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Neuropharmacological profiles of a novel atypical antipsychotic, NRA0562, in rats

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Abstract

Neuropharmacological profiles of 5-{2-[4-(6-fluoro-1*H*-indole-3-yl) piperidine-1-yl] ethyl}-4-(4-fluorophenyl) thiazole-2-carboxylic acid amide (NRA0562) in rats were examined using electrophysiological and immunohistochemical methods. The firing rates of the substantia nigra pars compacta (A9) and the ventral tegmental area (A10) dopamine neurons were inhibited by methamphetamine (1 mg/kg, i.v.). NRA0562 dose-dependently reversed the inhibitory effects of methamphetamine on A9 and on A10 dopamine neurons. NRA0562 was more potent to reverse the inhibitory effects of methamphetamine on A10 (ED_{50} = 0.3 mg/kg) than on A9 (ED_{50} = 0.9 mg/kg) dopamine neurons. NRA0562 produced significant increases in Fos-like immunoreactivity in both the nucleus accumbens and the dorsolateral striatum. The difference between the number of Fos-like immunoreactivity produced by NRA0562 in the nucleus accumbens vs. dorsolateral striatum, referred to as the atypical index, was positive. Similar results could be observed with risperidone, an atypical antipsychotic. These results suggest that NRA0562 may have the atypical antipsychotic activities seen with risperidone, but without the liability of motor side effects typical of classical antipsychotics.

Keywords: NRA0562; Risperidone; Dopamine neuron; Electrophysiology; Fos-like immunoreactivity

1. Introduction

Schizophrenia is a devastating mental illness for which there is currently no ideal therapy. Classical antipsychotics, which are presumed to act by antagonizing dopamine D_2 receptors (Snyder, 1981), are useful for the treatment of the positive symptoms, but are ineffective in the case of negative symptoms and there are various motor side effects (Baldessarini and Tarsey, 1980).

Therefore, searches for antipsychotics with different mechanisms of action, effective for negative symptoms of schizophrenia and with reduced liability of extrapyramidal side effects have been continuous. The atypical antipsychotic, clozapine, is effective for both positive and negative symptoms of schizophrenia (Clahgorn et al., 1987), is more efficacious than classical antipsychotics in treatment-refractory patients (Kane et al., 1988; Miller et al., 1994) and has a low incidence of extrapyramidal side effects (Clahgorn et al., 1987). Risperidone is also an effective antipsychotic drug with fewer extrapyramidal side effects than classic antipsychotics (Roose et al., 1988; Castelao et al., 1989; Chouinard et al., 1993). However, the use of clozapine has been compromised by a relatively high (as much as 2%) incidence of the potentially fatal blood disorder, agranulocytosis (Wagstaff and Bryson, 1995), hence, stringent monitoring of plasma levels is required. Risperidone prolongs the QT interval on electrocardiographic recordings, a phenomenon which, when severe, may facilitate the occurrence of complex ventricular arrhythmias, such as torsade de pointes (Drici et al., 1998). Thus, an atypical antipsychotic with fewer side effects is needed.

Electrophysiological studies have shown that amphetamine or methamphetamine causes a log-lasting inhibition of the firing rate of the substantia nigra pars com-

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pacta (A9) and the ventral tegmental area (A10) dopamine neurons, an event reversible with antipsychotic drugs (Bunney and Aghajanian, 1976; Okuyama et al., 1997). Differential reversible profiles of the activity of A9 vs. A10 dopamine neurons may play an important role in the efficacy and side effects of antipsychotics (Bunney, 1977; Chiodo and Bunney, 1983; White and Wang, 1983).

The ingestion of antipsychotics results in immediate-early gene c-*fos* expression in various brain regions (Deutch et al., 1992; Guo et al., 1995; Robertson et al., 1994). All clinically effective antipsychotics increase c-*fos* or its protein product, Fos, in the shell of the nucleus accumbens. Haloperidol induces extrapyramidal side effects, and there is an increased c-*fos* expression in the dorsolateral striatum (Deutch et al., 1992; Robertson et al., 1994). Because the dorsolateral striatum plays an important role in the regulation of movement (Carelli and West, 1991; Pisa, 1988), the extrapyramidal side effects potential of antipsychotics can be predicted on the basis of their potential to elevate these markers in the dorsolateral striatum (Merchant and Dorsa, 1993; Robertson et al., 1994). The difference between the number of Fos-like immunoreactivity in the nucleus accumbens vs. that in the dorsolateral striatum can be used to classify each compound (Robertson et al., 1994). This difference, referred to as the atypical index, was always positive for atypical antipsychotics and invariably negative for typical antipsychotics (Robertson et al., 1994).

