - C)/(B' - C)) \times 100 \qquad (2)$$

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

***In vitro* dopamine D_1, D_2 and mACh receptor binding study.** Preparation of the membrane sample was performed according to the method of Meltzer *et al*. (1989) and Haraguchi *et al*. (1998). The mice were decapitated, and the striatum was rapidly dissected. After weighing, 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. The homogenates were centrifuged (20,000 × g for 10 min at 4°C) twice with intermediate resus-

pension 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 receptor, respectively.

Aliquots of the membrane preparations were incubated with each drug and 0.3 nM [^{3}H]SCH23390 (for D_1 receptor binding) or 1 nM [^{3}H]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; $CaCl_2$, 2; and $MgCl_2$, 1. For mACh receptor binding, aliquots of the membrane preparations were incubated with each drug and 0.2 nM [^{3}H]QNB for 30 min at 37°C in 50 mM Tris-HCl buffer (pH 7.4). The final tissue concentrations were 1 mg of the original wet weight tissue per 1 ml for D_1 and D_2 receptor binding and 2 mg/3 ml for mACh receptor binding. The amounts of protein in the cells were measured by Lowry's methods (Lowry *et al*., 1951).

The incubation was terminated by rapid pouring of the contents of the tubes over Whatman GF/C glass fiber filters under vacuum. The filters were rinsed twice with 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and placed in glass scintillation vials; then 8 ml of Clea-sol I was added.

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 binding, respectively.

K_i values were calculated according to the following equation:

$$R = (Kd + D)/(Kd(1 + I/Ki) + D) \quad (3)$$

where R is the specific binding ratio (ratio of [^{3}H]count in the presence of drugs to that in the absence of drugs), and D is the concentration of [^{3}H]ligand, I is the concentration of the drugs as inhibitors and K_d is the dissociation constant of the [^{3}H]ligand obtained from Scatchard analysis of saturation experiment data. Data analysis and simulations used the nonlinear least-squares method MULTI (Yamaoka *et al*., 1981).

The tertiary structures analysis of drugs using computer graphics. The tertiary structures of each drug were analyzed by a Macintosh, using program CAChe by Sony Tectolonics (Tokyo, Japan). The tertiary structures of each drug were superimposed on that of haloperidol, and a highly similar side chain was found by eye-fit.

Statistical analysis. Statistical analysis was performed by Student's *t*-test. Statistical significance was considered at a P value of $<.05$.

Results

***In vivo* induction of catalepsy.** Time courses of the intensity of catalepsy induced by various doses of amiodarone aprindine, procaine, matoclopramide, tiapride and haloperidol after intraperitoneal injection are shown in figure 2, A–F. The intensities of drug-induced catalepsy at 30 min after the administration were dose dependent (fig. 3). Drugs-induced catalepsy was observed for several hours in all of the test drugs with a difference in dose dependency among the drugs. The relative intensity was defined as the ratio of the inverse of the dose at 20 sec as intensity of catalepsy in various drugs to that of haloperidol (fig. 3). The relative intensity of haloperidol, amiodarone, aprindine, procaine, metoclopramide and tiapride was 1.0, 0.002, 0.01, 0.0005, 0.02 and 0.03, respectively (table 1).

Effect of central and peripheral anticholinergic drugs on catalepsy. Biperiden, a central anticholinergic drug, completely reduced the catalepsy induced by any tested drugs to the base line level (fig. 4, A–F). However, there was no change in catalepsy in the presence of propantheline, a peripheral anticholinergic drug (fig. 5, A–F).

***In vivo* dopamine D_1, D_2 and mACh receptor occupancy and catalepsy.** The intensities of catalepsy measured at 30 min after the administration of amiodarone (200 mg/kg), aprindine (30 mg/kg), procaine (150 mg/kg), metoclopramide (25 mg/kg) or tiapride (50 mg/kg) and at 90 min after the administration of haloperidol (0.5 mg/kg) and *in vivo* dopamine D_1, D_2 and mACh receptor occupancies of the various drugs are shown in table 1. The *in vivo* occupancies to both D_1 and D_2 receptors were 2% to 89% with any drug. Moreover, the *in vivo* occupancies to the mACh receptor were

