

Neurotensin Receptor Antagonist SR 142948A Alters Fos Expression and Extrapyramidal Side Effect Profile of Typical and Atypical Antipsychotic Drugs

Elisabeth B Binder^{1,2}, Becky Kinkead¹, Michael J Owens¹ and Charles B Nemeroff^{*1}

¹Laboratory of Neuropsychopharmacology, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA

Antipsychotic drugs (APDs) have previously been shown to alter Fos expression in a regionally specific manner. Increases in Fos expression in the nucleus accumbens (NAcc) are common to all clinically effective APDs. In contrast, APD-induced Fos expression increases in the caudate-putamen (CPU) and prefrontal cortex (PFC) are associated with the extrapyramidal side effect liability of typical APDs or the effectiveness against negative symptoms of atypical APDs, respectively. Considerable evidence suggests that the neuropeptide neurotensin (NT) mediates some of the effects of APDs. To determine whether NT neurotransmission is also involved in APD-induced Fos expression in brain regions relevant for therapeutic efficacy, the NT receptor antagonist SR 142948A (10 or 100 µg/kg i.p.) was coadministered with APDs (haloperidol (2.0 mg/kg s.c.), olanzapine (5 mg/kg i.p.), or clozapine (20 mg/kg s.c.)). Fos expression was evaluated in the PFC, NAcc shell, dorsomedial, and dorsolateral CPU and the lateral septum. SR 142948A attenuated haloperidol-induced Fos expression in the CPU but, in contrast, increased olanzapine-induced Fos expression in this brain region. The effects of the NT receptor antagonist were paralleled by its effects on catalepsy in olanzapine—but not haloperidol—treated animals.

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INTRODUCTION

Schizophrenia is a devastating psychiatric disease with a worldwide prevalence of approximately 1%. Antipsychotic drugs (APDs) have repeatedly been shown to be effective in reducing many of the cardinal symptoms of schizophrenia, and yet the underlying neurochemical actions responsible for their clinical efficacy remain poorly understood. Mapping of specific APD-induced changes in immediate early gene expression has been used to identify brain regions and neurotransmitter systems involved in mediating the action of APDs. To date, all clinically effective APDs increase the expression of the immediate early gene Fos in the nucleus accumbens (NAcc) shell (Deutch *et al*, 1992; Fibiger, 1994; Robertson and Fibiger, 1992). Increased Fos

expression in this brain region is thus considered to be predictive of antipsychotic activity. In contrast, only typical (eg haloperidol) but not atypical (eg clozapine and olanzapine) APDs increase Fos in the dorsolateral caudate/putamen (CPU) (Deutch *et al*, 1992; Fibiger, 1994; Robertson and Fibiger, 1992), and this has been attributed to the typical APD propensity for eliciting extrapyramidal side effects (EPSEs). Additionally, atypical APDs with purported superior efficacy against negative symptoms of schizophrenia (eg amotivation and anhedonia) increase Fos in the medial prefrontal cortex (PFC), specifically in the deep layers of the infralimbic and ventral prelimbic cortices (Deutch, 1994; Fibiger, 1994; Merchant *et al*, 1996; Robertson and Fibiger, 1996). APDs have also been reported to increase Fos expression in other, mostly limbic brain regions, with increases in the lateral septum being the most consistent finding (Cohen and Wan, 1996; Deutch *et al*, 1995; Sebens *et al*, 1995). Manipulating APD-induced Fos expression could therefore serve as an indicator as to which neurotransmitter pathways are involved in the specific effects of APDs.

A concatenation of data points to the fact that the tridecapeptide neurotensin (NT) may be involved in the therapeutic efficacy and side effect profile of APDs (Binder *et al*, 2001b; Kinkead *et al*, 1999). Typical and atypical APDs

*Correspondence: Dr CB Nemeroff, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Suite 4000 WMRB, 1639 Pierce Dr, Atlanta, GA 30322, USA, Tel +1 404 727 8382, Fax +1 404 727 3233, E-mail: cnemero@emory.edu

²Current address: Max-Planck Institute of Psychiatry, Kraepelinstr. 10, 80804 Munich, Germany.

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increase NT/Neuromedin N (NT/NN) mRNA expression, NT tissue concentrations and NT release in the NAcc, an effect seen following both acute and chronic drug administration (Huang and Hanson, 1997; Kilts *et al*, 1988; Kinkead *et al*, 2000; Merchant *et al*, 1992; Radke *et al*, 1989, 1998). Only typical APDs increase NT/NN mRNA expression, NT tissue concentrations and NT release in the CPu. There is therefore a striking overlap between APD-induced Fos expression and APD-induced NT release. Moreover, NT receptor activation has been shown to induce Fos expression both *in vivo* and *in vitro* (Ehlers *et al*, 1998; Lambert *et al*, 1996; Portier *et al*, 1998).

Increased NT neurotransmission following APD administration may therefore contribute to some extent to the observed APD-induced increases in Fos expression and by extension the therapeutic efficacy and side effect profile of these drugs. Two recent studies have confirmed that NT neurotransmission partly mediates haloperidol-induced Fos expression in the CPu, but not in the NAcc (Fadel *et al*, 2001; Dobner *et al*, 2001). This was shown using the NT receptor antagonist SR 48692 and transgenic mice lacking a functional NT/NN gene, respectively. Interestingly, even though NT-deficient mice showed an attenuated Fos response to haloperidol, the catalepsy-inducing effects of the drug remained unchanged in these animals.

