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

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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 D1 and D2 receptor occupancies of the six drugs to the striatum were observed. In vitro binding affinity (Ki) of these drugs to the D1 and D2 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 Ki values for the dopamine D1 and D2 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 D1 and D2 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 D2 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 (Gershnik, 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 D1 and D2 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 D1, D2 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).

ABBREVIATIONS: mACh, muscarinic acetylcholine; SCH23390, (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; [3H]QNB, [3H]-quinuclizinyl benzilate; 5-HT, 5-hydroxytryptamine; GABA, γ-aminobutyric acid.
**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); aprindine hydrochloride, procaine hydrochloride, tiapride hydrochloride, propantheline bromide and atropine (50 mg/kg) at 25 min before the administration of [3H]QNB. The activity or absence of drugs, respectively. C is the nonspecific binding where A and B are the ratio of radioactivities (striatum/cerebellum)

\[
\Phi_1(\%) = \left(1 - \frac{(A - 1)}{(B - 1)}\right) \times 100
\]

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.

\[
\Phi_2(\%) = \left(1 - \frac{(A' - C)}{(B' - C)}\right) \times 100
\]

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 [3H]QNB.

**In vitro dopamine D1, D2 and mACh receptor binding study.** Preparation of the membrane sample was performed according to the method of Melzer et al. (1989) and Haraguchi et al. (1998). 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% H2O2 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:

- **Dopamine D1, D2 and mACh receptor binding study.** Preparation of the membrane sample was performed according to the method of Melzer et al. (1989) and Haraguchi et al. (1998). The mice were decapitated, and the striatum and cerebellum were 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-
pensation 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 [3H]SCH23390 (for D1 receptor binding) or 1 nM [3H]raclopride (for D2 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 the original wet weight tissue per 1 ml for D1 and D2 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.

Non-specific binding was determined in the presence of 100 nM SCH23390, 1 μM nemonapride and 1 μM atropine for D1, D2 and mACh receptor binding, respectively.

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, metoclopramide, 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 D1, D2 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 D1, D2 and mACh receptor occupancies of the various drugs are shown in table 1. The in vivo occupancies to both D1 and D2 receptors were 2% to 89% with any drug. Moreover, the in vivo occupancies to the mACh receptor were...
10% to 57%, except for aprindine, tiapride and haloperidol with low occupancies (0–10%).

In vitro dopamine D₁, D₂ and mACh receptor binding affinity to striatum nervous membrane. Figure 6 shows the inhibition curves for the in vitro binding of dopamine D₁, D₂ or mACh receptor-selective radioligands to the striatum membrane in the presence of tested drugs. The $K_i$ 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 D₁, D₂ 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 D₁ receptor next to haloperidol. Haloperidol, metoclopramide, tiapride and aprindine showed very strong binding affinity to the D₂ receptor. For the mACh receptor, metoclopramide, tiapride and haloperidol showed significantly weak binding affinity as compared with that to the dopamine D₁ and D₂ receptors, although aprindine, amiodarone and procaine exhibited binding affinity to the mACh receptor comparable with that to the dopamine D₁ and D₂ receptors.

Relationship between in vitro $K_i$ values for dopamine D₁ or D₂ 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 D₁ or D₂ 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 anesthetic 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...
induced by these drugs using catalepsy as an index of behavioral pharmacology. In vivo and in vitro specific binding to the dopamine D₁, D₂ 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. A–F). However, the peripheral anticholinergic drug protriptyline (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 D₁, D₂ and mACh receptor was carried out (tables 1 and 2). The previously reported in vitro Ki value of haloperidol in rats was 76 nM for the D₁ receptor (Andersen, 1988), and 2.6 nM (Andersen, 1988) and 4.0 nM (Syvalahti, 1988) for the D₂ receptor. The Ki of metoclopramide was 150 nM for the D₂ receptor (Syvalahti, 1988). Tiapride showed high binding affinity (114 nM) for the D₂ receptor (Woodward et al., 1994), although it scarcely bound to the D₁ receptor (Arima et al., 1986). The Ki of amiodarone was 6.5 μM for the mACh receptor in the rat brain (Cohen-Armon et al., 1984). The Ki 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 D₁ and D₂ receptors based on in vivo and in vitro experiments, suggesting that dopamine D₁ and D₂ receptors were related to this drug-induced catalepsy 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.
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 ta-}

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**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; \( n = 3 \)).

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**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.
netic monkeys after chronic levodopa or D2 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-HT2 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 D1 and D2 receptors. This discrepancy was explained by its relatively potent antagonistic activity in the dopaminergic D1 and D2 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 Kᵢ values for the dopamine D1 or D2 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.

References