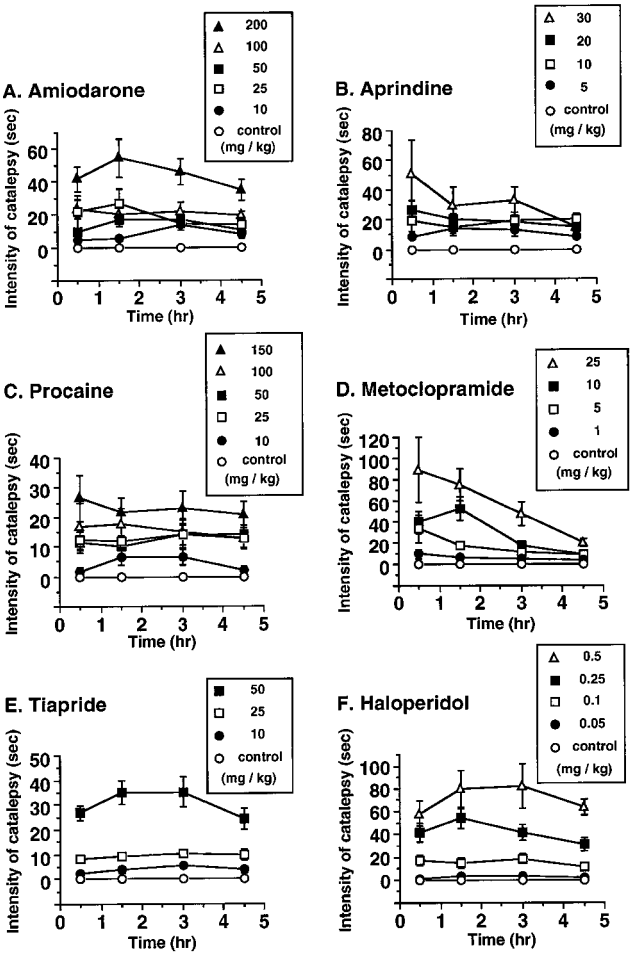

Fig. 2. Time courses of catalepsy induced by 6 drugs. The intensity of catalepsy was assessed at 0.5, 1.5, 3 and 4.5 hr for (A) amiodarone, (B) aprindine, (C) procaine, (D) metoclopramide, (E) tiapride and (F) haloperidol after i.p. administration. The catalepsy was measured as described in Materials and Methods. Data are mean ± S.E. (n = 6–10).

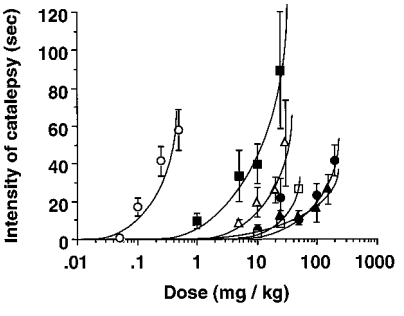

Fig. 3. Dose-dependent induction of catalepsy. ●, amiodarone-induced, △, aprindine-induced, ▲, procaine-induced, ■, metoclopramide-induced, □, tiapride-induced and ○, haloperidol-induced catalepsy 30 min after i.p. administration. Data are mean ± S.E. (n = 6–10).