In the following set of experiments we addressed two main questions. First, is NT neurotransmission involved in the induction of Fos-expression in limbic brain regions (NAcc, PFC, LS) thought to be associated with the therapeutic efficacy of APDs? Our group has shown that NT-neurotransmission mediates the effects of typical as well as atypical APDs on sensorimotor gating in rats (Binder *et al*, 2001a). It has been proposed that the restoration of pre-existing deficits in sensorimotor gating in schizophrenic patients may be an important factor for the therapeutic efficacy of these drugs (Braff and Geyer, 1990; Swerdlow and Geyer, 1998). The assessment of changes in APD-induced Fos-expression following administration of the NT receptor antagonist may help to identify which brain regions are involved in these behavioral effects. To test whether NT neurotransmission is involved in the induction of Fos-expression in limbic brain regions, we examined the effects of the NT receptor antagonist SR 142948A at two dose levels on Fos expression induced by typical (haloperidol) and atypical (clozapine and olanzapine) APDs in the dorsomedial and dorsolateral CPu, NAcc shell, PFC, and LS.

Second, does the dissociation of APD-induced Fos expression and catalepsy observed in transgenic mice lacking the NT/NN gene (Dobner *et al*, 2001) reflect the physiology in intact animals or is it due to developmental adaptations in NT/NN knockout mice? To that aim, we examined the effect of the NT receptor antagonist SR 142948A on haloperidol-induced catalepsy and on cataleptic behavior in clozapine and olanzapine-treated rats, at doses that do not induce catalepsy by themselves.

METHODS

Animals and Housing

Adult male Sprague-Dawley rats (300–400 g, Charles River Laboratories, Raleigh, NC) were housed three per cage on

reverse dark–light cycle (lights on 2200, lights off 1000). Food and water were available *ad libidum*. All animal protocols were approved by The Emory University Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Drug Administration

The NT receptor antagonist SR 142948A (2-[[5-(2,6-dimethoxyphenyl)-1-(4-(N-(3-dimethylaminopropyl)-N-methylcarbamoyl)-2-isopropylphenyl)-1H-pyrazole-3-carbonyl]amino]adamantane-2-carboxylic acid, hydrochloride) was a generous gift of Sanofi Recherche (Toulouse, France). SR 142948A was suspended in several drops of Tween 20 and brought to volume with 0.9% NaCl (drug vehicle = 0.9% NaCl plus several drops of Tween 20). Haloperidol (Sigma Chemical Co., St Louis, MO) was dissolved in 0.3% tartaric acid (drug vehicle), clozapine (Sandoz Pharmaceuticals) and olanzapine (Eli Lilly Co.) in 0.3% tartaric acid with a few drops of glacial acetic acid (drug vehicle). All drugs were administered in a fixed volume of 1.0 ml/kg body weight. Haloperidol and clozapine were administered subcutaneously (s.c.) and SR 142948A and olanzapine were administered intraperitoneally (i.p.).

Immunohistochemistry-Drug Administration and Brain Preparation

Drug treatments were all conducted in the dark phase between 1100–1600 on six experimental days. Each treatment group consisted of 13–15 animals. SR 142948A (10 and 100 µg/kg) or its vehicle was administered 1 h before the administration of haloperidol (2.0 mg/kg s.c.), olanzapine (5 mg/kg i.p.), clozapine (20 mg/kg s.c.), or their respective vehicles. At 2 h after APD administration animals used for Fos immunohistochemistry were deeply anesthetized with Euthanasia 5 solution (E. Schein Inc., Port Washington, NY, 1.0 ml/kg) and immediately perfused transcardially at 4°C with saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.6). Each brain was removed immediately after perfusion and placed in fresh 4% paraformaldehyde for 24 h at 4°C on a rotating shaker. Brains were then switched to a 20% sucrose solution in 0.1 M sodium phosphate buffer and left on the rotating shaker at 4°C for another 48 h. Brains were then rinsed, patted dry, frozen and kept at –70°C until further use.

Fos Immunohistochemistry

Brains were sectioned coronally at 35 µm on a freezing microtome and slices were collected into cryoprotectant solution (50% 0.05 M sodium phosphate buffer, 30% ethylene glycol, 20% glycerol) and stored at –20°C until use. All steps of the immunohistochemistry were carried out on a rotating shaker table. Brain slices were rinsed (4 × 15 min) in 0.05 M potassium phosphate-buffered saline (KPBS), pH 7.2 and then incubated for 20 min in methanol containing 1% hydrogen peroxide to block endogenous peroxidase activity. Brain sections were then transferred into KPBS containing 4% normal goat serum, 1% bovine

