British Journal of Pharmacology (1997) 121, 515–525

The atypical antipsychotic profile of NRA0045, a novel dopamine D_4 and 5-hydroxytryptamine$_{2A}$ receptor antagonist, in rats

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1 The atypical antipsychotic profile of (**R**)-(+)-2-amino-4-(4-fluorophenyl)-5-[1-[4-(4-fluorophenyl)-4-oxobutyl] pyrrolidin-3-yl] thiazole (NRA0045), a potent dopamine D_4 and 5-hydroxytryptamine (5-HT)$_{2A}$ receptor antagonist, was examined in rats.

2 Spontaneous locomotor activity was decreased dose-dependently with i.p. administration of clozapine (ED_{50} 3.7 mg kg^{-1}), haloperidol (ED_{50} 0.1 mg kg^{-1}) and chlorpromazine (ED_{50} 0.9 mg kg^{-1}), whereas inhibition of this type of behaviour induced by i.p. administration of NRA0045, at doses up to 10 mg kg^{-1}, did not exceed 50%.

3 Locomotor hyperactivity induced by methamphetamine (MAP, 2 mg kg^{-1}, i.p.) in rats (a model of antipsychotic activity) was dose-dependently antagonized by NRA0045 (ED_{50} 0.4 mg kg^{-1}, i.p., and 0.3 mg kg^{-1}, p.o., respectively), clozapine (ED_{50} 0.3 mg kg^{-1}, i.p. and 0.8 mg kg^{-1}, p.o., respectively), haloperidol (ED_{50} 0.02 mg kg^{-1}, i.p. and 0.1 mg kg^{-1}, p.o., respectively), chlorpromazine (ED_{50} 0.3 mg kg^{-1}, i.p. and 3.3 mg kg^{-1}, p.o., respectively). In contrast, the MAP (3 mg kg^{-1}, i.v.)-induced stereotyped behaviour in rats (a model of extrapyramidal symptoms) was not affected by NRA0045 or clozapine, at the highest dose given (30 mg kg^{-1}, i.p.). Haloperidol (ED_{50} 0.3 mg kg^{-1}, i.p.) and chlorpromazine (ED_{50} 4.8 mg kg^{-1}, i.p.) strongly blocked the MAP-induced stereotyped behaviour. NRA0045 and clozapine selectively blocked behaviour associated with activation of the mesolimbic/mesocortical dopamine neurones rather than nigrostriatal dopamine neurones.

4 Extracellular single-unit recording studies demonstrated that MAP (1 mg kg^{-1}, i.v.) decreased the firing rate in the substantia nigra (A9) and ventral tegmental area (A10) dopamine neurones in anaesthetized rats. NRA0045 completely reversed the inhibitory effects of MAP on A10 dopamine neurones (ED_{50} 0.1 mg kg^{-1}, i.v.), whereas the inhibitory effects of MAP on A9 dopamine neurones were not affected by NRA0045, in doses up to 1 mg kg^{-1} (i.v.). Clozapine completely reversed the inhibitory effects of MAP on A10 dopamine neurones (ED_{50} 1.9 mg kg^{-1}, i.v.) and on A9 dopamine neurones (ED_{50} 2.5 mg kg^{-1}, i.v.). Haloperidol completely reversed the inhibitory effects of MAP on A10 (ED_{50} 0.03 mg kg^{-1}, i.v.) and on A9 dopamine neurones (0.02 mg kg^{-1}, i.v.). NRA0045, like clozapine, was more potent in reversing the effects of MAP on A10 than A9 dopamine neurones.

5 Prepulse inhibition (PPI) is impaired markedly in humans with schizophrenia. The disruption of PPI in rats by apomorphine (0.5 mg kg^{-1}, s.c.) was reversed significantly by NRA0045 (3 mg kg^{-1}, i.p.), clozapine (3 mg kg^{-1}, i.p.) and haloperidol (0.3 mg kg^{-1}, i.p.).

6 Phencyclidine (PCP) elicits predominantly psychotic symptoms in normal humans and in schizophrenics. NRA0045 (0.03–0.3 mg kg^{-1}, i.p.) and clozapine (0.1–1 mg kg^{-1}, i.p.) significantly and dose-dependently shortened the PCP(1.25 mg kg^{-1}, i.p.)-induced prolonged swimming latency in rats in a water maze task, whereas haloperidol (0.01–0.1 mg kg^{-1}, i.p.) did not significantly alter swimming latency.

7 These findings suggest that NRA0045 may have unique antipsychotic activities without the liability of motor side effects typical of classical antipsychotics.

Keywords: Dopamine D_4 receptor antagonist; 5-hydroxytryptamine$_{2A}$ receptor antagonist; antipsychotic activities; NRA0045; clozapine; haloperidol; behavioural pharmacology; electrophysiology; phencyclidine; methamphetamine; apomorphine

Introduction

Schizophrenia is a mental illness for which present therapy leaves much to be desired. Classical antipsychotics, which are presumed to act by antagonism of dopamine D_2 receptors (Snyder, 1981), are useful for the treatment of the positive symptoms, but have various motor side effects (Aldessarini & Tarsey, 1980). The atypical antipsychotic clozapine is used to treat subjects with both positive and negative symptoms of schizophrenia and induces few extrapyramidal side effects (Wagstaff & Bryson, 1995). Van Tol *et al*. (1991) have shown that clozapine had a much higher affinity for dopamine D_4 receptors than for other dopamine receptors. The dissociation constant of about 9 nM for clozapine at dopamine D_4 receptors matches the plasma water concentration of clozapine, under conditions of clinical treatment (Seeman, 1992; 1995; Seeman & Van Tol, 1994). However, the use of clozapine has been compromised by a relatively high (up to 2%) incidence of the potentially fatal blood disorder agranulocytosis (Wagstaff & Bryson, 1995), necessitating stringent monitoring of plasma levels.

Extrapyramidal side effects are associated with dopamine receptor blockade in the nigrostriatal dopaminergic system,

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whereas blockade of mesolimbic/mesocortical dopaminergic function is thought to be important in alleviating symptoms of schizophrenia (Evenden & Ryan, 1990; Hoffman & Donovan, 1995). Relatively higher levels for the dopamine D_4 mRNA were observed in the mesolimbic/mesocortical dopaminergic system than in nigrostriatal dopaminergic system (Van Tol *et al*., 1991; O'Malley *et al*., 1992). The observed higher dopamine D_4 mRNA density in the frontal cortex and mesolimbic area is an important characteristic of this receptor, because this area of the brain is directly related to schizophrenia (Weinberger, 1988; Tamminga *et al*., 1992).

A number of dopamine D_4 ligands, including 5-(4-chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)piperidin-4-yl) isoxazole (Rowley *et al*., 1996), 3-[[4-(4-chlorophenyl)piperazin-1-yl]-methyl]-1H-pyrrolol[2,3-b]pyridine (Kulagowski, *et al*., 1996), (**S**)-(−)-4-[4-[2-(isochroman-1-yl) ethyl]-piperazin-1-yl] benzonesulphonamide (TenBrink *et al*., 1996), JL18 (Leigeois *et al*., 1995), 2-naphthoate esters (Boyfield *et al*., 1996) and YM-43611 (Hidaka *et al*., 1996; Ohmori *et al*., 1996) have been developed. However, *in vivo* dopamine D_4 receptor antagonism, potency and antipsychotic activity of these compounds have not been demonstrated.