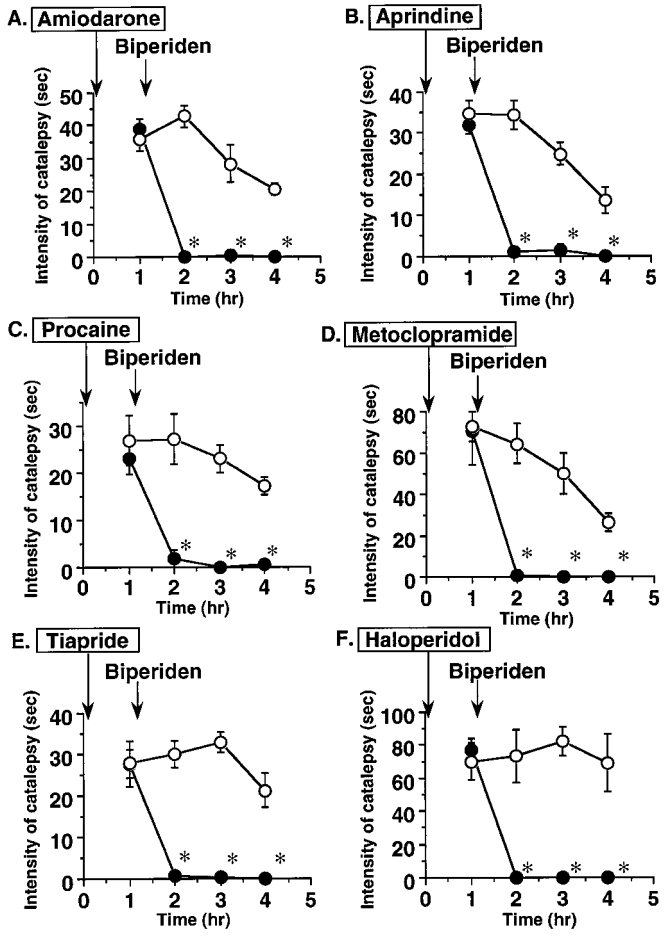

Fig. 4. Effect of biperiden on drug-induced catalepsy. Biperiden (10 mg/kg; ●) or the solvent alone (○) was administrated 60 min after i.p. administration of (A) amiodarone (200 mg/kg), (B) aprindine (30 mg/kg), (C) procaine (150 mg/kg), (D) metoclopramide (25 mg/kg), (E) tiapride (50 mg/kg) and (F) haloperidol (0.5 mg/kg). Data are mean ± S.E. (n = 5–6). Significant differences from the drug alone (*P < .05).

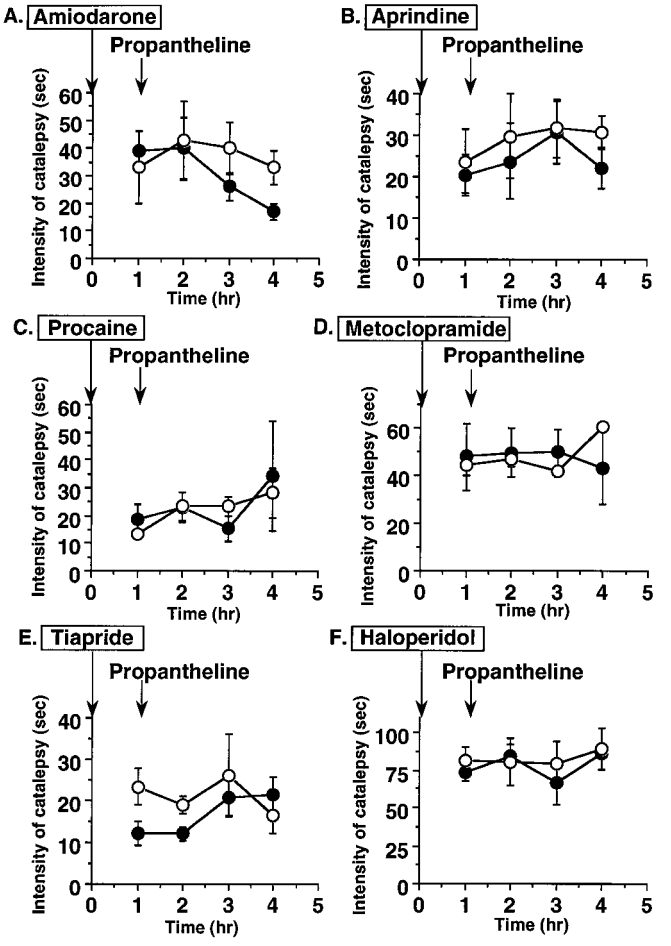

Fig. 5. Effect of propantheline on drug-induced catalepsy. Propantheline (2.5 mg/kg; ●) or the solvent alone (○) was administrated 60 min after i.v. administration of (A) amiodarone (200 mg/kg), (B) aprindine (30 mg/kg), (C) procaine (150 mg/kg), (D) metoclopramide (25 mg/kg), (E) tiapride (50 mg/kg) and (F) haloperidol (0.5 mg/kg). Data are mean ± S.E. (n = 3). No significant differences from the drug alone.