serum albumin (BSA) and 0.4% Triton X-100 and incubated for 1 h. Sections were subsequently incubated at 4°C for 72 h with the primary rabbit Fos antibody (Oncogene Research Products) diluted to 1:20 000 in KPBS containing 1% normal goat serum, 1% BSA, and 0.4% Triton X-100. This rabbit polyclonal Fos antiserum was raised against residues 4–17 of human Fos and recognizes c-fos but not Fos-related antigens. Sections were then rinsed (5 × 15 min) in KPBS containing 0.02% Triton X-100 and 0.25% BSA, and incubated with a biotinylated goat-antirabbit secondary antiserum (1:1000 dilution, Vector Laboratories) for 90 min in KPBS containing 0.02% Triton X-100 and 1% BSA. Brain slices were then washed with KPBS containing 0.25% BSA and incubated for 90 min in KPBS containing 1% BSA and 0.25% of the avidin-biotinylated horseradish peroxidase (Vectastain AbC Elite Kit, Vector Laboratories). Sections were then rinsed in KPBS (2 × 10 min) and 0.175 M sodium acetate, pH 7.2 (2 × 10 min). The peroxidase conjugated antibody was visualized using a nickel (II) sulfate intensification of the chromagen 3,3-diaminobenzidine tetrachloride (DAB) staining. Sections were then washed in 0.175 M sodium acetate (10 min) and KPBS (10 min) and mounted on Superfrost™-plus coated slides, dried for 48 h, counterstained with methyl-green, and coverslipped with DPX™ mountant.

Counting of Labeled Cells and Data Analysis

The number of Fos-like immunoreactive neurons in the prelimbic/infralimbic cortex, NAcc shell, dorsomedial and dorsolateral CPU, and lateral septum (according to the Paxinos and Watson, 1986) in both hemispheres was determined using an image analysis system equipped with Image 1.60 software (developed at the US National Institutes of Health and available on the internet at: <http://rsb.info.nih.gov/nih-image>) (see Figure 1). Due to tissue loss during brain preparation, the effective *n* per group/per brain region varies by up to three animals, leaving an *n* of 10–13 animals per group. An image was relayed from a light microscope (100 × magnification) to a computer screen. Once digitized, the contrast was set such that only cell nuclei identified by the observer as Fos-positive in the microscope were counted as positive. Constant density threshold and target acceptance criteria were used to quantify Fos-positive neurons. For all brain regions, Fos-positive neurons were counted in one or two distinct 600 × 400 μm² areas in each hemisphere (see Figure 1). The number of Fos-positive neurons is expressed as the average number of positive neurons/mm².

Catalepsy

Catalepsy was measured using the wood block test as well as the bar test in two separate sets of male Sprague–Dawley rats. Rats (*n* = 6–7/group for the wood block test and 5–10/group for the bar test) received a single injection of SR 142948A (100 μg/kg i.p.) or vehicle 1 h before administration of haloperidol (2.0 mg/kg s.c.), olanzapine (5.0 mg/kg i.p.), clozapine (20.0 mg/kg s.c.), or vehicle. For the wood block test, a rectangular wooden block of 6 cm height was used (Ellenbroek *et al.*, 1987). Rats were placed with both forelimbs on the edge of the block and latency to move

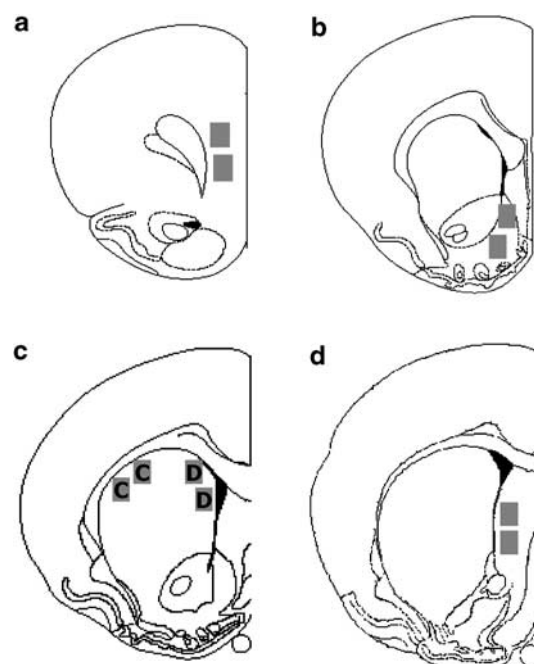


Figure 1 Brain regions in which Fos-like immunoreactivity was determined (according to Paxinos rat brain atlas). (a) prelimbic/infralimbic cortex (+ 3.20 mm of bregma), (b) nucleus accumbens shell (+ 1.70 mm of bregma), (c) dorsolateral (C) and dorsomedial (D) striatum (+ 1.00 mm of bregma) and (d) lateral septum (+ 0.20 mm of bregma).

one foot off the block was measured (descent latency in seconds) 5, 15, 30, and 60 min after administration of the APD or its vehicle. For the bar test, a plastic bar fixed in an animal cage at a height of 6 cm was used and rats were placed with both forelimbs on the bar. Latency to move one foot off the bar was measured in a similar manner than for the wood block test.

Statistical Analysis

There were no significant differences among the control animals treated with different vehicles (0.3% tartaric acid or 0.3% tartaric acid with a few drops of glacial acetic acid), so control animals were combined for further analysis. Data were analyzed by two-way analysis of variance (ANOVA) with APD treatment × SR 142948A dose as factors. To account for possible effects of the experimental day on basal and drug-induced changes in Fos expression, the experimental day was added as covariate in these analyses. The cataleptic effect of a combination of compounds was expressed as the area under the curve for the descent latency values measured at four time points (5, 15, 30, and 60 min after injection). For olanzapine effects, a one-way ANOVA for each time point (vehicle/vehicle, vehicle/olanzapine, and SR142948A/olanzapine as groups) was used to determine differences in the onset of cataleptic behavior. The Fischer LSD test was used as multiple comparison procedure for *post hoc* analysis. *p* < 0.05 was considered the limit for statistical significance.

