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Effect of a dopamine D1/5 receptor antagonist on haloperidol-induced inhibition of the acquisition of conditioned fear

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Abstract

This study examined the effects of combined treatment with the typical antipsychotic drug haloperidol and dopamine D1/5 receptor antagonist SCH 23390 on the acquisition of contextual conditioned fear (re-exposure to an environment paired previously with inescapable electric footshocks), compared with those of various antipsychotic adjuvants, which may increase the effects of antipsychotic drugs. Rats were treated subcutaneously with haloperidol (3 mg/kg) combined with SCH 23390 (0.03 mg/kg) and were given fear conditioning by 5 min footshocks in shock chambers 30 min after the injection. One week after the footshocks, the rats were tested in the same shock chamber without shocks and freezing behavior was observed as an index of fear and anxiety. Haloperidol significantly inhibited the acquisition of conditioned freezing. SCH 23390 combined with haloperidol inhibited the acquisition of conditioned freezing more than either drug alone did. These results suggest that combined dopamine D2-like receptor antagonism and dopamine D1-like receptor antagonism is a promising and effective strategy to increase antipsychotic effects.

Keywords: Dopamine D1/5 receptor; Dopamine D2 receptor; Anxiety; Conditioned fear

1. Introduction

Our previous study (Inoue et al., 1996) showed that typical and atypical antipsychotic drugs reduce the acquisition of contextual conditioned fear when drugs are injected before conditioning by footshocks. However, these antipsychotic drugs did not affect the expression of contextual conditioned fear when drugs were injected 24 h after footshocks and before re-exposure to the context (Inoue et al., 1996). The inhibitory effects of selective dopamine D2-like receptor (D2, D3, and D4) antagonists on the acquisition of conditioned fear suggest that dopamine D2-like receptors are related to these effects of antipsychotic drugs (Inoue et al., 1996), although roles of other receptors are not entirely excluded. The antipsychotic drug effects on the acquisition of conditioned freezing are likely to be a reduction in the formation of aversive emotional memory (aversive conditioning) rather than an anxiolytic effect that inhibits the expression of conditioned freezing. Clinically, this common effect of typical and atypical antipsychotic drugs on emotional memory formation may be associated with clinical effects on the stress response or the development of anxiety observed in psychotic disorders. This effect is related to an index of antipsychotic action on emotional memory function, such as conditioned avoidance response, in which avoidance-suppressing activities of antipsychotic drugs are well correlated with the clinical daily doses of antipsychotic drugs used in the treatment of schizophrenia and are suggested to be related to the clinical antipsychotic strengths of antipsychotic drug effects (Kuribara and Tadokoro, 1981).

In addition to dopamine D2-like receptors, dopamine D1-like receptors (D1 and D5) also participate in the acquisition of contextual conditioned fear; the selective dopamine D1/5 receptor antagonist SCH 23390 reduces the

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acquisition, but not the expression, of the fear in the same way as antipsychotic drugs (Inoue et al., 2000). Some typical and atypical antipsychotic drugs have anti-D1-like receptor properties (Meltzer et al., 1989; Leysen et al., 1993; Bymaster et al., 1996), which are suggested to be relevant to the mechanism of unique action of the atypical antipsychotic drug clozapine (Altar et al., 1988). Clinically, the antagonism of dopamine D1/5 receptors may produce an additional effect to the effect of dopamine D2-like receptor antagonism that all conventional antipsychotic drugs have (Seeman, 1992). However, the effects of combined treatment with dopamine D2-like receptor antagonists and dopamine D1-like receptor antagonists on emotional memory function have not been reported experimentally.

A clinical guideline to schizophrenia recommends that, when schizophrenic patients fail to respond to one antipsychotic drug, this antipsychotic drug should be changed to another antipsychotic drug (usually an atypical drug) or lithium carbonate or other mood stabilizers may be added (American Psychiatric Association, 1997). Because atypical antipsychotic drugs have anti-5-HT_2 activities, blocking 5-HT_2 receptors may alleviate not only the occurrence of extrapyramidal side effects induced by antipsychotic drugs, but also the clinical efficacies for some psychotic symptoms (Meltzer, 1992). Until now, the effects of 5-HT_2 receptor blocking and lithium addition on antipsychotic drug-induced reduction in the acquisition of contextual conditioned fear have not been reported.