10% to 57%, except for aprindine, tiapride and haloperidol with low occupancies (0–10%).

***In vitro* dopamine D_1, D_2 and mACh receptor binding affinity to striatum nervous membrane.** Figure 6 shows the inhibition curves for the *in vitro* binding of dopamine D_1, D_2 or mACh receptor-selective radioligands to the striatum membrane in the presence of tested drugs. The K_d values of [^{3}H]SCH23390, [^{3}H]raclopride and [^{3}H]QNB obtained by the Scatchard analysis were 0.22, 1.0 and 0.075 nM, respectively. The calculated K_i values are listed in table 2. The K_i values of the tested drugs to dopamine D_1, D_2 and mACh receptor were over a range of 60 nM to 706 μM, 0.5 nM to 75 μM and 860 nM to 115 μM, respectively. Aprindine showed strong binding affinity to the D_1 receptor next to haloperidol. Haloperidol, metoclopramide, tiapride and aprindine showed very strong binding affinity to the D_2 receptor. For the mACh receptor, metoclopramide, tiapride and haloperidol showed significantly weak binding affinity as compared with that to the dopamine D_1 and D_2 receptors, although aprindine, amiodarone and procaine exhibited binding affinity to the mACh receptor comparable with that to the dopamine D_1 and D_2 receptors.

Relationship between *in vitro* K_i values for dopamine D_1 or D_2 receptor and the relative intensity of catalepsy. As shown in figure 7, the *in vivo* relative intensities of drug-induced catalepsies (table 1) were significantly correlated with *in vitro* K_i values for the dopamine D_1 or D_2 receptor (r = .911 or r = .896, respectively, P < .05).

The tertiary structures superimposed analysis of drugs by computer graphics. As shown in figure 8, the tertiary structures of amiodarone, aprindine, procaine, metoclopramide and tiapride were superimposed on that of haloperidol. Good fitting on the closed part (diethylaminoethyl side chain) was obtained, indicating that there was a high similarity among the drugs.

Discussion

It is well known that the antipsychotic drugs chlorpromazine and haloperidol, and drugs used in the treatment of cerebral blood flow disturbances such as flunarizine and cinnarizine, induce parkinsonian side effects (Chouza *et al*., 1986; Kuzuhara *et al*., 1989; Negrotti and Calzetti, 1997). Recently, it was reported that antiarrhythmic drugs amiodarone and aprindine and local anethetic drug procaine induced parkinsonism (Dotti and Federico, 1995; Itou *et al*., 1996; Marti Masso *et al*., 1993; Gjerris *et al*., 1971). We understood this investigation to predict the intensity of parkinsonism

TABLE 1
Intensity of catalepsy and *in vivo* D1, D2 and mACh receptor occupancies of the tested drugs

	Dose	Intensity of catalepsy	Relative intensity	*In vivo* receptor occupancy		
				D_1	D_2	mACh
	mg/kg	*sec*			%	
Amiodarone	200	54.3 ± 8.4	0.002	35.8 ± 3.5	46.6 ± 5.7	29.3 ± 7.9
Aprindine	30	51.8 ± 7.2	0.01	44.4 ± 4.4	30.2 ± 2.2	0
Procaine	150	26.6 ± 5.7	0.0005	49.2 ± 4.5	18.8 ± 3.7	57.4 ± 16.9
Metoclopramide	5	41.0 ± 4.3	0.02	2.3 ± 0.5	88.6 ± 0.6	11.5 ± 4.6
Tiapride	50	24.4 ± 4.8	0.003	36.2 ± 5.4	56.4 ± 3.3	0
Haloperidol	0.5	71.7 ± 7.9	1	25.0 ± 5.3	87.2 ± 6.5	9.7 ± 8.7

[^{3}H]SCH 23390, [^{3}H]raclopride and [^{3}H]QNB were used for *in vivo* labeling of dopamine D_1, D_2 and mACh receptor, respectively. Relative intensity is a ratio of the inverse of dose at 20 sec as intensity of catalepsy to that of haloperidol. Data are mean ± S.E. (*in vivo* receptor occupancy; n = 3–6, intensity of catalepsy; n = 11–15).

