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Effects of Typical and Atypical Antipsychotic Drugs on Freezing Behavior Induced by Conditioned Fear

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INOUE, T., K. TSUCHIYA AND T. KOYAMA. *Effects of typical and atypical antipsychotic drugs on freezing behavior induced by conditioned fear.* PHARMACOL BIOCHEM BEHAV **55**(2) 195–201, 1996.—Atypical antipsychotic drugs (atypical APDs), such as clozapine, ORG5222, and olanzapine, have been suggested to possess anxiolytic activity in the conflict test and elevated plus-maze test, while several studies have suggested that typical APDs are not anxiolytic in several models of anxiety. We investigated the effects of typical and atypical APDs on the acquisition and expression of conditioned fear-induced freezing. Drugs were administered subcutaneously to male Sprague–Dawley rats 30 min before foot shock stress (the VI60s schedule, 2.5 mA for 30 min). Twenty-four hours after foot shock, freezing behavior of rats was observed in the shock chamber without shocks. The atypical APD clozapine (0.3–10 mg/kg) dose-dependently inhibited the acquisition of conditioned freezing. Candidates for atypical APDs, ORG5222 (0.1–1 mg/kg), olanzapine (1–10 mg/kg), and raclopride (3–30 mg/kg), also reduced the acquisition of conditioned freezing in a dose-dependent manner. Typical APDs, haloperidol (3 mg/kg), spiperone (0.1–1 mg/kg) and nemonapride (1 mg/kg) had significant inhibitory effects on the acquisition of conditioned freezing, but their effects were reduced at higher doses. Chlorpromazine, a typical APD, showed about 50% inhibition of the acquisition of conditioned freezing at the dose of 10 mg/kg, but did not reveal significant inhibition at any of the doses (3–30 mg/kg). The ED_{50}s (mg/kg) for inhibiting the acquisition of conditioned freezing significantly correlated with the K_i values for D_4 dopaminergic receptors, but not with the K_i values for other monoamine and acetylcholine receptors. On the other hand, clozapine or haloperidol did not change the expression of conditioned freezing. These results suggest that protective effects of clozapine and other antipsychotic drugs on the acquisition of conditioned freezing may be mediated by blockade of D_4 receptors. **Copyright © 1996 Elsevier Science Inc.**

Dopamine Dopamine D_4 receptors Clozapine Haloperidol Conditioned fear stress Freezing behavior Atypical antipsychotic drug Schizophrenia

CLOZAPINE has been referred to as an atypical antipsychotic drug (atypical APD), which is characterized by potent antipsychotic effects and a relatively low level of extrapyramidal side effects (37). Recently, several new candidates for atypical APD have been developed in the search for a safe compound with a clozapine-like mode of action. Many investigations have suggested that the mechanism of action of clozapine is different from that of typical APDs. Several studies suggest that relatively low affinity for dopamine D_2 sites with a larger serotonin 5-HT_2/D2 pKi value than typical APDs (22,24), or anti-D_1 dopaminergic property may be relevant to the mechanism of action of clozapine (1,5,15). It has been also suggested that the preferential dopaminergic activation by clozapine in the medial prefrontal cortex might account for the clinical advantages of clozapine (20,25). More recently, it has been proposed that the D_4 receptor, for which clozapine has relatively high affinity (K_i = 9 nM), may be the primary dopamine receptor that mediates the antipsychotic action of clozapine (41).

Recent behavioral animal studies have revealed that clozapine has anxiolytic action in punished responding (26,36,42) and open-field test (4). Candidates for atypical APD, such as olanzapine (26) and ORG5222 (8), also have putative anxiolytic activity. On the other hand, several studies have failed to demonstrate that typical APDs have anxiolytic potential (7,23, 36,42), and some evidence suggests that they may even have anxiogenic properties (33). These suggest the possibility that anxiolytic potential of clozapine is relevant to the mecha-

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nism of action responsible for clozapine's unique clinical profile.

