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Short communication

Contrasting effects of SM-9018, a potential atypical antipsychotic, and haloperidol on c-fos mRNA expression in the rat striatum

Tadashi Ishibashi, Kazuhito Ikeda, Kumiko Ishida, Junko Yasui, Rie Tojima, Mitsutaka Nakamura, Yukihiro Ohno *

Discovery Research Laboratories II, Research Center, Sumitomo Pharmaceuticals Co., Ltd., 3-1-98 Kasugade-naka, Konohana-ku, Osaka 554, Japan

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Abstract

SM-9018 (*cis*-2-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)hexahydro-1*H*-isoindole-1,3(2*H*)-dione HCl) is a potential atypical antipsychotic with high affinity for 5-HT_2, dopamine D_2 and 5-HT_{1A} receptors. Northern blot analysis was performed to compare the effects of SM-9018 and of haloperidol on the striatal c-fos mRNA expression in rats. Haloperidol (0.3–30 mg/kg, p.o.) markedly increased the striatal c-fos mRNA levels (about eight-fold at 30 mg/kg), the increase being abolished by lesioning of dopamine neurons with 6-hydroxydopamine. In contrast, SM-9018 produced only a slight increase (about two-fold) in c-fos mRNA expression at doses up to 30 mg/kg (p.o.). The 5-HT_2 receptor antagonist, ritanserin (0.1–3 mg/kg, i.p.), dose-dependently attenuated the haloperidol-induced c-fos expression, but the putative 5-HT_{1A} receptor antagonist, NAN-190 (1-(2-methoxyphenyl)-4-(4-(2-phethalimmido)butyl)piperazine HBr; 1–10 mg/kg, i.p.), did not. These findings suggest that SM-9018 is weaker than haloperidol for induction of striatal c-fos mRNA expression, to which the 5-HT_2 receptor blocking activity of SM-9018 seems to contribute.

Keywords: Antipsychotic; SM-9018; Haloperidol; c-*fos* mRNA; 5-HT_2 receptor; Striatum

1. Introduction

The immediate early gene c-fos can be induced by various biological stimuli, such as stress, pain, seizure and drug treatments (Morgan and Curran, 1989). Previous studies have shown that antipsychotic agents, which commonly possess dopamine D_2 blocking activity, increase the c-fos mRNA level and Fos-like immunoreactivity in the striatum (Dragunow et al., 1990; Miller, 1990). The antipsychotic-induced c-fos expression appeared to occur in the striatal efferent neurons to the globus pallidus, which express enkephalin mRNA (Robertson et al., 1992). In addition, recent studies have shown that the ability of antipsychotics to enhance striatal c-fos expression can differentiate between typical and atypical antipsychotics (Fink-Jensen and Kristensen, 1994; Nguyen et al., 1992; Robertson and Fibiger, 1992). Regarding this, the typical antipsychotics (e.g., haloperidol), the clinical use of which is accompanied by severe extrapyramidal side effects (EPS) in humans, markedly enhanced c-fos expression in the striatum whereas the effects of the atypical antipsychotics (e.g., clozapine) with fewer EPS were very weak. Thus, striatal c-fos expression seems to be a useful biological marker for the evaluation of antipsychotic-induced EPS (Nguyen et al., 1992; Robertson et al., 1994).

SM-9018 (*cis*-2-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)hexahydro-1*H*-isoindole-1,3(2*H*)-dione HCl) is a newly developed antipsychotic agent that has potent blocking activities for both 5-HT_2 and dopamine D_2 receptors. It binds to 5-HT_2 and dopamine D_2 receptors with high affinity (K_i = 0.61 and 1.4 nM, respectively) and blocks various behaviors induced by dopamine and 5-HT receptor agonists (Hirose et al., 1990; Kato et al., 1990). SM-9018 also exhibits a relatively high affinity for 5-HT_{1A} receptors (K_i = 2.9 nM) while it interacts weakly with other receptors (Kato et al., 1990). In addition, SM-9018 was much weaker than the typical antipsychotics (e.g., haloperidol and chlorpromazine) for induction of the EPS signs (i.e., catalepsy and bradykinesia) in rats and mice, suggesting that SM-9018 could be classified as an atypical antipsychotic (Hirose et al., 1990; Meltzer and Nash, 1991; Ohno et al., 1994). To further characterize the atypical antipsychotic property of SM-9018, Northern blot analysis was used to compare the effects of SM-9018 with

* Corresponding author. Tel.: 81-6-466-5215; fax: 81-6-466-5218.