RESULTS

Effects of SR 142948A on APD-Induced Fos Expression

Prefrontal cortex. Two-way ANOVA revealed a significant APD treatment effect ($F_{3,323} = 50.3$, $p < 0.001$) and of the covariate experimental day ($F_{1,323} = 12.2$, $p = 0.001$), but no significant SR 142948A dose effect or interaction between the two factors. *Post hoc* analysis showed that only clozapine ($p < 0.001$) and olanzapine ($p < 0.001$), but not haloperidol, significantly increased the number of Fos positive neurons in the PFC (Figure 2). The effects of clozapine and olanzapine were not different from each other. The NTR antagonist had no significant effect in vehicle or APD treated animals.

NAcc Shell Subdivision

Two-way ANOVA revealed a significant APD treatment effect ($p < 0.001$; $F_{3,425} = 174.2$) and of the covariate experimental day ($p < 0.001$; $F_{1,425} = 28.4$), but no significant SR 142948A dose effect or interaction between the two factors. *Post hoc* analysis showed that haloperidol, olanzapine, and clozapine significantly ($p < 0.001$) increased the number of Fos positive neurons in the NAcc compared to vehicle (Figure 3). Although olanzapine and clozapine, and clozapine and haloperidol-induced Fos increases were not significantly different from each other, olanzapine had a significantly larger effect than haloperidol ($p = 0.01$). SR 142948A (10 or 100 $\mu\text{g/kg}$) had no significant effect on Fos expression in vehicle, haloperidol, or olanzapine treated animals.

Dorsomedial CPu

In the dorsomedial CPu, two-way ANOVA revealed a significant effect of APD treatment ($p < 0.001$; $F_{3,434} = 142.6$),

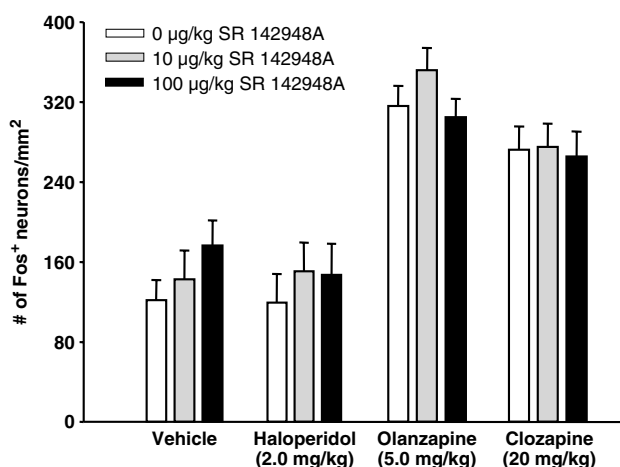


Figure 2 Effects of the neurotensin receptor antagonist SR 142948A on antipsychotic drug-induced Fos expression in the prefrontal cortex. SR 142948A (0, 10, or 100 $\mu\text{g/kg}$ i.p.) was administered 1 h before administration of antipsychotic drug. Fos-like immunoreactivity was measured 2 h after antipsychotic drug administration. Olanzapine and clozapine, but not haloperidol, significantly increase Fos expression from the vehicle/vehicle group independent of pretreatment with SR 142948A; $p < 0.001$ for all groups.

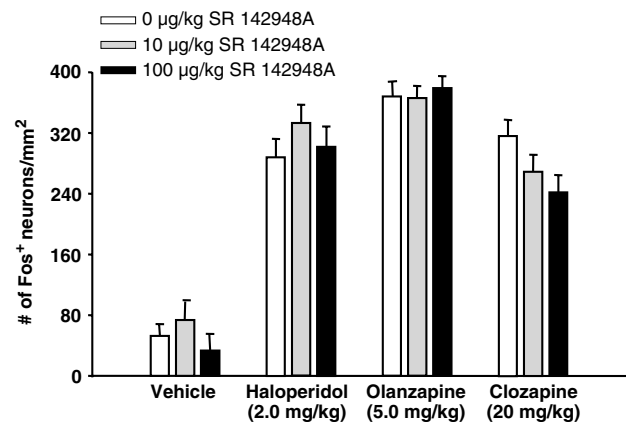


Figure 3 Effects of the neurotensin receptor antagonist SR 142948A on antipsychotic drug-induced Fos expression in the shell of the nucleus accumbens. SR 142948A (0, 10, or 100 $\mu\text{g/kg}$ i.p.) was administered 1 h before administration of antipsychotic drug. Fos-like immunoreactivity was measured 2 h after antipsychotic drug administration. All three antipsychotic drugs significantly increase Fos expression from the vehicle/vehicle group independent of pretreatment with SR 142948A; $p < 0.001$ for all groups.