We have found that conditioned fear stress (CFS; exposure to an environment paired previously with foot shock), an animal model of anxiety, increases both dopamine (DA) and serotonin (5-HT) metabolism in the medial prefrontal cortex (mPFC), and induces freezing behavior (16,17). We reported that CFS-induced freezing is attenuated by several classes of anxiolytics, such as benzodiazepines, serotonin 5-HT_{1A} agonists, and selective 5-HT reuptake inhibitors (18). Furthermore, foot shock per se also increases DA and 5-HT metabolism in the mPFC and other brain regions (14,16,17,40). Thus, previous neurochemical studies have suggested that both the acquisition and expression of CFS are related to DA activation of the mesocortical system, whereas there have been few pharmacological reports concerning effects of antipsychotic drugs on CFS-induced freezing.

The present study investigated the effects of the atypical APD clozapine on the acquisition and expression of conditioned fear-induced freezing. The results were compared to those obtained with typical APDs haloperidol, chlorpromazine, nemonapride, and spiperone, and candidates for atypical APDs olanzapine, raclopride, and ORG5222.

METHOD

Animals

Male Sprague–Dawley rats (the Shizuoka Laboratory Animal Center, Shizuoka, Japan) weighing 250–300 g were used. The rats were housed four per cage and maintained in a 12 L:12 D (light phase; 0630–1830 h), temperature-controlled environment, with free access to food and water. All experiments were performed between 0800 and 1300. Experiments began after a 14-day period of acclimatization.

Drugs

The following drugs were supplied by the manufacturers: ipsapirone HCl (Bayer Yakuhin Ltd., Japan), clozapine (Sandoz Ltd., Basel, Switzerland), ORG5222 maleate (Nippon Organon Co., Tokyo, Japan), olanzapine (Eli Lilly and Co., USA), haloperidol (Dainippon Pharmaceutical Co., Osaka, Japan), chlorpromazine (Yoshitomi Pharmaceutical Ind., Osaka, Japan), nemonapride (formerly YM-09151-2, Yamanouchi Pharmaceutical Co., Tokyo, Japan), raclopride tartrate (Astra, Sodertalje, Sweden), and spiperone (Eisai Co., Ltd., Tokyo, Japan). Ipsapirone, ORG5222, and raclopride were dissolved in saline. Other drugs were dissolved in 0.15% tartaric acid. Drugs were injected subcutaneously (SC) in a volume of 1 ml/kg.

General Procedure

Rats were individually subjected to inescapable electric foot shock [2.5 mA of scrambled shock (10 ms shock every 100 ms), on a variable interval schedule with a mean intershock interval of 60 s (35–85 s) and shock duration of 30 s] in a chamber with a grid floor (19 × 22 × 20 cm, Medical Agent Co., Kyoto, Japan). Electric shock was provided by a Model SGS-02D Shock Generator (Medical Agent Co., Kyoto, Japan). This provides a high-voltage, high-resistance circuit with resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5 mA, this generator actually gives the shock level equivalency of 0.2 mA for scrambled constant current to rats. Twenty-four hours after foot shock, rats were placed in the shock chamber without shocks. Behavior was videotaped to confirm the measurements and recorded using the time-sampling procedure during 5 min (11). Every 10 s the behavior that the animal was currently engaged in was classified as either freezing or activity. Freezing was defined as the lack of all observable movement of the body and the vibrissae, except those related to respiration. The percentage scores for the duration of freezing behavior (% freezing) were calculated for 5-min observation period. The results (% inhibition) were expressed as the percentage inhibition compared to the vehicle-treated controls. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee, and in compliance with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

Experiment 1

In the first experiment, the effects of typical and atypical antipsychotic drugs on the acquisition of conditioned freezing were investigated. Thirty minutes after receiving a single SC injection of test compounds or the vehicle in home cages, rats were individually subjected to 2.5 mA foot shock stress (the same parameters as above) for 30 min in the shock chambers, and then returned to home cages. Twenty-four hours after foot shock the rats were individually placed in the same shock chambers without shocks and observed for 5 min. Drug tests were conducted with haloperidol (0.25–10 mg/kg), chlorpromazine (3–30 mg/kg), nemonapride (0.1–2 mg/kg), spiperone (0.03–3 mg/kg), clozapine (0.3–10 mg/kg), olanzapine (1–10 mg/kg), ORG5222 (0.1–1 mg/kg), and raclopride (3–30 mg/kg). As a positive control, the effect of the new anxiolytic ipsapirone (0.1–10 mg/kg), a selective 5-HT_{1A} agonist (18), on the acquisition of conditioned freezing was also evaluated.