5-{2-[4-(6-fluoro-1*H*-indole-3-yl) piperidine-1-yl] ethyl}-4-(4-fluorophenyl) thiazole-2-carboxylic acid amide (NRA0562, Fig. 1) shows high affinities for human cloned dopamine D_1, D_2, D_3 and D_4 receptors with K_i values of 7.09, 2.49, 3.48 and 1.79 nM. NRA0562 also has potent high affinities for the 5-HT_{2A} receptor and the α_1-adrenoceptor when assessed on the basis of in vivo and ex vivo receptor binding in rats, while the occupancy of striatum dopamine D_2 receptor was moderate as with other atypical antipsychotics such as clozapine and risperidone. In behavioral studies, NRA0562 dose-dependently inhibited methamphetamine-induced locomotion hyperactivity in rats. At a higher dosage, NRA0562 significantly antagonized methamphetamine-induced stereotyped behavior and induced catalepsy, dose-dependently and significantly. The ED_{50} value for inhibiting methamphetamine-induced locomotion hyperactivity was 10 times lower than that for inhibiting methamphetamine-induced stereotyped behavior and 30 times lower than that for inducing catalepsy. These results suggested that NRA0562 exhibits atypical antipsychotic activities, without the liability of the extrapyramidal side effects.

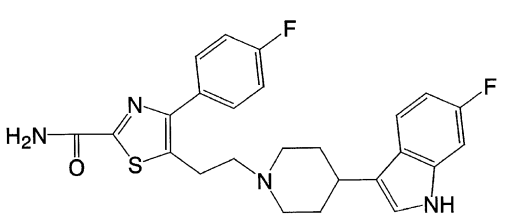


Fig. 1. Chemical structure of 5-{2-[4-(6-fluoro-1*H*-indole-3-yl) piperidine-1-yl] ethyl}-4-(4-fluorophenyl) thiazole-2-carboxylic acid amide (NRA0562).

In the present study, we examined in rats the neuropharmacological profiles of NRA0562, using both electrophysiological and immunohistochemical approaches.

2. Materials and methods

2.1. Animal

Male Wistar rats (Charls River, Japan) (weighing 300–400 g for electrophysiological studies, and 280–320 g for immunohistochemical studies) were housed three per cage, and were maintained under a 12-h light/dark cycle (lights on 07:00–19:00) in a temperature- and humidity-controlled holding room. Food and water were available ad libitum. All studies reported here have been reviewed by the Taisho Pharmaceutical, Animal Care Committee and have met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal experiments (1987).

2.2. Electrophysiological study

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and given supplements of this anesthetic, as required. The body temperature was maintained at 37 °C with a heating pad controlled by feedback from a rectal thermistor (KN-474, Natsume, Japan). An opening was made in the skin and skull (3×3 mm), and the dura mater was carefully removed. The area surrounding the wound was sprayed with xylocaine (Fujisawa, Japan). The electrode was placed stereotaxically in the A9 (AP: -5.2–-6.0 mm, L: 1.2–1.8 mm, H: 7.8–8.8 mm) and A10 (AP: -4.8–-5.2 mm, L: 0.5–0.8 mm, H: 7.5–8.5 mm), according to the atlas of Paxinos and Watson (1986). Only one neuron in each animal was used for recording. Dopamine neurons were identified as previously reported (Bunney and Aghajanian, 1973). A single-barrel glass electrode was filled with a solution of 1% (w/v) pontamine sky blue (Tokyo Kasei, Japan) in 0.5 M sodium acetate and resistance ranged between 2 and 10 MΩ measured at 135 Hz. At the end of each experiment, the recording sites were marked with a dye. The animal was perfused with 10% formalin and frozen 50 μm thick sections of the whole brain were cut on a cryostat microtome (MA-101, Komatsu, Japan), and stained with haematoxylin and eosin. Single-unit activities were monitored on an oscilloscope (VC-10, Nihon Kohden, Japan) and converted to a uniform voltage pulse by a window discrimina-

