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Effects of Perospirone, a Novel 5-HT$_2$ and D$_2$ Receptor Antagonist, on Fos Protein Expression in the Rat Forebrain

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ISHIBASHI, T., R. TAGASHIRA, M. NAKAMURA, H. NOGUCHI AND Y. OHNO. *Effects of perospirone, a novel 5-HT$_2$ and D$_2$ receptor antagonist, on Fos protein expression in the rat forebrain.* PHARMACOL BIOCHEM BEHAV **63**(4) 535–541, 1999.—The effects of perospirone, a novel 5-HT$_2$ and D$_2$ receptor antagonist, on Fos protein expression in the nucleus accumbens (NA) and dorsolateral striatum (DLSt) were compared with those of typical (i.e., haloperidol and fluphenazine) and atypical (i.e., clozapine and risperidone) antipsychotics using immunohistochemical techniques in rats. Perospirone and other antipsychotics tested at doses that exerted D$_2$ blocking actions increased Fos-like immunoreactivity both in the NA and DLSt. However, the levels of Fos expression in the DLSt induced by perospirone and clozapine were less than those induced by haloperidol and fluphenazine. When compared the differences in numbers of Fos-positive neurons between in the NA and DLSt, perospirone, clozapine, and risperidone preferentially increased Fos expression in the NA. These findings suggest that perospirone has a preferential action on the mesolimbic (vs. nigrostriatal) dopaminergic system in inducing Fos protein in the rat brain, which may be related to its atypical antipsychotic properties. © 1999 Elsevier Science Inc.

Perospirone Antipsychotic drugs Fos expression Nucleus accumbens Striatum Immunohistochemistry

ANTIPSYCHOTIC agents improve symptoms of schizophrenia, especially the positive symptoms (e.g., hallucination, delusion, and excitation), through blockade of the mesolimbic/cortical dopaminergic system. However, the use of typical or conventional antipsychotic agents (e.g., haloperidol and chlorpromazine) for treatment of schizophrenia is limited by intolerable adverse effects. Of primary concern is their propensity to cause extrapyramidal side effects (EPS) (e.g., parkinsonism, akathisia, and tardive dyskinesia), which are thought to be due to blockade of the nigrostriatal dopaminergic system. Recently, a series of drugs that possess combined 5-HT$_2$ and D$_2$ blocking actions (e.g., clozapine, risperidone, and olanzapine) have been developed as atypical antipsychotics, which show a lower propensity to induce EPS than the typical antipsychotics in humans (10). They also provide beneficial effects in schizophrenia treatment by improving the negative symptoms (e.g., flattening affect, apathy, and social withdrawal).

Neuronal expression of Fos, a product of the immediate early gene c-*fos*, is increased by various physiological and pharmacological stimuli (e.g., stress, pain, and psychostimulants) and is considered to be a marker for mapping neuronal activity (12). Several studies have shown that the typical antipsychotic agent haloperidol markedly increases Fos-like immunoreactivity (Fos-LI) and c-*fos* mRNA expression in the rat striatum (2,11). As Fos induction was also observed with other antipsychotics that commonly possess dopamine D$_2$ blocking activity, and was reversed by simultaneous treatment with D$_2$ agonist or dopaminergic denervation with 6-hydroxydopamine (11,19), the haloperidol-induced Fos expression is thought to be mediated by D$_2$ receptor blockade in the striatum. In addition, typical and atypical antipsychotics are

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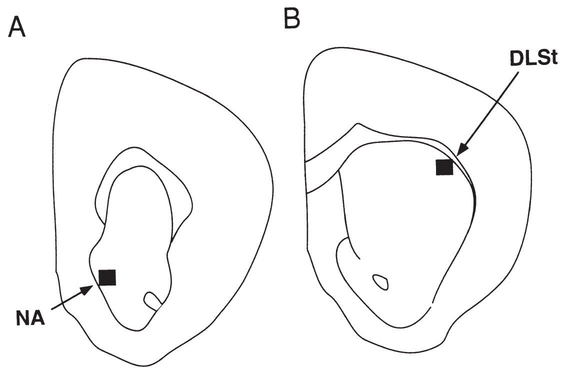