Experiment 2

In the second experiment, the effects of clozapine and haloperidol on the expression of conditioned freezing were investigated. Twenty-four hours after a single foot shock session for 30 min (the same parameters as above) rats were treated with haloperidol (0.01–1 mg/kg, SC), clozapine (0.25–10 mg/kg, SC), or the vehicle. Thirty minutes after the injection the rats were placed in the shock chamber without shocks and observed for 5 min.

Data Analysis

All the data are presented as the means ± SEM of the individual values of the rats from each group. The statistical analysis of the data was performed using a one-way analysis of variance followed by Bonferroni/Dunn's test for multiple comparisons. ED_{50} values were calculated according to Litchfield and Wilcoxon (21). Correlations between ED_{50} values of test compounds obtained in CFS and their affinities for various receptors were estimated by calculation of the linear regression correlation coefficient (r) using logarithmically transformed data.

RESULTS

Experiment 1

The new anxiolytic ipsapirone, a selective 5-HT_{1A} agonist, dose-dependently reduced the acquisition of conditioned freezing (Fig. 1), $F(4, 35) = 5.86$, $p < 0.001$. Ipsapirone, at the doses of 1 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.01$), significantly attenuated the acquisition of conditioned freezing.

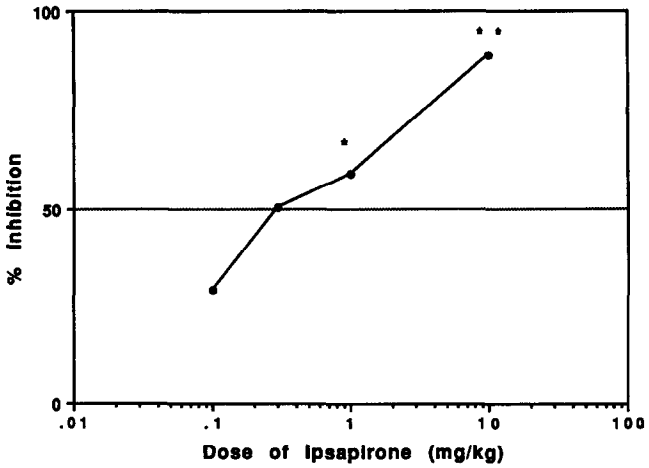

FIG. 1. Protective effects of the 5-HT_{1A} agonist ipsapirone on the acquisition of conditioned freezing. Thirty minutes after a single SC injection of drugs, rats were individually subjected to 2.5 mA foot shock stress for 30 min. Twenty-four hours after foot shock, rats were placed in the shock chamber without shocks and observed for 5 min. The results (% inhibition) were expressed as the percentage inhibition compared to the vehicle-treated controls. The data of % freezing in vehicle control was 76.7 ± 11.6 (the mean ± SEM). The number of rats/group was 8. $*p < 0.05$; $**p < 0.01$ vs. vehicle controls.

Figure 2 shows the dose–response curves for typical and atypical APD on inhibition of the acquisition of conditioned freezing. The atypical APD clozapine dose-dependently attenuated the acquisition of conditioned freezing, $F(4, 41) = 4.63$, $p < 0.005$. Clozapine, at the doses of 5 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.01$), significantly attenuated the acquisition of conditioned freezing. Candidates for atypical APD, ORG5222, and olanzapine, also dose-dependently attenuated the acquisition of conditioned freezing [ORG5222, $F(3, 36) = 6.8$, $p < 0.001$; olanzapine, $F(3, 36) = 7.51$, $p < 0.001$]. ORG5222 (1 mg/kg) produced a significant suppression of the acquisition of conditioned freezing ($p < 0.01$). Olanzapine significantly decreased the acquisition of conditioned freezing at the doses of 3 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.01$). Raclopride, a candidate for atypical APD, significantly suppressed the acquisition of conditioned freezing at the dose of 30 mg/kg ($p < 0.05$), $F(3, 28) = 3.06$, $p < 0.05$. The typical APD haloperidol at the doses of 1 mg/kg ($p < 0.05$) and 3 mg/kg ($p < 0.01$) had a significant protective effect on the acquisition of conditioned freezing, while 10 mg/kg had no significant effect, $F(4, 45) = 6.24$, $p < 0.001$. Like haloperidol, significant suppression of the acquisition of conditioned freezing was observed with 1 mg/kg ($p < 0.01$) but not with 2 mg/kg nemonapride, $F(4, 43) = 3.13$, $p < 0.05$. Chlorpromazine showed about 50% inhibition of the acquisition of conditioned freezing at the dose of 10 mg/kg ($p < 0.095$), but no significant inhibition at the dose of 30 mg/kg, $F(3, 34) = 1.74$, $p = 0.18$. Spiperone reduced the acquisition of conditioned freezing at the doses of 0.1 and 1 mg/kg ($p < 0.01$), $F(4, 39) = 7.02$, $p < 0.0003$. However, like haloperidol, the highest dose of spiperone (3 mg/kg) had no significant effect. Taken together, low doses of typical APDs significantly inhibited the acquisition of conditioned freezing, whereas higher doses of typical APDs, unlike atypical APDs, failed.