NRA0045, (**R**)-(+)-2-amino-4-(4-fluorophenyl)-5-[1-[4-(4-fluorophenyl) 4-oxobutyl]pyrrolidin-3-yl]thiazole (Figure 1), a novel thiazole derivative, has a high affinity for the human cloned dopamine $D_{4.2}$, $D_{4.4}$ and $D_{4.7}$ receptors, with K_i values of 2.54, 0.55 and 0.54 nM, respectively, and is approximately 91 fold more potent at dopamine $D_{4.2}$ than human cloned dopamine D_{2L} receptors (Okuyama *et al*., 1996). NRA0045 has also a high affinity for the 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptor (K_i value 1.9 nM) and the α_1 adrenoceptor (K_i value 1.4 nM), but weak affinities (IC_{50} values are approximately 1000 nM) for adenosine$_1$, 5-HT_{1A}, 5-HT_{1C}, α_{2A}- and α_{2B}-receptors and dopamine transporter, and negligible affinities (IC_{50} values are over 10^{-5} M) for 42 other receptors, including neurotransmitters and hormones, ion channels and second messenger systems. (Okuyama *et al*., 1996). NRA0045 antagonizes methamphetamine (MAP)-induced hyperactivity in mice, tryptamine-induced chronic seizure in rats and noradrenaline (NA)-induced lethality in rats; ED_{50} values are 0.5, 1.5 and 0.2 mg kg^{-1}, i.p., respectively (Okuyama *et al*., 1996). The inhibition, induced by the higher dose of NRA0045 (30 mg kg^{-1}, i.p.), of MAP-induced stereotyped behaviour in mice did not exceed 50% and this dose produced less than 50% catalepsy in rats (Okuyama *et al*., 1996). Thus, NRA0045 is a potent $D_4/5\text{-HT}_{2A}$ receptor antagonist with anti-NA activity. In addition, NRA0045 is rapidly absorbed after oral administration, and the brain concentration of NRA0045 is approximately 9 fold higher than the plasma level, in rats (unpublished observations). In this paper, we describe the atypical profile of NRA0045 in rats.

Methods

Animals

Male Wistar rats (Charles River, Japan) were used to assess spontaneous locomotor activity (150–250 g), MAP-induced hyperactivity (150 g–250 g), MAP-induced stereotyped behaviour (150 g–250 g), phencyclidine (PCP)-induced prolonged swimming latency in a water maze task (200–300 g) and electrophysiological experiments (320–400 g). Male Sprague-Dawley rats (280–310 g, Charles River, Japan) were used for the startle response. Animals were housed 3 per cage, and were maintained under a 12 h light:dark cycle (lights on 07 h 00 min) in a temperature and humidity controlled holding room. Food and water were available *ad libitum*.

All studies presented here have been reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and have met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal experiments (1987).

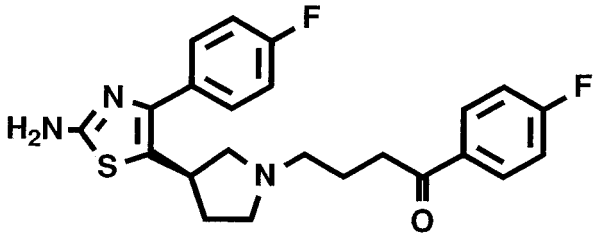

Figure 1 Chemical structure of (**R**)-(+)-2-amino-4-(4-fluorophenyl)-5-[1-[4-(4-fluorophenyl)-4-oxobutyl] pyrrolidin-3-yl]thiazole (NRA0045).

Spontaneous locomotor activity

Animals were housed individually in transparent acrylic cages ($40 \times 25 \times 20$ cm), and spontaneous locomotor activity was recorded every 5 min for 120 min by an Activity Monitor (ENV-510, MED Associates, Inc) placed in a sound-proof box. The rats were given NRA0045 (1–10 mg kg^{-1}), clozapine (1–10 mg kg^{-1}), haloperidol (0.03–1 mg kg^{-1}) or chlorpromazine (0.3–10 mg kg^{-1}) i.p. just before the start of measurements. Six rats, given the vehicle and each of 3–4 dosages of compounds, were used to determine dose-dependent effects. The total count in the vehicle-treated control group was defined as 100%, the % inhibition of each treatment group was calculated and ED_{50} values were determined.

MAP-induced locomotor hyperactivity

Animals were housed individually in transparent acrylic cages ($40 \times 25 \times 20$ cm), and acclimatized for 60 min in an Activity Monitor (ENV-510, MED Associates, Inc) placed in a sound-proof box. NRA0045 (0.1–1 mg kg^{-1}), clozapine (0.03–1 mg kg^{-1}), haloperidol (0.01–0.1 mg kg^{-1}) or chlorpromazine (0.1–1 mg kg^{-1}) was given i.p. 30 min before the i.p. administration of MAP (2 mg kg^{-1}). In the case of p.o. administration, NRA0045 (0.03–1 mg kg^{-1}), clozapine (0.1–1 mg kg^{-1}), haloperidol (0.03–1 mg kg^{-1}) or chlorpromazine (3–30 mg kg^{-1}) was given 60 min before the i.p. administration of MAP (2 mg kg^{-1}). Immediately after the MAP administration, locomotor activity was recorded every 5 min for 120 min. Six rats, given the vehicle and each of 3–4 doses of the compounds, were used to determine dose-dependent effects. The total count for the vehicle-treated control group was defined as 100%, the % inhibition in each group was calculated and ED_{50} values were determined.

MAP-induced stereotyped behaviour

MAP-induced stereotyped behaviour was determined in rats, as described previously (Watanabe *et al*., 1981). Animals were placed individually in a clear acrylic cage ($40 \times 25 \times 20$ cm) and allowed a minimum of 60 min to acclimatize to the new environment. The rats were given i.p. NRA0045 (3–30 mg kg^{-1}), clozapine (3–30 mg kg^{-1}), haloperidol (0.1–1 mg kg^{-1}) or chlorpromazine (1–10 mg kg^{-1}) 30 min before the i.v. administration of MAP (3 mg kg^{-1}). Immediately after MAP administration, stereotyped behaviour was scored every 30 min for 240 min, by use of the following scoring system: 0, normal behaviour; 1, discontinuous exploratory behaviour; 2, continuous exploratory behaviour and discontinuous sniffing and rearing; 3, continuous sniffing and rearing; 4, continuous severe sniffing and rearing; 5, continuous severe sniffing and discontinuous licking; 6, continuous biting and discontinuous gnawing; 7, slight biting; 8 mild biting; 9, severe biting; 10, self biting. Six rats, given the vehicle and each of 3 doses of the compounds, were used to determine dose-dependent effects. Total score in the vehicle-treated control group was defined as 100%, the % inhibition of each treatment group was calculated and ED_{50} values were determined.

MAP-induced inhibition of substantia nigra (A9) and ventral tegmental area (A10) dopamine neurones

Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and given supplements as required. The body temperature was maintained between 37 and 38°C with a heating pad controlled by feedback from a rectal thermistor (KN-474, Natsume).

A small hole 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). The electrode was placed stereotaxically in the A9 (AP: −5.2 to −6.0, L: 1.2 to 1.8, H: 7.8 to 8.8 mm) and A10 (AP: −4.8 to −5.2, L: 0.5 to 0.8, H: 7.5 to 8.5 mm), according to the atlas of Paxinos and Watson (1986). Single-barrel glass electrodes were filled with a solution of 1% (w/v) pontamine sky blue (Tokyo Kasei) in 0.5 M sodium acetate and resistances ranged between 2 to 10 MΩ measured at 135 Hz. At the end of each experiment, the recording sites were marked with a dye. The animal was anaesthetized with pentobarbitone and perfused with 10% formalin. Frozen 50 μm thick sections of the whole brain were cut on a cryostat microtome (MA-101, Komatsu), and stained with haematoxylin and eosin.

Single unit activities were monitored on an oscilloscope (VC-10, Nihon Kohden) and converted to a uniform voltage pulse by a window discriminator (DSE-325P, Dia Medical). The pulses were integrated at 10 s periods and displayed on an ink-writing oscilloscope (WT-645G, Nihon Kohden).

Detailed descriptions of the extracellular recording techniques and histological studies have been described elsewhere (Okuyama *et al*., 1986).

MAP (1 mg kg^{-1}, i.v.) or incremental doses of NRA0045 (the starting dose was 0.1 mg kg^{-1}, with sequential doses of 0.2 and 0.7 mg kg^{-1}), clozapine (starting dose 1 mg kg^{-1}, with sequential doses of 2 and 7 mg kg^{-1}) and haloperidol (starting dose 0.01 mg kg^{-1}, with sequential doses of 0.02 and 0.07 mg kg^{-1}) were administered every 2 min via an i.v. catheter implanted in the tail vein. A compound-induced change in neuronal activity was plotted as percentage changes from pre-injection baseline rate, which was calculated over a 10 min period and defined as 100%. The % inhibition of each dose was calculated and ED_{50} values were determined.