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those of haloperidol on striatal c-fos mRNA expression in rats. The effects of 5-HT_2 and 5-HT_{1A} receptor antagonists were also studied to explore the mechanisms underlying the differences between the actions of SM-9018 and haloperidol on c-fos mRNA expression.

2. Materials and methods

2.1. Animal treatments

Male Sprague-Dawley rats (Charles River Japan) weighing 190–220 g were given an oral dose of SM-9018 (1–30 mg/kg) or haloperidol (0.3–30 mg/kg). In experiments with ritanserin (0.1–3 mg/kg) and NAN-190 (1-(2-methoxyphenyl)-4-(4-(2-phthalimmido)butyl)piperazine HBr; 1–3 mg/kg), these agents were injected intraperitoneally 0.5 h before haloperidol administration.

For dopaminergic denervation, the animals were pretreated with desipramine (25 mg/kg, i.p.) and pargyline (40 mg/kg, i.p.; 45 min before), and then received an injection of 6-hydroxydopamine (370 μg in 10 μl of saline containing 0.1% ascorbic acid) into the lateral ventricle (coordinate, A: 8.2, L: 1.5, H: 6.5) under pentobarbital anesthesia (40 mg/kg, i.p.). The sham-operated rats were given an equivalent volume of the vehicle alone in a similar fashion. These animals were used for the experiments 6 days after the 6-hydroxydopamine injection.

SM-9018 and haloperidol were synthesized in our laboratories and all other drugs were purchased from a commercial source.

2.2. RNA isolation and Northern blotting

The animals were killed by decapitation 0.5 h after the administration of the antipsychotics, and the brains were rapidly removed from the skull. The bilateral striata were dissected out on an ice-cold Petri dish and homogenized in solution D (4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% sodium *N*-lauroylsarcosine and 0.1 M 2-mercaptoethanol). Total RNA from the striata was extracted with the AGPC (acid guanidinium thiocyanate-phenol-chloroform) method of Chomczynski and Sacchi (1987) and stored at −80°C until analysis.

20 μg of the total RNA were fractionated by electrophoresis on 1% agarose formaldehyde gels in MOPS (3-(*N*-morpholino)propanesulfonic acid) buffer and transferred to nylon membranes (Hybond-H^+; Amersham). The membranes were then fixed by UV transillumination and prehybridized in 40% formamide, 5 × standard saline citrate buffer (SSC), 1 × Denhardt's solution, 10% dextran sulfate, 20 mM Tris (pH 7.5), 1% sodium dodecyl sulfate (SDS), 20 μg/ml yeast tRNA and 100 μg/ml salmon sperm DNA at 42°C for 2 h. The membranes were then hybridized at 42°C for 16 h with ^{32}P-3′-end-labeled c-fos oligonucleotide probe (5′-GCA GCG GGA TGA GGC CTC GTA GTC CGC GTT GAA ACC CGA GAA CAT-3′). The hybridized membranes were washed to a stringency of 0.5 × SSC in 0.1% SDS for 30 min at 65°C, and exposed to X-ray film (Fuji RX) at −80°C. The c-fos mRNA levels were evaluated by measuring the optical density of the band on the film, using an image analyzer (TIF-256R; Toyojozo).

