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σ Receptor antagonists block the development of sensitization to cocaine

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Abstract

The effects of putative σ receptor antagonists, BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine), rimcazole and SR-31742A (*cis*-3-(hexahydroazepin-1-yl)1-(3-chloro-4-cyclohexylphenyl)propene-1), on the development of behavioral sensitization induced by repeated administration of cocaine were investigated. Acute intraperitoneal injection of 15 mg/kg cocaine in rats induced moderate hyperactivity which mainly consisted of sniffing and rearing. These acute effects of cocaine were hardly affected by co-administration of the σ receptor antagonists, except that BMY-14802 enhanced, but not significantly, cocaine-induced locomotion. While repeated cocaine administration induced a progressive increase in stereotyped behaviors and resulted in sensitization, every σ receptor antagonists tested attenuated the development of sensitization to cocaine. These prophylactic effects of σ receptor antagonists against cocaine-induced sensitization were confirmed by the challenge test with cocaine alone after an abstinence. These results were consistent with results of our previous study which revealed that BMY-14802 blocked the sensitization to methamphetamine, another psychostimulant. Therefore, σ receptors play a crucial role in the development of the psychostimulant-induced sensitization phenomenon, which is a pharmacological model of schizophrenia.

Keywords: σ Receptor; Behavioral sensitization; Cocaine; BMY-14802; SR-31742A; Schizophrenia

1. Introduction

It has been suggested that the σ receptors could be involved in the various physiological functions of the central nervous system and neuropsychiatric disorders (Ferris et al., 1991; Su, 1991; Walker et al., 1990). Benzomorphans such as (+)-pentazocine, which are presumed to work as σ receptor agonists, have psychotomimetic effects in man (Bellville and Forrest, 1968). Psychostimulants such as amphetamine and cocaine also possess a weak but significant affinity for σ receptors (Sharkey et al., 1988). On the other hand, most neuroleptics with few exceptions showed potent affinity for these σ receptors (Largent et al., 1984; Su, 1982; Weber et al., 1986). Haloperidol, one of the strongest neuroleptics, showed the highest affinity to σ receptors among neuroleptics (Walker et al., 1990). In addition, postmortem studies revealed the down-regulation of σ receptors in the cortices of schizophrenic brains (Reynolds et al., 1991; Weissman et al., 1991). These findings suggest that σ receptors may contribute to the pathogenesis of psychosis and/or the therapeutic mechanism of neuroleptics.

The behavioral sensitization phenomenon or reverse tolerance induced by repeated administration of psychostimulants has been recognized as an animal model of schizophrenia (Ellinwood et al., 1973; Post, 1975, 1977; Robinson and Becker, 1986). Using this model, we previously reported that BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine), a putative σ receptor antagonist, prevented the development of sensitization to methamphetamine (Ujike et al., 1992a). On the other hand, the animals that were sensitized by repeated methamphetamine injections showed supersensitivity to a putative σ receptor agonist, (+)-3-PPP ((+)-3-(3-hydroxyphenyl)-*N*-(1-propyl) piperidine) (Ujike et al., 1992b). These findings indi-

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cate that the σ receptors plays a crucial role in the sensitization phenomenon induced by psychostimulants. To confirm this hypothesis, we previously investigated and revealed the involvement of σ receptors in the expression process of sensitization to cocaine, another type of psychostimulant (Ujike et al., 1992c). The present study was then aimed at clarifying the roles of σ receptors in the induction process of cocaine sensitization, using several kinds of putative σ receptor antagonists.

2. Materials and methods

2.1. Animals and treatments

Male Sprague-Dawley rats weighing 160–180 g at the beginning of drug administration were used. The animals were housed three per cage, with free access to food and water, at constant temperature (24°C) and humidity (65%) under a standard 12–12-h light-dark cycle (lights on at 08:00 h). Following a 1-week accommodation period for handling, the rats were randomly divided into seven groups, each consisting of six to ten rats. The rats of each group received an intraperitoneal (i.p.) injection of either 1 ml/kg sterile water or one of the σ receptor antagonists dissolved in the same volume of sterile water. They received a subsequent i.p. injection of saline or 15 mg/kg cocaine 30 min later. Three separate σ receptor antagonists, BMY-14802 (15 or 30 mg/kg), rimcazole (50 mg/kg) and SR-31742A (*cis*-3-(hexahydroazepin-1-yl)-1-(3-chloro-4-cyclohexylphenyl)propene-1, 5 or 15 mg/kg), were used. The rats received these treatments once daily for 10 consecutive days. To confirm the prophylactic effects of σ receptor antagonists, all rat groups were withdrawn for 10 days, and then were challenged with 15 mg/kg cocaine alone.