no effect of SR 142948A dose, but a significant interaction between the two factors ($p < 0.05$; $F_{6,434} = 2.5$) (Figure 4a). There was no significant effect of the covariate experimental day. *Post hoc* analysis showed that all APDs significantly increased the number of Fos positive neurons in the dorsomedial CPu ($p < 0.001$ for haloperidol and olanzapine and $p < 0.005$ for clozapine). Increases in Fos expression following haloperidol were significantly larger than the ones observed with olanzapine and clozapine, $p < 0.005$ and < 0.001 respectively. The effects of olanzapine were also significantly larger ($p < 0.001$) than the effects of clozapine. Pretreatment with the NTR antagonist significantly increased olanzapine-induced Fos expression at both doses (38% with $p < 0.005$ at 10 $\mu\text{g/kg}$ and 57% with $p < 0.001$ at 100 $\mu\text{g/kg}$ of SR 142948A of Fos-positive neurons induced by olanzapine alone). SR 142948A had no significant effects in vehicle, haloperidol, and clozapine treated animals.

Dorsolateral CPu

Two-way ANOVA revealed a significant APD treatment effect ($p < 0.001$; $F_{3,429} = 159.0$), a significant SR 142948A dose effect ($p < 0.05$; $F_{2,429} = 3.4$) and a significant interaction between the two factors ($p < 0.001$; $F_{6,429} = 4.2$) (Figure 4b). There was no significant effect of the covariate experimental day. *Post hoc* analysis showed that all APDs significantly increased the number of Fos positive neurons in the dorsolateral CPu ($p < 0.001$ for haloperidol and olanzapine and $p < 0.05$ for clozapine). Increases in Fos expression following haloperidol, however, were markedly and significantly higher ($p < 0.001$) than the ones observed with the two atypical APDs; 469% higher than olanzapine- and 977% higher than clozapine-induced increases in Fos expression in this brain region. The effects of olanzapine on Fos expression in this brain region were also significantly higher ($p < 0.05$) than the effects of clozapine. Pretreatment with the NTR antagonist significantly decreased ($p < 0.001$)

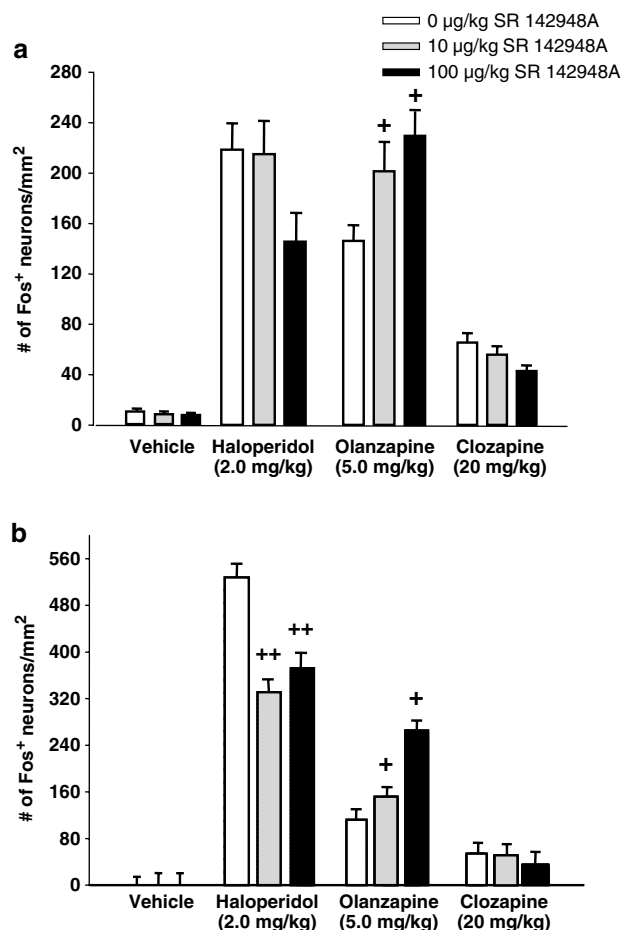


Figure 4 Effects of the neurotensin receptor antagonist SR 142948A on antipsychotic drug-induced Fos expression in the dorsomedial (a) and dorsolateral (b) caudate/putamen. SR 142948A (0, 10, or 100 µg/kg i.p.) was administered 1 h before administration of antipsychotic drug. Fos-like immunoreactivity was measured 2 h after antipsychotic drug administration. (a) All three antipsychotic drugs significantly increase Fos expression from the vehicle/vehicle group independent of pretreatment with SR 142948A; $p < 0.005$ for all groups. + $p < 0.05$ compared to olanzapine alone. (b) Haloperidol and olanzapine significantly increase Fos expression from the vehicle/vehicle group independent of pretreatment with SR 142948A; $p < 0.001$ for all groups, for clozapine, only the vehicle/clozapine group is significantly different from vehicle/vehicle; $p < 0.05$. + $p < 0.05$ compared to vehicle/haloperidol; ++ $p < 0.05$ compared to vehicle/olanzapine.

haloperidol-induced Fos expression by about a third (37% decrease at 10 µg/kg and 29% decrease at 100 µg/kg of SR 142948A). In contrast, SR 142948A significantly increased ($p < 0.001$) olanzapine-induced Fos expression in this brain region. The number of Fos positive neurons was 35 and 135% higher in animals pretreated with 10 or 100 µg/kg of SR 142948A, respectively, than in animals that received only olanzapine. The NTR antagonist did not significantly affect Fos immunoreactivity in vehicle and clozapine treated animals.