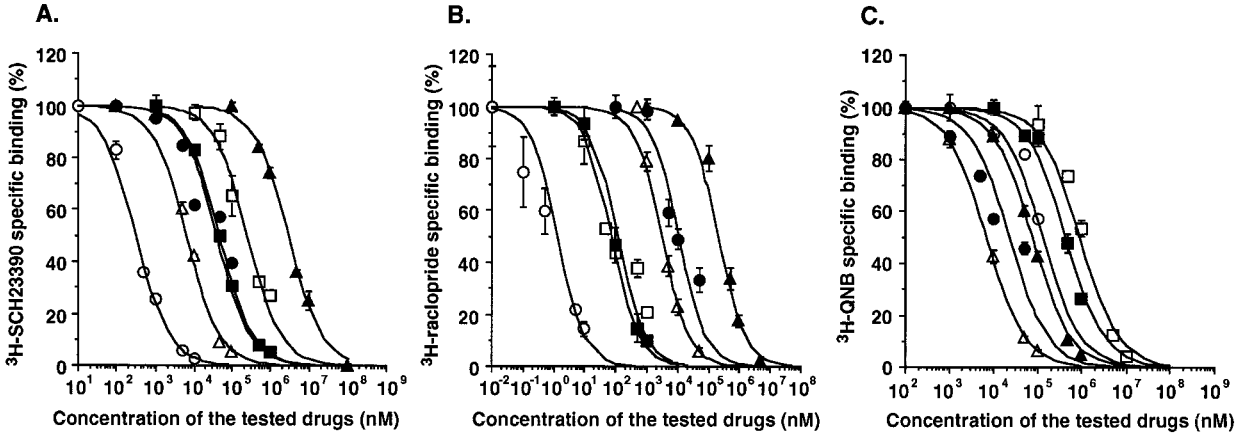

Fig. 6. Inhibition curves for binding of (A) [^{3}H]SCH23390, (B) [^{3}H]raclopride and (C) [^{3}H]QNB to mouse striatal membranes in the presence of amiodarone (●), aprindine (△), procaine (▲), metoclopramide (■), tiapride (□) or haloperidol (○). Data are mean ± S.E. (n = 3).

TABLE 2
In vitro K_i values of the tested drugs for D_1, D_2 and mACh receptor

	K_i		
	D_1	D_2	mACh
		nM	
Amiodarone	12,000 ± 2160	4000 ± 614	2850 ± 621
Aprindine	1400 ± 87.2	1220 ± 89.3	860 ± 28.5
Procaine	706,000 ± 33,700	75,400 ± 11,100	9430 ± 283
Metoclopramide	14,200 ± 413	28.8 ± 3.95	55,000 ± 2790
Tiapride	77,000 ± 8410	49.1 ± 11.8	115,000 ± 11,600
Haloperidol	60.0 ± 3.12	0.515 ± 2.89	16,300 ± 610

[^{3}H]SCH 23390, [^{3}H]raclopride and [^{3}H]QNB were used for *in vitro* labeling of dopamine D_1, D_2 and mACh receptor, respectively. Data are mean ± S.D. (n = 3).

induced by these drugs using catalepsy as an index of behavioral pharmacology. *In vivo* and *in vitro* specific binding to the dopamine D_1, D_2 receptor and mACh receptor was also investigated to predict the intensity of catalepsy induced by drugs. Moreover, a comparison of the tertiary structures among the drugs was performed.

Antipsychotic drugs, including haloperidol, induced catalepsy in a dose-dependent manner (Ossowska *et al*., 1990; Haraguchi *et al*., 1997). We found that catalepsy was also induced by amiodarone, aprindine, procaine, metoclopramide and tiapride in a dose-dependent manner (figs. 2 and 3).

To confirm whether the observed catalepsies were caused by enhanced cholinergic central nervous system, the effects of a central anticholinergic agent biperiden (Yokogawa *et al*., 1986), which was transported into the brain *in vivo* (Syvalahti *et al*., 1987), on those drugs that induced catalepsies were investigated after subcutaneous administration. The catalepsies induced by the tested drugs were almost completely reduced in the presence of biperiden (fig. 4, A–F). However, the peripheral anticholinergic drug propantheline (Davis *et al*., 1983) did not reduce catalepsies (fig. 5, A–F). These results suggested that the observed catalepsy was due to the enhanced cholinergic central nervous system by the blockade of the dopaminergic receptor.

