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Effects of antipsychotic drugs on neurotoxicity, expression of fos-like protein and c-*fos* mRNA in the retrosplenial cortex after administration of dizocilpine

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Abstract

In this study, we examined the effect of clozapine, olanzapine, risperidone and haloperidol on the neuropathology (i.e. neuronal vacuolization) and the expression of Fos-like protein and c-*fos* mRNA in the retrosplenial cortex of female Sprague–Dawley rats induced by the NMDA receptor antagonist dizocilpine. Pretreatment (15 min) with clozapine or olanzapine, but not risperidone or haloperidol, blocked the neuronal vacuolization produced by dizocilpine (0.5 mg/kg, s.c.) in the rat retrosplenial cortex in a dose-dependent manner. Furthermore, pretreatment (15 min) with clozapine or olanzapine, but not risperidone or haloperidol, significantly attenuated the expression of Fos-like protein in the retrosplenial cortex induced by dizocilpine (0.5 mg/kg, s.c.) in a dose-dependent manner. The marked expression of c-*fos* mRNA in the rat retrosplenial cortex induced by the administration of dizocilpine (0.5 mg/kg, s.c.) was significantly attenuated by pretreatment (15 min) with clozapine (10 mg/kg) or olanzapine (10 mg/kg), but not risperidone (10 mg/kg) or haloperidol (10 mg/kg). The present results suggest that pharmacologically relevant doses of clozapine or olanzapine, but not risperidone or haloperidol, block the neuropathological changes and the expression of Fos-like protein and c-*fos* mRNA in the rat retrosplenial cortex elicited by the administration of dizocilpine. It is possible that the blockade of dizocilpine-induced neuropathological changes by clozapine and olanzapine may be related to the unique antipsychotic actions of these drugs in schizophrenic patients, although this remains to be verified.

Keywords: Dizocilpine ((+)-MK-801); NMDA receptor antagonist; Neurotoxicity; Retrosplenial cortex; Clozapine; Olanzapine; Immediate early gene

1. Introduction

Several lines of evidence suggest that a dysfunction of glutamatergic neurotransmission may contribute to the pathophysiology of schizophrenia (reviews by Coyle, 1996; Javitt and Zukin, 1991; Olney and Farber, 1995; Tamminga et al., 1995). For example, phencyclidine (PCP) and its analog ketamine have psychotomimetic properties in healthy human subjects and have been shown to cause a prolonged worsening of symptoms in stabilized, chronic schizophrenic patients (Luby et al., 1959; Krystal et al., 1994). It has been shown that the psychotomimetic properties of PCP and ketamine are due to a blockade of the NMDA subtype of the glutamate receptor as these agents act as antagonists at a site within the ion channel of the NMDA receptor (Javitt and Zukin, 1991). Very recently, it has been demonstrated that mice expressing only 5% of the normal levels of the essential NMDA receptor NR1 subunit survive to adulthood and display behavioral abnormalities, including increased motor activity and stereotypy and deficits in social and sexual interactions, which suggests that reduced NMDA receptor activity results in schizophrenia-like behavior (Mohn et al., 1999). Furthermore, it has been demonstrated that PCP and other NMDA receptor antagonists such as dizocilpine ((+)-MK-801) induce neuropathological changes in the retrosplenial cortex of the rat brain (Olney et al., 1989). The retrosplenial cortex is a component of the limbic system and is bidirectionally interconnected with anterior fields of the cingulate gyrus and the anterior nuclear complex of the thalamus, a relay station of the Papez circuit (e.g. entorhinal cortex, subiculum, mamillary body, anterior thalamic nucleus, cin-

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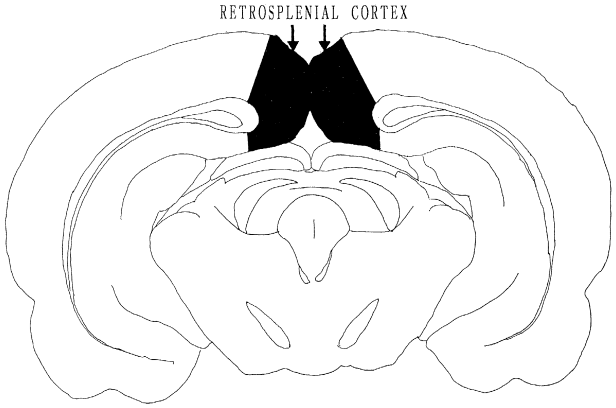

Fig. 1. Rat brain showing the location of the retrosplenial cortex. The arrows are the place of the retrosplenial cortex of rat brain. The number of vacuolized neurons and the amount of Fos-like protein in the layers III and IV of the retrosplenial cortex of each side of brain were determined. The map represents the brain level of bregma −5.80 mm from the atlas of rat brain (Paxinos and Watson, 1997).

gulate cortex) (MacLean, 1993). Furthermore, it has been suggested that the retrosplenial cortex plays a pivotal role in various learning and memory processes such as spatial memory and discriminative avoidance learning (Sutherland and Hoesing, 1993; Maddock, 1999). Recently, it has been reported that spatial learning impairment and neuronal injury in the mouse retrosplenial cortex induced by dizocilpine occur in parallel, suggesting that injury of retrosplenial cortical neurons may play a role in the spatial learning impairments induced by NMDA receptor antagonists in mice (Brosnan-Watters et al., 1999). Therefore, it is possible that the cognitive dysfunction produced by NMDA receptor antagonists is associated with the neuropathological changes in the retrosplenial cortex produced by NMDA receptor antagonists. Furthermore, it has been demonstrated that the atypical antipsychotic drugs clozapine and olanzapine, which are more effective than typical antipsychotic drugs in treating the positive, negative and cognitive dysfunction of schizophrenic patients (reviews by Ashby and Wang, 1996; Borison, 1997; Sharma and Mockler, 1998), block the neuropathological changes in rat brain caused by administration of dizocilpine (Farber et al., 1996). However, the precise mechanisms underlying the neuropathological changes elicited by NMDA receptor antagonists are currently unclear (reviews by Ellison, 1995; Fix et al., 1994; Olney and Farber, 1995).

