Journal of Neuroscience Research 60:783–794 (2000)

Brain-Derived Neurotrophic Factor and Tyrosine Kinase Receptor TrkB in Rat Brain Are Significantly Altered After Haloperidol and Risperidone Administration

Francesco Angelucci,[1,2] Aleksander A. Mathé,[1] and Luigi Aloe[2]*

[1]Karolinska Institutet, Department of Clinical Neuroscience, St. Göran's Hospital, Stockholm, Sweden
[2]Institute of Neurobiology, Rome, Italy

The antipsychotics haloperidol and risperidone are widely used in the therapy of schizophrenia. The former drug mainly acts on the dopamine (DA) D_2 receptor whereas risperidone binds to both DA and serotonin (5HT) receptors, particularly in the neurons of striatal and limbic structures. Recent evidence suggests that neurotrophins might also be involved in antipsychotic action in the central nervous system (CNS). We have previously reported that haloperidol and risperidone significantly affect brain nerve growth factor (NGF) level suggesting that these drugs influence the turnover of endogenous growth factors. Brain-derived neurotrophic factor (BDNF) supports survival and differentiation of developing and mature brain DA neurons. We hypothesized that treatments with haloperidol or risperidone will affect synthesis/release of brain BDNF and tested this hypothesis by measuring BDNF and TrkB in rat brain regions after a 29-day-treatment with haloperidol or risperidone added to chow. Drug treatments had no effects on weight of brain regions. Chronic administration of these drugs, however, altered BDNF synthesis or release and expression of TrkB-immunoreactivity within the brain. Both haloperidol and risperidone significantly decreased BDNF concentrations in frontal cortex, occipital cortex and hippocampus and decreased or increased TrkB receptors in selected brain structures. Because BDNF can act on a variety of CNS neurons, it is reasonable to hypothesize that alteration of brain level of this neurotrophin could constitute one of the mechanisms of action of antipsychotic drugs. These observations also support the possibility that neurotrophic factors play a role in altered brain function in schizophrenic disorders. J. Neurosci. Res. 60:783–794, 2000.
© 2000 Wiley-Liss, Inc.

Key words: neurotrophins; nerve growth factor; dopamine; antipsychotic drugs

The therapeutic effects of antipsychotic drugs are believed to be due to their blockade of dopamine (DA) and serotonin (5HT) receptors in the central nervous system (CNS) (Joyce et al., 1997). A variety of studies have indicated that antipsychotic drugs block the activity of DA neurons localized particularly in the striatal and limbic systems (Reynold, 1997). Correlative structural and biochemical studies revealed that DA antagonist action occurs mostly through the blockade of DA-D_2 receptors (Gerlach, 1991). Additional evidence supporting the hypothesis that haloperidol and risperidone affect DA system is suggested by the findings that neurons of the substantia nigra pars reticulata, corpus striatum, the mesencephalic reticular formation, and the anterior cortex are functionally affected in the catalepsy induced by antipsychotic response (Yntema and Korf, 1987).

In addition to these effects on D_2 receptor, haloperidol affects neuronal plasticity (Kerns et al., 1992), neurotrophic-receptor expression (Alberch et al., 1991), and synthesis or release of neurotrophins (Alleva et al., 1996; Aloe et al., 1997). Indeed, there is considerable both in vitro and in vivo evidence that growth and functionality of DA neurons are regulated by brain-derived neurotrophic factor (BDNF) (Hyman et al., 1991), a member of the neurotrophin family, that includes NGF, neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) (Levi-Montalcini, 1987; Ebendal, 1992; Nawa et al., 1995). BDNF is more abundantly expressed in the brain than NGF (Hohon et al., 1990). The biological activity of BDNF is mediated by a low affinity receptor, called p75, and by a high affinity receptor, the TrkB that seems to play a crucial role in the survival and differentiation of developing and mature brain DA neurons (Ebendal, 1992; Benisty et al., 1998). Specifically, BDNF plays an important role in maintaining functional activity of DA mesencephalic neurons (Hyman et al., 1991; Spenger et al., 1995), cholinergic septal neurons (Alderson et al., 1990), cortical and hippocampal neurons (Ghohs et al., 1994), striatal neurons (Ventimiglia et al., 1995), and spinal motor neurons (Yan et al., 1994). BDNF also promotes survival and recovery of

Contract grant sponsor: Swedish Medical Research Council; Contract grant number: 10414; Contract grant sponsor: Italian National Research Council (CNR); Contract grant sponsor: Karolinska Institutet.