The *in vivo* and *in vitro* binding assay of the six drugs to the dopamine D_1, D_2 and the mACh receptor was carried out (tables 1 and 2). The previously reported *in vitro* K_i value of haloperidol in rats was 76 nM for the D_1 receptor (Andersen, 1988), and 2.6 nM (Andersen, 1988) and 4.0 nM (Syvalahti, 1988) for the D_2 receptor. The K_i of metoclopramide was 150 nM for the D_2 receptor (Syvalahti, 1988). Tiapride showed high binding affinity (114 nM) for the D_2 receptor (Woodward *et al*., 1994), although it scarcely bound to the D_1 receptor (Arima *et al*., 1986). The K_i of amiodarone was 6.5 μM for the mACh receptor in the rat brain (Cohen-Armon *et al*., 1984). The K_i of procaine was 3.8 μM for the mACh receptor in the rat hippocampus (Sharkey *et al*., 1988). Our findings in this study in mice were almost in agreement with these results. Each drug used in this study blocked both the D_1 and D_2 receptor based on *in vivo* and *in vitro* experiments, suggesting that dopamine D_1 and D_2 receptors were related to this drug-induced catelepsy as drug-induced parkinsonism. Because there was a possibility to reduce the catalepsy by binding as an antagonist to the mACh receptor, we measured mACh receptor binding. Procaine showed both *in vivo* and *in vitro* binding to the mACh receptor (tables 1 and 2), suggesting that procaine-induced catalepsy may be reduced by the mACh receptor blockade.

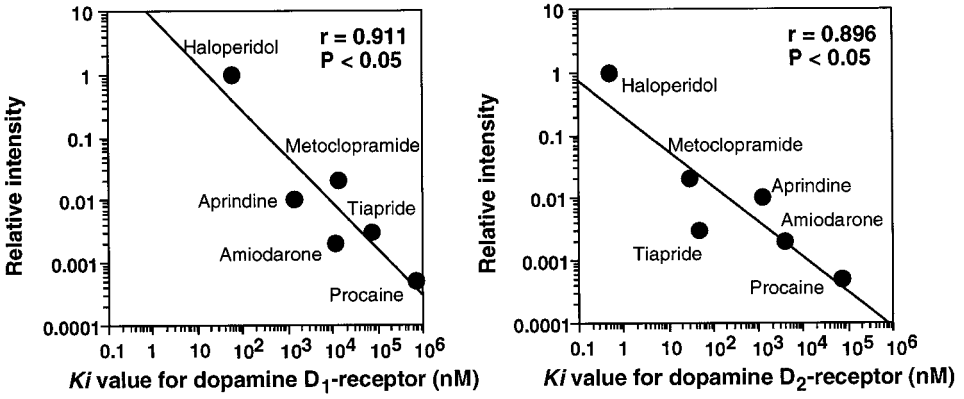

Fig. 7. Relationship between K_i values for dopamine (A) D_1 or (B) D_2 receptor and relative intensity. Relative intensity is the ratio of the inverse of dose of various drugs at 20 sec as catalepsy to that of haloperidol (relative intensity; n = 6–10, K_i values; n = 3).