FIG. 1. Drawings showing the counting grids (filled boxes) of Fos-positive neurons in the shell regions of NA and the DLSt.

known to exhibit a distinctive pattern of Fos expression in the rat forebrain (9,14,19). The typical antipsychotics (e.g., haloperidol), which often induce severe EPS in humans, enhance Fos expression in both the nucleus accumbens (NA) and dorsolateral striatum (DLSt), whereas atypical antipsychotics (e.g., clozapine) with fewer EPS preferentially induced Fos expression in the NA. Because the DLSt has been implicated in the regulation of extrapyramidal motor function (18), the antipsychotic-induced Fos expression in the DLSt was considered to reflect their EPS potential (20). In this context, Robertson et al. (20) compared Fos protein expression induced by various types of antipsychotics and proposed the "atypical index," as revealed by the difference in numbers of Fos-positive neurons between the NA and DLSt, which would serve as a useful marker for discrimination of atypical from typical antipsychotics.

Perospirone is a newly developed antipsychotic agent that has potent blocking activities both for 5-HT_2 and D_2 receptors (3,6). Our previous studies demonstrated that perospirone was weaker than typical antipsychotics (e.g., haloperidol and chlorpromazine) in inducing EPS signs (i.e., catalepsy and bradykinesia) in rodents (3,16). Perospirone also showed lower propensity than haloperidol to cause dopaminergic behavioral supersensitivity and upregulation of striatal D_2 receptors after repeated treatment (15,17). These findings suggest that perospirone belongs to the class of atypical antipsychotics. In the present study, to determine whether perospirone exhibits atypical antipsychotic effects on Fos protein expression, we compared the effects of perospirone on Fos protein expression in NA and DLSt with those of typical (i.e., haloperidol and fluphenazine) and atypical (i.e., clozapine and risperidone) antipsychotics using immunohistochemical techniques in rats.

METHOD

Animals and Drug Treatments

Male Sprague–Dawley rats (Charles River Japan, Inc.) weighing 160–250 g were used. The animals were kept in air-conditioned rooms at 23 ± 2°C and 55 ± 10% relative humidity under a 12 L: 12 D cycle (dark period; 2000–0800). Standard rat chow and tap water were given ad lib.

The animals were orally administered perospirone (1, 3, and 10 mg/kg), clozapine (30, 100, and 300 mg/kg), risperidone (1, 3, and 10 mg/kg), haloperidol (0.3, 1, 3, and 10 mg/kg), fluphenazine (3 and 10 mg/kg), or vehicle (0.5% methylcellulose (MC) solution). The dosage of each drug was chosen on the basis of their D_2 antagonistic activity (i.e., inhibition of 2 mg/kg IP methamphetamine-induced hyperactivity in rats), which has been suggested to reflect their clinical efficacy in the treatment of schizophrenia (8). The ED_{50} values (mg/kg, PO) of the test drugs were as follows; 2.2 for perospirone, 130 for clozapine, 1.1 for risperidone, 0.56 for haloperidol, and 1.4 for fluphenazine [(3); and unpublished experimental data]. Two hours after drug administration, animals were deeply anesthetized with pentobarbital (100 mg/kg, IP) and perfused with saline solution (100 ml) followed by 10% formalin neutral buffer solution (200 ml). The brain was then removed from the skull and placed in fresh fixative for at least 24 h.