Apomorphine-induced disruption of prepulse inhibition (PPI)

The method used was based on that of Geyer and Braff (1990) and was similar to that described by others (Swerdlow *et al*., 1991). The chambers (SR-LAB, San Diego, CA) were housed in a sound-attenuated room with 60 dB (A) ambient noise level. A chamber consisted of a Plexiglas cylinder 8.8 cm in diameter resting on 5 × 15 cm Plexiglas frame within a ventilated enclosure. Noise bursts were presented through a speaker mounted 24 cm above the rat. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced motion within the cylinder. Stimulus delivery was controlled by the SR-LAB microcomputer and interface assembly, which also digitized (0-4095), rectified and recorded stabilimeter readings, with 100 one-ms readings collected beginning at stimulus onset. Startle amplitude was defined as the average of the 100 readings. Background noise and all acoustic stimuli were delivered through one Radio Shack Superweeter (frequency response predominantly between 5–16 KHz) in a chamber. Sound levels were measured and calibrated with a Quest Sound Level Meter, A scale (relative to 20 $\mu N/M^2$), with the microphone placed inside the Plexiglas cylinder; response sensitivities were calibrated by use of an SR-LAB Startle Calibration System.

The rats were administered (i.p.) NRA0045 (0.3–3 mg kg^{-1}), clozapine (1–10 mg kg^{-1}) or haloperidol (0.03–0.3 mg kg^{-1}) 30 min before the s.c. administration of apomorphine (0.5 mg kg^{-1}), then immediately placed in a startle chamber for a 5 min acclimatization period with 70 dB (A) background noise. The test session consisting of six different trial types was utilized for all experiments: a 40 ms broadband 118 dB burst ('pulse' alone trial), three 'prepulse + pulse' trials in which 20 ms noises of either 73, 75 or 80 dB preceded the 118 dB burst by 100 ms, a 40 ms broadband 80 dB burst ('prepulse alone' trial), and a no stimulus trial. Thus, prepulses were 3, 5, 10 dB above the 70 dB background. All of these trial types were presented 6 times in a pseudo-random order during the test session for a total of 36 trials. An average of 30 s (20–40 s) was allowed between consecutive trials.

PPI was defined as the % reduction in startle amplitude in the presence of the prepulse compared to the amplitude in the absence of the prepulse [100-(100 × amplitude on prepulse trial/amplitude on 'pulse' trial)].

Phencyclidine (PCP)-induced prolonged swimming latency in a water maze task

A circular tank (140 cm in diameter and 45 cm high) was used. A transparent platform (10 cm in diameter and 25 cm high) was set inside the tank and the tank was filled to a height of 27 cm with water at approximately 23°C (the platform's surface was 2 cm below the surface of the water). The pool was located in a large test room and many external cues within the room could be used by the rat for spatial orientation. The positions of the cues were unchanged throughout the training. The rats were placed at one of three starting positions for each training session, but the sequence of positions was selected at random. The platform was located in a constant position in the middle of one quadrant, equidistant from the centre and the edge of the pool. In each training session, the time to escape onto the hidden platform was reduced. If the rat found the platform within 100 s, it was allowed to remain there for 30 s, then it was returned to its home cage. If the rat was unable to find the platform within 100 s, it was placed on the platform for 30 s and a maximum score of 100 s was assigned. The training session was conducted on 2 different days and the rat underwent 4 trials at 2 h intervals, each day. The swimming latency, swimming speed and swimming pattern used to find the platform were monitored by a TV camera (Video Image Motion Analyzer AXIS 30; Neuroscience Inc. Japan) and analysed by computer (PC-9801, NEC, Japan). NRA0045 (0.03–0.3 mg kg^{-1}), clozapine (0.1–1 mg kg^{-1}) or haloperidol (0.01–0.1 mg kg^{-1}) was administered i.p. 30 min before each trial. PCP was administered i.p. 15 min before each trial.

Detailed descriptions of the PCP-induced prolonged swimming latency in a water maze task have been described elsewhere (Ogawa *et al*., 1994a,b; Okuyama *et al*., 1995).

Statistical analysis

Data from spontaneous locomotor activity and MAP-induced hyperactivity were analysed by one-way analysis of variance (ANOVA) and significant differences between groups were determined by Dunnett's test. Data from MAP-stereotyped behaviour were analysed by the Kruskall-Wallis test, with significant differences between groups determined by non-parametric Dunnett's test. Data from PCP-induced prolonged swimming latency in a water maze task were analysed by two-way ANOVA and significant differences between groups were determined with Dunnett's test. Data from PPI and the electrophysiological study were analysed by two-way ANOVA with repeated measures and significant differences between groups were determined by Dunnett's test. Data from pulse amplitude were analysed by one-way ANOVA and significant differences between groups were determined by Dunnett's test. The ED_{50} values were calculated by Allfit analysis by use of % inhibition.

Compounds

Apomorphine HCl (Sigma Chemicals), methamphetamine (MAP) HCl (Dainippon Pharmaceuticals), chlorpromazine

HCl (Wako) and haloperidol (Serenase injections, Dainippon Pharmaceutical) were dissolved in 0.9% saline with the addition of 0.1% ascorbic acid for apomorphine. Clozapine (Gee Lawson Chemicals) 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. Phencyclidine (PCP) and NRA0045 were synthesized in the laboratories of Taisho Pharmaceutical Co., Ltd. NRA0045 was suspended in 5% arabic gum. In the electrophysiological study, NRA0045 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.

Results

Effect on spontaneous locomotor activity in rats

The inhibition of spontaneous locomotor activity induced by administration of NRA0045 (1–10 mg kg^{-1}) did not exceed 50%. However, a reduction of spontaneous locomotor activity

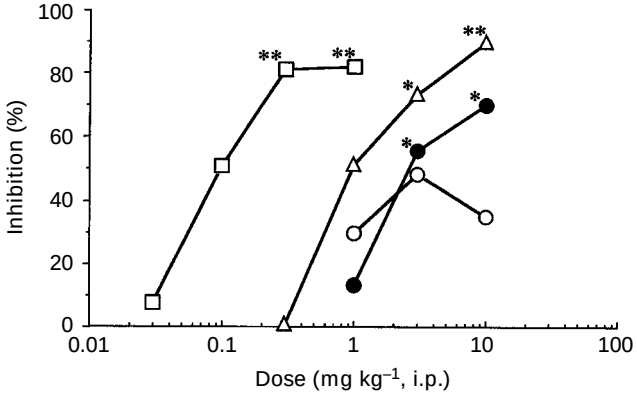

Figure 2 Effects of (○) NRA0045, (●) clozapine, (□) haloperidol and (△) chlorpromazine on spontaneous locomotor activity in rats. Each group consisted of six rats. $*P<0.05$ and $**P<0.01$ vs control group (Dunnett's test).

Table 1 Behavioural profile of rats given NRA0045: comparison with clozapine, haloperidol and chlorpromazine

		ED_{50} (mg kg^{-1})			
	Route	*NRA0045*	*Clozapine*	*Haloperidol*	*Chlorpromazine*
Spontaneous locomotor activity	i.p.	> 10	3.7	0.1	0.9
MAP-induced locomotor hyperactivity	i.p.	0.4	0.3	0.02	0.3
MAP-induced locomotor hyperactivity	p.o.	0.3	0.8	0.1	3.3
MAP-induced stereotyped behaviour	i.p.	> 30	> 30	0.3	4.8