Table 1 shows comparison of pharmacological potencies of the test compounds in blocking the acquisition of conditioned

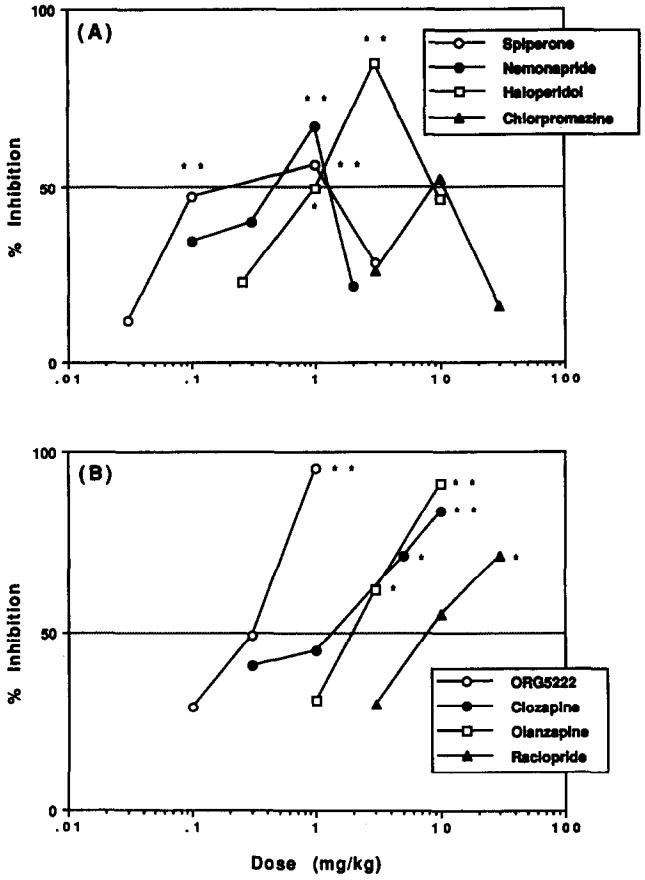

FIG. 2. Protective effects of typical (A) and atypical (B) antipsychotics on the acquisition of conditioned freezing. Thirty minutes after a single SC injection of drugs, rats were individually subjected to 2.5 mA foot shock stress for 30 min. Twenty-four hours after foot shock rats were placed in the shock chamber without shocks and observed for 5 min. The results (% inhibition) were expressed as the percentage inhibition compared to the vehicle-treated controls. The data of % freezing in vehicle controls were: haloperidol, 61.1 ± 5.9; nemonapride, 62.3 ± 5.8; chlorpromazine, 57.1 ± 7.1; spiperone, 80.8 ± 5.5; clozapine, 57.1 ± 7.1; olanzapine, 57.1 ± 7.0; ORG5222, 57.1 ± 7.0; raclopride, 65.8 ± 14.1 (the mean ± SEM). The number of rats/group for each experiment were: haloperidol, 8–18; nemonapride, 8–16; chlorpromazine, 8–14; spiperone, 8–12; clozapine, 8–14; olanzapine, 8–16; ORG5222, 8–16; and raclopride, 8. $*p < 0.05$; $**p < 0.01$ vs. vehicle controls.

freezing with affinities for various monoamine and acetylcholine receptors in vitro, which are derived from published results (6,10,27,30,34,39,41). The ED_{50} values (mg/kg) of the test compounds for blocking the acquisition of conditioned freezing were positively correlated with the K_i values for D_4 dopaminergic receptors (Fig. 3, $r = 0.88$, $p < 0.01$), but not with the K_i values for other monoamine and acetylcholine receptors.