Lateral Septum

Two-way ANOVA revealed a significant APD treatment effect ($p < 0.001$; $F_{3,394} = 112.2$) and of the covariate experimental day ($p < 0.001$; $F_{1,394} = 12.5$), but no significant SR

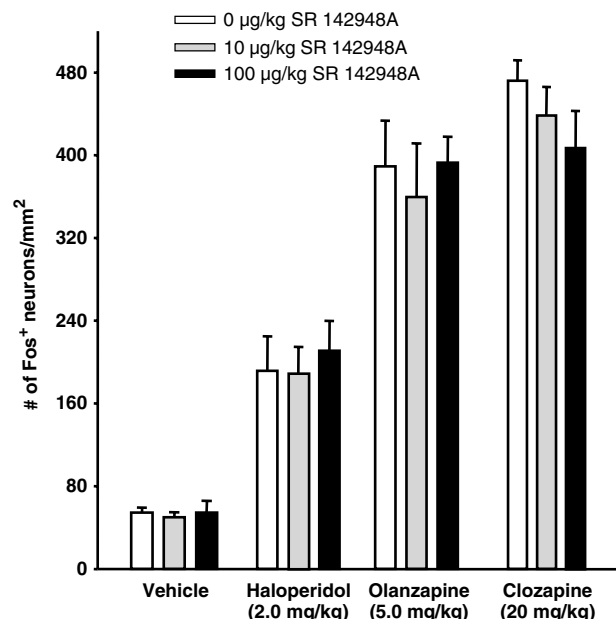


Figure 5 Effects of the neurotensin receptor antagonist SR 142948A on antipsychotic drug-induced Fos expression in the lateral septum. SR 142948A (0, 10, or 100 µg/kg i.p.) was administered 1 h before administration of antipsychotic drug. Fos-like immunoreactivity was measured 2 h after antipsychotic drug administration. All three antipsychotic drugs significantly increase Fos expression from the vehicle/vehicle group independent of pretreatment with SR 142948A ($p < 0.001$ for olanzapine and clozapine and $p < 0.05$ for haloperidol).

142948A dose effect or interaction between the two factors (Figure 5). *Post hoc* analysis showed that all APDs significantly increased the number of Fos positive neurons in the lateral septum ($p < 0.001$ for olanzapine and clozapine and $p = 0.04$ for haloperidol). Olanzapine and clozapine-induced Fos expression was significantly higher ($p < 0.001$) than haloperidol-induced Fos expression in this brain region. The NTR antagonist had no significant effects in vehicle or APD treated animals.

Effects of SR 142948A on APD-Induced Catalepsy

Wood block test. Two-way ANOVA of the area under the curve of the descent latencies over all four time points revealed a significant APD effect ($p < 0.001$, $F_{3,50} = 24.9$), but no significant effect of SR142948A ($p = 0.12$) or interaction between the two factors ($p = 0.41$). *Post hoc* analysis showed that only haloperidol ($p < 0.001$) but not clozapine, olanzapine, or SR142948A led to a significant increase in descent latency (catalepsy) compared to vehicle treated animals. The NT receptor antagonist had no significant effect on haloperidol- or clozapine-induced catalepsy, but significantly increased descent latency following olanzapine administration ($p < 0.05$), so that in the SR142948A/olanzapine group catalepsy was significantly increased compared to vehicle treated animals ($p = 0.01$) (see Figure 6a–d). When the four time points were analyzed separately, the SR142948A/olanzapine groups had significantly higher catalepsy scores ($p < 0.05$) than vehicle treated animals at the 15 and 30 min time-point while olanzapine treated animals did not differ from vehicle treated animals at any time point.

Bar test. Two-way ANOVA of the area under the curve of the descent latencies over all four time points revealed a significant APD effect ($p < 0.001$, $F_{3,55} = 89.02$), but no significant effect of SR142948A ($p = 0.71$) or interaction