It has been demonstrated that NMDA receptor antagonists such as PCP and dizocilpine increase glucose utilization in various brain structures associated with the limbic system (Gao et al., 1998; Kurumaji et al., 1989; McCulloch and Iversen, 1991; Weissman et al., 1987). The increased glucose utilization induced by NMDA receptor antagonists closely mirrors the pattern of neurotoxicity induced by NMDA receptor antagonists, which suggests that prolonged hyperactivity in these regions may contribute to the neuropathological changes (review by Ellison, 1995).

The c-*fos* gene belongs to an inducible class of genes called immediate early genes, which encode transcription factors (reviews by Herrera and Robertson, 1996; Hughes and Dragunow, 1995). Such genes are rapidly and transiently induced in the central nervous system after a variety of extracellular stimuli. Furthermore, the Fos protein encoded by the c-*fos* gene acts on stimulus-transcription coupling to transduce extracellular signals into intracellular function changes by regulating late response or target genes (reviews by Herrera and Robertson, 1996; Hughes and Dragunow, 1995). This has led to the hypothesis that the expression of Fos-like protein is a marker of neuronal activation (Sagar et al., 1988; Herrera and Robertson, 1996; Hughes and Dragunow, 1995). Interestingly, it has been reported that NMDA receptor antagonists, such as dizocilpine, produce a marked expression of Fos-like protein in the corticolimbic regions, including the retrosplenial cortex in the rat brain (Dragunow and Faull, 1990; Gao et al., 1993, 1998; Gass et al., 1993; Hashimoto et al., 1997a; Hughes et al., 1993; Näkki et al., 1996). In addition, it has been shown that the continuous expression of Fos, beginning hours or days before the morphological demise of the cell, appears to be a hallmark of terminal differentiation and a harbinger of death (Smeyne et al., 1993). Taken together, it is likely that the marked expression of Fos-like protein in these regions produced by dizocilpine is associated with the neuropathological changes in these regions produced by the drug.

Currently, there have been no published studies regarding the effect of antipsychotic drugs on the neuropathology

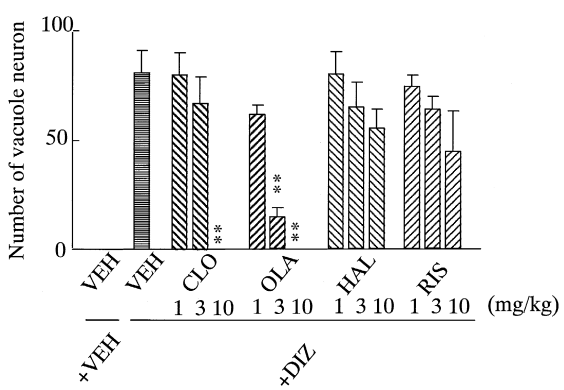

Fig. 2. Effect of antipsychotic drugs on the neuropathological changes in rat retrosplenial cortex produced by the administration of dizocilpine. Vehicle (VEH, 1 ml/kg), clozapine (CLO, 1, 3 or 10 mg/kg), olanzapine (OLA, 1, 3 or 10 mg/kg), haloperidol (HAL, 1, 3 or 10 mg/kg) or risperidone (RIS, 1, 3 or 10 mg/kg) was administered i.p. into rats. Fifteen min after the i.p. administration of vehicle or drug, vehicle (VEH, 1 ml/kg, s.c.) or dizocilpine (DIZ, 0.5 mg/kg, s.c.) was injected. The evaluation of the neuropathological changes was performed 4 h after the administration of dizocilpine, as described in Section 2.4. Each value is the mean ± S.E.M. from six rats. $^{**}P < 0.01$; Significantly less than the vehicle-dizocilpine treatment group by Dunnett's method.

and induction of Fos-like protein and c-*fos* mRNA in the same animal following the administration of dizocilpine. Therefore, in this study, we examined the effect of the antipsychotic drugs clozapine, olanzapine, risperidone and haloperidol on the neuropathological changes (i.e. neuronal vacuolization) and on the expression of Fos-like protein and c-*fos* mRNA in the rat retrosplenial cortex induced by the administration of dizocilpine.