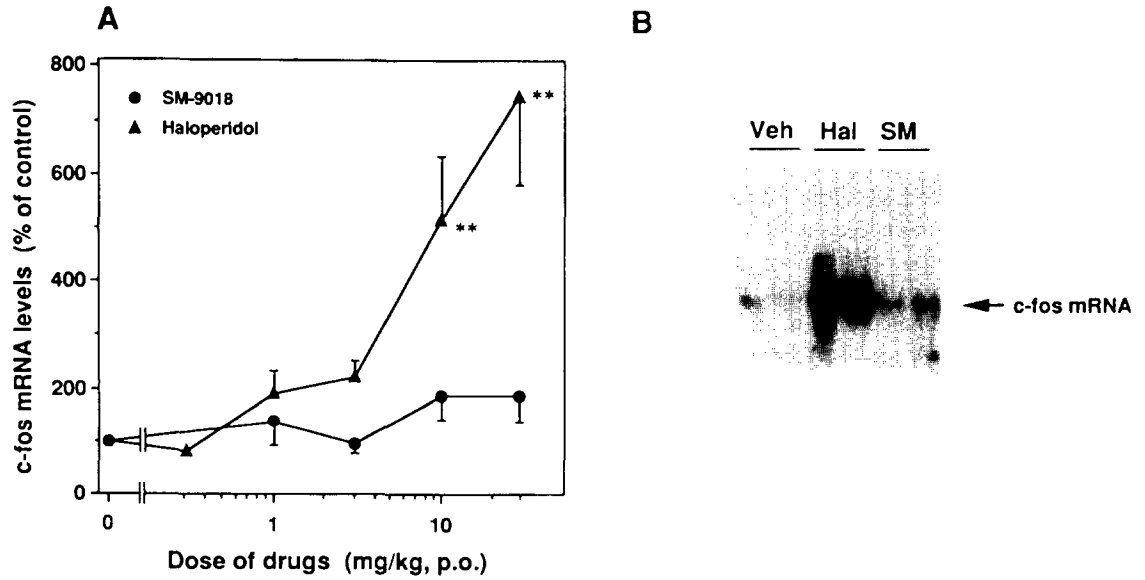

Fig. 1. The effect of haloperidol and SM-9018 on c-fos mRNA expression in the rat striatum. *A*: Dose-response curves of SM-9018- and haloperidol-induced c-fos mRNA expression in the rat striatum. The amount of striatal c-fos mRNA was measured by Northern blot analysis as described in the text. The results are expressed as percentages of the control value with vehicle alone. ** $P < 0.01$; significantly different from the control value (one-way ANOVA and Dunnett's test). *B*: Autoradiogram of Northern blot analysis. Each lane contains 20 μg of total RNA of the striata of rats which were killed 0.5 h after the administration of vehicle (Veh, 0.5% methylcellulose), haloperidol (Hal, 10 mg/kg) or SM-9018 (SM, 10 mg/kg).

2.3. Data analysis

The data were expressed as percentages of the control value (vehicle administration). In the 6-hydroxydopamine experiments, the c-fos mRNA levels of the sham-operated rats, which were treated with vehicle alone, were used as controls. The statistical significance of the differences among drug treatments was determined by one-way ANOVA (analysis of variance) followed by a two-tailed Dunnett multicomparison test.

3. Results

Oral administration of haloperidol at 1–30 mg/kg (p.o.) markedly enhanced striatal c-fos mRNA expression in a dose-dependent manner (Fig. 1). The c-fos mRNA levels increased significantly by about five and eight times the control levels following administration of haloperidol at 10 and 30 mg/kg, respectively. In contrast, SM-9018 at 1–30 mg/kg produced only slight increases in striatal c-fos mRNA expression (Fig. 1). The c-fos mRNA levels increased to about twice the control levels in response to 10–30 mg/kg of SM-9018. The action of SM-9018 appeared transiently, with a maximum effect at 0.5 h and complete recovery to the control level within 1 h while haloperidol exhibited a longer action with complete recovery at 2–4 h (data not shown).

The haloperidol- and SM-9018-induced c-fos mRNA expression in the striatum was blocked by lesioning of dopamine neurons with 6-hydroxydopamine. In sham-operated rats, 10 mg/kg (p.o.) haloperidol significantly increased the c-fos mRNA levels (479.3 ± 225.1% of the control, $n = 3$); however, this increase was greatly diminished by the pretreatment of animals with 6-hydroxydopamine (208.3 ± 34.7% of the control, $n = 4$). Similarly, SM-9018 produced a slight (not significant) increase in the c-fos mRNA levels (260.2 ± 54.4% of the control, $n = 3$) in the sham-operated group, but this increase was almost abolished by the 6-hydroxydopamine pretreatment (112.2 ± 2.8% of the control, $n = 4$). 6-Hydroxydopamine pretreatment alone did not significantly change the c-fos mRNA levels as compared to those in the sham-operated group.