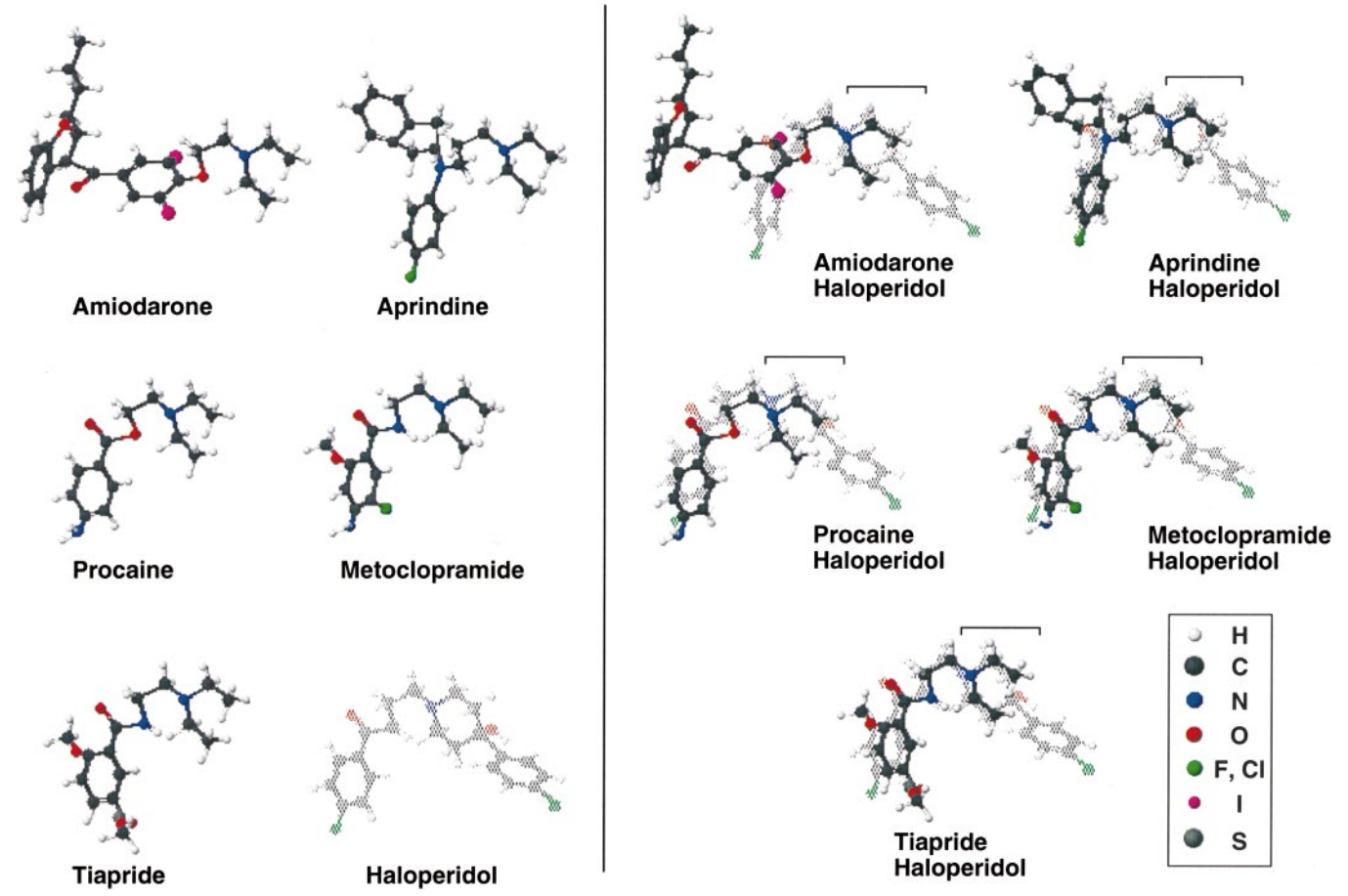

Fig. 8. Tertiary structures of amiodarone, aprindine, procaine, metoclopramide, tiapride and haloperidol and their superimposition among test drugs and haloperidol by computer graphics as described in Materials and Methods.

The *in vivo* relative intensity of drugs-induced catalepsy significantly correlated with *in vitro* K_i values for the dopamine D_1 or D_2 receptor, suggesting that the D_1 and D_2 receptors were intensely involved in the catalepsy in mice. These findings indicated that parkinsonism by amiodarone, aprindine and procaine in humans was due to the dopaminergic neural system blockade.

The occurrence mechanism of aprindine-induced tremor was similar to that of drugs used as local anesthetics such as lidocaine, and it was considered that aprindine might suppress GABA release from the inhibitory GABA neuron (Kamiya *et al*., 1985). Therefore, not only the blockade of the dopaminergic neural system but also the blockade of the GABAergic system might be involved in the tremor. It was reported that the GABA receptor binding in the substantia nigra was significantly decreased in the brains of subjects with Parkinson's disease (Rinne *et al*., 1978; Lloyd *et al*., 1991). The GABAergic neurons were involved in dopaminergic functional control in the basal ganglia, which agrees with earlier reports (Gerlach *et al*., 1996; Rosales *et al*., 1997), and hypofunctions of the GABAergic system may play a role in the generation of L-dopa-induced dyskinesia (Nishino *et al*., 1984). It was also reported that stimulation of $GABA_A$ receptors in the substantia nigra pars reticulata could block tacrine-induced tremulous jaw movements (Finn *et al*., 1997); the $GABA_A$ receptor was significantly up-regulated in dyski-