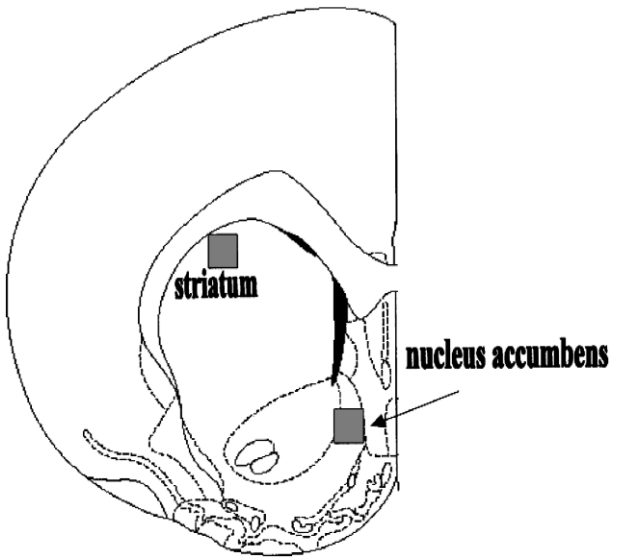


Fig. 2. Fos-like positive nuclei areas in rat brain. Camera lucida drawings of coronal rat brain sections depict areas used for counting Fos-like positive nuclei (gray shaded boxes). The areas shown are located in the nucleus accumbens and the dorsolateral striatum.

tor (DES-325P, Dia Medical, Japan). The pulses were integrated as 10-s periods and displayed on an ink-writing oscilloscope (WT-645G, Nihon Kohden).

Methamphetamine (1 mg/kg, i.v.) and incremental doses of NRA0562 (the starting dose was 0.1 mg/kg, with sequential doses of 0.2, 0.7 mg/kg) or risperidone (the starting dose was 0.01 mg/kg, with sequential doses of 0.02, 0.07 mg/kg) were administered every 2–3 min (compound-induced changes usually reached a plateau in 2–3 min) via an i.v. catheter implanted into the femoral vein. Compound-induced changes (after reaching a plateau) in neuronal activities were plotted as percentage changes from the pre-injection baseline rate, which was recorded over a 5-min period and defined as 100%. The % control was calculated and ED_{50} values were determined. The ED_{50} values were analyzed by fitting it to the four parametric logistic function, using non-linear least square regression.

2.3. Immunohistochemical study

Six groups with eight rats each were used for drug treatment, and one group of 10 rats was given only saline. All drugs were administered intraperitoneally. After 3 h, the animals were anesthetized with pentbarbital-Na (50 mg/kg, i.p.) and decapitated. The time point was selected on the basis of the finding that Fos protein was maximally induced between 2 and 4 h after drug administration (Deutch and Duman, 1996). The brain of each rat was excised and frozen immediately. The frozen sections were cut 10 μm thick from Optimal Cutting Temperature compound (Miles, USA)-embedded blocks of the brain. After fixation with 4% paraformaldehyde in 0.1 M phosphate buffer for 10 min on ice, an immunohistochemical study of Fos-like protein was done and counting of labeled cells was performed immunohistochemically, according to Fink-Jensen and Kristensen (1994). The effects of each drug were compared with the response to saline. The atypical index, which is the difference between the number of Fos-like positive nuclei in the nucleus accumbens vs. the dorsolateral striatum, was also calculated to predict atypical profiles (Robertson et al., 1994). We used the rabbit polyclonal antibody against Fos (Santa Cruz Biotechnology, (c-*fos* 4: catalog no. sc-52), diluted 1:250). Fos-like positive nuclei were counted manually by the same person, one section per area per rat, and the positive nuclei were marked on each section. The nucleus accum-