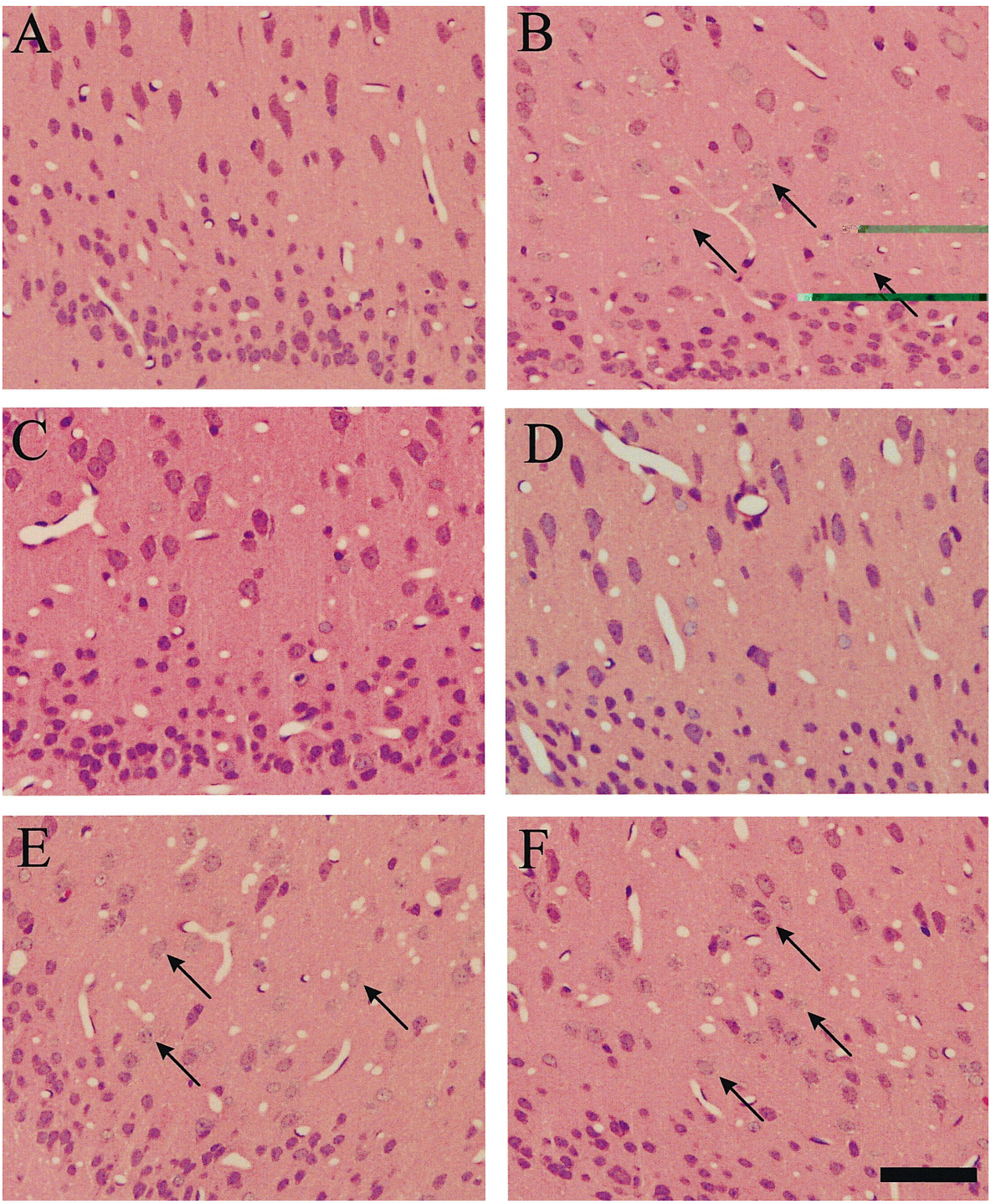

Fig. 3. Effect of antipsychotic drugs on the neuropathological changes in the rat retrosplenial cortex produced by the administration of dizocilpine. Fifteen min after the i.p. administration of vehicle or drugs, dizocilpine (0.5 mg/kg, s.c.) was administered. The evaluation of the neuropathological measurements was performed 4 h after the administration of dizocilpine, as described in Section 2.4. Scale bar: 400 μm. (A) Vehicle (1 ml/kg) + vehicle (1 ml/kg), (B) vehicle (1 ml/kg) + dizocilpine (0.5 mg/kg), (C) clozapine (10 mg/kg) + dizocilpine (0.5 mg/kg), (D) olanzapine (10 mg/kg) + dizocilpine (0.5 mg/kg), (E) haloperidol (10 mg/kg) + dizocilpine (0.5 mg/kg), (F) risperidone (10 mg/kg) + dizocilpine (0.5 mg/kg). The arrows mark the vacuolized neurons in the retrosplenial cortex of rat brain.

2. Methods

2.1. Animals

Female Sprague–Dawley rats were used in all experiments (10–12 weeks olds, 200–250 g, Japan Clea, Tokyo, Japan) and were housed under a 12-h light–12-h dark cycle with free access to food and water. In this study, female rats were used as they are more sensitive than male rats to the neurotoxic effects of NMDA receptor antagonists (Olney and Farber, 1995). All experiments were carried out in accordance with the Research Laboratories, Yoshitomi Pharmaceutical Industries, Guide far the Care and Use of Laboratory Animals.

2.2. Drugs

The following drugs were obtained from the following sources: dizocilpine maleate (Research Biochemicals International, Natick, MA, USA), sodium pentobarbital (Nembutal® Injection) (Dainippon Pharmaceutical, Osaka, Japan). Clozapine, olanzapine, haloperidol and risperidone were synthesized in the Research Laboratories, Yoshitomi Pharmaceutical Industries. All other chemicals were purchased commercially.

2.3. Drug treatment

Animals were injected intraperitoneally (i.p.) with vehicle (0.8% acetic acid, 1 ml/kg), clozapine, olanzapine, haloperidol or risperidone (1, 3 or 10 mg/kg). Fifteen minutes later, the animals were injected subcutaneously (s.c.) with vehicle (0.9% NaCl, 1 ml/kg) or dizocilpine (0.5 mg/kg, 1 ml/kg).