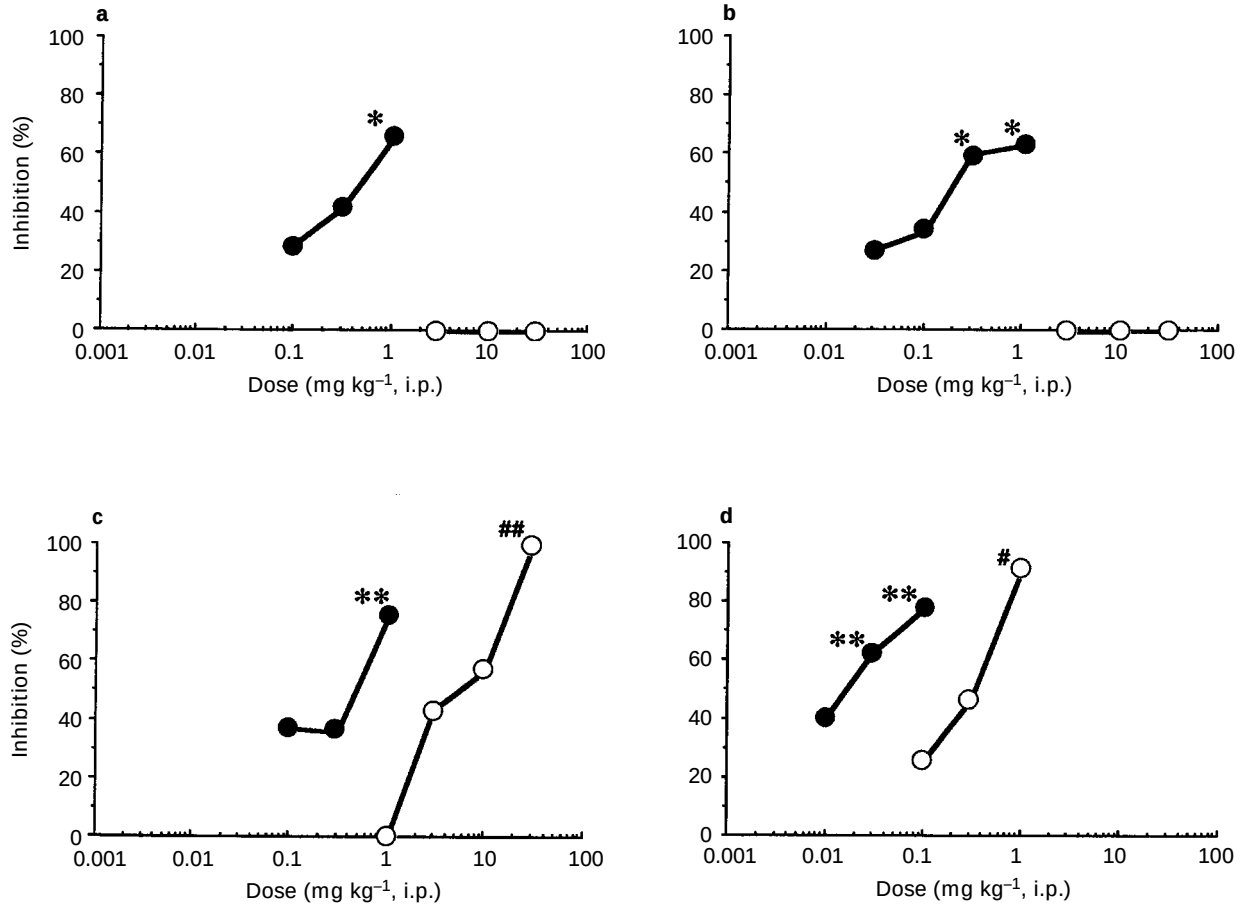

Figure 3 Effects of (a) NRA0045, (b) clozapine, (d) haloperidol and (c) chlorpromazine on methamphetamine (MAP, 2 mg kg^{-1}, i.p.)-induced hyperactivity (●) and MAP (3 mg kg^{-1}, i.v.)-induced stereotyped behaviour (○) in rats. Each group consisted of six rats. $*P<0.05$ and $**P<0.01$ vs control group (Dunnett's test). $\#P<0.05$ and $\#\#P<0.01$ vs control group (non-parametric Dunnett's test).

was recorded after i.p. administration of clozapine (ED_{50} 3.7 mg kg^{-1}), haloperidol (ED_{50} 0.1 mg kg^{-1}) or chlorpromazine (ED_{50} 0.9 mg kg^{-1}) (Figure 2 and Table 1).

Effect of MAP-induced locomotor hyperactivity

The MAP-induced locomotor hyperactivity was attenuated dose-dependently and significantly after i.p. (ED_{50} 0.4 mg kg^{-1}) or p.o. (ED_{50} 0.3 mg kg^{-1}) administration of NRA0045 (Figure 3 and Table 1). A dose-dependent blockade of MAP-induced locomotor hyperactivity was also observed in rats pretreated with clozapine (ED_{50} 0.3 mg kg^{-1}, i.p. and ED_{50} 0.8 mg kg^{-1}, p.o., respectively), haloperidol (ED_{50} 0.02 mg kg^{-1}, i.p. and ED_{50} 0.1 mg kg^{-1}, p.o., respectively), chlorpromazine (ED_{50} 0.3 mg kg^{-1}, i.p. and ED_{50} 3.3 mg kg^{-1}, p.o., respectively) (Figure 3 and Table 1).

Effect on MAP-induced stereotyped behaviour

This stereotyped behaviour was attenuated dose-dependently and significantly after i.p. administration of haloperidol (ED_{50} 0.3 mg kg^{-1}) or chlorpromazine (ED_{50} 4.8 mg kg^{-1}) (Figure 3 and Table 1). In contrast, the i.p. administration of NRA0045 and clozapine, at doses up to 30 mg kg^{-1}, did not exceed 50% inhibition (Figure 3).

Effect on MAP-induced inhibition of A9 and A10 dopamine neurones

The activity of A9 and A10 dopamine neurones displayed action potentials of long-duration (2.5–4.2 ms), at a rate of 1–7 spikes s^{-1}. These neurones also exhibited a notch on the rising phase of the spike and typically fired in bursts of 3–8 spikes of steadily decreasing amplitude. These features seem to be characteristic of dopamine neurones (Bunney *et al*., 1973; Okuyama *et al*., 1986; Stockton & Rasmussen, 1996).

In 36 of the 48 dopamine neurones, the firing rate of the A9 or A10 dopamine neurones was inhibited by at least 50% or more by MAP (1 mg kg^{-1}, i.v.). A9 dopamine neurones were inhibited significantly by MAP (Figures 4 and 5a,b). A10 dopamine neurones were also significantly inhibited by MAP (Figures 4, 5a,b).

NRA0045 administration dose-dependently reversed the effects of MAP on A10 dopamine neurones (Figure 4). In contrast, NRA0045, at doses up to 1 mg kg^{-1}, did not significantly reverse MAP effects on A9 dopamine neurones; the maximal reversal achieved was about 40% of baseline (Figure 4). ED_{50} values for the effects of NRA0045 on MAP-inhibited A10 and A9 neurones were 0.1 mg kg^{-1} and >1.0 mg kg^{-1}, respectively. When administered alone, NRA0045 (0.1, 0.3 and 1 mg kg^{-1}, i.v.) did not change baseline firing rates of A9 ($n=3$) or A10 ($n=3$) dopamine neurones (data not shown).

Clozapine administration dose-dependently reversed the effects of MAP on A10 dopamine and A9 dopamine neurones (Figure 5a). Clozapine was more potent in reversing the effects of MAP on A10 (ED_{50} 1.9 mg kg^{-1}) than A9 (ED_{50} 2.5 mg kg^{-1}) dopamine neurones.

Haloperidol administration dose-dependently reversed the effects of MAP on A10 dopamine and A9 dopamine neurones

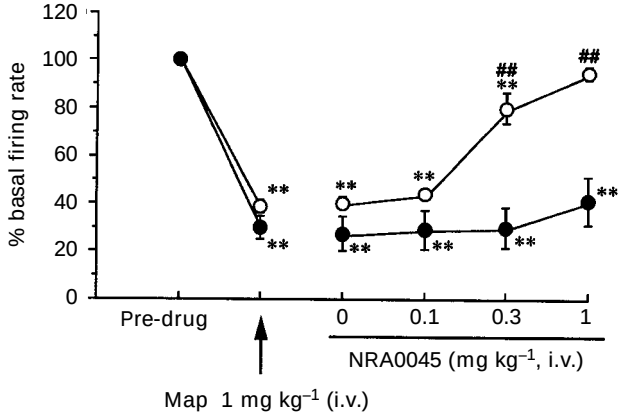

Figure 4 Comparison of the potential of NRA0045 to reverse the inhibitory effects of methamphetamine (MAP, 1 mg kg^{-1}, i.v.) on A9 (●) and A10 (○) dopamine neurones. The results are presented as mean with vertical lines showing s.e. Each group consisted of six rats. Starting dose of NRA0045 was 0.1 mg kg^{-1}, i.v. $^{**}P<0.01$ vs pre-drug value (Dunnett's test). $^{\#\#}P<0.01$ vs MAP + vehicle-treated group (Dunnett's test). ANOVA showed an effect for site (A9 vs A10), $F(1,10)=15.36$, $P<0.01$; an effect for treatment (MAP vs NRA0045), $F(3,30)=48.72$, $P<0.01$; an effect for site × treatment interaction, $F(3,30)=22.58$, $P<0.01$. Basal firing rate (10 s^{-1}): A9, 41.0±9.8 ($n=6$); A10, 43.2±4.4 ($n=6$).