Fos Immunohistochemistry

After postfixation, sections 30 μm thick were cut from each brain using a microslicer. Sections were washed three times, incubated in phosphate-buffered saline (PBS) containing 0.3% Triton X-100 and 5% normal rabbit serum (NRS) for 2 h, and incubated in PBS containing 0.3% Triton X-100, 5% NRS and Fos primary antisera (diluted 1 : 2000; Cambridge Research Biochemicals, OA-11-824) for 36 h. The sections were washed three times with PBS and incubated with biotinylated rabbit antisheep IgG secondary antibody (Vector Laboratories; diluted 1:500) for 2 h. The sections were then incubated in PBS containing 0.3% hydrogen peroxide for 0.5 h to block endogenous peroxidase activity. After washes with PBS, the sections were incubated for 1 h with PBS containing avidin–biotinylated–horseradish peroxidase complex (Vectastain ABC kit). The sections were stained using 3, 3′-diaminobenzidine (1 mg/ml), H_2O_2 (0.03%) and NiCl (0.04%). Staining was terminated by washing with PBS, and the sections were mounted on gelatin-coated slides, dehydrated, and prepared for microscopic observation. To ensure the specificity of immunoreactivity, some sections were incubated with Fos-antisera that had been preabsorbed with Fos peptide (CRB OP-11-3210). We confirmed that preabsorption of the antibody with N-terminal antigenic sequence eliminated Fos-LI. In addition, omission of the primary antibody from the immunohistochemical procedure prevented staining.

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Perospirone hydrochloride, haloperidol, risperidone, and clozapine were synthesized in our laboratory. Fluphenazine was purchased from Sigma Chemical Co. (St. Louis, MO). All antipsychotics were suspended in 0.5% MC. Vectastain ABC and DAB substrate kits were purchased from Vector Laboratories (Burlingame, CA). Normal rabbit serum and 10% formalin neutral buffer solution were purchased from Wako Pure Chemicals (Osaka, Japan). All other drugs were purchased from commercial sources.

Data Analysis

Fos-LI was quantified by counting the number of Fos-positive nuclei per 500 × 500-μm^2 fields in the shell regions of NA and the DLSt (Fig. 1). Statistical significance of the changes in numbers of Fos-positive neurons was determined by one-way analysis of variance (ANOVA) followed by two-tailed Dunnett's post hoc test. The atypical index, based on difference in the number of Fos-positive neurons in the NA from that in the DLSt, was calculated by the method of Robertson et al. (20). In that study, the number of Fos-positive neurons in each region after a vehicle treatment was first subtracting from the number produced by the drug treatment. Then, the corrected value in the DLSt was subtracted from the corresponding value in the NA for each drug dose. This manipula-