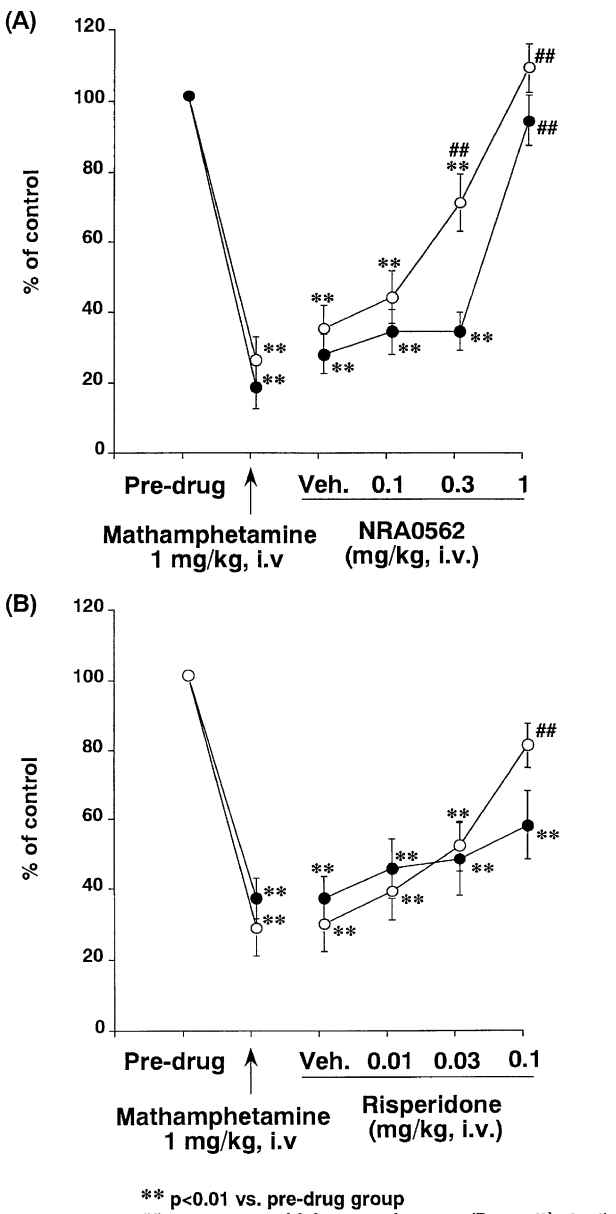


Fig. 3. Reversal by NRA0562 of the inhibitory effects of methamphetamine (1 mg/kg, i.v.) on A10 (A) and A9 (B) dopamine neurons.

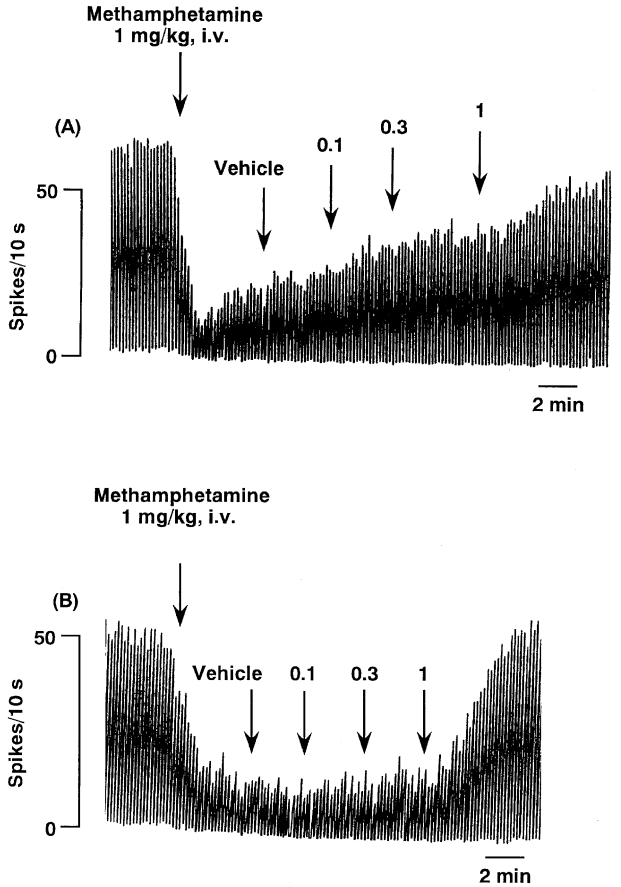


Fig. 4. Comparison of the potential of NRA0562 (A) and risperidone (B) to reverse the inhibitory effects of methamphetamine (1 mg/kg, i.v.) on A10 (○) and A9 (●) dopamine neurons. The results are presented as means with vertical lines showing S.E. ($n = 6$). Starting doses of NRA0562 and risperidone were 0.1 and 0.01 mg/kg, i.v., respectively. $^{**}P < 0.01$ vs. pre-drug value (Dunnett's test). $^{\#\#}P < 0.01$ vs. methamphetamine + vehicle-treated group (Dunnett's test).

bens (shell area) and the dorsolateral striatum were identified with the use of a 10× objective on a microscope equipped with a TV camera. The nucleus accumbens and the dorsolateral striatum counting areas were 525 × 525 μm. The counting areas are depicted as gray shaded boxes in Fig. 2.