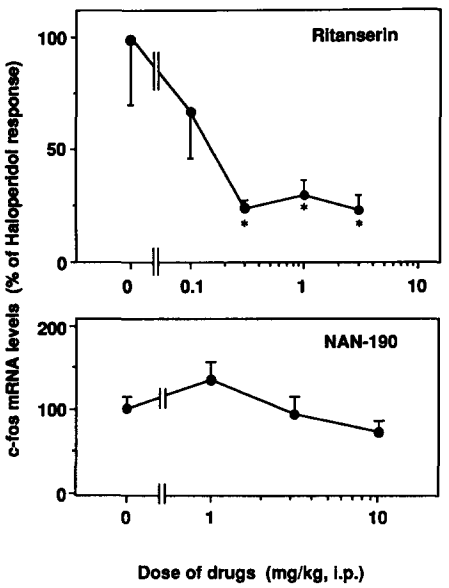

Fig. 2. Effects of ritanserin and NAN-190 on haloperidol-induced c-fos mRNA expression in the rat striatum. Various doses of ritanserin and NAN-190 or the vehicle (0.5% methylcellulose) were intraperitoneally injected 0.5 h before haloperidol (10 mg/kg, p.o.) administration. The c-fos mRNA levels were expressed as percentages of the value stimulated by haloperidol. * $P < 0.05$; significantly different from the control values (one-way ANOVA and Dunnett's test).

Fig. 2 shows the effects of ritanserin (a 5-HT_2 receptor antagonist) and NAN-190 (a putative 5-HT_{1A} receptor antagonist) on the haloperidol-induced c-fos mRNA expression. The administration of ritanserin did not itself alter the c-fos mRNA levels (data not shown), but it significantly attenuated the haloperidol-induced c-fos mRNA expression in a dose-dependent manner. The level of c-fos mRNA was reduced to about 20% of that with haloperidol (10 mg/kg, p.o.) alone by 0.3–3 mg/kg of ritanserin. On the other hand, NAN-190 at doses up to 10 mg/kg (i.p.) failed to affect haloperidol-induced c-fos mRNA expression (Fig. 2).

4. Discussion

Previous studies have shown that antipsychotics, including haloperidol, increase the c-fos mRNA levels and the Fos-like immunoreactivity in the rat striatum (Dragunow et al., 1990; Miller, 1990). The antipsychotic-induced c-fos expression was most prominent in the dorsolateral part of the striatum which contains a high density of dopamine D_2 receptors (Nguyen et al., 1992). The increase could be reversed by the administration of dopamine D_2 receptor agonists (Miller, 1990). The present study confirmed that haloperidol markedly enhanced c-fos mRNA expression in the rat striatum. The time course and the extent of the haloperidol-induced increase of c-fos mRNA levels were similar to those reported previously (Miller, 1990). In addition, the haloperidol-induced c-fos mRNA expression was suppressed by the dopaminergic denervation with 6-hydroxydopamine. These findings suggest strongly that haloperidol increased the striatal c-fos mRNA levels by blocking endogenous dopamine which tonically inhibits c-fos expression via striatal D_2 receptors.

In contrast to haloperidol, a newly developed antipsychotic, SM-9018, was very weak for induction of striatal c-fos mRNA expression. The c-fos mRNA levels at their maximal increase by SM-9018 (10–30 mg/kg) were about twice the control levels whereas those increased by haloperidol (30 mg/kg) were about eight-fold higher. The dose range of SM-9018 used herein was sufficient to block

various dopaminergic behaviors, in that SM-9018 inhibited the methamphetamine-induced hyperactivity and the apomorphine-induced climbing behavior with ED_{50} values of 2.2–3.5 mg/kg (p.o.) (Hirose et al., 1990). In addition, SM-9018-induced c-fos mRNA expression was also abolished by the 6-hydroxydopamine pretreatment. Our results suggest that SM-9018 is much weaker than haloperidol to block the striatal D_2 receptor-induced increase in c-fos mRNA expression. We have previously demonstrated that SM-9018 was weaker than the typical antipsychotics (e.g., haloperidol and chlorpromazine) for induction of the EPS signs (i.e., catalepsy and bradykinesia) in rats and mice (Hirose et al., 1990; Ohno et al., 1994), suggesting that SM-9018 belongs to the class of atypical antipsychotics. SM-9018 also showed a low propensity to cause up-regulation and supersensitivity of the striatal D_2 receptors after its repeated administration (Ohno et al., 1995). The present results, taken together with the previous behavioral findings, support the hypothesis that striatal c-fos mRNA expression could serve as a useful marker for differentiating the atypical from the typical antipsychotic agents.