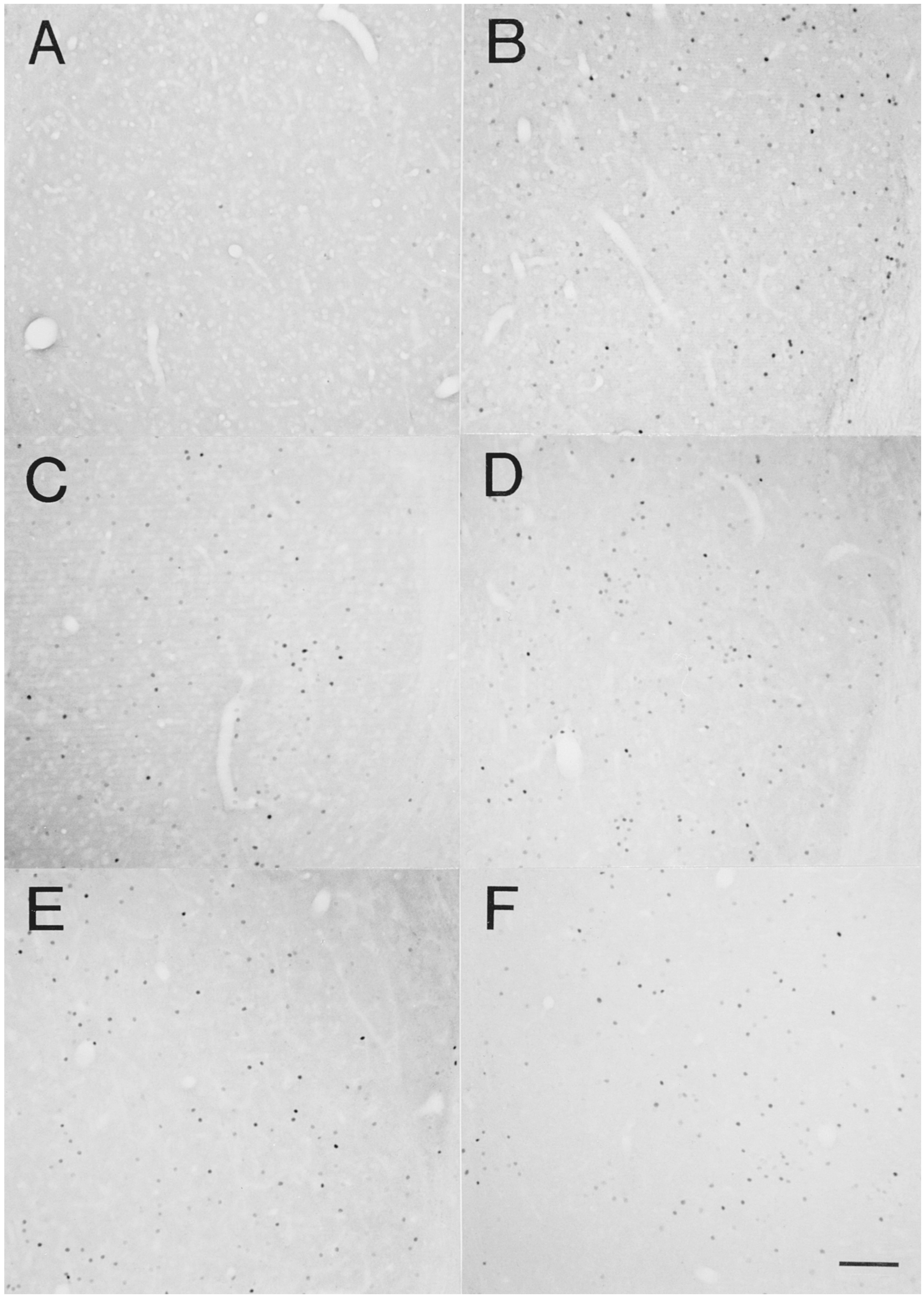

FIG. 2. Photomicrographs of antipsychotic-induced Fos-LI in the NA in rats. Fos-LI was induced by vehicle [0.5% MC (A)], haloperidol [3 mg/kg (B)], fluphenazine [3 mg/kg (C)], perospirone [10 mg/kg (D)], clozapine [100 mg/kg (E)], and risperidone [3 mg/kg (F)]. Scale bar = 100 μm.

tion gave the atypical index defined as the number of Fos-positive neurons in the NA minus the number in the DLSt.

RESULTS

Fos Expression in the NA

In the NA, vehicle-treated controls showed a few Fos-positive neurons (14.1 ± 2.1 cells/grid; Figs. 2 and 4A). Perospirone, at 1–10 mg/kg, markedly increased the Fos-LI in a dose-dependent manner (Fig. 4A), and the effects at 3 and 10 mg/kg were statistically significant compared to the control group. Similarly, the atypical antipsychotics clozapine and risperidone, and typical antipsychotics haloperidol and fluphenazine, significantly increased the number of Fos-positive neurons in a dose-dependent manner (Figs. 2 and 4A). The numbers of Fos-positive neurons induced by the highest doses of test drugs used were comparable (ca. 70–80 cells/grid) among perospirone (10 mg/kg), risperidone (10 mg/kg), halo-