Experiment 2

Although clozapine (0.25–10 mg/kg) or haloperidol (0.01–1 mg/kg), administered to rats 30 min before the test, had no significant effect on the expression of conditioned freezing, there appeared to be a trend ($p < 0.06$) for 1 mg/kg of haloperidol to enhance the expression of conditioned freezing. These

TABLE 1

RECEPTOR BINDING PROFILES OF ANTIPSYCHOTIC DRUGS USED IN THE PRESENT STUDY AND PROTECTIVE EFFECTS OF THOSE DRUGS ON THE ACQUISITION OF CONDITIONED FREEZING

	Anti-CFS ED_{50} (mg/kg)	K_i (nM)									
		D_1	D_2	D_3	D_4	D_5	Ach-M	H_1	5-HT_2	α_1	α_2
ORG5222	0.21						5270[b]	7.7[c]	1.3[b]	0.42[c]	13[c]
Nemonapride	0.37		0.1[a]	0.1[a]	0.1[a]		1300[d]	2100[d]	100[d]	230[d]	4000[d]
Spiperone	0.42	258[g]	0.1[g]	0.6[g]	0.1[g]	4500[g]	1600[d]	1200[d]	1.3[d]	5.6[d]	>10000[d]
Haloperidol	0.78	60[g]	1.3[g]	3[g]	5.1[c]	48[g]	2200[g]	5900[c]	110[c]	2.8[g]	3200[b]
Clozapine	0.84	172[g]	182[g]	479[g]	9[g]	250[g]	24[c]	9.6[c]	24[c]	4.6[c]	71[c]
Olanzapine	1.92				27[a,h]			7[b]	5[b]	19[b]	228[b]
Chlorpromazine	9.1‡	96[g]	8.5[g]	6[g]	37[g]	133[g]	160[c]	10.1[c]	39[c]	4.3[f]	490[c]
Raclopride	8.65	18000[g]	2.9[g]	3.5[g]	237[g]		>10000†[c]	>10000†[c]	26100†[c]	32900†[c]	>10000†[c]

[a] data from ref. 41; [b] data from ref. 27; [c] data from ref. 10; [d] data from ref. 39; [e] data from ref. 6; [f] data from ref. 30; [g] data from ref. 34.
* Personal communication from K. Hidaka (Yamanouchi Pharmaceutical Co. Ltd., Japan).
† IC_{50} (nM)
‡ ED_{50} value was calculated by linear regression.
Only the linear portion of the dose response curve was used for fitting regression lines and determination of ED_{50} values.

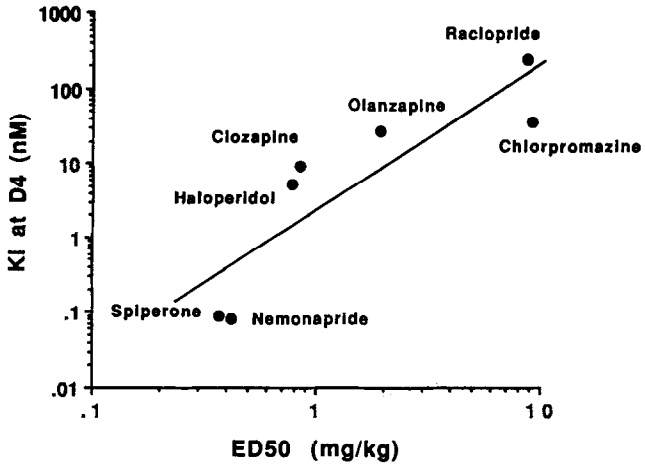

FIG. 3. Correlation between ED_{50} values for inhibition of the acquisition of conditioned freezing and the neuroleptic dissociation constants (K_i values) at the dopamine D_4 receptor ($r = 0.88$, $p < 0.01$). The K_i values of the test compounds for D_4 dopaminergic receptors are derived from published results (shown in Table 1).

drugs did not attenuate the expression of conditioned freezing (Fig. 4).