2.4. Assessment of neuropathology

Four hour after administration of dizocilpine, the animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Ten minutes later, the animals were transcardially perfused with 100 ml of isotonic saline, followed by 400 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Subsequently, the brain was removed, processed by graded ethanol dehydration, embedded in paraffin and sectioned into 4-μm-thick coronal slices and stained with hematoxylin and eosin. The number of vacuolized neurons in the layers III and IV of the retrosplenial cortex of each side of the brain was counted by an individual who was blind to the drug treatment administered to the animal. The number of vacuolized neurons per slide from two sections was averaged for each subject. Although the posterior-most portion of the cingulate cortex is given different designations in various anatomic references, it will be referred to as the retrosplenial cortex according to Paxinos and Watson (1997). In this study, the brain level of bregma −5.80 mm from the atlas of the rat brain (Paxinos and Watson, 1997) was used (Fig. 1).

2.5. Immunohistochemistry

Immunohistochemistry was performed using a Vectastain *Elite* ABC kit (Vector Laboratories, Burlingame, CA, USA), as previously described (Hashimoto et al., 1997a,b). Four hours after the administration of dizocilpine, the animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Ten minutes later, the animals were transcardially perfused with 100 ml of isotonic saline followed by 400 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was removed, post-fixed overnight at 4°C in the same fixative, and 50-μm-thick coronal sections of brains were cut in the ice-cold 0.01 M phosphate buffer saline (PBS; pH 7.4) using a Microslicer (DTK-3000, Dosaka EM, Kyoto, Japan). The slices were placed in ice-cold 0.01 M PBS and washed twice in ice-cold PBS. Free-floating sections were placed in 0.01 M PBS buffer containing 2% goat serum, 0.2% Triton X-100 and 0.1% bovine serum albumin (GS-PBST) for 1 h at room temperature. The samples were incubated for 48 h at 4°C with a primary antibody to c-*fos* (Oncogene Research Products, Cambridge, MA, USA) diluted 1/1000 in GS-PBST. The sections were washed twice in PBS, incubated for 1 h with a second antibody (biotinylated goat anti-rabbit immunoglobulin G; diluted 1/200) and incubated in an avidin-biotinylated horseradish peroxidase solution prepared from the kit for 1 h at room temperature. The sections were washed twice in ice-cold PBS and antibody reaction was developed with 3,3′-diaminobenzidine (0.015%) and 0.001% hydrogen peroxide

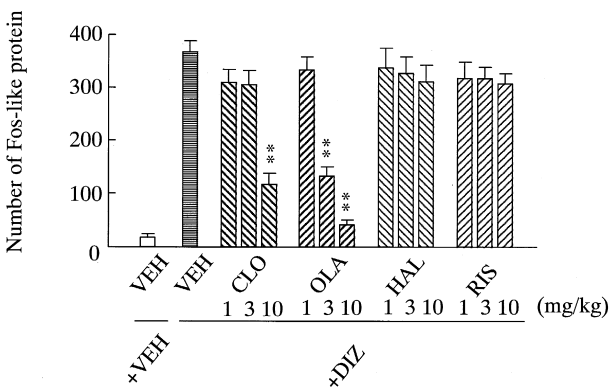

Fig. 4. Effect of antipsychotic drugs on the expression of Fos-like protein in the rat retrosplenial cortex following the administration of dizocilpine. Vehicle (VEH, 1 ml/kg), clozapine (CLO, 1, 3 or 10 mg/kg), olanzapine (OLA, 1, 3 or 10 mg/kg), haloperidol (HAL, 1, 3 or 10 mg/kg) or risperidone (RIS, 1, 3 or 10 mg/kg) was administered i.p. Fifteen min later, vehicle (VEH, 1 ml/kg, s.c.) or dizocilpine (DIZ, 0.5 mg/kg, s.c.) was injected. Immunohistochemistry was performed 4 h after the administration of dizocilpine as described in Section 2.5. Each value is the mean ± S.E.M. for six rats. $^{**}P < 0.01$; Significantly less than the vehicle-dizocilpine treatment group by Dunnett's method.

in 50 mM Tris–HCl (pH 7.4). Following several rinses in PBS, sections were mounted on gelatinized slides, dehydrated through an ethanol gradient and cleared in xylene and coverslipped with Permount® (Fisher scientific, Fair Lawn, NJ, USA). The number of Fos-like protein in the layers III and IV of the retrosplenial cortex on each side of the brain was counted by an individual who was blind to the drug treatment administered to the animal. The number of neurons containing Fos-like protein per slide from four sections was averaged for each subject. Alternate sections, incubated in the absence of primary antibody, served as an immunocytochemical control and showed no immunostaining.

2.6. In situ hybridization

One h after the administration of dizocilpine, animals were euthanized by exposure to CO_2 and decapitated. The