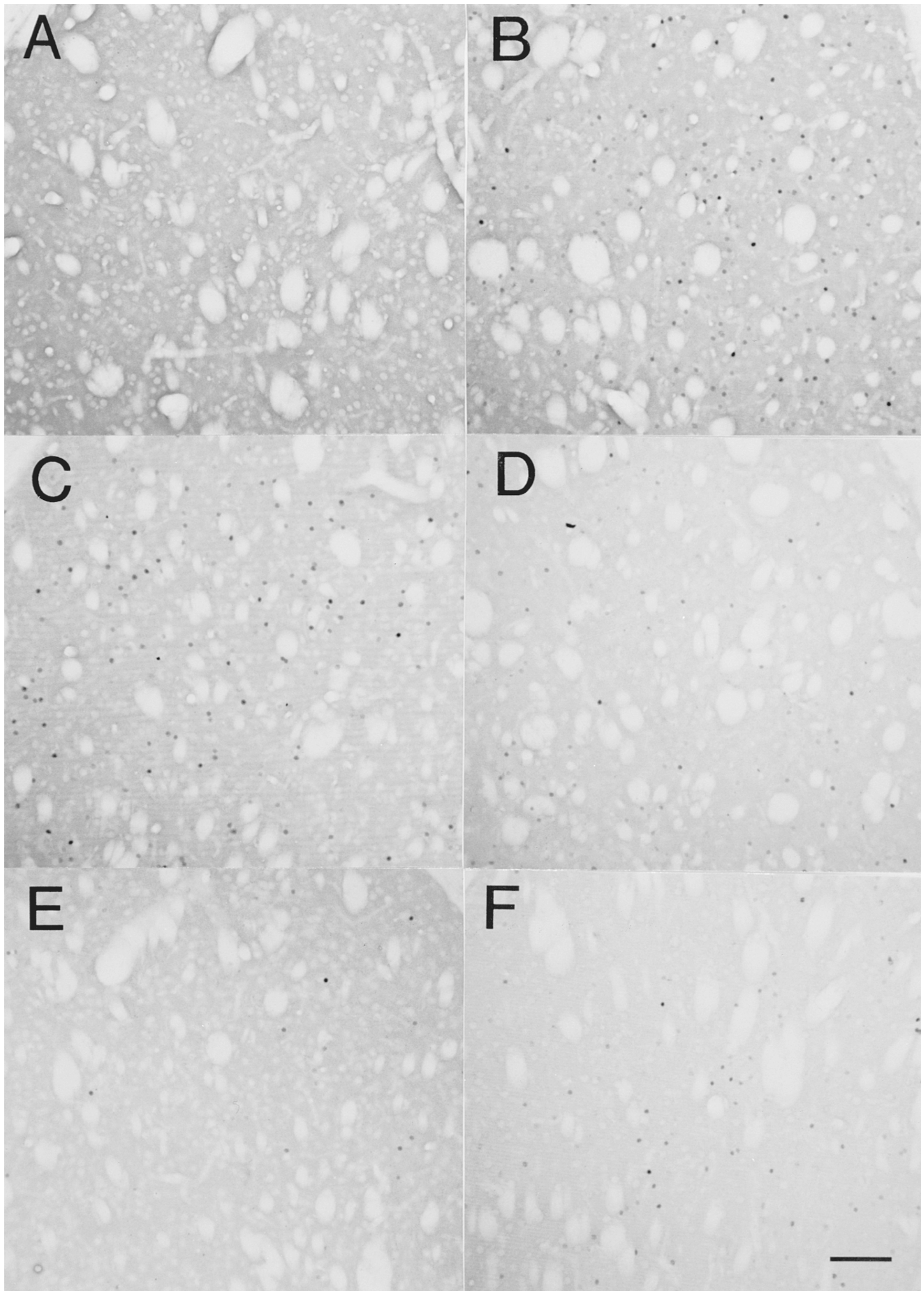

FIG. 3. Photomicrographs of antipsychotic-induced Fos-LI in the DLSt in rats. Fos-LI was induced by vehicle [0.5% MC (A)], haloperidol [3 mg/kg (B)], fluphenazine [3 mg/kg (C)], perospirone [10 mg/kg (D)], clozapine [100 mg/kg (E)], and risperidone [3 mg/kg (F)]. Scale bar = 100 μm.

peridol (10 mg/kg), and fluphenazine (10 mg/kg), whereas the magnitude of Fos expression evoked by clozapine (300 mg/kg) was slightly lower (ca. 50 cells/grid).

