Evaluation of Perospirone (SM-9018), a Novel Serotonin-2 and Dopamine-2 Receptor Antagonist, and Other Antipsychotics in the Conditioned Fear Stress-Induced Freezing Behavior Model in Rats

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ABSTRACT—We studied the effects of perospirone (SM-9018), a novel serotonin-2 (5-HT2) and dopamine-2 (D2) receptor antagonist (SDA), on conditioned fear stress (CFS)-induced freezing behavior in rats and compared its actions with those of other antipsychotics. Exposure of rats to the environment previously paired with foot shock induced marked freezing behavior, which was reduced by the anxiolytic diazepam (0.1–3 mg/kg, p.o.) or antidepressants, desipramine and imipramine (10–100 mg/kg, p.o.). Perospirone at 0.3–3 mg/kg, p.o. significantly attenuated the CFS-induced freezing behavior in a dose-dependent manner, while the effect was reduced at the higher dose of 6 mg/kg. Other SDA-type antipsychotics, clozapine (1–30 mg/kg, p.o.) and risperidone (0.03–1 mg/kg, p.o.), and selective 5-HT2 antagonists, ritanserin (0.1–1 mg/kg, p.o.) and ketanserin (0.3–1 mg/kg, p.o.), all reduced the freezing behavior with U-shaped dose-response curves. However, neither conventional antipsychotic, haloperidol (0.1–3 mg/kg, p.o.), chlorpromazine (3–100 mg/kg, p.o.), thioridazine (3–100 mg/kg, p.o.), mosapramine (3–100 mg/kg, p.o.) nor tiapride (30–1000 mg/kg, p.o.) reduced the CFS-induced freezing behavior. In addition, subacute treatment of rats with perospirone (1–10 mg/kg/day) or imipramine (30 mg/kg/day) for 2 weeks prevented the induction of the freezing behavior by CFS. These findings suggest that SDA-type antipsychotics including perospirone are effective for the treatment of mood disturbances such as anxiety and depressive mood associated with schizophrenia and have a broader efficacy profile as compared with the conventional antipsychotics.

Keywords: Perospirone, Antipsychotic, Conditioned fear stress, Freezing behavior, Schizophrenia

Schizophrenia is a heterogeneous disorder that displays diverse symptoms, course, prognosis and probably also etiology. The patients show not only positive symptoms (e.g., hallucination, delusion and excitation), but also exhibit negative or deficit symptoms (e.g., flattening affect, apathy and social withdrawal) and dysphoric mood disturbances (e.g., anxiety and depression) (1). The conventional antipsychotics (e.g., haloperidol and chlorpromazine) effectively improve the positive symptoms through antagonism of dopamine-2 (D2) receptors. However, these drugs are poorly effective against the negative symptoms and frequently induce extrapyramidal side effects (e.g., parkinsonism, akathisia and dyskinesia). Previous studies have shown that the serotonin-2 (5-HT2) receptor antagonists produce thymosthenic actions and improve the negative symptoms in patients with schizophrenia (2–4). These agents also reduced the extrapyramidal side effects induced by conventional antipsychotics. In addition, clozapine, which possesses both 5-HT2 and D2-blocking actions, has been shown to improve the negative schizophrenic symptoms with a reduced liability for the extrapyramidal side effects (5). Based on these clinical findings, several 5-HT2 and D2 antagonists (SDA) are now being developed as novel type antipsychotics.

Perospirone (SM-9018; cis-N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl) butyl)cyclohexane-1,2-dicarboximide) is a newly developed SDA-type antipsychotic agent that has high affinities for both 5-HT2 and D2 receptors (Ki=0.61 and 1.4 nM, respectively) (6–9). Perospirone, like haloperidol, blocks various behaviors induced by

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Animals

Male Sprague-Dawley rats (Nihon SLC, Inc., Shizuoka) each weighing 175-255 g were used. The rats were housed in a temperature (23±2°C), and humidity (55±10%)-controlled room under the standard lighting condition (light/dark: 8 AM/8 PM) for at least one week before being tested. They were given tap water and standard rat chow ad libitum.