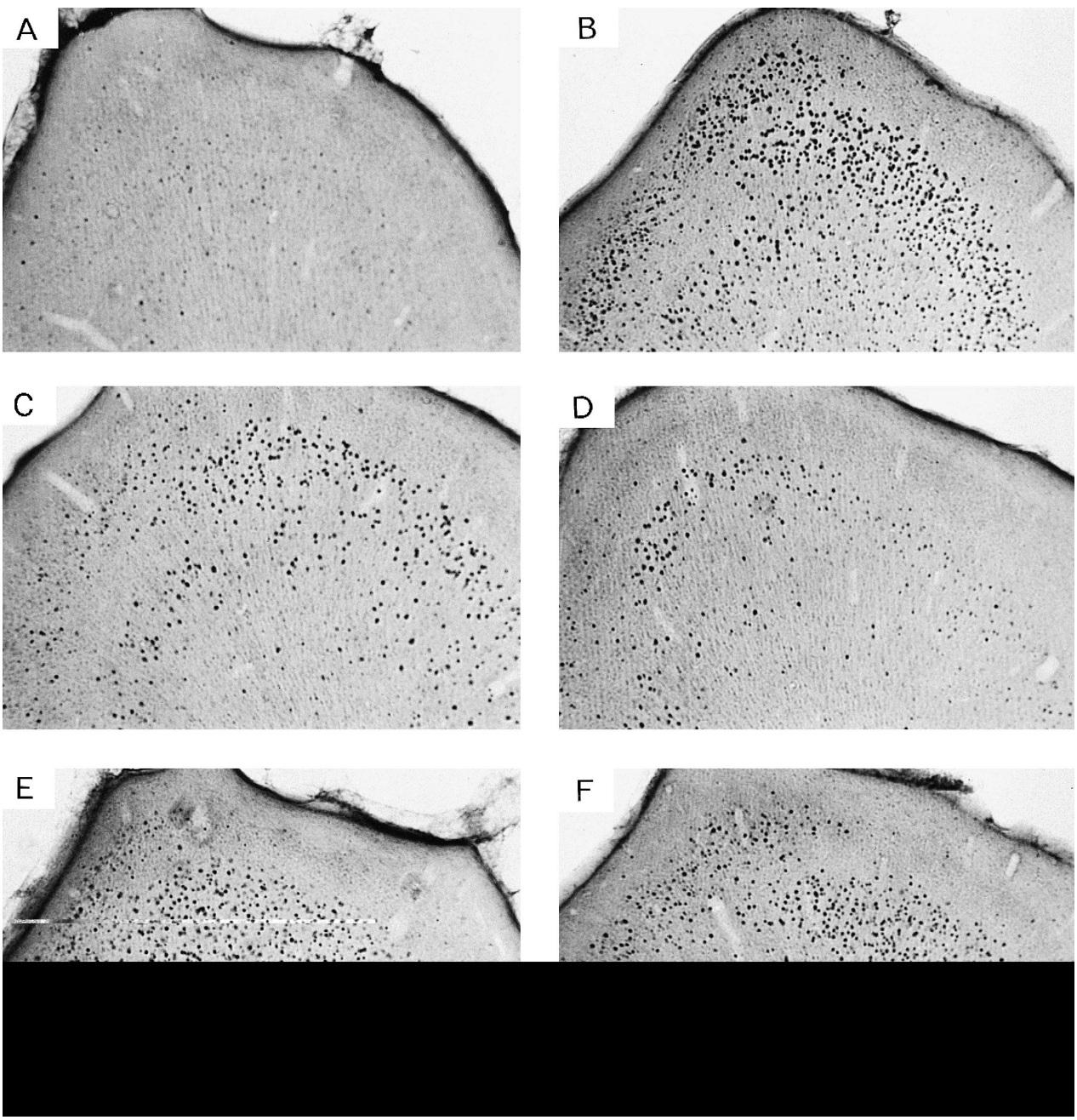

Fig. 5. Effect of antipsychotic drugs on the expression of Fos-like protein in the rat retrosplenial cortex following the administration of dizocilpine. Fifteen min after the i.p. administration of vehicle or drugs, dizocilpine (0.5 mg/kg, s.c.) was injected. Immunohistochemistry was performed 4 h after the administration of dizocilpine, as described in Section 2.5. Scale bar: 100 μm. (A) Vehicle (1 ml/kg) + vehicle (1 ml/kg), (B) vehicle (1 ml/kg) + dizocilpine (0.5 mg/kg), (C) clozapine (10 mg/kg) + dizocilpine (0.5 mg/kg), (D) olanzapine (10 mg/kg) + dizocilpine (0.5 mg/kg), (E) haloperidol (10 mg/kg) + dizocilpine (0.5 mg/kg), (F) risperidone (10 mg/kg) + dizocilpine (0.5 mg/kg).

brain was rapidly removed and frozen on dry ice. Coronal sections, 15-μm thick, were made using a cryostat (Bright Instrument, Hungtingdon, UK) kept at −15°C and mounted on silanized slides (DAKO, Kyoto, Japan). The sections were fixed for 30 min in 4% paraformaldehyde in 0.1 M phosphate buffer, acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0), dehydrated in ethanol, delipidated in chloroform and then rehydrated in a descending ethanol gradient series. Slides were air-dried. The oligonucleotide (45 mer) used for in situ hybridization of c-*fos* mRNA was 5′-GCAGCGGGAGGATGACGC-CTCGTAGTCCGCGTTGAAACCCGAGAA-3′ complementary to bp 141–185 of rat c-*fos* (Curran et al., 1987). Furthermore, control sections hybridized with a sense oligonucleotide probe showed no evidence of specific hybridization. The oligonucleotide probe was labeled at the 3′ end with [^{35}S]dATP (>30 TBq/mmol, Amersham, UK) using the oligonucleotide 3′-end labeling system (DuPont/New England Nuclear, MA, USA) and purified over a NENSORB™ 20 cartridge (DuPont/New England Nuclear). The probe then was diluted in hybridization buffer (Hybribuffer ISH, Biognostik, Göttingen, Germany) and 0.1 M dithiothreitol. One hundred μl of probe ($5–10 \times 10^6$ cpm/ml) in hybridization buffer was applied to each slide, which was then covered with a glass coverslip. Slides were hybridized for 12 h at 42°C. Following five washes in 1× saline–sodium citrate solution (SSC), the sections were dehydrated and apposed to an imaging plate (Fuji Film, Tokyo, Japan) for 2 days. The c-*fos* mRNA signal was quantified by measuring the photo-stimulated luminescence (PSL) radioactivity emitted from the imaging plate containing the retrosplenial cortical sections after scanning with a laser beam. The c-*fos* mRNA signal in four sections of the rat retrosplenial cortex was averaged for each subject. The resulting PSL was analyzed using a microcomputer interfaced to an image analyzing system (BAS-5000, Fuji Film, Tokyo, Japan).