Fos Expression in the DLSt

The control group showed little Fos-LI (0.3 ± 0.2 cells/grid) in the DLSt (Figs. 3 and 4B). Perospirone increased the number of Fos-positive neurons in a dose-dependent manner (Figs. 3 and 4B). However, the actions of perospirone at 1–3 mg/kg were marginal, and only the highest dose (10 mg/kg) significantly increased Fos-LI (Fig. 4B). The atypical antipsychotic clozapine (30–300 mg/kg) also showed small increases in Fos-LI that were statistically significant at 100 and 300 mg/kg (Figs. 3 and 4B). In contrast, risperidone, haloperidol, and fluphenazine produced more prominent increases in Fos-LI

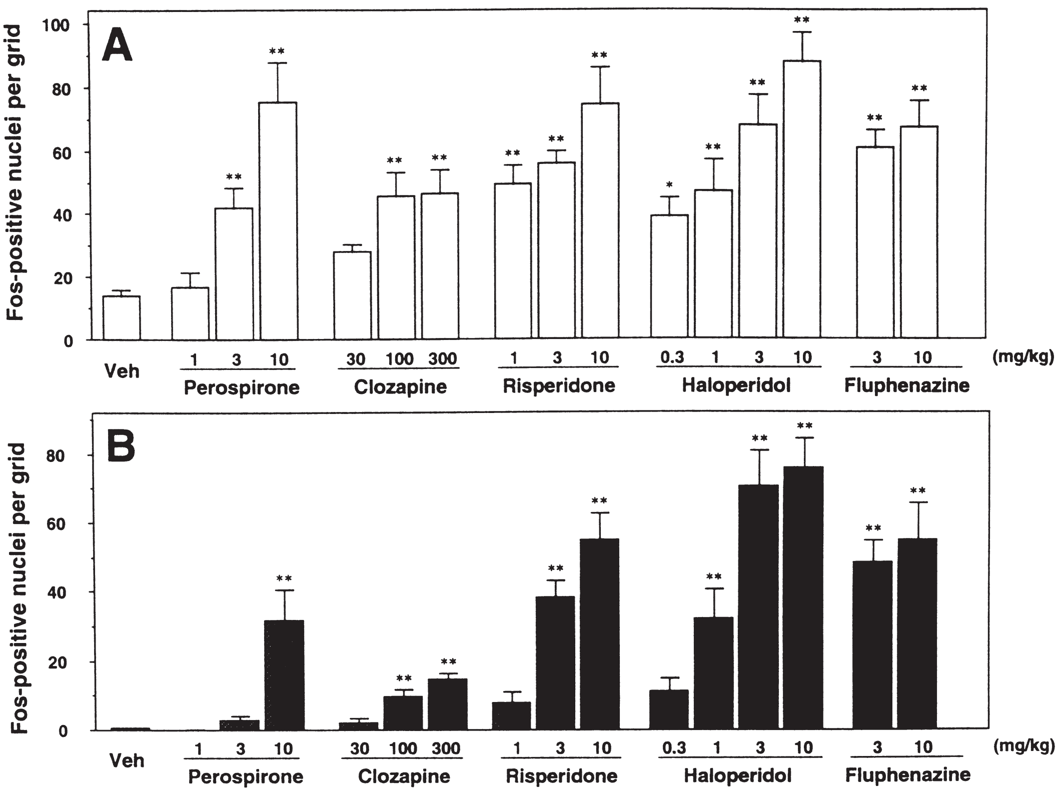

FIG. 4. Effects of perospirone, other atypical and typical antipsychotics on Fos-LI in the rat NA (A) and DLSt (B). Results are mean numbers ± SEM of the Fos-positive neurons per grid (500 × 500 μm^2) of 5–15 rats. *$p < 0.05$, **$p < 0.01$ vs. the vehicle administration (ANOVA, followed by Dunnett's test).

than perospirone or clozapine (Figs. 3 and 4B). The number of Fos-positive neurons increased to about 50 to 80 cells/grid following administration of risperidone (10 mg/kg), haloperidol (3 and 10 mg/kg), and fluphenazine (3 and 10 mg/kg), whereas those with perospirone (10 mg/kg) and clozapine (300 mg/kg) were about 30 and 20 cells/grid, respectively (Fig. 4B).