CFS-induced freezing behavior

Experiments were carried out according to the method of Inoue et al. (23) with slight modifications. On the training day, animals in the conditioning groups were individually placed in the experimental chamber (30 x 20 x 30 cm) with a grid floor and subjected to an unconditioned stimulus (US) (2 mA of scramble foot shock) for 30 min (Scrambler SGS-004; Tokai Irika, Tokyo). Animals in the no-conditioning group (No-cond) were also placed individually in the chamber for 30 min, but received no US. On the testing day, scheduled at 24 hr after the training session, the rats were again placed in the identical chamber, in which US was previously applied. The CFS-induced freezing behavior of the animals was measured for 5 min in the absence of US using a timesampling procedure (test session). Each rat was rated as either being frozen or active every 10 sec in a total of 30 sampling periods (5 min). Freezing behavior was defined as the complete lack of all observable movements of the body and vibrissae except those related to respiration, and the animal was judged as being frozen if it kept the behavioral status of freezing for a whole 10-sec period in each sampling period. All other behavior seen in the sampling period was judged as active. The freezing score of each rat was calculated as the sum of the number of sampling periods in which the animal was rated as being frozen. Perospirone and all other test drugs were administered 60 min before the test session.

Subacute treatment

Perospirone (1, 3 and 10 mg/kg) and imipramine (30 mg/kg) were orally administered to the rats once a day (between 9 AM-11 AM) for 14 days. The training session with US was carried out 60 min before the last drug administration, and the CFS-induced freezing behavior was recorded in the same manner as in the acute experiment 24 hr after the conditioning session.

Drugs

Perospirone hydrochloride, haloperidol, clozapine, risperidone, diazepam, mosapramine dihydrochloride, thioridazine and tiapride were synthesized in our laboratory. Chlorpromazine hydrochloride, imipramine hydrochloride and desipramine hydrochloride were purchased from Sigma Chem. Co. (St. Louis, MO, USA), and ketanserin tartrate and ritanserin were from Research Biochem., Inc. (Natick, MA, USA). All drugs were suspended in 0.5% methylcellulose.

Data analyses

The data are expressed as the mean±S.E.M. Differences in the freezing scores among multiple treatment groups were analyzed by the non-parametric Kruskal-
Wallis test followed by Steel’s test. Comparisons between two groups were made by the Mann-Whitney U-test. Percent changes in the CFS-induced freezing behavior was also obtained by the following equation: (the mean score of test group – the mean score of No-cond group) / (the mean score of vehicle-treated group – the mean score of No-cond group) x 100. The apparent dosage of a drug that reverses the CFS-induced freezing behavior by 50% (ED50) was determined by the method of Litchfield and Wilcoxon.

RESULTS

Effects of perospirone and other antipsychotics on CFS-induced freezing behavior

The No-cond group normally exhibited a low freezing score of 6.19 ± 0.80 (N = 36), probably due to the habituation to the test environment (Fig. 1). CFS (an exposure to the same environment previously paired with foot shock) induced marked freezing behavior in rats, which was characterized by a complete suppression of motility with occasional defecation and urination. The freezing behavior score was usually increased to about 19 to 22 by the CFS in most experiments.

Oral administration of perospirone at 0.3–6 mg/kg significantly attenuated the CFS-induced freezing behavior without affecting the activity in the No-cond group (Fig. 1). The inhibitory action of perospirone appeared in a dose-dependent manner at doses of 0.3–3 mg/kg, while it tended to be reduced at a higher dose (6 mg/kg). The maximal inhibition rate of the CFS-induced freezing by
perospirone was 79.8% at 3 mg/kg, and the apparent dose (ED50 value) that reversed the freezing by 50% was 1.3 mg/kg. Similarly, other SDA-type antipsychotics, clozapine (1–30 mg/kg, p.o.) and risperidone (0.03–1 mg/kg, p.o.), also reduced the incidence of the CFS-induced freezing with U-shaped dose-response curves (Fig. 2). The maximal inhibition rates of freezing behavior by clozapine and risperidone were 46.7% and 61.4%, respectively, and the ED50 value of risperidone was 0.13 mg/kg. In contrast, none of the administered conventional antipsychotics, haloperidol (0.1–3 mg/kg, p.o.), chlorpromazine (3–100 mg/kg, p.o.), thioridazine (3–100 mg/kg, p.o.), mosapramine (3–100 mg/kg, p.o.) and tiapride (30–100 mg/kg, p.o.), significantly affected the CFS-induced freezing behavior (Table 1). Conversely, high doses of haloperidol and chlorpromazine rather augmented the CFS-induced freezing behavior (Table 1).