netic monkeys after chronic levodopa or D_2 agonist administration (Calon *et al*., 1995). Therefore, a loss of the GABAergic system was involved in human Parkinson's disease (Kawabata and Tachibana, 1997). Moreover, serotonin 5-HT_2 receptor antagonists have been reported to reduce catalepsy (Balsara *et al*., 1979; Hicks, 1990; Neal-Beliveau *et al*., 1993). Clozapine, an atypical neuroleptic, scarcely induced extrapyramidal adverse effects, although there was the specific binding to both D_1 and D_2 receptors. This discrepancy was explained by its relatively potent antagonistic activity in central serotonergic receptor functions (Meltzer *et al*., 1989; Okuyama *et al*., 1997; Andree *et al*., 1997). Therefore, the contribution of 5-HT_2 receptor binding should be taken into consideration for drugs that have a high affinity for the 5-HT_2 receptor. The binding affinity of haloperidol to the 5-HT_2 receptor measured using [^{3}H]spiperone is 46 nM (Okuyama *et al*., 1997), 95 nM (Terai *et al*., 1989) in rats and 36 nM in humans (Wander *et al*., 1987), respectively.

It is known that drug-induced parkinsonism has a similar structure (Ogawa *et al*., 1990). To function as dopamine D_2 receptor antagonist, the aminoethyl moiety at the N-alkyl substituent of the amide side chain is contained and the methoxy moiety at the 2-position of the benzoyl substituent is necessary (Pannatiar *et al*., 1981). There were many reports about the conformational analysis between the N-alkyl substituent of the amide side chain and the methoxy moiety at the 2-position of the benzoyl substituent (van de Waterbeemd and Testa, 1983), tertiary structures of aminoethyl moiety (Pettersson and Liljefors, 1992) and structure-activity relationship in various N-alkyl substituents of the amide side chain to antidopaminergic activity (Usuda, 1987). However, there were few reports about the diethylaminoethyl substituent for metoclopramide or tiapride. Harrold *et al*. (1993) reported that metoclopramide and sulpiride required a basic nitrogen atom in their charged molecular form for binding the D_2 receptor, and then differences in their biological profiles did not appear to be due to any appreciable differences in the binding of the basic nitrogen atom. The tertiary structure of amiodarone, aprindine and procaine is similar to that of metoclopramide and tiapride in the position of the diethylaminoethyl substituent (figs. 1 and 8). These findings suggested that the diethylaminoethyl substituent might be involved in the antidopaminergic activity. In the future, it would be necessary to investigate the conformational analysis between the dopamine D_2 receptor and the interaction of each drug as antagonists.

Drugs that possess the diethylaminoethyl group or a similar structure to that may possibly to induce catalepsy and/or parkinsonism. For example, a clinical case of parkinsonism by an anticancer drug cyclophosphamide, possessing a similar structure has been recently reported (Fleming and Mangino, 1997). The risk of induction of parkinsonism may be increased when antipsychotics as dopamine antagonists are used concurrently. Thus, the occurrence of drug-induced parkinsonism may be partially predicted from the tertiary structures of drugs.

In conclusion, the occurrence of catalepsy by amiodarone, aprindine and procaine was mainly caused by the blockade of the dopaminergic D_1 and D_2 receptors and the enhancement of the central cholinergic nervous system. The importance of possessing a part of an analogous structure to haloperidol, the diethylaminoethyl substituent was suggested. Moreover, a good correlation between the *in vivo* relative intensity of drugs-induced catalepsy and *in vitro* K_i values for the dopamine D_1 or D_2 receptor indicated that the *in vivo* intensity of catalepsy could be predicted from *in vitro* receptor binding affinity. Thus, the intensity of catalepsy and parkinsonism may be predicted from both the biochemical and physicochemical information on drugs.

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