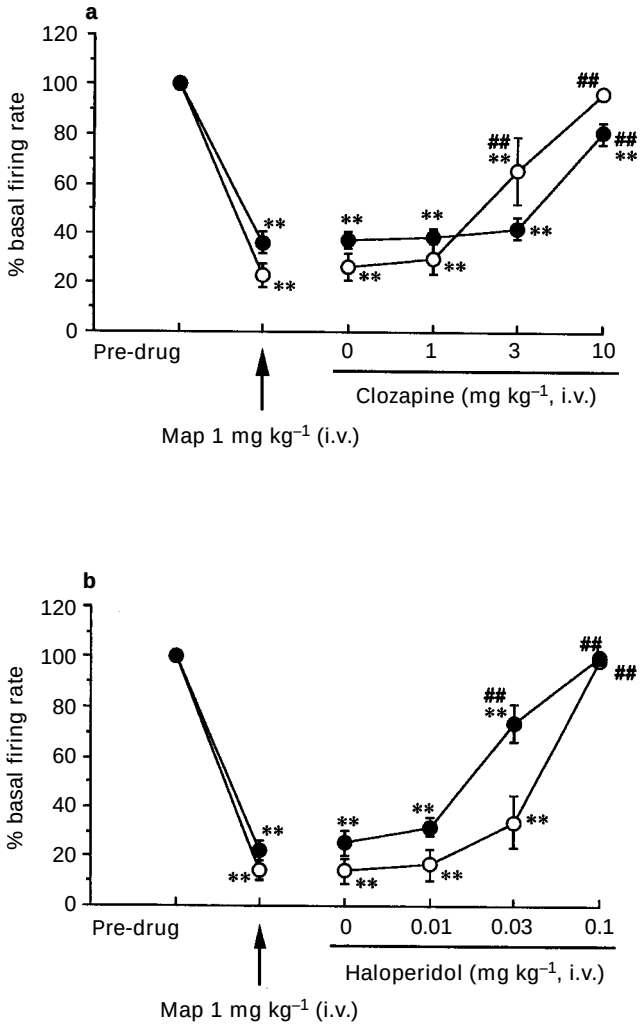

Figure 5 Comparison of the potential of clozapine (a) and haloperidol (b) to reverse the inhibitory effects of methamphetamine (MAP, 1 mg kg^{-1}, i.v.) on A9 (●) and A10 (○) dopamine neurones. The results are presented as mean with vertical lines showing s.e. Each group consisted of six rats. Starting doses of clozapine and haloperidol were 1 and 0.01 mg kg^{-1}, i.v., respectively. $^{**}P<0.01$ vs pre-drug value (Dunnett's test). $^{\#\#}P<0.01$ vs MAP + vehicle-treated group (Dunnett's test). In the case of clozapine, ANOVA showed an effect for site (A9 vs A10), $F(1,10)=8.07$, $P<0.05$; an effect for treatment (MAP vs clozapine), $F(3,30)=118.46$, $P<0.01$; effects for site × treatment interaction, $F(3,30)=5.96$, $P<0.05$. In the case of haloperidol, ANOVA showed no effect for site (A9 vs A10), $F(1,10)=0.83$; an effect for treatment (MAP vs haloperidol), $F(3,30)=40.32$, $P<0.01$; an effect for site × treatment interaction, $F(3,30)=4.41$, $P<0.05$. Basal firing rates (10 s^{-1}): (a) A9, 43.2±2.7 ($n=6$); A10, 38.5±5.1 ($n=6$); (b) A9, 53.1±4.6 ($n=6$); A10, 38.8±4.5 ($n=6$).

(Figure 5b). Haloperidol was more potent in reversing the effects of MAP on A9 (ED_{50} 0.02 mg kg^{-1}) than A10 (ED_{50} 0.03 mg kg^{-1}) dopamine neurones.

Effect on apomorphine-induced disruption of PPI

The acoustic startle amplitude was potentiated significantly (Figures 6a and 7a,c), and PPI was decreased significantly (Figures 6b and 7b and d) in apomorphine-treated animals.

Pretreatment with NRA0045 (0.3–3 mg kg^{-1}, i.p.) dose-dependently blocked the apomorphine-induced disruption of PPI (Figure 6b). NRA0045, 3 mg kg^{-1} i.p., significantly ($P<0.05$) blocked the apomorphine-induced disruption of PPI. NRA0045 alone had no effect on PPI in vehicle-treated animals.

NRA0045 had no significant effects on either potentiated acoustic startle amplitude in apomorphine-treated animals (Figure 6a) or on acoustic startle amplitude in vehicle-treated animals.

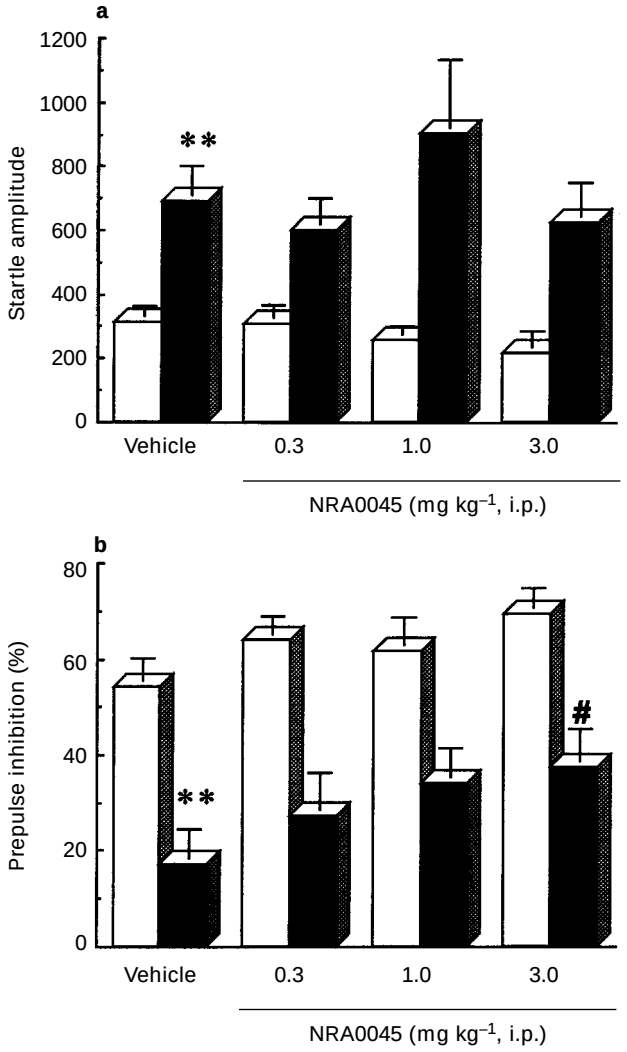

Figure 6 Effects of NRA0045 on (a) startle amplitude and (b) prepulse inhibition (PPI) in vehicle (open columns)- and apomorphine (0.5 mg kg^{-1}, s.c., solid columns)-treated rats. The results are presented as mean ± s.e. of three separate experiments ($n=12$), one for each dose of compound, where the rats received vehicle + vehicle, compound + vehicle, vehicle + apomorphine in a randomized order and were tested every third or fourth day. Control conditions (no compounds) are represented by data pooled from all three experiments ($n=21-26$). $^{**}P<0.01$ vs vehicle + vehicle treated group (Dunnett's test). $\#P<0.05$ vs vehicle + apomorphine-treated group (Dunnett's test). Analysis of PPI in rats pretreated with NRA0045, ANOVA showed no effect for treatment (apomorphine vs NRA0045), $F(3,56)=1.16$; an effect for trial (3, 5, 10 dB), $F(2,112)=108.53$, $P<0.01$; an effect for treatment × trial interaction, $F(6,112)=3.45$, $P<0.05$.