This study examined the effects of combined therapy of a dopamine D1-like receptor antagonist and a 5-HT_2 antagonist or lithium carbonate on the dopamine D2-like receptor antagonist haloperidol on the acquisition of contextual conditioned freezing, using freezing behavior as an index of fear or anxiety.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan). They weighed 230–250 g, were housed in groups of four, and were maintained in a 12 h light : 12 h dark cycle (light phase, 06:30–18:30 h), temperature-controlled environment (22 ± 1 °C) with free access to food and water. Experiments began after 2 weeks of acclimatization. The rats were tested between 8:00 and 13:00.

2.2. Drugs

The selective dopamine D1/5 receptor antagonist SCH 23390 [*R*-(+)-7-chloro-8-hydroxy-3-methyl-2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzazepine] maleate (Schering-Plough, Bloomfield, NJ, USA) and 5-HT_2 receptor antagonist ICI 169,369, 2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride (Imperial Chemical Industries, Macclesfield, UK) were each dissolved in saline (SCH 23390: 0.01 and 0.03 mg/kg; ICI 169,369: 10 mg/kg) and were injected subcutaneously in a volume of 1 ml/kg (Middlemiss and Tricklebank, 1992; Seeman, 1992; Hoyer et al., 1994). The typical antipsychotic drug haloperidol (0.3 and 3 mg/kg) (Dainippon Pharmaceutical Co., Osaka, Japan) was dissolved in 0.15% tartaric acid and injected subcutaneously in a volume of 1 ml/kg (Inoue et al., 1996). The doses of SCH 23390, haloperidol, and ICI 169,369 were chosen to sufficiently occupy dopamine D1 receptors (SCH 23390, 30% occupation for 0.01 mg/kg, 50% for 0.03 mg/kg), dopamine D2 receptors (haloperidol, 50% occupation for 0.3 mg/kg, 100% for 3 mg/kg), and 5-HT_2 receptors (ICI 169,369, 68% occupation for 10 mg/kg) in vivo (Nowak et al., 1988; Saller et al., 1990; Matsubara et al., 1993).

Lithium carbonate (Li_2CO_3) was given in a diet of rat chow containing 0.2% Li_2CO_3 for 7 days. In the lithium experiments, the lithium-treated rats and the control rats were given 10 mM NaCl instead of tap water to prevent lithium-induced hyponatremia (Thomsen and Olesen, 1974). The dose of Li_2CO_3 was chosen to achieve therapeutic plasma lithium levels. In our previous study, plasma lithium levels in rats treated with 0.2% Li_2CO_3 for 7 days were 0.71 ± 0.05 mEq/L (Muraki et al., 2001).

2.3. Procedures

2.3.1. Conditioned fear stress-induced freezing

The total duration of the conditioning session was 5 min. As described previously (Inoue et al., 2000), rats were individually subjected to inescapable electric footshocks for a total of 2.5 min [five shocks (2.5 mA scrambled shock, 30 s duration), which were delivered at intershock intervals of 35–85 s (mean 60 s)] in a chamber with a grid floor (19 × 22 × 20 cm, Medical Agent Co., Kyoto, Japan). The electric shocks were provided by a Model SGS-02D shock generator (Medical Agent Co., Kyoto, Japan), which has a high-voltage, high-resistance circuit with the resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5 mA, this generator gave a shock intensity of 0.2 mA to the rats. Seven days after the footshocks, the rats were again placed in the shock chamber and were observed for 5 min without shocks. Conditioned fear, as measured by freezing, develops from the contextual stimuli of the conditioned chamber with these procedures (Fanselow, 1980). During the observation period, the duration of freezing behavior was recorded by using a time-sampling procedure (Fanselow, 1980) modified as previously described (Inoue et al., 2000). The rat behavior was classified as either freezing or active throughout the entire 10-s period: freezing was defined as the absence of all observable movement of the skeleton and vibrissae, except related to respiration; all other behavior was scored as activity. The percentage of freezing represented the number of 10-s periods during which the animal froze for the entire 10 s. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee and complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.3.2. Effect of SCH 23390 or ICI 169,369 on haloperidol-induced inhibition of the acquisition of conditioned fear