Differences in Fos Expression Between the NA and DLSt

We compared the atypical index calculated by subtracting the number of Fos-positive neurons of the DLSt from that of the NA in each animal (20). Perospirone showed a dose-dependent increase in the atypical index, which remained positive over the dose range tested (1–10 mg/kg) (Fig. 5). Clozapine (30–300 mg/kg) and risperidone (1–10 mg/kg) also produced positive atypical indexes (Fig. 5). The atypical index of perospirone was similar to that of clozapine, but that of risperidone reduced with increasing dose. In contrast to the atypical antipsychotics, haloperidol and fluphenazine showed a negative or near "zero" atypical index in Fos expression, except for the lowest dose of haloperidol (Fig. 5).

DISCUSSION

The present study confirmed previous findings (14,19,20) that typical and atypical antipsychotics exhibit different patterns of increases in Fos-LI in the NA and DLSt. The atypical antipsychotics (i.e., clozapine and risperidone) show a propensity to increase the Fos-LI in the NA with a reduced action in the DLSt. Furthermore, a newly developed antipsychotic agent, perospirone, mimicked the action of clozapine and enhanced Fos protein expression preferentially in the NA rather than in the DLSt.

The NA is considered to be a potential site for antipsychotic actions, and various antipsychotics commonly enhance the Fos expression in this structure (20). In this study, all antipsychotic agents tested, including perospirone, markedly increased Fos-LI in the NA at a dose range that effectively inhibited methamphetamine-induced hyperactivity in rats. The extent of the increases in Fos-LI were mostly consistent (ca. five- to sixfold over the control level) among the antipsychotics tested except for clozapine, which showed a weaker effect (ca. threefold). Because the inhibitory action of antipsychotics in the methamphetamine-induced hyperactivity (D_2 receptor antagonism) is considered to reflect their clinical activity in the treatment of schizophrenia (8), Fos expression in the NA appears to be a good marker for predicting the therapeutic action of antipsychotics.

In contrast, the antipsychotics showed a different pattern of Fos expression in the DLSt. Typical antipsychotics, haloperidol and fluphenazine, produced a large increase in Fos-LI in DLSt, the magnitude of which was comparable to or more prominent that those in the NA. However, the atypical antipsychotic clozapine-induced Fos expression to a much lesser extent than the typical antipsychotics. Perospirone did not significantly induce Fos expression at doses up to 3 mg/kg, but increased Fos-LI at 10 mg/kg. The increase in Fos-LI by 10

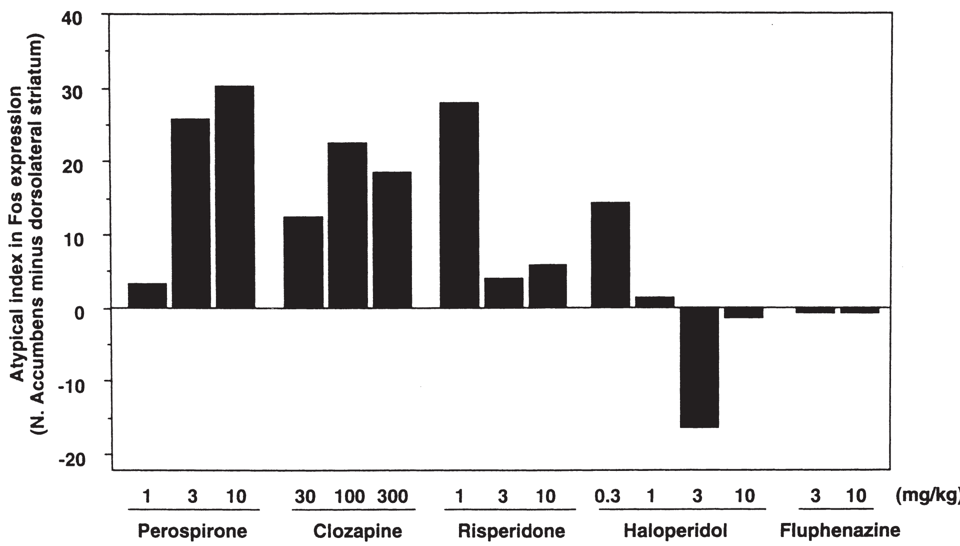

FIG. 5. Comparison of the "atypical index" of antipsychotics in Fos expression in the rat NA and DLSt. The atypical indexes of test drugs were obtained by subtracting the number of Fos-positive neurons in the DLSt from that of the NA in the same animals according to the method of Robertson et al. (20).