Analysis of PPI in animals pretreated with clozapine (1–10 mg kg^{-1}, i.p.) suggests that this compound has biphasic dose-response properties (Figure 7b). Thus, at a low dose (3 mg kg^{-1}, i.p.) clozapine significantly ($P<0.05$) reversed the apomorphine-induced inhibition of PPI, whereas at higher doses (10 mg kg^{-1}, i.p.) it failed to reverse this apomorphine effect. Clozapine (10 mg kg^{-1}) also significantly ($P<0.01$) decreased PPI in vehicle-treated animals.

At a higher dose, clozapine (10 mg kg^{-1}, i.p.) significantly ($P<0.01$) decreased the potentiated acoustic startle amplitude in apomorphine-treated animals (Figure 7a). Clozapine (10 mg kg^{-1}) also significantly ($P<0.01$) decreased the acoustic startle amplitude in vehicle-treated animals.

Pretreatment with haloperidol (0.03–0.3 mg kg^{-1}, i.p.) dose-dependently and significantly blocked the apomorphine-induced disruption of PPI (Figure 7d). The apomorphine-induced inhibition of PPI was significantly ($P<0.05$) decreased by the highest dose of haloperidol (0.3 mg kg^{-1}). Haloperidol had no significant effects on PPI in vehicle-treated animals.

The apomorphine-induced potentiated acoustic startle amplitude was also significantly ($P<0.01$) decreased by the highest dose of haloperidol (0.3 mg kg^{-1}). Haloperidol had no significant effects on acoustic startle amplitude in vehicle-treated animals (Figure 7c).

Effect on PCP-induced prolonged swimming latency in a water maze task

Hyperactivity, ataxia and stereotyped behaviour were not induced by PCP 1.25 mg kg^{-1} (Ogawa *et al*., 1994a,b).

The swimming latency for all groups decreased with training. The swimming latencies of PCP-treated rats on the first trial did not differ from those of the control group (Figures 7 and 8). However, throughout the training, the PCP-treated rats were slower to find the platform than controls. By the end of the training session, the mean latency in the PCP-treated group was approximately 70 s, while that of controls was approximately 20 s. Swimming speed during the 8 trial blocks did not differ between groups (approximately 28 cm s^{-1}). The behavioural trace pattern revealed that the PCP-treated group swam around the side wall of the pool and did not approach the platform.

There were no differences in swimming latencies between NRA0045-treated (0.03 mg kg^{-1}, i.p.) and PCP-treated groups. NRA0045 (0.1 and 0.3 mg kg^{-1}, i.p.) was not effective on day 1, but did significantly reduce the PCP impairment on day 2 (Figure 8).

Clozapine (0.3 and 1 mg kg^{-1}, i.p.) was not effective on day 1, but did significantly reduce the PCP-induced impairment on day 2 (Figure 9a). The swimming latencies of haloperidol (0.01 and 0.1 mg kg^{-1}, i.p.)-treated groups showed no differences from those of the PCP-treated group (Figure 9b).

Discussion

The present study is apparently the first to show *in vivo* an index of antipsychotic activity for a potent dopamine D_4, 5-HT_{2A} and α_1-adrenoceptor antagonist, NRA0045, in laboratory animals. NRA0045 and clozapine exhibited a behavioural profile distinct from that of haloperidol and chlorpromazine in rodent models commonly used to predict antipsychotic potential and side effect probability in man. A classical preclinical test for antipsychotic potential is antagonism of D-amphetamine (or methamphetamine)-stimulated locomotor hyperactivity (Kehne *et al*., 1981; Evenden & Ryan, 1990). NRA0045, clozapine, haloperidol and chlorpromazine produced dose-related decreases in MAP-induced hyperactivity in rats. NRA0045 and clozapine failed to attenuate stereotyped behaviour induced by MAP in rats. In contrast, haloperidol and chlorpromazine blocked MAP-induced stereotyped beha-

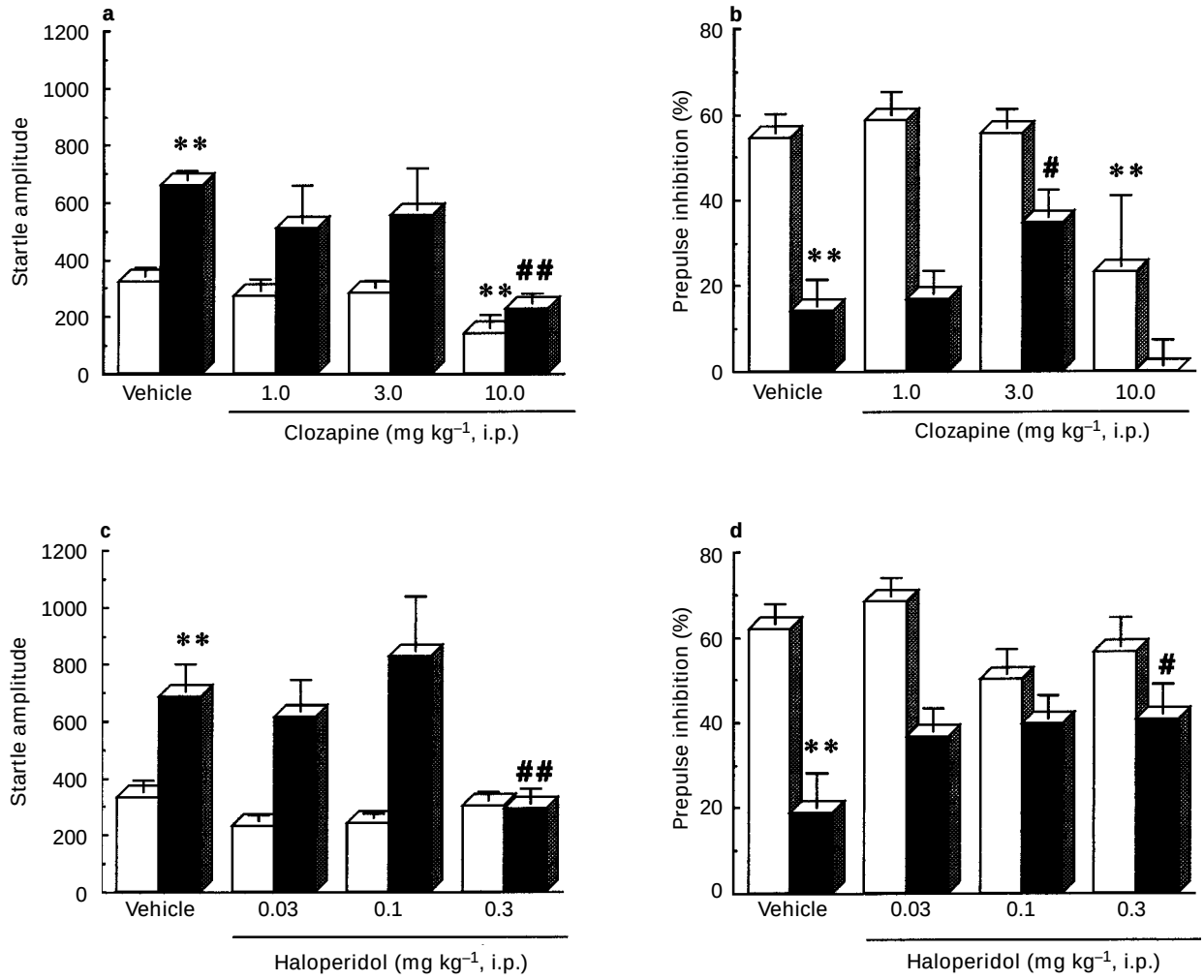

Figure 7 Effects of (a,b) clozapine and (c,d) haloperidol on (a, c) startle amplitude and (b,d) prepulse inhibition (PPI) in vehicle (open columns)- and apomorphine (0.5 mg kg^{-1}, s.c., solid columns)-treated rats. The results are presented as mean ± s.e. of three separate experiments ($n=12$), one for each dose of compound, where the rats received vehicle + vehicle, compound + vehicle, vehicle + apomorphine in a randomized order and were tested every third or fourth day. Control conditions (no compounds) are represented by data pooled from all three experiments ($n=21-26$). $^{**}P<0.01$ vs vehicle + vehicle treated group (Dunnett's test). $\#P<0.05$ and $\#\#P<0.01$ vs vehicle + apomorphine-treated group (Dunnett's test). Analysis of PPI in animals pretreated with clozapine, ANOVA showed no effect for treatment (apomorphine vs clozapine), $F(3,58)=2.02$; an effect for trial (3, 5, 10 dB), $F(2,116)=58.88$, $P<0.01$; an effect for treatment × trial interaction, $F(6,116)=2.78$, $P<0.05$. In the case of haloperidol, ANOVA showed no effect for treatment (apomorphine vs haloperidol), $F(3,52)=1.02$; an effect for trial (3, 5, 10 dB), $F(2,104)=88.56$, $P<0.01$; an effect for treatment × trial interaction, $F(6,104)=2.45$, $P<0.05$.