### Table 1. The effects of antipsychotics on the conditioned fear stress (CFS)-induced freezing behavior in rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg, p.o.)</th>
<th>CFS-induced freezing (score/5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>18.7 ± 2.9</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.1</td>
<td>20.7 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>14.7 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>23.8 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>28.7 ± 0.7</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>3</td>
<td>23.2 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>22.2 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>27.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>28.8 ± 0.4</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>3</td>
<td>19.5 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>14.7 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>18.3 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>17.5 ± 1.1</td>
</tr>
<tr>
<td>Mosapramine</td>
<td>3</td>
<td>13.5 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>15.5 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>14.5 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16.5 ± 3.2</td>
</tr>
<tr>
<td>Tiapride</td>
<td>30</td>
<td>19.5 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>14.7 ± 1.5</td>
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<tr>
<td></td>
<td>300</td>
<td>17.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>19.8 ± 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.7 ± 1.8</td>
</tr>
</tbody>
</table>

Each value shows the mean ± S.E.M. of 6 rats. Drugs were orally administered 1 hr before observation of the freezing behavior.

### Effects of other CNS drugs and 5-HT2 antagonists on CFS-induced freezing behavior

We next examined the effects of the anxiolytic diazepam, the antidepressants imipramine and desipramine, and the selective 5-HT2 antagonists ritanserin and ketanserin on the CFS-induced freezing behavior. As shown in Fig. 3, the administration of diazepam (0.3–3 mg/kg, p.o.) significantly reduced the CFS-induced freezing behavior in a dose-dependent manner, while the effect was
diminished at a dose of 10 mg/kg. Imipramine (10–100 mg/kg, p.o.) and desipramine (10–100 mg/kg, p.o.) also reduced the incidence of CFS-induced freezing with the maximum inhibition rates of 58.9% and 52.4% (at 100 mg/kg), respectively (Fig. 3). On the other hand, both 5-HT2 antagonists, ritanserin (0.1–1 mg/kg, p.o.) and ketanserin (0.3–1 mg/kg, p.o.), reduced the CFS-induced freezing behavior with U-shaped dose response curves (Fig. 4). The maximal inhibition rates of ritanserin and ketanserin were 60% to 65%, and apparent ED50 values were 0.28 and 0.54 mg/kg, respectively.

Subacute effects of perospirone on CFS-induced freezing behavior

The animals were treated once a day with an oral dose of perospirone (1–3 mg/kg/day), imipramine (30 mg/kg/day) or vehicle for 2 weeks. The training session was carried out 60 min before the last treatment and the CFS-induced freezing behavior was observed after a 24-hr withdrawal. Under these conditions, CFS induced a marked freezing behavior in control animals treated with the vehicle alone (Fig. 5). However, the induction of the CFS-induced freezing behavior was attenuated by the subacute perospirone treatment in a dose-dependent manner (Fig. 5). The perospirone treatment at 3 or 10 mg/kg/day reduced the CFS-induced freezing scores by about 75%, the scores not being significantly different from that in the No-cond group. A similar attenuation in the induction of freezing behavior by the CFS was also obtained by the subacute treatment with imipramine (30 mg/kg/day) (Fig. 5).
DISCUSSION

The present study demonstrated that the exposure of rats to a distinctive environment previously paired with foot shock (US) induces a marked freezing behavior characterized by the complete suppression of motility with occasional defecation and urination. The CFS-induced freezing behavior was significantly ameliorated by administration of the anxiolytic diazepam. In addition, single and repeated administrations of the tricyclic antidepressants, desipramine or imipramine, reduced the incidence of the CFS-induced freezing behavior. These findings are consistent with previous studies (18–21) and suggest that the CFS-induced freezing responses in rats are closely associated with the emotional states of anxiety, fear and/or depressive mood in humans.

In this study, the newly developed antipsychotic perospirone at moderate doses significantly improved the CFS-induced freezing with an ED50 value of 1.3 mg/kg. The freezing score was reduced to a level similar to that of the No-cond group by the administration of 3 mg/kg perospirone, while its action tended to be reduced with increasing dosage (6 mg/kg). Perospirone has been shown to exert anti-dopaminergic actions (e.g., the antagonisms for the methamphetamine-induced hyperactivity and apomorphine-induced climbing behavior) with ED50 values of 2.2 to 3.5 mg/kg (6). Thus the present findings suggest that perospirone at clinical doses is effective for mood disturbances (i.e., anxiety and depression) associated with schizophrenia. The anxiolytic potential of perospirone was also observed in our recent study using the rat social interaction test, where perospirone, like diazepam, significantly increased the time spent in active social interaction by the pair of Lister-hooded rats (unpublished observation). In addition, a recent clinical study revealed that perospirone effectively improved not only the positive symptoms but also the negative symptoms and anxiety-depression in patients with schizophrenia (13).