DISCUSSION

The present study demonstrated that the atypical APD clozapine and candidates for atypical APD inhibited the acquisition of conditioned freezing but did not change the expression of conditioned freezing. However, because typical APDs also prevented the acquisition, but not expression, of conditioned freezing, clear distinction between typical and atypical APDs in behavioral effects on CFS could not be shown in the present study, although typical but not atypical APDs showed bell-shaped dose–response curves for the effect on the acquisi-

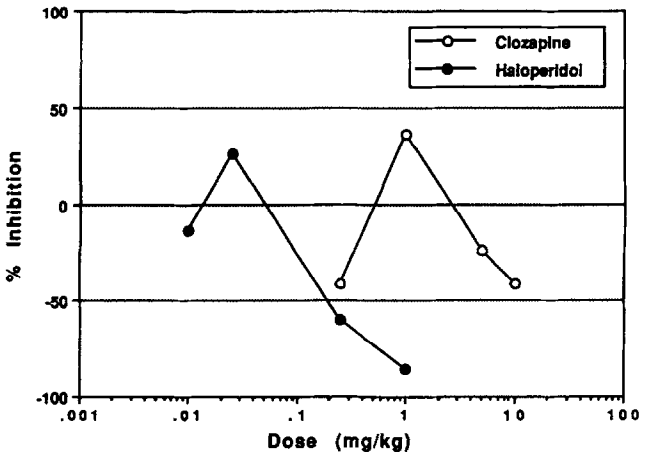

FIG. 4. Effects of clozapine and haloperidol on the expression of conditioned freezing. Twenty-four hours after a single foot shock session (2.5 mA for 30 min), rats were treated with drugs or the vehicle. Thirty minutes after injection, the rats were placed in the shock chamber without shock and observed for 5 min. The results (% inhibition) were expressed as the percentage inhibition compared to the vehicle-treated controls. The data of % freezing in vehicle controls was 52.5 ± 11.3 (the mean ± SEM). The number of rats/group for each drug were: haloperidol, 4–12; clozapine, 4–12. In no case were statistical significant differences observed.

tion of conditioned freezing. In the conditioned fear paradigm, benzodiazepines, 5-HT_{1A} agonists and 5-HT reuptake inhibitors have been reported to inhibit both the acquisition and expression of conditioned freezing [(12,18,32), our unpublished data]. In the present study, the new anxiolytic ipsapirone, a selective 5-HT_{1A} agonist (18), dose-dependently reduced the acquisition of conditioned freezing. Thus, the effects of APDs on CFS were different from those of the standard anxiolytics benzodiazepines and 5-HT_{1A} agonists with respect to the effects on the expression of conditioned freezing. The effect of APDs on the acquisition of conditioned freezing is likely to be an effect on the perception of the noxious foot shock (unconditioned stimulus), rather than anxiolytic effect, which should inhibit the expression of conditioned freezing.

Several recent reports have shown that clozapine and other candidates for atypical APDs differ from typical APDs in their effects on schedule-controlled behavior and ethological models of anxiety. Clozapine (26,36,42) and olanzapine (26), a candidate for atypical APD, have been shown to increase punished responding while the typical APDs haloperidol and chlorpromazine failed to increase punished responding (36,42). As Cook and Davidson (7) and Pich and Samanin (31) described, APDs typically do not attenuate the behavioral suppressant effects of punishment, with only few exceptions (e.g., low doses of trifluoperazine and haloperidol). In addition, in the mouse light/dark discrimination test and the rat elevated plus-maze test, another candidate for atypical APD ORG5222 was reported to release exploratory behavior suppressed by the aversive white or elevated environments (8). Thus, the previous studies have focused on the anxiolytic profiles of atypical APDs and a distinction between typical and atypical APDs in antianxiety effects. However, as mentioned above, the present study did not show the anxiolytic effect of atypical APDs. This difference between previous studies and the present study appears to be due to the differences between these paradigms. The clinical significance of this protective effect of APDs on the acquisition of conditioned freezing is unclear, but it may be associated with clinical effect of APDs on the stress response or the development of anxiety observed in psychotic disorders (3).