2.2. Behavior rating

Rats were individually placed in an observation cage of transparent plastic with dimensions 310 × 360 × 175 mm, and allowed 1 h for settling down prior to the drug treatments. Each rat was videotaped for 30-s epochs every 6 or 12 min for 1 h after the injection of cocaine. Behavior was scored by trained raters, who were unaware of the pretreatment conditions, according to a rating system reported previously (Ujike et al., 1992a). Briefly, grooming and rearing were estimated in terms of the total duration for which these forms of behavior were evident. Locomotion was scored as the number of times an animal moved from one corner of the cage to the other. Hyperactivity and stereotypy of sniffing and repetitive head movement were rated using a 0–5 activity/stereotypy score scale.

2.3. Statistical analysis

In the experiment on acute interaction of cocaine and σ receptor antagonists, each score taken at 8 time points after the cocaine injections was summed and analyzed for statistical significance using a one-way analysis of variance (ANOVA). Fisher's protected least significant difference (PLSD) test was used for a post-hoc multiple comparison when one-way ANOVA was significant, and the Bonferroni and Dunn test was used when it was not. In the experiment involving repeated treatment sessions and challenge tests, two-way ANOVA of repeated measures followed by Fisher's PLSD test was used. Values of $P < 0.05$ were considered significantly different.

3. Results

3.1. Effects of σ receptor antagonists on acute motor activities induced by single injection of 15 mg/kg cocaine (Fig. 1)

Acute intraperitoneal injection of 15 mg/kg cocaine induced moderate hyperactivity consisting mainly of sniffing and rearing. Average scores of activity/stereotypy and rearing were 1.1 ± 0.3° and 7.8 ± 6.2 s, respectively. Locomotion induced by cocaine 15 mg/kg was as low as 0.7 ± 0.6 of the cage side. These acute behavioral effects of cocaine, except for locomotion, were hardly affected by co-administration of σ receptor antagonists, BMY-14802 (15 or 30 mg/kg), rimcazole (50 mg/kg) and SR-31742A (5 or 15 mg/kg). Cocaine-induced locomotion was moderately enhanced by co-administration of BMY-14802, however, the enhancement was not significant because individual variance was large.

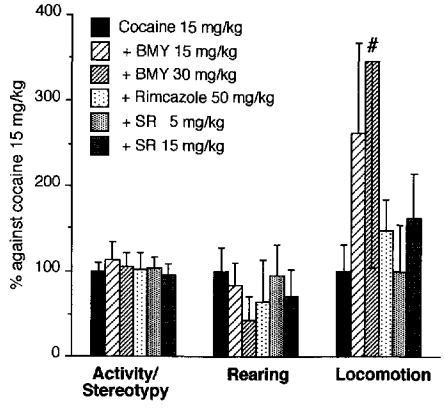

Fig. 1. Effects of σ receptor antagonists on acute motor activity induced by cocaine in rats. Rats received a σ receptor antagonist injection followed by injection of 15 mg/kg cocaine 30 min later. Means ± S.E.M., $n = 6–9$. # $P = 0.0598$ by the Bonferroni and Dunn test.