2.7. Statistical analysis

Statistical analyses of the data were performed by Dunnett's method and *t*-test. The criteria for significance were $^{*}P < 0.05$, $^{**}P < 0.01$.

3. Results

3.1. Effect of clozapine, olanzapine, haloperidol and risperidone on the neuropathological changes in the rat brain following the administration of dizocilpine

Dizocilpine (0.5 mg/kg, s.c.) caused neuronal vacuolization in layers III–IV of the retrosplenial cortex of the rat brain 4 h after its administration (Figs. 2 and 3). In contrast, no vacuolized neurons were detected in other brain areas (e.g. subiculum, prefrontal cortex, nucleus accumbens, and dorsolateral striatum; data not shown). In addition, the administration of vehicle did not produce neuronal vacuolization. Pretreatment of animals with clozapine (1, 3 or 10 mg/kg, i.p.) or olanzapine (1, 3 or 10 mg/kg, i.p.) produced a significant dose-dependent decrease in the number of vacuolized neurons in the retrosplenial cortex produced by dizocilpine compared to the number in vehicle-treated animals (Figs. 2 and 3). In contrast, pretreatment of animals with haloperidol (1, 3 or 10 mg/kg, i.p.) or risperidone (1, 3 or 10 mg/kg, i.p.) did not significantly alter the number of vacuolized neurons detected in the retrosplenial cortex produced by dizocilpine compared to the number in vehicle-treated animals (Figs. 2 and 3).

3.2. Effect of clozapine, olanzapine, haloperidol and risperidone on the dizocilpine-induced expression of Fos-like protein in the rat retrosplenial cortex

The administration of dizocilpine (0.5 mg/kg, s.c.) produced a significant increase in the expression of Fos-like protein in the layers III–IV of the retrosplenial cortex of rat brain compared to that in vehicle-treated animals (Fig. 5). Pretreatment of animals with clozapine (1, 3 or 10 mg/kg, i.p.) or olanzapine (1, 3 or 10 mg/kg, i.p.) produced a significant dose-dependent decrease in Fos-like protein in the retrosplenial cortex induced by the administration dizocilpine compared to that in vehicle-treated animals (Fig. 4). In contrast, neither pretreatment with haloperidol (1, 3 or 10 mg/kg, i.p.) nor risperidone (1, 3, or 10 mg/kg, i.p.) produced a significant block of the dizocilpine-induced increase in Fos-like protein in the retrosplenial cortex (Figs. 4 and 5).

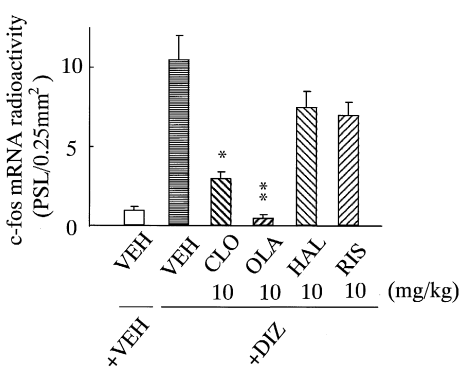

Fig. 6. Effect of antipsychotic drugs on the expression of c-*fos* mRNA in the rat brain induced by administration of dizocilpine. Fifteen minutes after the administration of vehicle (VEH, 1 ml/kg, s.c.) or dizocilpine (DIZ, 0.5 mg/kg, s.c.), clozapine (CLO, 10 mg/kg), olanzapine (OLA, 10 mg/kg), haloperidol (HAL, 10 mg/kg) or risperidone (RIS, 10 mg/kg) was injected i.p. Animals were killed 1 h after the administration of dizocilpine. The measurement of the expression of c-*fos* mRNA in the rat retrosplenial cortex was performed using in situ hybridization, as described in Section 2.6. Each value is the mean ± S.E.M. for six rats. $^{*}P < 0.05$, $^{**}P < 0.01$; Significantly less than the vehicle-dizocilpine treatment group by *t*-test.

3.3. Effect of clozapine, olanzapine, haloperidol and risperidone on the dizocilpine-induced expression of c-fos mRNA in rat retrosplenial cortex

Figs. 6 and 7 show the effect of clozapine, olanzapine, haloperidol and risperidone on the dizocilpine-induced expression of c-*fos* mRNA in the rat retrosplenial cortex. The administration of dizocilpine (0.5 mg/kg, s.c.) produced a significant increase in the expression of c-*fos* mRNA in the retrosplenial cortex compared to that in vehicle-treated animals. The pretreatment of animals with clozapine (10 mg/kg, i.p.) or olanzapine (10 mg/kg, i.p.) significantly decreased the dizocilpine-induced expression of c-*fos* mRNA in the retrosplenial cortex compared to that in vehicle-treated animals (Figs. 6 or 7). In contrast, pretreatment with haloperidol (10 mg/kg, i.p.) or risperidone (10 mg/kg, i.p.) did not significantly alter the dizocilpine-induced expression of c-*fos* mRNA in the retrosplenial cortex compared to that in vehicle-treated animals (Figs. 6 or 7).