2.4. Statistics

Data from electrophysiological studies were analyzed by one-way analysis of variance (ANOVA) and significance of differences between groups was determined by Dunnett's test. Significance of differences between groups from immunohistochemical studies was determined with a non-parametric Newman–Keuls test.

2.5. Drug

Methamphetamine HCl (Dainippon Pharmaceuticals, Japan) was dissolved in 0.9% saline and NRA0562 and risperidone were synthesized in the laboratories of Taisho Pharamceutical (Ohmiya, Japan). NRA0562 was dissolved in 20% HCO50 (Japan Chemicals, Japan). Risperidone was dissolved in a minimal amount of 0.5 N HCl and saline, and then titrated with 0.5 N NaOH to a final pH of 5.

3. Results

3.1. Effects on methamphetamine-induced inhibition of A9 and A10 dopamine neurons

A9 and A10 dopamine neurons displayed action potentials of wide duration (2.5–4.5 ms) at a rate of 1–7 spikes/s. These neurons also exhibited a triphasic form containing a notch in the initial rising phase of the first positive peak and typically fired in bursts of 3–8 spikes of steadily decreasing amplitude. These features seem to be characteristic of dopamine neurons (Bunney and Aghajanianm, 1973).

Both A9 and A10 dopamine neurons were significantly ($P < 0.01$) inhibited by methamphetamine (1 mg/kg, i.v., Figs. 3 and 4). NRA0562 dose-dependently and significantly reversed the effects of methamphetamine on both A9 and A10 dopamine neurons (Figs. 3 and 4). NRA0562 was more potent to reverse the effects of methamphetamine on A10 ($ED_{50} = 0.3$ mg/kg) than on A9 ($ED_{50} = 0.9$ mg/kg) (Figs. 3 and 4). Similar effects on dopamine neurons were observed with risperidone ($ED_{50} = 0.5$

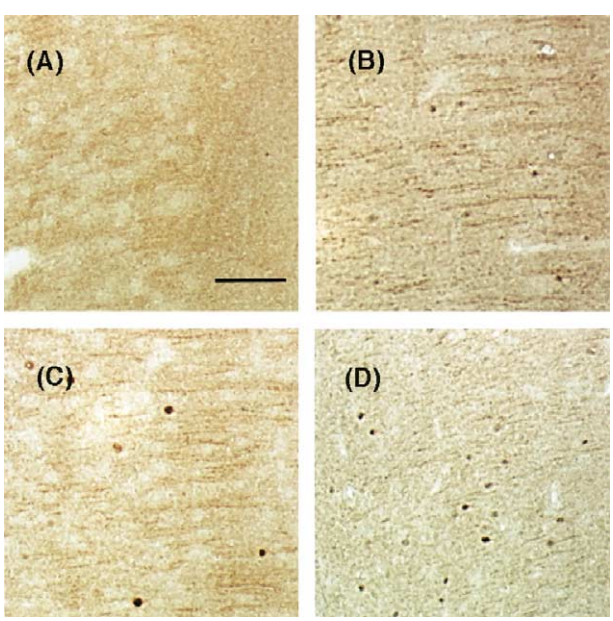


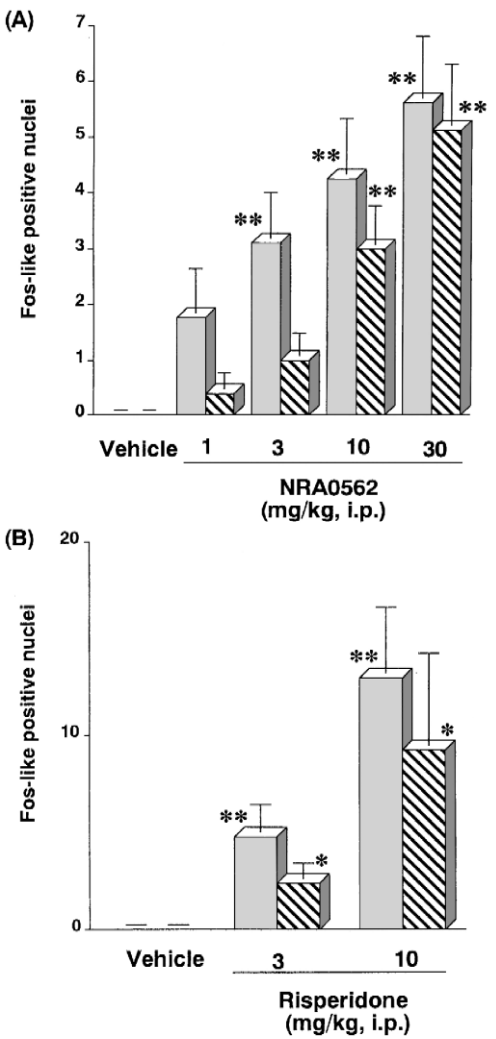
Fig. 5. Photomicrographs of Fos-like immunoreactivity in the nucleus accumbens after injection of saline (A); NRA0562 3 mg/kg (B); 10 mg/kg (C); 30 mg/kg (D). Bars indicate 100 μm.