*Correspondence to: Dr. Luigi Aloe, Institute of Neurobiology, CNR, Viale C. Marx, 15/43, 00137, Rome, Italy. E-mail: aloe@in.rm.cnr.it

Received 25 October 1999; Revised 18 January 2000; Accepted 19 January 2000

© 2000 Wiley-Liss, Inc.

serotonergic neurons (Mamounas et al., 1995), reduces glutamate-induced neuronal damage in rat cortical cultures (Shimohama et al., 1993), and prevents dopaminergic neuronal damage (Nishio et al., 1998). Cumulatively, these observations point to a potential role of BDNF in functional activity of DA neurons during neurological insult (Lindwall et al., 1994). Thus, involvement of BDNF and NGF in stress and related affective illnesses (Aloe et al., 1994; Duman et al., 1995), epileptic response (Ernfors et al., 1991) and limbic seizures (Lindwall et al., 1992) has been reported. Moreover, BDNF exerts a prominent action on entorhinal cortex, a limbic region implicated in pathogenesis of schizophrenia (Weinberger and Lipska, 1995). Recent studies have also shown that basal expression of brain BDNF and TrkB mRNA are significantly affected by chronic electroconvulsive seizure and by administration of antidepressant drugs (Nibuya et al., 1995).

These observations led us to hypothesize that treatment with haloperidol and risperidone might interfere with functional activity of BDNF. To test this hypothesis we investigated the effect of chronic haloperidol and risperidone treatment on constitutive level of BDNF and expression of TrkB in the rat brain.

MATERIALS AND METHODS

Animals

Adult Male Wistar rats weighing 200–250 g were used. They were housed 4/cage at 21°C on a 12-hr light-dark cycle and allowed free access to food and water. All experimental procedures were approved by the Ethical Committee on Animal Protection and the animals were taken care of in line with the Karolinska Institute's Guidelines for Animal Care.

Antipsychotic Drugs

Because one of the study aims was to explore possible differences in the mechanism(s) of action of typical and atypical antipsychotics, a standard dose of haloperidol and two doses of risperidone were used. Thus, rat chow was supplemented with haloperidol (1.15 mg/100 g food), risperidone (1.15 or 2.3 mg/100 g food), or vehicle. These dosages were identical to those used in our previous experiments and resulting in adequate plasma and brain haloperidol and risperidone concentrations as blindly determined in Janssen Pharmaceutica Laboratories by High Performance Liquid Chromatography (HPLC) (see Results).

Drug Treatments

The rats were randomly divided into 4 groups, 15 animals in each group. Groups 1 to 3 received 1.15 mg haloperidol, 1.15 mg risperidone or 2.30 mg risperidone/100 g food pellet, whereas Group 4 received pellet to which only vehicle was added. Two doses of risperidone were used to better evaluate pharmacological properties of this more recent atypical drug. The treatments did not induce changes in body or brain weight (see Results).

BDNF Measurement by Enzyme-Linked Immunosorbent Assay (ELISA)

After 29 days of treatment, animals used for analysis of BDNF (n = 10/group) were sacrificed by decapitation starting around 10:00 AM. All brains were removed and dissected on ice into hypothalamus, hippocampus, striatum, frontal cortex and occipital cortex according to Glowinski and Iversen (1965), and stored at −80°C until neurochemical analysis. The brain tissues were homogenized with ultrasonication in extraction buffer containing 100 mM Tris-HCl, pH 7.2, 400 mM NaCl, 4 mM EDTA, 0.2 mM PMSF, 0.2 mM benzethonium chloride, 2 mM benzamidine, 40 U/ml aprotinin, 0.05% sodium azide, 2% BSA, 0.5% gelatin, and 0.2% Triton X-100 (Sigma Chemical, MO). The homogenates were centrifuged at 10,000 × *g* for 20 min, the supernatants were collected, and processed for quantification of endogenous BDNF using a two-site enzyme immunoassay kit (Promega, USA). Briefly, 96-well immunoplates (NUNC) were coated with 100 μl per well of monoclonal anti-mouse-BDNF antibody. After an overnight incubation at 4°C, the plates were washed three times with wash buffer and the samples were incubated in the coated wells (100 μl each) for 2 hr at room temperature with shaking. After an additional five washes the immobilized antigen was incubated with an anti-human BDNF antibody for 2 hr at room temperature with shaking. The plates were washed again with wash buffer, and then incubated with an anti-IgY HRP for 1 hr at room temperature. After another wash the plates were incubated with a TMB/Peroxidase substrate solution for 15 min. and phosphoric acid 1 M (100 μl/well) was added to the wells. The colorimetric reaction product was measured at 450 nm using a microplate reader (Dynatech MR 5000, Germany). BDNF concentrations were determined from the regression line for the BDNF standard (ranging from 7.8–500 pg/ml-purified mouse BDNF) incubated under similar conditions in each assay. As reported by the manufacture's instruction the sensitivity of the assay is about 15 pg/ml of BDNF and the cross-reactivity with other related neurotrophic factors (NGF, NT-3 and NT-4) is considered not relevant. BDNF concentration is expressed as pg of BDNF/mg of total proteins measured according to the methods described by Lowry et al., (1951). All assays were carried out in triplicate. This ELISA method is comparable to that used by our group for evaluation of NGF after antipsychotic treatment (Angelucci et al., 2000).