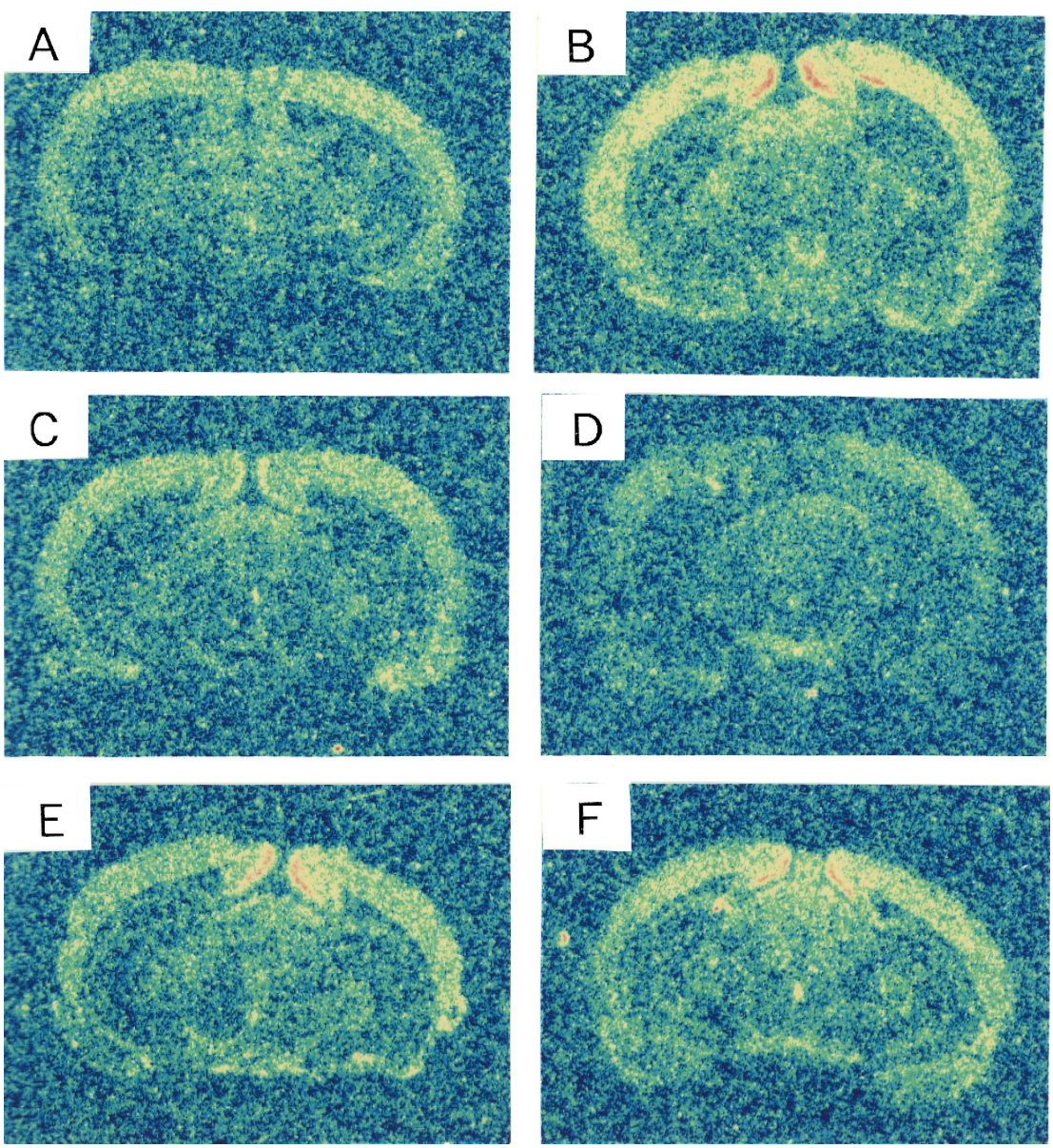

Fig. 7. Effect of antipsychotic drugs on the expression of c-*fos* mRNA in rat brain induced by administration of dizocilpine. Fifteen minutes after the i.p. administration of vehicle or drugs, vehicle (1 ml/kg) or dizocilpine (0.5 mg/kg, s.c.) was injected. Animals were killed 1 h after administration of dizocilpine. The measurement of the expression of c-*fos* mRNA in the rat retrosplenial cortex was performed using in situ hybridization, as described in Section 2.6. Red color shows high level of c-*fos* mRNA, and yellow color shows low level of c-*fos* mRNA. (A)Vehicle (1 ml/kg) + vehicle, (B)vehicle (1 ml/kg) + dizocilpine, (C) clozapine (10 mg/kg) + dizocilpine, (D) olanzapine (10 mg/kg) + dizocilpine, (E) haloperidol (10 mg/kg) + dizocilpine, (F) risperidone (10 mg/kg) + dizocilpine.

4. Discussion

The major finding of this study was that pretreatment of female Sprague–Dawley rats with clozapine and olanzapine, but not risperidone and haloperidol, significantly blocked the neuropathological changes, as well as the expression of Fos-like protein and c-*fos* mRNA, in the rat retrosplenial cortex elicited by the administration of dizocilpine. The order of potencies for olanzapine and clozapine to block the neuropathological changes produced by dizocilpine are consistent with those previously published (Farber et al., 1996). However, in our study, only a partial blockade of the dizocilpine-induced vacuolization was produced by pretreatment with risperidone or haloperidol. Indeed, the ED_{50} value for haloperidol to block the dizocilpine-induced neuronal vacuolization was more than 10 mg/kg. This value is significantly higher than that previously reported ($ED_{50} = 5.1$ mg/kg) (Farber et al., 1996). At present, the reasons underlying the discrepancy are unknown, particularly since the dose and route of administration of dizocilpine and the species and sex of the animals used in this study were identical to those used in previous studies (Farber et al., 1996). It should be pointed out that the doses of haloperidol and risperidone used in this and other studies were significantly higher than the doses required to block apomorphine and amphetamine-induced locomotor activity, produce catalepsy, inhibit conditioned avoidance responding and suppress spontaneous activity (Janssen et al., 1988; Megens et al., 1996; Van Cauteren et al., 1996). Consequently, it is likely that, at pharmacological doses, neither haloperidol nor risperidone could block the dizocilpine-induced neuropathological changes. Finally, the administration of haloperidol 1 or 2 h, as opposed to 15 min, before the administration of dizocilpine did not alter neuronal vacuolization (data not shown), suggesting that pretreatment time does not explain the lack of effect of haloperidol. Furthermore, these compounds did not significantly block neuropathological changes or the expression of Fos-like protein and c-*fos* mRNA in the rat retrosplenial cortex after the administration of PCP (unpublished data).