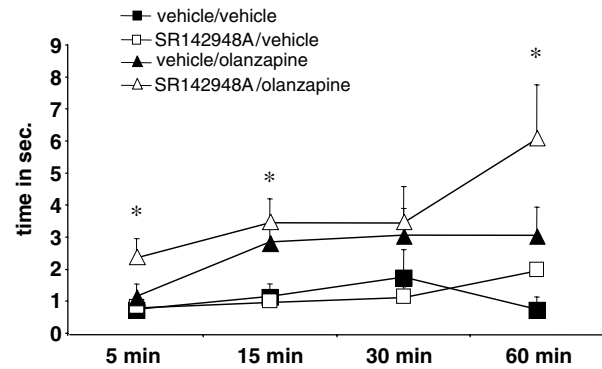
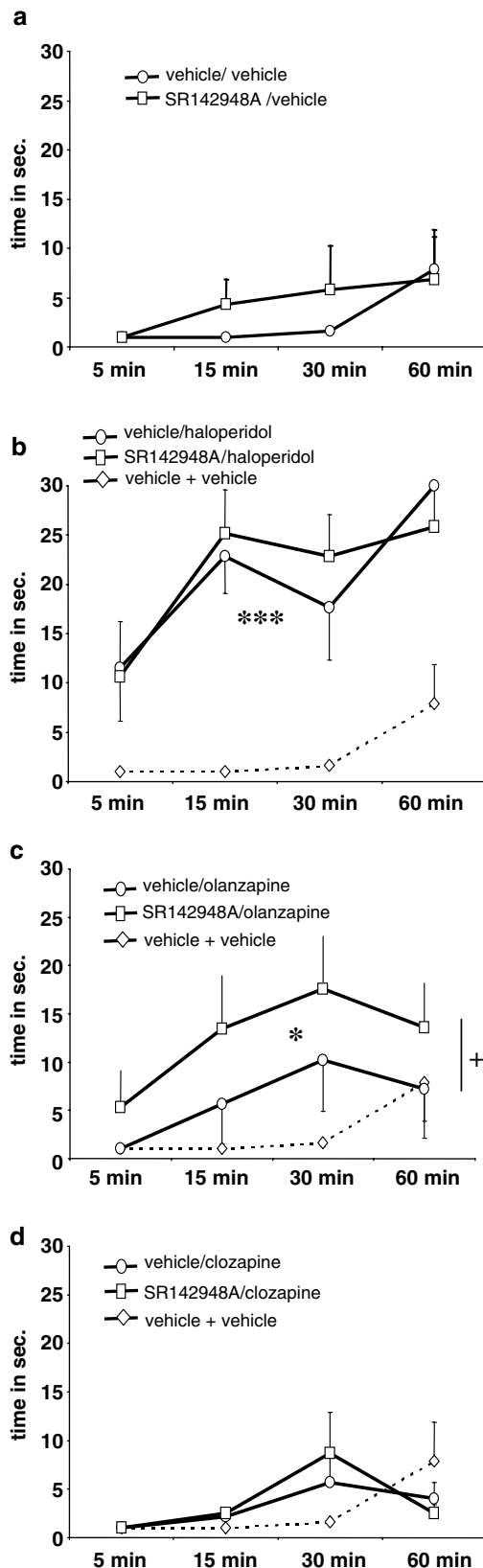


Figure 7 Effects of the neurotensin receptor antagonist SR 142948A on olanzapine-induced catalepsy in the bar test. SR 142948A (0 or 100 $\mu\text{g/kg}$ i.p.) was administered 1 h before administration of olanzapine. * $p < 0.05$ for difference from vehicle/vehicle group at each time point.

between the two factors ($p = 0.48$). *Post hoc* analysis showed that only haloperidol ($p < 0.001$) but not clozapine, olanzapine, or SR 142948A led to a significant increase in descent latency (catalepsy) compared to vehicle treated animals. The NT receptor antagonist had no significant effect on haloperidol- or clozapine-induced catalepsy, but showed a trend to increase descent latency following olanzapine administration ($p < 0.1$). When the four time points were analyzed separately, the SR142948A/olanzapine groups had significantly higher catalepsy scores ($p < 0.05$) than vehicle treated animals at the 5, 15, and 60 min time-point while olanzapine treated animals did not differ from vehicle treated animals at any time point (see Figure 7).

DISCUSSION

Our results confirm and extend the previous findings of Fadel *et al* (2001) and Dobner *et al* (2001) concerning the involvement of NT neurotransmission in APD-induced Fos expression in the CPu. We did not, however, observe any common effects of the NT receptor antagonist on APD-induced Fos-expression in limbic brain regions. Disruption of NT neurotransmission using the NT receptor antagonist SR 142948A changed the regional pattern of APD-induced Fos expression for the tested APDs, but no unitary effect was observed, which is in contrast to the effects of the NT receptor antagonist on APD-induced effects in sensorimotor gating (Binder *et al*, 2001a).

In agreement with the findings reported for SR 48692, pretreatment with SR 142948A reduced haloperidol-induced Fos expression in the dorsolateral CPu (Fadel *et al*, 2001). SR 48692 is a NT receptor antagonist that is relatively selective for NT₁, the receptor subtype that has so far been

Figure 6 Effects of the neurotensin receptor antagonist SR 142948A on antipsychotic drug-induced catalepsy in the wood block test. SR 142948A (0 or 100 $\mu\text{g/kg}$ i.p.) was administered 1 h before administration of antipsychotic drug (a: vehicle, b: haloperidol, c: olanzapine, d: clozapine). Stars indicate that both treatment groups are significantly different from vehicle/vehicle. * $p < 0.05$ compared to vehicle/vehicle. *** $p < 0.001$ compared to vehicle/vehicle. + $p < 0.05$ SR142948A/olanzapine compared to vehicle/olanzapine.