The pharmacological profile of SM-9018 differs from that of haloperidol since it is a more potent antagonist for 5-HT_2 receptors than for D_2 receptors (Hirose et al., 1990). The affinity of SM-9018 for 5-HT_2 receptors (K_i = 0.61 nM) is about 200 times that of haloperidol while its affinity for D_2 receptors (K_i = 1.4 nM) is similar to that of haloperidol (K_i = 1.7 nM). In addition, SM-9018 also showed a high affinity (K_i = 2.9 nM) for 5-HT_{1A} receptors, where it seemed to act as an antagonist, as described previously (Kato et al., 1990). We therefore examined the effects of ritanserin (a 5-HT_2 receptor antagonist) and NAN-190 (a putative 5-HT_{1A} receptor antagonist) on the haloperidol-induced c-fos mRNA expression to determine whether the 5-HT_2 and 5-HT_{1A} receptor blocking activities of SM-9018 contribute to the difference from haloperidol in c-fos mRNA expression. In the present study, ritanserin significantly inhibited haloperidol-induced c-fos mRNA expression, but NAN-190 did not. Similar inhibitory effects were also observed on administration of another 5-HT_2 receptor antagonist, ketanserin (data not shown). Our findings suggest that the blockade of 5-HT_2 receptors, but not of 5-HT_{1A} receptors, can reduce the striatal c-fos mRNA induction associated with D_2 receptor antagonism. Although previous studies had shown that muscarinic receptor antagonists (e.g., scopolamine) or *N*-methyl-D-aspartate receptor antagonists (e.g., dizocilpine) also reduced haloperidol-induced c-fos expression (Dragunow et al., 1990; Guo et al., 1992), SM-9018 lacks the binding affinity to these receptors (Kato et al., 1990). Thus, the 5-HT_2 receptor blocking activity of SM-9018 seems to contribute to its reduced action on striatal c-fos mRNA expression.

This is the first report of the reversal by the 5-HT_2 receptor antagonists of the dopamine D_2 receptor antagonist-induced c-fos mRNA elevation in the striatum. Since several atypical antipsychotics with mixed 5-HT_2 and dopamine D_2 receptor blocking activities (e.g., clozapine and sertindole) are also known to have a reduced effect on striatal c-fos expression (Fink-Jensen and Kristensen, 1994; Nguyen et al., 1992; Robertson and Fibiger, 1992), their 5-HT_2 receptor blocking activity may have a role in reducing the c-fos mRNA elevation associated with dopamine D_2 receptor antagonism. Our findings are in line with results of previous studies which demonstrated that 5-HT_2 receptor blockade counteracts the dopamine D_2 receptor blocking actions of antipsychotics in the striatum and thereby reduce the EPS in animals and humans (Saller et al., 1990; for review see Meltzer and Nash, 1991; Ohno et al., 1994; Ishida et al., 1996). Although the mechanisms underlying the interaction between the 5-HT_2 and dopamine D_2 receptor antagonists are still uncertain, the 5-HT_2 receptor antagonists are known to increase firing of the nigral dopamine neurons, enhance dopamine release and/or turnover, and reduce the in vivo occupancy by ^{11}C-raclopride of dopamine D_2 receptors in the striatum (Meltzer and Nash, 1991; Dewey et al., 1995). Thus, the 5-HT_2 receptor antagonists might attenuate the haloperidol-induced c-fos mRNA elevation by modifying the activity of nigrostriatal dopamine neurons. Further studies are required to define the mechanisms of the interaction between 5-HT_2 and D_2 receptor antagonists in striatal c-fos expression.

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