Thirty minutes after receiving a single subcutaneous injection of drugs (SCH 23390 or ICI 169,369 with haloperidol) or the vehicle, rats were individually subjected to a single 5-min session of footshocks in the shock chambers, and then were returned to their home cages. Seven days after the footshocks, the rats were

individually placed in the same shock chambers without shocks and were observed for 5 min. The interval between the footshock and the test was set at 7 days to exclude the possibility that any drugs remained at testing (Bernardi et al., 1981).

2.3.3. Effect of subchronic lithium treatment on haloperidol-induced inhibition of the acquisition of conditioned fear

In the lithium experiments, after the 7 days during which the rats were maintained on a diet of standard rat chow or rat chow containing 0.2% Li_2CO_3, the rats received a subcutaneous injection of haloperidol or the vehicle 30 min before a 5-min shock session. After the footshocks, rat chow containing 0.2% Li_2CO_3 was stopped and standard rat chow was given to the lithium-treated rats. Seven days after the footshocks, the rats were individually placed in the same shock chambers without shocks and were observed for 5 min.

2.4. Data analysis

All the data are the means±S.E.M. of the individual values of the rats from each group. Multiple group comparisons were made using one-way analysis of variance (ANOVA) followed by Duncan's test, or two-way ANOVA.

3. Results

3.1. Effect of haloperidol on the acquisition of conditioned fear

Haloperidol (3 mg/kg) given before conditioning significantly reduced conditioned freezing in the conditioned fear test 7 days after conditioning [1-way ANOVA, $F(2,45)=4.932$, $P=0.0116$] (Fig. 1A).

3.2. Effect of the drugs on haloperidol-induced inhibition of the acquisition of conditioned fear

3.2.1. SCH 23390

The selective dopamine D1/5 receptor antagonist SCH 23390 (0.03 mg/kg) administered before the footshocks reduced conditioned freezing in the test observed 7 days after shock and further increased the inhibitory effect of haloperidol (3 mg/kg) on the acquisition of conditioned freezing (Fig. 1B). Two-way ANOVA showed significant main effects of SCH 23390 and haloperidol [effect of SCH 23390, $F(1,28)=20.383$, $P=0.0001$; effect of haloperidol, $F(1,28)=40.759$, $P<0.0001$], but no significant interaction [effect of interaction, $F(1,28)=1.176$, $P=0.2873$].

The combined treatment with low doses of haloperidol (0.3 mg/kg) and SCH 23390 (0.01 mg/kg), both having marginal effects on the acquisition of conditioned freezing, showed a less prominent effect (Fig. 2). Two-way ANOVA showed a significant main effect of haloperidol [$F(1,28)=11.223$, $P=0.0023$], but no significant main effect of SCH 23390 [$F(1,28)=0.424$, $P=0.5203$] and no significant interaction [$F(1,28)=0.246$, $P=0.6235$].

3.2.2. ICI 169,369

The selective 5-$HT_{2A/2C}$ receptor antagonist ICI 169,369 (10 mg/kg) failed to influence the acquisition of conditioned freezing and did not affect haloperidol (3 mg/kg)-induced inhibition of the acquisition of conditioned freezing (Fig. 3). Two-way ANOVA