Immunocytochemistry

After 29 days of treatment animals (n = 5 for group) were deeply anaesthetized with Nembutal and perfused via aorta first with 0.1M PBS to remove circulating blood elements, and then with paraformaldehyde 4% in 0.1 M PBS to fix the brains. For immunocytochemistry, brains were left one day in paraformaldehyde, cryoprotected in 0.1 M PBS with 20% sucrose, and then coronally cut on a cryostat (20 μm in thickness) at the temperature of −18 ± 2°C. To quench the endogenous peroxidase activity, the brain sections were washed in 1 ml of PBS 0.1% triton X-100, 2% phenylhydrazine, 10% of goat serum for 45 min and then incubated overnight at 4°C with a rabbit polyclonal anti-TrkB (sc-12-G, Santa Cruz, CA), specific for BDNF high affinity receptor (TrkB gp145). As reported by the manufacturer, gp145 does not cross-react with the TrkB gp95, TrkA or TrkC. The sections were then washed 3 times in PBS 0.1% triton X-100, followed by incubation for 2 hr with a secondary antibody solution. This solution was prepared by adding one drop of biotinylated goat anti-rabbit IgG (Vectastain Kit, Vector Laboratories, CA), to 1 ml of PBS 0.1% triton

X-100, 5% of goat serum. The sections were then washed 3 times in PBS 0.1% triton X-100, and incubated for 2 hr in an avidin horseradish peroxidase solution (Vectastain Kit, Vector Laboratories). Sections were again washed 3 times in PBS 0.1% triton X-100 and reacted with a mixture of 15 mg 3,3′-diaminobenzidine (DAB) (Sigma Chemical, MO) dissolved in 10 ml of PBS 0.1% triton X-100, followed by 0.001% hydrogen peroxide solution added a drop at a time. Sections were washed several times in PBS, mounted, dehydrated and coverslipped. The specificity of the primary antibody was assessed by incubating tissue sections from drugs treated and vehicle treated rats with antibody preabsorbed with an excess of antigen (TrkB), or in absence of primary antibody.

Double Immunostaining

To assess whether dopaminergic neurons express BDNF receptors, sections of the ventral mesencephalon were double stained with the rabbit anti-TrkB (Santa Cruz, CA, see previous section) and a polyclonal goat anti-dopamine D_2 receptor (N-19, sc-7522, Santa Cruz, CA). This antibody does not cross-react with dopamine D_1-, D_3-, D_4-, D_5-receptors. The first procedure was identical to that used for localization of TrkB immunopositive cells alone (see above). Between the two immunostaining we used an avidin-biotin blocking step (SP-2006, Vector Laboratories) to avoid nonspecific cross-reactions. The second procedure was identical to the first regarding the sequence of the steps and time of incubations, but it differed for the composition of the secondary antibody solution [biotinylated rabbit anti-goat IgG (Vectastain Kit, Vector Laboratories)]. As first chromogen we used DAB-nickel ammonium sulfate for a black precipitate, and as the second chromogen DAB alone, that gives an orange-brown precipitate. Controls for double labeling included reversing the order of the primary antibodies, as well as omitting the first or second primary antibody (for further details see Goutan et al., 1998).

Topographic Localization and Evaluation of TrkB-Immunopositive Cells

To evaluate the distribution of TrkB-positive neurons comparable sections from each animal were counted unilaterally with a Zeiss Axiophot microscope equipped with a Vidas Kontron images analysis system. Only intact neurons with a rounded nucleus were included and the mean values from all sections of a group were calculated and used in the statistical analysis.