Recently, it has been reported that clozapine (5 or 10 mg/kg) completely blocks the changes in glucose utilization in all brain regions following administration of a subanesthetic dose (30 mg/kg) of ketamine, and that the administration of 0.5 mg/kg of haloperidol, a dose that produces a substantial cataleptic response, potentiates rather than blocks the increase of glucose utilization produced by ketamine (Duncan et al., 1998). The blockade by clozapine of ketamine-induced brain metabolic activation suggests that the antagonism of the consequences of reduced NMDA receptor function could contribute to the superior therapeutic effects of clozapine (Duncan et al., 1998). In addition, it has been recently demonstrated that clozapine and olanzapine, but not haloperidol, prevent PCP-induced blockade of NMDA responses in pyramidal neurons of rat medial prefrontal cortical slices (Arvanov and Wang, 1999; Wang and Liang, 1998), suggesting that clozapine and olanzapine may be useful for treating the PCP-induced psychotomimetic state that closely resembles schizophrenia. Furthermore, it has been suggested that clozapine and olanzapine might be effective in ameliorating the schizophrenic symptoms, including cognitive and neuropsychological deficits, seen in people who abuse PCP (Wang and Liang, 1998). Moreover, it has also been reported that clozapine, but not haloperidol, blunts ketamine-induced psychosis in humans (Malhotra et al., 1997). It seems that the inability of haloperidol to affect dizocilpine-induced neurotoxicity is consistent with the observations that typical antipsychotic drugs such as haloperidol are not very effective in treating PCP-induced psychosis (Allen and Young, 1978).

It has been proposed that the cognitive dysfunction in schizophrenia might result from underlying brain structural damage and that cognitive symptoms are associated with a poor outcome of schizophrenia (Sharma and Mockler, 1998). The ability of PCP and ketamine to induce reversible neuropathological abnormalities in human subjects suggests that hypofunction of NMDA receptors could be critically linked to cognitive and neuropsychological deficits in schizophrenia. Recently, it has been reported that spatial learning impairment and neuronal injury in the mouse retrosplenial cortex induced by dizocilpine occur in parallel, suggesting that injury of retrosplenial cortical neurons may play a role in the spatial learning impairments induced by NMDA receptor antagonists in mice (Brosnan-Watters et al., 1999). As pointed out earlier, the retrosplenial cortex is a part of the limbic system which plays a significant role in emotion and learning and memory, and it has been suggested that the retrosplenial cortex plays a pivotal role in various learning and memory process (Sutherland and Hoesing, 1993; Maddock, 1999). Taken together, it seems that the ability of NMDA receptor antagonists such as PCP and ketamine to induce neuropathological changes in the retrosplenial cortex might be associated with cognitive dysfunction induced by these agents. Thus, in combination with our findings, atypical antipsychotic drugs such as clozapine and olanzapine might be useful to treat cognitive dysfunction in schizophrenic patients.

It has been hypothesized that dizocilpine and other NMDA receptor antagonists produce neuronal injury by disinhibiting γ-aminobutyric acid (GABA) neurons that subserve two underlying neurotransmitter systems (Olney and Farber, 1995), and ultimately leading to the release of acetylcholine and glutamate, the latter interacting with non-NMDA receptors (Jevtovic-todorovic et al., 1999; Corso et al., 1997). This hypothesis is supported by recent data indicating that a muscarinic agonist, pilocarpine (10–100 mg/kg), could attenuate the neuroprotective effects of clozapine and olanzapine against dizocilpine-induced neurotoxicity (Jevtovic-todorovic et al., 1999). Olanzapine is

structurally related to clozapine, but not risperidone and haloperidol (Borison, 1997). It is known that clozapine and olanzapine interact with a wide range of many neuroreceptors, including dopamine (D_1, D_2, D_3, D_4) receptors, 5-HT (5-HT_2, 5-HT_3, 5-HT_6, 5-HT_7) receptors, muscarinic acetylcholine (M_1, M_2, M_3, M_4, M_5) receptors, adrenoceptor (α_1, α_2) receptors, and histamine H_1 receptors (Bolden et al., 1992; Leysen et al., 1996). However, it is unclear whether any of the above pharmacological characteristics, or some combination thereof, account for the unique clinical efficacy of these drugs. Further detailed studies will be necessary to clarify the mechanism underlying the blockade of dizocilpine-induced neuropathological changes.

In summary, the present study indicates that, at a pharmacological dose, clozapine and olanzapine, but not risperidone and haloperidol, could block the neuropathological changes and the expression of Fos-like protein and c-*fos* mRNA induced in the rat retrosplenial cortex by administration of dizocilpine. The blockade by clozapine and olanzapine of dizocilpine-induced neuropathological changes suggests that the antagonism of the consequences of reduced NMDA receptor function could contribute to the superior therapeutic effects of clozapine and olanzapine in schizophrenic patients.

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