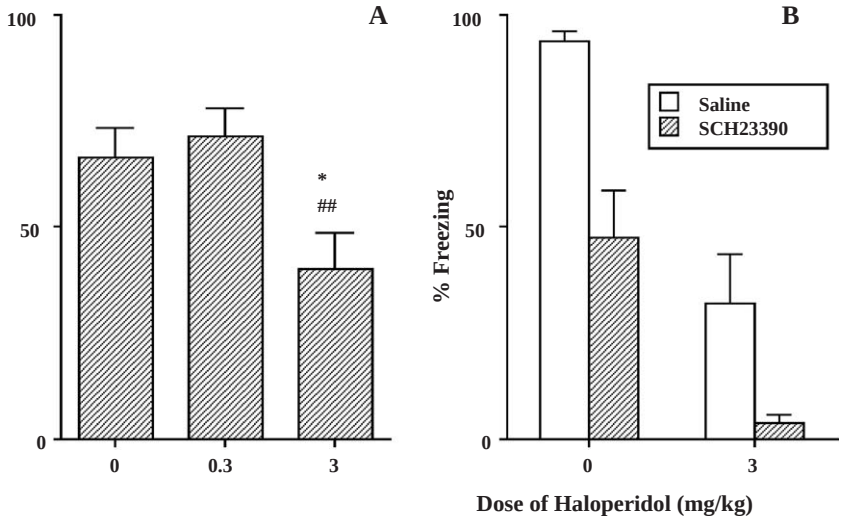

Fig. 1. (A) Effect of the typical antipsychotic drug haloperidol administered before footshocks on conditioned freezing in the test observed 7 days after footshocks. Thirty minutes after a single subcutaneous injection of haloperidol, rats were individually subjected to 2.5 mA shock stress for 5 min. Seven days after footshocks, rats were placed in the shock chamber without shocks and were observed for 5 min. Represented are the mean percentages±S.E.M. of freezing scored for 5 min. The number of rats in each group was 16. One-way ANOVA: $F(2,45)=4.932$, $P=0.0116$, $^{*}P<0.05$ vs. vehicle controls, $^{\#\#}P<0.01$ vs. 0.3 mg/kg group. (B) Effect of combined treatment with SCH 23390 (0.03 mg/kg) and haloperidol (3 mg/kg) on the acquisition of conditioned freezing. Thirty minutes after a single subcutaneous injection of haloperidol and SCH 23390, rats were individually subjected to 2.5 mA shock stress for 5 min. Seven days after the footshocks, the rats were placed in the shock chamber without shocks and were observed for 5 min. Represented are the mean percentages±S.E.M. of freezing scored for 5 min. The number of rats in each group was 8. Two-way ANOVA: effect of SCH 23390, $F(1,28)=20.383$, $P=0.0001$; effect of haloperidol, $F(1,28)=40.759$, $P<0.0001$; effect of interaction, $F(1,28)=1.176$, $P=0.2873$.

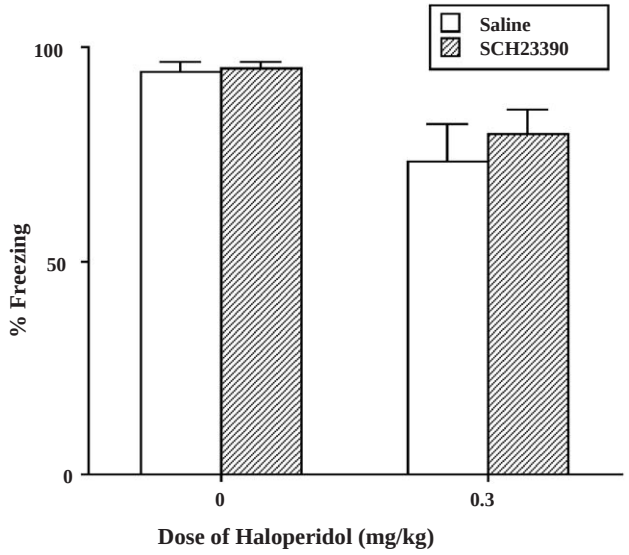

Fig. 2. Effect of combined treatment with SCH 23390 (0.01 mg/kg) and haloperidol (0.3 mg/kg) on the acquisition of conditioned freezing. The experimental procedure, percentages, and number of rats in each group were as for Fig. 1B. Two-way ANOVA: effect of SCH 23390, $F(1,28)=0.424$, $P=0.5203$; effect of haloperidol, $F(1,28)=11.223$, $P=0.0023$; effect of interaction, $F(1,28)=0.246$, $P=0.6235$.

showed a significant main effect of haloperidol [$F(1,28)=10.273$, $P=0.0034$], but no significant main effect of ICI 169,369 [$F(1,28)=1.248$, $P=0.2735$] and no significant interaction [$F(1,28)=0.029$, $P=0.8652$].

