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).

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]-quinuclidinyl 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). Procaine hydrochloride and Clea-sol I 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 D1, D2 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, D1-selective antagonist [3H]SCH23390 (2 μCi/body), D2-selective antagonist [3H]raclorpride (2 μCi/body) or mACh specific antagonist [3H]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% 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:

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

and

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

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.

In vitro dopamine D1, D2 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 [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/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 D1, D2 and mACh receptor binding, respectively.

\[ K_i = \frac{(K_d + D)(1 + I/K_i) + D}{R} \]  

where \( R \) is the specific binding ratio (ratio of [3H]count in the presence of drugs to that in the absence of drugs), and \( D \) is the concentration of [3H]ligand, \( I \) is the concentration of the drugs as inhibitors and \( K_i \) is the dissociation constant of the [3H]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, 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 $K_d$ values of $[3H]$SCH23390, $[3H]$raclopride and $[3H]$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...
Table 1: Intensity of catalepsy and in vivo D1, D2 and mACh receptor occupancies of the tested drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Intensity of catalepsy (sec)</th>
<th>Relative intensity</th>
<th>In vivo receptor occupancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>54.3 ± 8.4</td>
<td>0.002</td>
<td>D1</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aprindine</td>
<td>30</td>
<td>51.8 ± 7.2</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Procare</td>
<td>150</td>
<td>26.6 ± 5.7</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>5</td>
<td>41.0 ± 4.3</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Tiapride</td>
<td>50</td>
<td>24.4 ± 4.8</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.5</td>
<td>71.7 ± 7.9</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± S.E. (n = 3–6, intensity of catalepsy; n = 11–15).

**Table 2**

<table>
<thead>
<tr>
<th>Drug</th>
<th>in vitro Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>12,000 ± 2160</td>
</tr>
<tr>
<td>Aprindine</td>
<td>1400 ± 87.2</td>
</tr>
<tr>
<td>Procare</td>
<td>706,000 ± 33,700</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>14,200 ± 413</td>
</tr>
<tr>
<td>Tiapride</td>
<td>77,000 ± 11,600</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>60.0 ± 3.12</td>
</tr>
</tbody>
</table>

in-vivoKi values of the tested drugs for D1, D2 and mACh receptor. 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 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. 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 D1, D2 and the 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 K 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 K of amiodarone was 6.5 μM for the mACh receptor in the rat brain (Cohen-Armon et al., 1984). The K 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 receptor 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- crine-induced tremulous jaw movements (Finn *et al*., 1997); the GABA$_A$ receptor was significantly up-regulated in dysk-
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 central serotonergic receptor functions (Meltzer et al., 1989; Okuyama et al., 1997; Andree et al., 1997). Therefore, the contribution of 5-HT₂ receptor binding should be taken into consideration for drugs that have a high affinity for the 5-HT₂ receptor. The binding affinity of haloperidol to the 5-HT₂ receptor measured using [³H]spiperone is 46 nM (Okuyama et al., 1997), 95 nM (Tseri 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 D₂ receptor antagonist, the aminoethoxy moiety at the N-alkyl substituent of the amide side chain is contained, and the methoxy moiety at the 2-position of the benzyl substituent is necessary (Panniatia 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 benzyl substituent is necessary (Panniatia et al., 1981). There were many 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 D₂ 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 diethylaminoethyl and triapride in the position of the diethylaminoethyl substituent (figs. 1 and 8). These findings suggested that the diethylaminoethyl substituent might be involved in the antiparkinsonian activity. In the future, it would be necessary to investigate the conformational analysis between the dopamine D₂ receptor and the interaction of each drug as antagonists.

Drugs that possess the diethylaminoethyl group or a similar structure to that may possibly 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 structure of drugs.

In conclusion, the occurrence of catalepsy by amiodarone, aprindine and procaine was mainly caused by the blockade of 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 Kᵣ 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