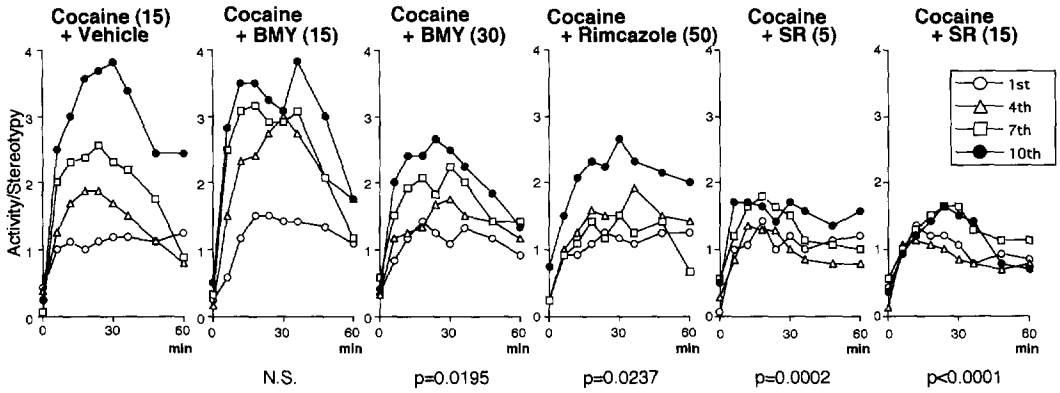

Fig. 2. Effects of σ receptor antagonists on serial changes in stereotypy induced by repeated cocaine administration. Rats received repeated injections of 15 mg/kg cocaine alone or in combination with the σ receptor antagonist once daily for 10 days. Induced behaviors were observed on the 1st, 4th, 7th and 10th treatment day. BMY; BMY-14802, SR; SR-31742A. Numbers in parentheses following drugs indicate their doses (mg/kg). S.D. bars are omitted for legibility of the figures. Two-way ANOVA revealed that the stereotypy induced on the 10th treatment day (closed circle) was significantly different among six groups ($F(5,272) = 8.868$, $P < 0.0001$), and the subsequent post-hoc test by Fisher's PLSD showed a difference from the cocaine + vehicle group on the 10th day (each P value is shown under the corresponding figure).

3.2. Serial changes in stereotypy induced by repeated administration of 15 mg / kg cocaine alone and in combination with σ receptor antagonists (Fig. 2)

Repeated treatments with 15 mg/kg cocaine induced a progressive augmentation of stereotyped behaviors and resulted in the development of behavioral sensitization ($F(3,224) = 20.53$, $P < 0.0001$). BMY-14802 in the dose of 30 mg/kg, but not 15 mg/kg, co-administered with cocaine significantly attenuated the development of sensitization to cocaine. The other σ receptor antagonists, rimcazole and SR-31742A, also blocked the cocaine-induced sensitization. Among the σ receptor antagonists, SR-31742A was most effective to prevent the sensitization to cocaine.

3.3. Challenge test with 15 mg / kg cocaine alone after abstinence (Fig. 3)

To confirm the prophylactic effects of the σ receptor antagonists against cocaine-induced sensitization, the challenge dose of cocaine alone was given to all rats of seven groups after a 10-day abstinence period, which is considered enough for withdrawal of cocaine and σ receptor antagonists. The challenge dose of cocaine 15 mg/kg revealed that the sensitized rats