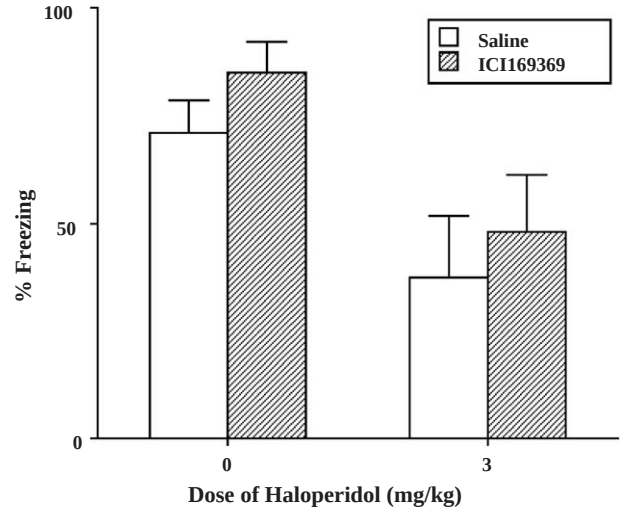

Fig. 3. Effect of ICI 169,369 on haloperidol-induced inhibition of the acquisition of conditioned freezing. Thirty minutes after a single subcutaneous injection of haloperidol (3 mg/kg) and ICI 169,369 (10 mg/kg), rats were individually subjected to 2.5 mA shock stress for 5 min. Seven days after the footshocks, the rats were placed in the shock chamber without shocks and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for 5 min. The number of rats in each group was 8. Two-way ANOVA: effect of ICI 169,369, $F(1,28)=1.248$, $P=0.2735$; effect of haloperidol, $F(1,28)=10.273$, $P=0.0034$; effect of interaction, $F(1,28)=0.029$, $P=0.8652$.

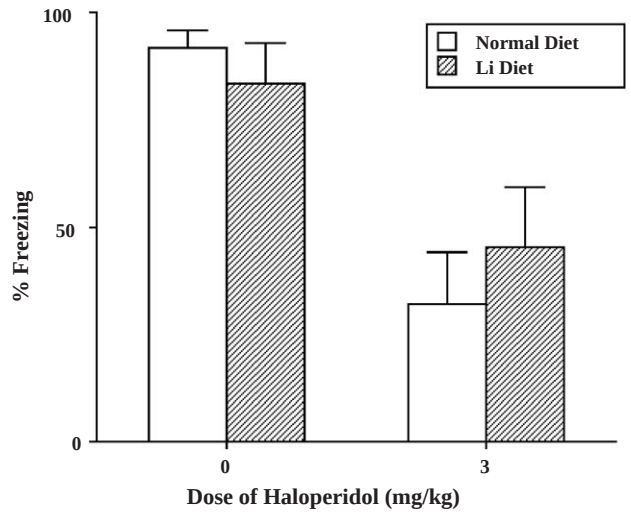

Fig. 4. Effect of subchronic lithium pretreatment on haloperidol-induced inhibition of the acquisition of conditioned freezing. Lithium carbonate (0.2% Li_2CO_3 in food) was administered orally for 7 days before the footshocks. Thirty minutes after a single subcutaneous injection of haloperidol (3 mg/kg), rats were individually subjected to 2.5 mA shock stress for 5 min. Seven days after the footshocks, the rats were placed in the shock chamber without shocks and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. The number of rats in each group was 8. Two-way ANOVA: effect of lithium, $F(1,28)=0.057$, $P=0.8136$; effect of haloperidol, $F(1,28)=21.452$, $P<0.0001$; effect of interaction, $F(1,28)=1.059$, $P=0.3123$.