Among the antipsychotics examined in this study, only the SDA-type antipsychotics with combined 5-HT2 and D2-blocking actions improved the CFS-induced freezing behavior. Clozapine and risperidone, like perospirone, significantly attenuated the freezing behavior with U-shaped dose-response curves. In contrast, administration of any of the conventional antipsychotics, haloperidol, chlorpromazine, thioridazine, mosapramine or tiapride, failed to improve the CFS-induced freezing behavior. The former two drugs rather potentiated the freezing at higher doses. The SDA-type antipsychotics commonly exhibit a higher affinity for 5-HT2 receptors than D2 receptors, and they are expected to block the 5-HT2 receptors at clinical doses (4). For example, perospirone inhibited the striatal 3H-spiperone (D2) binding with a potency similar to that of haloperidol (K, value = 1.4 and 1.8 nM, respectively), whereas it inhibits the cortical 3H-ketanserin (5-HT2) binding with more than 100 times the potency of haloperidol (K, value = 0.61 and 116 nM, respectively) (6). These findings suggest that the blockade of 5-HT2 receptors contributes to the inhibition of the CFS-induced freezing behavior by the SDA-type antipsychotics. In this study, the selective 5-HT2 antagonist ritanserin significantly reduced the CFS-induced freezing behavior. Ketanserin, which has 5-HT2- and a1-blocking activities, also tended to reduce the freezing behavior. Thus the 5-HT2 receptor blockade might be at least partly involved in the amelioration of the CFS-induced freezing behavior by the SDA-type antipsychotics. Our findings are in agreement with the previous neurochemical studies (23, 24) that showed that an increased activity of the serotonergic system is involved in the freezing behavior induced by psychological stress (e.g., CFS). The reason why most of the drugs (i.e., the SDA-type antipsychotics, the 5-HT2 antagonists and diazepam) inhibited the freezing behavior with the common characteristic of U-shaped dose-response curves is presently uncertain. In the rat open-field test, we observed that the antipsychotics perospirone, clozapine, risperidone, haloperidol and chlorpromazine inhibited the line-crossing activity with ED50 values of 5.9, 21, 0.59, 0.48 and 9.1 mg/kg (p.o.), respectively (unpublished observation). These doses were relatively consistent with the dose range for the efficacy-decline of the former three drugs and for the freezing-potentiation of the latter two drugs in the CFS model. Thus, the inhibitory effects of the SDA-type antipsychotics on locomotor activity might inhibit or mask their ameliorative actions in the CFS-induced freezing.

We have recently demonstrated that the subacute treatment of rats with perospirone (10 mg/kg/day, for 2 weeks) produced a significant reduction (about 20%) in the density (Bmax) of the cortical 5-HT2 receptors (12). However, such changes were not observed in animals treated with haloperidol. Antidepressants also produce the down-regulation of 5-HT2 receptors after repeated administration, which has been suggested to be one of the possible mechanisms of the antidepressant action (25–27). We therefore compared the subacute effects of perospirone and imipramine on the CFS-induced freezing behavior model. The animals were treated with perospirone (1–10 mg/kg/day) or imipramine (30 mg/kg/day) for 2 weeks, and the CFS-induced freezing behavior was observed 24 hr after the last administration of each drug. Under these conditions, both perospirone and imipramine prevented the induction of the freezing behavior by CFS. Interestingly, the dose-response curve of perospirone was no longer U-shaped at these doses. These
findings suggest that perospirone at clinical doses exerts imipramine-like mood stabilizing actions under repeated administration. Since perospirone down-regulated the cortical 5-HT₂ receptors under the same treatment as in the present study (12), the 5-HT₂ receptor changes might contribute to the ameliorative effects of subacute perospirone on the CFS-induced freezing behavior.

It is of interest that the 5-HT₂ antagonists and SDA-type antipsychotics, but not conventional ones, improved the CFS-induced freezing behavior. Previous studies have shown that SDA-type antipsychotics including perospirone, clozapine and risperidone effectively improved the negative symptoms of schizophrenia, which do not respond well to the conventional antipsychotics (1, 4, 13, 14). In addition, selective 5-HT₂ antagonists (e.g., ritanerin and setoperone) have also been shown to produce the thymosthenic effects in schizophrenia patients and improve the negative symptoms (2, 3). Thus, the differential suppression of the CFS-induced freezing behavior by the SDA-type antipsychotics may reflect their efficacy for the negative schizophrenic symptoms. There are so far only a few animal models proposed that can predict the pathophysiological mechanisms of the negative symptoms are still unknown, the CFS-induced freezing behavior in rats may serve as a useful model that can differentiate the antipsychotics with a potential efficacy against the negative symptoms. Further studies are required to clarify the actions of SDA-type antipsychotics in mood disturbances and their clinical relevance in schizophrenia treatment.

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