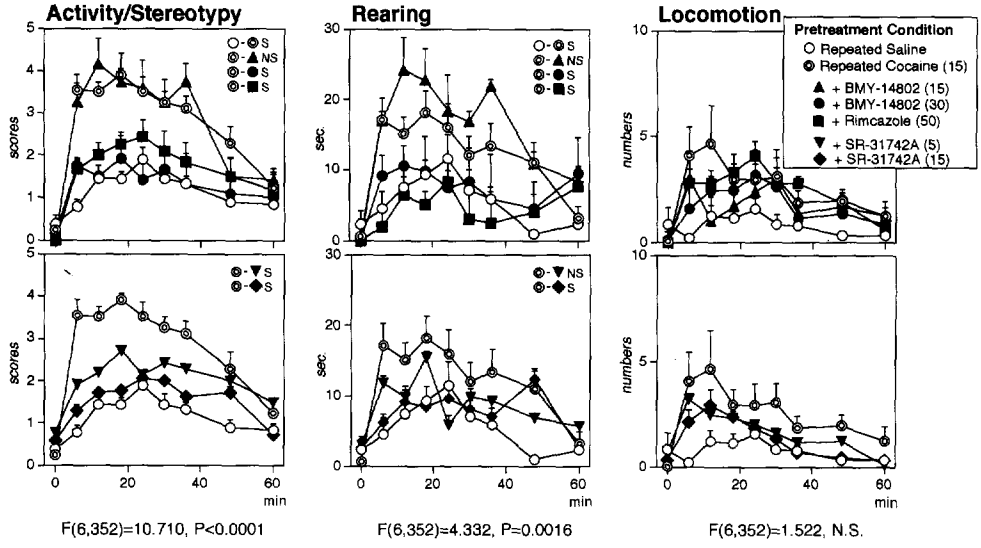

Fig. 3. Challenge test with 15 mg/kg cocaine alone. After the repeated treatment session with cocaine and σ receptor antagonists, all groups were withdrawn for 10 days and received 15 mg/kg cocaine injection alone. Means ± S.E.M., $n = 6–10$. Numbers in parentheses following drugs in legend indicate their doses (mg/kg) used in the repeated treatment session. F and P values of significant differences among seven groups by two-way ANOVA of repeated measures are shown under the figures. The results of multiple comparison are shown in the right upper corner of each figure. S: significant, N.S.: not significant.

continued to show supersensitivity of stereotypy and rearing to the subsequent cocaine when compared with saline-pretreated rats. Locomotion also seemed to be more enhanced in the sensitized rats than the controls, but not significantly so. The rats pretreated with cocaine and BMY-14802 at dose of 15 mg/kg did not show any prophylactic effect on the development of sensitization to cocaine, because the challenge with cocaine alone showed that these rats were not different from the rats sensitized to cocaine as to intensity of any type of behavior. However, the rats pretreated with cocaine and 30 mg/kg BMY-14802 showed prevention of supersensitivity to cocaine for stereotyped behaviors and rearing. These rats showed no difference from the saline-pretreated control rats in their response to the subsequent cocaine. Earlier pretreatment with rimcazole and SR-31742A also prevented the development of supersensitivity to cocaine. SR-31742A was more effective at the dose of 15 mg/kg than at that of 5 mg/kg. The larger dose of SR-31742A showed complete prevention of sensitization for stereotyped behaviors, with no significant difference from the saline-pretreated rats. However, the rats pretreated with cocaine and a smaller dose of SR-31742A (5 mg/kg) showed partial prevention with significantly reduced stereotypy compared to the cocaine-pretreated rats ($P = 0.0103$), and also with significantly greater stereotypy than the saline-pretreated control rats ($P = 0.0044$).

4. Discussion

In the present study, we demonstrated that combined treatment with cocaine and with each kind of putative σ receptor antagonist, BMY-14802, rimcazole or SR-31742A, prevented the development of sensitization to cocaine. These prophylactic effects of σ receptor antagonists against cocaine-induced sensitization were confirmed by a subsequent challenge test with cocaine alone after long enough abstinence for withdrawal of σ receptor antagonists. BMY-14802 is known to be a relatively selective σ receptor antagonist (Largent et al., 1988; Taylor et al., 1991), but it also has a significant affinity for 5-HT_{1A} receptors (Bristow et al., 1991). Preclinical study has suggested that 5-HT_{1A} receptors may participate in antipsychotic effects of neuroleptics (Wadenberg and Ahlenius, 1991). Although BMY-14802 was shown to reduce the locomotion induced by acute amphetamine administration, this effect is supposed to be mediated by its antagonism at 5-HT_{1A} but not σ receptors (Bristow et al., 1991). Therefore, it is possible that the prophylactic effects of BMY-14802 against cocaine sensitization may result from the action at the 5-HT_{1A} receptors. However, this is unlikely because the other σ receptor antagonists, rimcazole and SR-31742A, which lack affinity for 5-HT_{1A} receptors (unpublished data), also prevented the development of cocaine sensitization. The rank order of potency for the prophylactic effects of σ receptor antagonists was SR-31742A > BMY-14802 > rimcazole. This order was consistent with that of their affinities for the σ receptors (Largent et al., 1988; Poncelet et al., 1993). In addition, the prophylactic effects of BMY-14802 and SR-31742A showed dose dependence. The doses of σ receptor antagonists used in the present study were considered within the physiological range because the compounds at these doses elicited no toxic effects such as convulsion and ataxia in the present and previous studies (Ujike et al., 1992a; Menkel et al., 1991; Poncelet et al., 1993). Therefore, the prophylactic effects of σ receptor antagonists against cocaine sensitization shown in the present study are most likely to result from their antagonistic actions at σ receptors.