mg/kg on A9 and 0.06 mg/kg on A10, respectively) (Fig. 4).

NRA0562 (0.1–1 mg/kg, i.v.) and risperidone (0.01–0.1 mg/kg, i.v.) alone had no apparent effects on the baseline firing rate of either A9 or A10 dopamine neurons (data not shown).

3.2. Effects on Fos-like immunoreactivity

NRA0562 (1, 3, 10 and 30 mg/kg) dose-dependently and significantly increased Fos-like immunoreactivity in both the nucleus accumbens and the dorsolateral striatum (Figs. 5 and 6). Risperidone (3 and 10 mg/kg) also significantly increased Fos-like immunoreactivity in both the nucleus accumbens and the dorsolateral striatum signif-



*p<0.05 and **p<0.01 vs. vehicle-treated group (Newman-Keuls test)

Fig. 6. Effects of NRA0562 (A) and risperidone (B) in the nucleus accumbens (dotted columns) and the dorsolateral striatum (hatched columns). Results are presented as mean numbers of Fos-positive nuclei with vertical line showing S.E. ($n = 8$). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle-treated group ($n = 10$) (Newman–Keuls test).

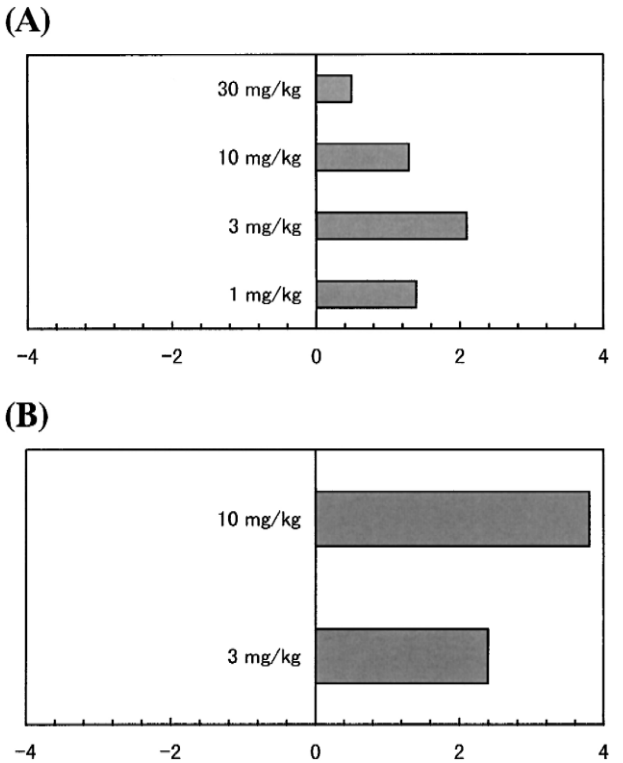


Fig. 7. The atypical index of NRA0562 (A) and risperidone (B). This value is the difference between the number of Fos-like positive nuclei in the nucleus accumbens vs. the dorsolateral striatum. The atypical index was calculated for each dose (mg/kg, i.p.). Both compounds are considered to have atypical antipsychotic properties.

icantly (Fig. 6). The atypical indices of both NRA0562 and risperidone were positive (Fig. 7).

4. Discussion

In the present study, we examined the effects of NRA0562 on methamphetamine-induced inhibition of both A9 and A10 dopamine neurons and on Fos-like protein expression in rats.