3.2.3. Subchronic lithium treatment

Subchronic lithium treatment (0.2% Li_2CO_3 in food) for 1 week before conditioning did not influence the acquisition of conditioned freezing or the haloperidol (3 mg/kg)-induced inhibition of the acquisition of conditioned freezing (Fig. 4). Two-way ANOVA showed a significant main effect of haloperidol [$F(1,28)=21.452$, $P<0.0001$], but no significant main effect of lithium [$F(1,28)=0.057$, $P=0.8136$] and no significant interaction [$F(1,28)=1.059$, $P=0.3123$].

4. Discussion

This study showed that combined administration of the selective dopamine D1/5 receptor antagonist SCH 23390 had a marked effect on haloperidol-induced inhibition of the acquisition of conditioned fear, but adding lithium or the 5-HT_2 receptor antagonist ICI 169,369 did not. We reported previously (Inoue et al., 1996, 2000) that both a dopamine D1-like receptor antagonist and dopamine D2-like receptor antagonist, such as the typical antipsychotic drug haloperidol, reduced the acquisition of conditioned fear, using freezing behavior as an index of fear. This study showed that combined dopamine D1-like receptor antagonism and dopamine D2-like receptor antagonism inhibited the acquisition of conditioned fear more than either drug alone did.

A previous study of in vivo receptor binding showed that 3 mg/kg haloperidol occupies 100% of dopamine D2-like

receptors in the rat brain (Matsubara et al., 1993). In our previous behavioral study (Inoue et al., 1996), haloperidol showed a bell-shaped dose–response curve for the effect on the acquisition of conditioned freezing and 3 mg/kg haloperidol produced a maximal effect. In contrast, another of our previous studies (Inoue et al., 2000) showed that the effects of SCH 23390 on the acquisition of conditioned freezing were dose-dependent at or above 0.01 m/kg. In this study, SCH 23390 at a dose of 0.03 mg/kg, which occupies 50% of dopamine D1-like receptors in the brain (Nowak et al., 1988), produced 50% inhibition of freezing compared with vehicle controls. Thus, the maximal effect of haloperidol does not completely block the acquisition of conditioned freezing (66% inhibition), but this incomplete blocking of the acquisition of conditioned freezing by the sufficient dose of haloperidol was completed by moderate dopamine D1/5 receptor blocking. However, in this study, insufficient doses of haloperidol (0.3 mg/kg) and SCH 23390 (0.01 mg/kg), which occupy 50% of dopamine D2-like receptors (Matsubara et al., 1993) and 30% of dopamine D1-like receptors (Nowak et al., 1988), respectively, did not increase each drug effect in a combined treatment.

Preclinical data (Altar et al., 1988) suggested that dopamine D1-like receptor antagonism might produce an atypical profile for antipsychotic drugs, especially clozapine. However, a previous clinical trial indicated that a selective dopamine D1 receptor antagonist does not have an apparent antipsychotic effect in acutely ill schizophrenic patients treated with monotherapy (Karlsson et al., 1995). Several typical and atypical antipsychotic drugs have dopamine D1 receptor antagonist properties (Meltzer et al., 1989; Leysen et al., 1993), but whether adding dopamine D_1-like receptor antagonism may clinically produce the additional psychotropic effect of antipsychotic drugs, generally having dopamine D2-like receptor antagonism, is unclear. This study suggests from the rationale of the mechanism of action supported by the experimental data that adding dopamine D1-like receptor antagonism to dopamine D2-like receptor antagonism may be effective in managing emotional symptoms, and that switching from dopamine D2-like receptor antagonists to mixed dopamine D1/D2-like receptor antagonists is a promising treatment strategy.

In conclusion, both the selective dopamine D1/5 receptor antagonist SCH 23390 and the typical antipsychotic drug haloperidol (dopamine D2-like receptor antagonist) inhibited the acquisition of conditioned fear. These drugs when combined completely blocked the acquisition of conditioned fear and their effect was more than the maximal effect of haloperidol alone, which occupies 100% of dopamine D2-like receptors in vivo. Although monotherapy with a dopamine D1-like receptor antagonist is ineffective in the treatment of schizophrenia, adding a dopamine D1-like receptor antagonistic property to antipsychotic drugs may produce more ameliorative effects on emotional symptoms of schizophrenia.

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