The σ receptor antagonists showed only minor interaction with acute motor effects of cocaine. SR-31742A and rimcazole never affected the motor stimulant effects of acute cocaine, such as sniffing, up-down head movement, rearing and locomotion. BMY-14802 at the higher dose showed a tendency to enhance cocaine-induced locomotion but not the other kinds of movement. This result did not agree with those of the study which showed that BMY-14802 and rimcazole significantly attenuated the locomotion induced by cocaine at the dose of 10-50 mg/kg in mice (Menkel et al., 1991). As the present study involved rats, this inconsistency as to acute interaction between σ receptor antagonists and cocaine may result from the species difference, but further studies are necessary.

The manner of prevention of cocaine-induced sensitization by the σ receptor antagonists was quite unique. Although the σ receptor antagonists did not affect the motor stimulant effects of acute cocaine as mentioned above, they blocked the reverse tolerance of cocaine motor effects that developed during repeated administration. This characteristic feature of preventing sensitization by σ receptor antagonists was also shown in our previous study of the sensitization to methamphetamine, another psychostimulant (Ujike et al., 1992a). Co-administration of BMY-14802 at the doses of 15 and 30 mg/kg blocked fully the development of sensitization to methamphetamine during repeated administration, with little interaction of motor stimulant effects of acute methamphetamine. This feature of σ receptor antagonists to prevent sensitization is quite different from that of dopamine receptor antagonists. Haloperidol and selective dopamine D_1 or D_2 receptor antagonists were shown to block the development of sensitization to cocaine and amphetamine/methamphetamine (Kuczenski and Leith, 1981; Tella, 1994; Ujike et al., 1989; Weiss et al., 1989). However, they

also completely suppressed the motor stimulant effects of psychostimulants at each treatment. Therefore, dopamine receptor antagonists work through interference with dopamine in the synaptic cleft, which is increased by psychostimulants, activating the postsynaptic dopamine receptors. In contrast, σ receptor antagonists did not influence the actions of postsynaptic dopamine receptors because they hardly affect the dopamine-mediated behaviors induced by psychostimulants. Thus, the mechanisms of prevention of sensitization by the σ receptor antagonists must be distinct from those involved with the dopamine antagonists. We also reported previously that the rats sensitized in advance by repeated treatment with cocaine or methamphetamine showed an enhanced sensitivity to a subsequent dose of (+)-3-PPP, a putative σ receptor agonist (Ujike et al., 1992b, c). These results indicate that the repeated administration of psychostimulants may induce the development of supersensitivity in the σ receptors. As cocaine and amphetamine have a weak but significant affinity for the σ receptors (Sharkey et al., 1988), they can act directly at the σ receptors. Repeated treatment with psychostimulants may affect or activate the σ receptors repeatedly and this process may result in supersensitivity of σ receptors, which should contribute to the development of behavioral sensitization. Accordingly, it is supposed that the σ receptor antagonists blocked direct effects of psychostimulants at the σ receptors and prevented the psychostimulant-induced sensitization. As our present and previous studies showed cross-sensitization between σ receptor agonist and psychostimulants and prevention of sensitization by σ receptor antagonists, the σ receptors must play a crucial role in both the induction process and the expression process in the behavioral sensitization to psychostimulants. As psychostimulant-induced sensitization in rodents is recognized as an animal model for schizophrenia, especially for the relapse of chronic schizophrenia (Post, 1977; Sato et al., 1992), our results suggest that abnormal functioning of σ receptors may participate in the susceptibility to relapse of schizophrenic patients. However, a recent clinical trial using BMY-14802 failed to improve psychiatric symptoms of schizophrenia (Gewirtz et al., 1994). As the subjects in the trial were acute exacerbated schizophrenic patients, this clinical fact was not inconsistent with our preclinical findings. BMY-14802 and other σ receptor antagonists did not attenuate acute motor effects induced by psychostimulants, which correspond to acute symptoms of schizophrenia. Our findings predict that σ receptor antagonists may prevent schizophrenia from deteriorating or becoming susceptible to relapse, which may occur over time. Further clinical trials designed to assess this aspect of σ receptor antagonists should be done to clarify the exact clinical role of σ receptor antagonism by σ receptor antagonists and ordinary neuroleptics possessing σ receptor affinities in therapy of schizophrenia.

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