mg/kg perospirone was similar to that induced by a low dose (1 mg/kg) of haloperidol and about half that evoked by 3 mg/kg fluphenazine. Thus, perospirone seems to mimic the actions of clozapine in striatal Fos expression. As the DLSt has been implicated in the regulation of extrapyramidal motor function (18) and the striatal Fos expression is considered to reflect EPS liability of antipsychotics (20), our findings suggest that perospirone has an atypical antipsychotic property characterized by fewer EPS in humans. This is consistent with our previous findings that perospirone was weaker than the typical antipsychotics (e.g., haloperidol and chlorpromazine) in inducing catalepsy and bradykinesia-like behavior in rodents (3,16). Furthermore, recent double-blind, and comparative studies with schizophrenic patients have revealed that perospirone is superior to the typical antipsychotics haloperidol and mosapramine with regard to EPS, as well as efficacy for treatment of negative schizophrenia symptoms (7,13).

The atypical characteristics of risperidone were not clear by simple comparison of Fos-LI intensity in the DLSt. Although the action of risperidone in the DLSt was weaker than that of haloperidol, its action resembled that of the typical antipsychotic fluphenazine and was greater than those of perospirone and clozapine. To predict the EPS potential of these antipsychotic drugs more precisely, Robertson et al. (20) proposed comparison of the atypical index that is based on the differences between the number of neurons that displayed antipsychotic-induced Fos-LI in the NA and DLSt. If a compound preferentially enhanced Fos expression in the NA compared to the DLSt, the atypical index would be positive or higher. Such a compound would be classified as an atypical antipsychotic. Conversely, a compound exhibiting a large increase in Fos expression in the DLSt would have a smaller or negative atypical index and would be classified as typical antipsychotic. Using the atypical index in the Fos expression, Robertson et al. (20) demonstrated that many of the antipsychotics are correctly assigned to the category of typical or atypical antipsychotics. In addition, this classification scheme can identify a compound that selectively produces EPS at higher doses. The atypical index of such compounds would be reduced with increasing dosage. In the present study, perospirone, clozapine, and risperidone all had a higher atypical index than haloperidol and fluphenazine, suggesting lower propensity of these compounds to induce EPS. However, consistent with a previous study (20), the atypical index of risperidone reduced with increasing dosage. No such reduction of the atypical index was observed with perospirone and clozapine. Therefore, it is consistent with the clinical finding that risperidone shows a relatively narrow therapeutic window in the treatment of schizophrenia and induced EPS with increasing dose (1).

The mechanisms underlying the differential actions of atypical and typical antipsychotics on Fos expression are still uncertain. However, the atypical antipsychotics tested here (i.e., perospirone, clozapine, and risperidone) commonly possess a relatively potent 5-HT_2 blocking activity compared to the typical antipsychotics. For example, perospirone inhibits striatal ^{3}H-spiperone (D_2) binding with similar potency to haloperidol (K_i = 1.4 and 1.8 nM, respectively), whereas it inhibits the cortical ^{3}H-ketanserin (5-HT_2) binding with potency about 200-fold greater than that of haloperidol (K_i = 0.61 and 120 nM) (3). In addition, we have previously shown that the selective 5-HT_2 antagonists ritanserin and ketanserin attenuate the elevation of c-*fos* mRNA induced by haloperidol in the striatum (4). These findings suggest that the 5-HT_2 blocking activity of perospirone might contribute to the reduced Fos protein expression in the DLSt. This is consistent with other studies showing that the blockade of 5-HT_2 receptors may counteract the D_2 blocking action of antipsychotics in the nigrostriatal dopaminergic system (5,10,16,21). Further studies are required to clarify the mechanism of D_2 and 5-HT_2 receptor interaction in the Fos expression.

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