In electrophysiological studies, NRA0562 dose-dependently and significantly reversed the effects of methamphetamine on both A9 and A10 dopamine neurons. The ED_{50} value for reversing methamphetamine-induced inhibition of A10 dopamine neurons was three times lower than that for A9 dopamine neurons. The effect of reversing amphetamine-induced inhibition of A10 has been hypothesized to be predictive of clinical antipsychotic efficacy (Bunney, 1977). Clinically active antipsychotics, such as haloperidol, chlorpromazine, clozapine and seroquel, but not clinically inactive antipsychotics, such as metpazine, promethazine, diethazine and tricyclic antidepressants, can reverse the inhibitory effects of amphetamine on A10 dopamine neurons (Bunney and Aghajanian, 1973; Bunney, 1977; Goldstein et al., 1993). It was reported that atypical antipsychotics, such as clozapine and olanzapine,

were more potent to reverse the inhibitory effects of amphetamine on A10 than on A9 dopamine neurons (Stockton and Rasmussen, 1996; Okuyama et al., 1997). In contrast, a typical antipsychotic, such as haloperidol, with a high incidence of extrapyramidal side effects is more potent to reverse the effects of amphetamine on A9 than on A10 dopamine neurons (Bunney and Aghajanian, 1976; Goldstein et al., 1993; Okuyama et al., 1997). Actually, the ED_{50} value of risperidone on A10 dopamine neurons was lower than that for A9 dopamine neurons in the present study. This result supports the atypical antipsychotic properties of risperidone. Thus, NRA0562 may have the atypical antipsychotic activities seen with risperidone.

In immunohistochemical studies, NRA0562 dose-dependently and significantly increased Fos-like immunoreactivity in both the nucleus accumbens and the dorsolateral striatum. Moreover, the atypical index of NRA0562 was positive. The nucleus accumbens is thought to be an important site of antipsychotic activity (Deutch et al., 1992; Robertson et al., 1994). In contrast, the dorsolateral striatum is an important brain area in relation with extrapyramidal side effects produced by typical antipsychotics (Robertson and Fibiger, 1992; Merchant and Dorsa, 1993). Risperidone, known as an atypical antipsychotic in the clinic, increased Fos-like immunoreactivity not only in the nucleus accumbens but also in the dorsolateral striatum in the present study. These results are in agreement with reported data (Robertson et al., 1994). These authors compared the difference between Fos-like immunoreactivity in the nucleus accumbens and in the dorsolateral striatum to classify each antipsychotic. This difference, referred to as the atypical index, was positive for atypical antipsychotics including clozapine and risperidone and negative for typical antipsychotics including haloperidol (Robertson et al., 1994). According to this classification, the atypical index of risperidone in the present study was also positive. Thus, NRA0562 may be an atypical antipsychotic, like risperidone.

We observed that NRA0562 exhibited atypical antipsychotic properties in behavioral studies. NRA0562 potently inhibited methamphetamine-induced locomotion hyperactivity like other antipsychotics, such as clozapine, risperidone and olanzapine, while it showed a lower potency on stereotyped behavior and a lower incidence of catalepsy induction. The potency of NRA0562 to reverse methamphetamine-induced locomotion hyperactivity was greater than that of clozapine, risperidone and olanzapine. NRA0562 moderately occupied dopamine D_2 receptors in the striatum, and potently occupied 5-HT_{2A} receptors and α_1-adrenoceptors in the frontal cortex as did clozapine and risperidone. There are several lines of evidence that pharmacological actions of antipsychotics are ascribable to blockade of both the dopamine D_2 receptor and the 5-HT_{2A} receptor. The predominance of 5-HT_{2A} receptor antagonism over dopamine D_2 receptor antagonism is an important criterion for determining the therapeutic properties of atypical antipsychotics (Meltzer and Matsubara, 1989). While the role of the α_1-adrenoceptor is unclear, some reports support the significance of the α_1-adrenoceptor in the etiology of schizophrenia (Andersson et al., 1994; Svensson et al., 1995; Bakshi and Geyer, 1997).

In addition to our recent data, neuropharmacological results obtained with NRA0562 in the present study suggested that NRA0562 has profiles similar to those obtained with risperidone and may clinically have atypical antipsychotic properties without the liability of motor side effects. Potent occupancies of both 5-HT_{2A} and α_1-adrenoceptor in the frontal cortex may be involved in the effects of NRA0562.

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