viour in rats. Dopamine-agonist induced locomotor hyperactivity (a model of antipsychotic activity) and stereotyped behaviour (a model of extrapyramidal symptoms) are mediated through stimulation of the mesolimbic/mesocortical dopaminergic system and nigrostriatal dopaminergic system, respectively (Kehne *et al*., 1981; Evenden & Ryan, 1990; Hoffman & Donovan, 1995). Thus, NRA0045 selectively blocked behaviour associated with activation of the mesolimbic/mesocortical dopaminergic system and showed similarities to the atypical antipsychotic clozapine. Clozapine is the only known antipsychotic that produces a very low incidence of extrapyramidal side effects and virtually no tardive dyskinesia (Casey, 1989).

Sertindole has high affinity for dopamine D_2, 5-HT_{2A} receptors and α_1-adrenoceptors (Sanchez *et al*., 1991; Andersen *et al*., 1996). Sertindole has atypical antipsychotic activity with a low incidence of extrapyramidal side effects in laboratory animals (Sanchez *et al*., 1991) and in man (Weiden *et al*., 1996). The *in vivo* results obtained with sertindole indicate that it is a very potent and long-acting 5-HT antagonist (Sanchez *et al*., 1991). NRA0045 and sertindole have a high affinity for 5-HT_{2A} receptors and α_1-adrenoceptors but, in contrast to sertindole, NRA0045 has a low affinity for dopamine D_2 receptors and a high affinity for dopamine D_4 receptors.

The behavioural profile of NRA0045 on dopamine agonist-induced behaviour is similar to MDL 100,907, a selective 5-HT_{2A} receptor antagonist (Kehne *et al*., 1996a). A hypothesis to explain the mechanism by which 5-HT_{2A} receptor antagonism indirectly reduces the effects of D-amphetamine was summarized by Schmidt *et al*. (1995). Briefly, it was proposed that 5-HT_{2A} receptors are 'permissive' for stimulated dopamine release. Thus, normal 5-HT_{2A} receptor activation supports increased synthesis of dopamine, under conditions of accelerated demand, such as after D-amphetamine. 5-HT_{2A} receptor blockade with MDL 100,907 blocks this permissive role, thereby attenuating D-amphetamine-induced locomotor stimulation. NRA0045 has a high affinity for the 5-HT_{2A} receptor and dose-dependently antagonized tryptamine-induced chronic seizures (Okuyama *et al*., 1996). Thus, NRA0045 may have dual antagonistic actions on the mesolimbic/mesocortical dopaminergic system, induced by both a direct action on the dopamine D_4 receptors and indirect action on 5-HT_{2A} receptors. Moreover, 5-HT_2 receptor antagonists such as ritanserin improved the negative symptoms of schizophrenia

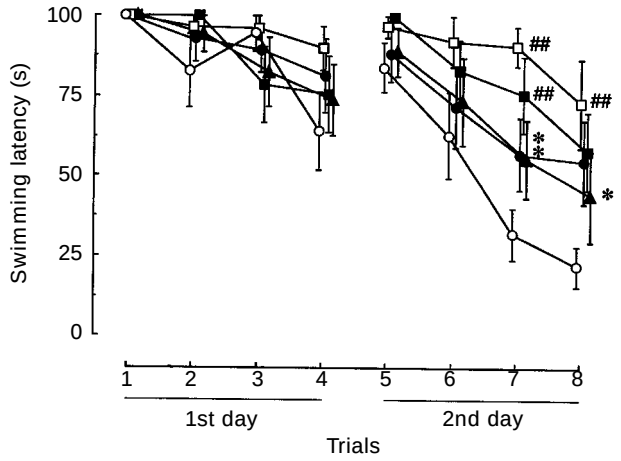

Figure 8 Effects of NRA0045 ((●) 0.03, (■) 0.1 and (▲) 0.3 mg kg^{-1}) on phencyclidine (PCP, 1.25 mg kg^{-1}, i.p., □)-induced prolonged swimming latency in a water maze task. Each group consisted of ten rats. The results are presented as mean with vertical lines showing s.e. $\#\#P<0.01$ vs control group (○) (Dunnett's test). $*P<0.05$ vs PCP-treated group (Dunnett's test). ANOVA showed an effect for trials, day 1 [$F(3,144)=5.95$, $P<0.01$], day 2 [$F(3,144)=8.14$, $P<0.01$]; an effect for treatment (control vs PCP), day 1 [$F(1,72)=4.44$, $P<0.05$], day 2 [$F(1,72)=35.38$, $P<0.01$], (PCP vs NRA0045), day 1 [$F(3,144)=0.98$, not significant], day 2 [$F(3,144)=3.87$, $P<0.05$].

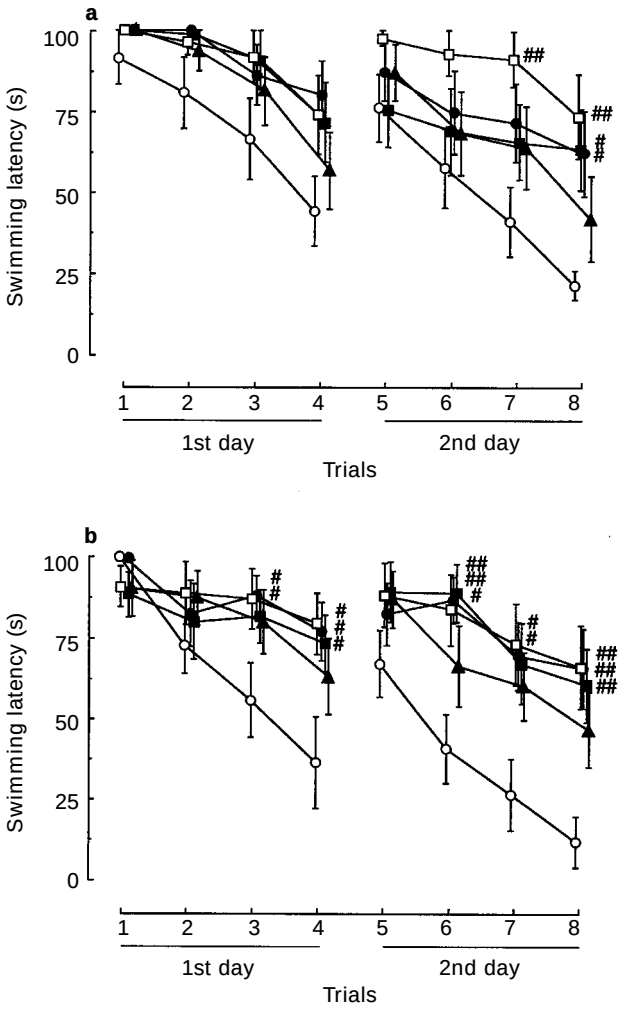

Figure 9 Effects of (a) clozapine ((●) 0.1, (■) 0.3 and (▲) 1 mg kg^{-1}) and (b) haloperidol ((●) 0.01, (■) 0.03 and (▲) 0.1 mg kg^{-1}) on phencyclidine (PCP, 1.25 mg kg^{-1}, i.p., □)-induced prolonged swimming latency in a water maze task. Each group consisted of ten rats. The results are presented as mean with vertical lines showing s.e. $\#P<0.05$ and $\#\#P<0.01$ vs control group (○) (Dunnett's test). In the case of clozapine, ANOVA showed an effect for trials, day 1 [$F(3,144)=10.09$, $P<0.01$], day 2 [$F(3,144)=3.79$, $P<0.05$]; an effect for treatment (control vs PCP), day 1 [$F(1,72)=8.95$, $P<0.01$], day 2 [$F(1,72)=39.12$, $P<0.01$], (PCP vs clozapine), day 1 [$F(3,144)=1.67$, not significant], day 2 [$F(3,144)=3.45$, $P<0.05$]. In the case of haloperidol, ANOVA showed an effect for trials, day 1 [$F(3,144)=5.28$, $P<0.01$], day 2 [$F(3,144)=1.87$, not significant; an effect for treatment (control vs PCP), day 1 [$F(1,72)=11.36$, $P<0.01$], day 2[$F1$, $72)=20.26$, $P<0.01$], (PCP vs haloperidol), day 1 [$F(3,144)=0.22$, not significant], day 2 [$F(3,144)=0.16$, not significant].