implicated in the pre- and postsynaptic effects of NT on the mesolimbic and nigrostriatal dopamine system (Gully *et al*, 1993; Tyler *et al*, 1998; Vincent *et al*, 1999). NT₂, another G-protein coupled NT receptor, is also widely expressed in the brain including dopaminergic nuclei (the ventral tegmental area and substantia nigra) and the NAcc and its activation could be involved in APD-induced Fos expression in some brain regions (Walker *et al*, 1998). SR 142948A has equal affinity for NT₁ and NT₂ in the nanomolar range and several studies indicate that SR 48692 and SR 142948A may also distinguish between different pharmacological NT receptor subtypes (Gully *et al*, 1997; Vincent *et al*, 1999). We chose to use SR 142948A, as it cannot be ruled out that effects of NT at NT₂ may also contribute to APD-induced Fos expression. The results of this study are similar to those reported for SR 48692 indicating that disruption of NT₁ receptor mediated transmission is crucial for the observed reduction in haloperidol-induced Fos expression in the CPU and that there is no additional contribution of NT₂. The documented enhanced NT release in this brain region following haloperidol administration (Huang and Hanson, 1997; Radke *et al*, 1998) may thus partially mediate haloperidol-induced Fos expression, by direct activation of NT₁ in striatal neurons (Ehlers *et al*, 1998; Lambert *et al*, 1996; Portier *et al*, 1998) or by acting on NT₁ receptors located on glutamatergic corticostriatal projections or dopaminergic projections (Boudin *et al*, 1996; Goedert *et al*, 1984).

Pretreatment with the NTR antagonist SR 142948A increased Fos expression in the dorsomedial and dorsolateral CPU in combination with olanzapine, but had no effect by itself in these brain regions. Olanzapine increases NT peptide concentrations and NT release in the NAcc but not the CPU (Kinkead *et al*, 1997; Radke *et al*, 1998). The effects of the NTR antagonist on olanzapine-induced Fos expression may therefore be mediated by antagonizing basal NT transmission in the CPU. Several groups have reported detectable, albeit low, basal NT release in this brain region (Radke *et al*, 1998; Wagstaff *et al*, 1996).

The NT receptor antagonist had no effect on clozapine-induced Fos expression in these brain regions. Disrupting NT neurotransmission thus has no common effect on APD-induced Fos expression in the dorsolateral CPU. This underlines the fact that NT serves as neuromodulator and can exert opposite effects depending on the level of activity of other neurotransmitter systems.

Both increased Fos expression and NT neurotransmission in the dorsolateral striatum have been linked to the EPSE liability of APDs (Adams *et al*, 1997; Decker *et al*, 1995; Shibata *et al*, 1987; Ward and Dorsa, 1999). NT receptor antagonism and a genetically disrupted NT neurotransmission both decrease haloperidol-induced Fos expression in the dorsolateral CPU but do not have an effect on haloperidol-induced catalepsy (Dobner *et al*, 2001). The fact that developmental adaptations in transgenic mice may be responsible for that discrepancy can be ruled out as we observed identical results with the NT receptor antagonist in wild-type animals.

We also examined the effects of disrupting NT neurotransmission on motor effects of the atypical APDs clozapine and olanzapine. Although clozapine does not induce catalepsy up to very high doses (100 mg/kg), olanzapine can induce catalepsy at doses over 10 mg/kg

(Ellenbroek, 2000). Such doses of olanzapine are several fold higher than those needed for behavioral effects thought to reflect therapeutic efficacy, such as the prepulse inhibition of the acoustic startle reflex or latent inhibition (Ellenbroek, 2000). We observed that the NT receptor antagonist did not produce any effect on descent latency either by itself or in combination with clozapine. Pretreatment with SR 142948A, however, induced catalepsy in rats that were administered 5.0 mg/kg olanzapine, a dose that did not induce catalepsy by itself. This observation was made when using the wood-block as well as the bar test to measure catalepsy (see Figures 6c and 7). The enhancement of catalepsy was paralleled by an increase in Fos expression in the dorsomedial and dorsolateral CPU. This molecular and behavioral concordance is contrary to the findings with haloperidol. As suggested above, the effects of the NT receptor antagonist on both olanzapine-induced Fos expression and catalepsy may stem from counteracting the neuromodulator activity of basal NT release in the CPU. Basal NT release may therefore be necessary for the favorable motor side effect profile of some atypical APDs.

Pretreatment with the NT receptor antagonist did not alter APD-induced Fos expression for any of the investigated compounds in the limbic brain regions, the NAcc, the PFC, or the LS. The common induction of Fos expression by APDs in the NAcc, CPU or PFC has been attributed to their common therapeutic or side effect profile. For a neurotransmitter system to be involved in a specific therapeutic aspect of APDs, one would expect to observe a consistent change in APD-induced Fos expression pattern following its disruption. In our experiments we did not observe any consistent influence of SR 142948A pretreatment on APD-induced Fos expression. In contrast, there is evidence for a common role of NT neurotransmission in the effects of both a typical and an atypical APD on behavioral tests for antipsychotic activity. SR 142948A was able to prevent haloperidol- and quetiapine-induced restoration of prepulse inhibition of the acoustic startle reflex in isolation reared rats (Binder *et al*, 2001a), and this is most likely mediated by the antagonism of NT neurotransmission in the NAcc (Feifel *et al*, 1997). This difference could be explained by the divergence in APD doses inducing sensorimotor gating effects (up to 20 times less) compared to those inducing Fos-expression. Nonetheless, our findings challenge the view that manipulating APD-induced Fos expression in the NAcc provides insight into the neurotransmitter systems involved in the therapeutic efficacy of APDs. Caution may thus be warranted in using regionally specific APD-induced Fos expression as a screening tool for antipsychotic activity.

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