The present study shows the positive correlation between anti-CFS effect (ED_{50}; inhibitory effect on the acquisition of conditioned freezing) and the K_i values for D_4 dopaminergic receptors. Interestingly, Seeman (34) has indicated that the dissociation constant of about 9 nM for clozapine at the D_4 receptors, but not D_2 receptors, matches the plasma water concentration of clozapine under therapeutic conditions. Furthermore, Seeman et al. (35) reported that the density of dopamine D_4 receptors in the striatum is sixfold elevated in schizophrenia. These findings suggest that the D_4 receptors may be the primary dopamine receptor that mediates the antipsychotic action of clozapine. In addition, these might explain the clozapine's unique mechanism of action: very weak affinity of clozapine for D_2 receptors and its potent affinity for D_4 receptors may relate to few extrapyramidal side effects and potent antipsychotic effects, respectively. Nevertheless, the functional role of D_4 receptors has not been elucidated yet. The present data suggest that D_4 receptor blockade is associated with inhibition of the acquisition of conditioned freezing. This effect might be partly associated with antipsychotic effects of clozapine and other APDs.

The putative role of D_4 blockade for clozapine's anti-CFS effect is further supported by the present data that anti-CFS effect of the $D_{2/3/4}$ antagonist nemonapride (38,39) is more potent (23-fold) than that of the $D_{2/3}$ antagonist raclopride (6,34). The ED_{50} values of nemonapride and raclopride for anti-CFS effect were 0.37 and 8.65 mg/kg, respectively, while both 0.5 mg/kg of nemonapride and 1 mg/kg of raclopride have been reported to exhibit similar in vivo occupation (70–80%) of D_2-like receptors in the rat striatum (22,28). Potency mismatch for anti-CFS effects and in vivo D_2 occupation might be explained by the differences between nemonapride and raclopride in affinities for D_4 or 5-HT_{1A} receptors. Nemonapride has high affinities for D_4 (K_i = 0.09 nM) and 5-HT_{1A} receptors (K_i = 8.1 nM) (39,41) whereas raclopride has no affinity for 5-HT_1 receptors and very weak affinity for D_4 receptors (K_i = 237 nM) (6,34). Because our unpublished data demonstrated inability of a selective 5-HT_{1A} receptor antagonist (WAY100135) to protect the acquisition of conditioned freezing, the difference between nemonapride and raclopride in anti-CFS effect can be explained more likely by the difference in D_4 receptor binding affinity.

One might account for the effects of typical and atypical APDs on the acquisition of conditioned freezing by a State-Dependent Learning (SDL) hypothesis (29). This SDL hypothesis postulates that acquisition of a task under a drug may require the same or similar drug state for recall. There has been few evidence that APDs produce SDL. Nevertheless, additional experiments will be needed to further examine the role of SDL in the effects of APDs on the acquisition of conditioned freezing. In addition, the question may arise as to whether the APD-induced inhibition of the acquisition of conditioned freezing is due to motor effects produced by APDs. In the present study, freezing tests were conducted 24 h after drug-conditioning trials. Previous studies reported that neuroleptics did not produce the marked motor effect, either hyperactivity or hypoactivity, 24 h after a single injection, although only very high doses of neuroleptics (10 mg/kg of haloperidol) reduced locomotion but not grooming or rearing (2). Therefore, it is unlikely that freezing tests were subject to motoric nonspecific actions of the neuroleptic treatments.

In humans, it is classically accepted that neuroleptics act primarily by inhibiting subcortical psychomotor functions, leaving higher cognitive functions largely intact (19). Recent studies reported that chlorpromazine spared explicit memory in healthy volunteers (9). On the other hand, in nonhuman laboratory animals, dopamine antagonists have been reported to interfere with associative learning of the classical conditioning type by blocking the conditioned and unconditioned excitatory properties of CS (13). This finding, however, cannot explain the present findings because neuroleptics injected before testing did not block conditioned freezing in previously shocked rats. Taken together, the marked inhibition of the acquisition of conditioned freezing by APDs cannot be explained by their effects on memory.

In summary, CFS is a simple and useful model in the development of APD and a search for the mechanism of action of clozapine. Protective effects of clozapine and other antipsychotic drugs on the acquisition of conditioned freezing may be mediated by blockade of D_4 receptors. The mechanism of the protective effect of clozapine on the acquisition of CFS requires further investigation.

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