(Leysen *et al*., 1985; Reyntjens *et al*., 1986) as well as reducing extrapyramidal symptoms resulting from chronic treatment with haloperidol (Bersani *et al*., 1986). A compound with combined dopamine D_4 and 5-HT_2 receptor antagonist activity may be useful as a novel antipsychotic drug for the treatment of both the positive and negative symptoms of schizophrenia.

In situ hybridization studies have shown that mRNA for dopamine D_2 receptor is predominantly localized in the striatum, nucleus accumbens and olfactory tubercles, with lower levels in various cortical areas (Bouthenet *et al*., 1991; Giros *et al*., 1991). In contrast, the dopamine D_4 receptor is most highly concentrated in the cortical and limbic areas with lesser amounts in the striatum (Van Tol *et al*., 1991). The dopamine D_4 receptor blocking effects of NRA0045 and clozapine in the cortical limbic areas may contribute to their behavioural effects. In contrast, haloperidol and chlorpromazine blocked MAP-induced hyperactivity and MAP-induced stereotyped behaviour, which result from blockade of the dopamine D_2 receptors in the nucleus accumbens and striatum, respectively. Furthermore, the inhibitory effects of haloperidol and chlorpromazine, which have significant dopamine D_4 activity *in vitro* (Lahti *et al*., 1993), on MAP-induced locomotor hyperactivity may be related to block of dopamine D_4 receptors as well as dopamine D_2 receptors.

The electrophysiological study also indicated that NRA0045 and clozapine selectively attenuate MAP-induced inhibition of the neuronal activity of A10 dopamine neurones. Clozapine was more potent in reversing the effects of MAP on A10, compared to A9 dopamine neurones (Goldstein *et al*., 1993; Stockton & Rasmussen, 1996). Differential effects on the activity of A10 versus A9 dopamine neurones may play an important role in the efficacy and side effects of antipsychotics, respectively (Bunney, 1977; Chiodo & Bunney, 1983; White & Wang, 1983). In fact, haloperidol, a classical antipsychotic with a high incidence of extrapyramidal side effects, is more potent in reversing the effects of MAP on A9 versus A10 dopamine neurones. Administration of NRA0045 reversed the MAP-induced inhibition of A10 but not A9 dopamine neurones. Therefore, NRA0045 was more selective and potent in reversing the effects of MAP on A10 dopamine neurones than clozapine.

It is interesting to note that NRA0045 and clozapine (Stockton & Rasmussen, 1996) alone did not increase the baseline firing rate of A10 dopamine neurones, yet did so following MAP treatment. While the mechanism for this effect is unknown, one possibility is that MAP, by releasing dopamine throughout the brain, affects input to A10 (e.g., decreased activity of an inhibitory input, increased activity of an excitatory input), an activity which is unmasked by the addition of NRA0045 or clozapine.

Clozapine had a weaker effect on MAP-induced inhibition of A10 firing (ED_{50} 1.9 mg kg^{-1}, i.v.) than on MAP-induced locomotor hyperactivity (ED_{50} 0.3 mg kg^{-1}, i.p.). This finding is in keeping with that of previous studies (Kehne *et al*., 1996a; Stockton & Rasmussen, 1996). Although the reason for this is unknown, one possibility is that there are many factors that could contribute to a reduction of MAP-induced locomotor

hyperactivity, including dopamine D_2, dopamine D_4, 5-HT_{2A} and α_1-adrenoceptor antagonistic action of a compound.

Studies of PPI in rats provide a valuable model for testing hypotheses on neural substrates of deficient sensory gating in schizophrenic patients (Geyer *et al*., 1990; Swerdlow *et al*., 1991), as well as providing a useful preclinical screening method (Geyer *et al*., 1990; Swerdlow *et al*., 1994). The findings that apomorphine-induced loss of PPI is reversed by clozapine and haloperidol suggest that this assay is sensitive to clinically effective antipsychotics. As disruption of PPI by apomorphine is also reversed by NRA0045, NRA0045 might be expected to have antipsychotic actions in man.

The potential of clozapine to reverse the decrease in PPI deficit induced by apomorphine follows an inverted-U shaped dose-response curve. This finding is in keeping with that of previous studies (Swerdlow *et al*., 1991). The biphasic dose-response properties might be ascribed to the activity of clozapine within non-dopaminergic transmitter systems.

Clozapine and haloperidol but not NRA0045 significantly decreased the apomorphine-induced potentiated startle amplitude. Since PPI was calculated by use of a percentage score, such a decrease in startle amplitude would not be expected to increase artificially the level of PPI; if anything, a possible 'floor' effect might be expected artificially to decrease the observed PPI, which is opposite to the effect noted with clozapine and haloperidol.

Ritanserin, a 5-HT_2 receptor antagonist, does not significantly impair apomorphine-induced loss of PPI (Geyer & Braff, 1990). In contrast, MDL 100,907, a selective 5-HT_{2A} antagonist, antagonized the disruptions of PPI produced by the 5-HT releasing agents fenfluramine and 3,4-methylenedioxymethamphetamine (MDMA) (Kehne *et al*., 1996b; Padich *et al*., 1996). The direct acting 5-$HT_{2A/2C}$ receptor agonist/hallucinogen (+)1-4-iodo-2.5-dimethoxyphenyl-2-aminopropane (DOI) consistently disrupted PPI, and this effect was blocked by MDL 100,907 (Kehne *et al*., 1996b; Padich *et al*., 1996). Thus, NRA0045 may be effective in central nervous system disorders in which an excessive 5-HT_{2A} receptor tone disrupts sensory gating processes.

PCP psychosis may provide a model for negative and positive symptoms of schizophrenia (Javitt & Zukin, 1991; Ogawa *et al*., 1994a;b; Okuyama *et al*., 1995). Administration of PCP to schizophrenic patients leads to exacerbation of thought and increases in assertiveness and hostility (Javitt & Zukin, 1991). It should be noted that the water maze task can detect a change in behaviour in the acquisition of the spatial proficiency task. In the present study, a low dose of PCP produced prolonged swimming latency in a water maze task and this was shortened by administration of NRA0045 and clozapine, but not by haloperidol, on day 2. Since the doses of all the compounds administered were based on the range that does not alter locomotor activity or interfere with ability to perform required tasks, it was considered that the effects of haloperidol were not influenced by sedation resulting from a dopamine D_2 receptor antagonistic action.

Although classical antipsychotics improve positive symptoms in schizophrenia (Hirschowitz *et al*., 1991; Reynolds, 1992; Chouinard *et al*., 1993), they do not improve negative symptoms such as dysfunction of cognition (Boyer *et al*., 1990; Levinson, 1991). In contrast, the greater improvement in negative symptoms seen in patients on clozapine compared with classical antipsychotics may be associated with the greater improvement in positive symptoms and fewer extrapyramidal symptoms (Wagstaff & Bryson, 1995). Moreover, in contrast to classical antipsychotics, clozapine improves cognitive function in patients with schizophrenia (including treatment-resistant schizophrenia), especially attention, verbal fluency and retrieval from reference memory (Wagstaff & Bryson, 1995).

In summary, NRA0045 is a potent ligand for dopamine D_4, 5-HT_2 receptors and α_1-adrenoceptors (Okuyama *et al*., 1996). The pharmacological profile suggests that NRA0045 may have unique antipsychotic activity without the probability of motor side effects typical of other antipsychotics.

We thank M. Ohara for helpful comments.

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(Received November 4, 1996
Revised February 28, 1997
Accepted March 3, 1997)