Within the hippocampal formation, we examined the TrkB-immunoreactive neurons present in the polymorph layer of the dentate gyrus, as depicted in the Atlas of Paxinos and Watson (Paxinos and Watson, 1986) (Bregma: −3.80 mm, Interaural 5.20 mm, Plates 33–36), and in the lacunosum molecular layer of the hippocampus, subregions CA1, CA2, CA3 (Bregma: −3.80 mm, Interaural 5.20 mm, Plates 33–36). Within the ventral mesencephalon, we evaluated the TrkB-positive neurons in the pars reticulata of substantia nigra (Bregma: −4.80 mm, Interaural 4.20 mm, Plates 37–39). In the adjacent ventral tegmental area (Bregma: −4.80 mm, Interaural 4.20 mm, Plates 37–39) we counted TrkB immunopositive neurons present in mm^2 of the section to limit the possibility of mistake because this brain region has a less defined contour compared to the other regions.

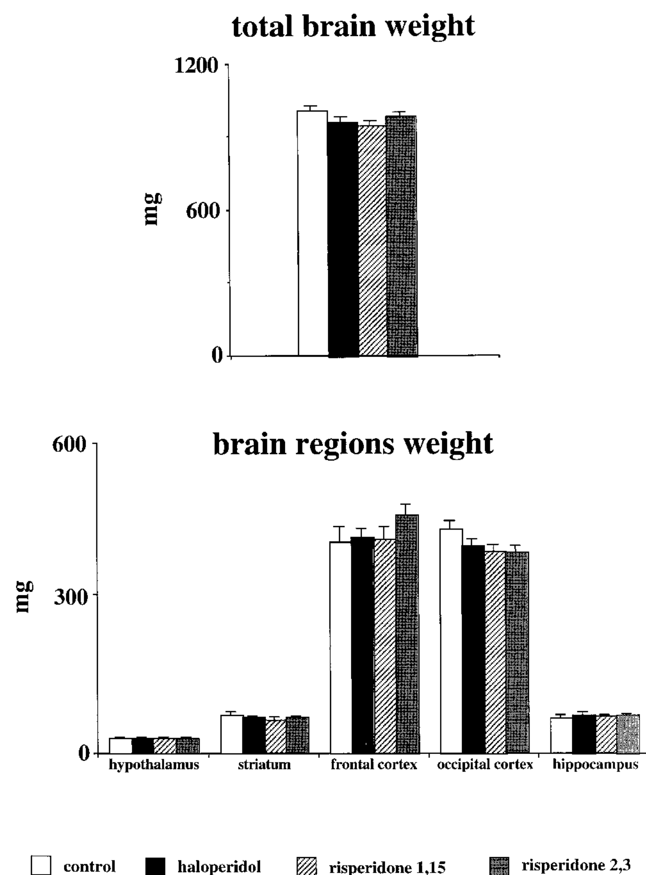

Fig. 1. Weights of brain and brain regions (expressed as mg of wet weight tissue) of haloperidol, risperidone and vehicle-treated rats. Results presented as mean ± SEM (n = 10 animals/group).

Statistical Analysis

Data were analyzed by analyses of variance (ANOVA) considering the treatments with haloperidol and risperidone (two doses) as between subject variables. When significant differences were obtained, post-hoc test within logical sets of means were carried out using the Newman–Keuls's test.

RESULTS

Effect of Chronic Haloperidol and Risperidone on Body and Brain Weight

Weight of haloperidol or risperidone treated animals did not differ from animals that were fed chow to which the vehicle was added; the mean ± SEM weights for the four groups at the end of the treatment were: 367 g ± 21.2 (controls); 371 g ± 19.8 (haloperidol 1.15); 359 ± 23.7 (risperidone 1.15); and 353 ± 17.2 (risperidone 2.30). Likewise, treatment with these drugs does not result in changes of brain weight. The mean ± SEM total brain weights and the brain regions weights are presented in Figure 1. No differences between the four groups were observed. These results are consistent with the findings reported by others showing that haloperidol has a limited facilitatory effect on body

TABLE I. Plasma and Brain Concentrations of Haloperidol and Risperidone After 29 Days of Oral Administration

	Haloperidol	Risperidone	
	1.15 mg/100 g food/day	1.15 mg/100 g food/day	2.30 mg/100 g food/day
Plasma	1.61 ± 1.09	13.02 ± 2.26	19.37 ± 2.58
Brain			
Frontal cortex	78.75 ± 17.47	4.59 ± 0.35	3.67 ± 1.11
Occipital cortex	92.20 ± 25.2	2.49 ± 0.35	4.52 ± 0.15
Striatum	86.0 ± 29.7	3.09 ± 1.46	3.02 ± 0.50

Data are expressed as mean ± SD ng/g brain tissue and ng/ml plasma.

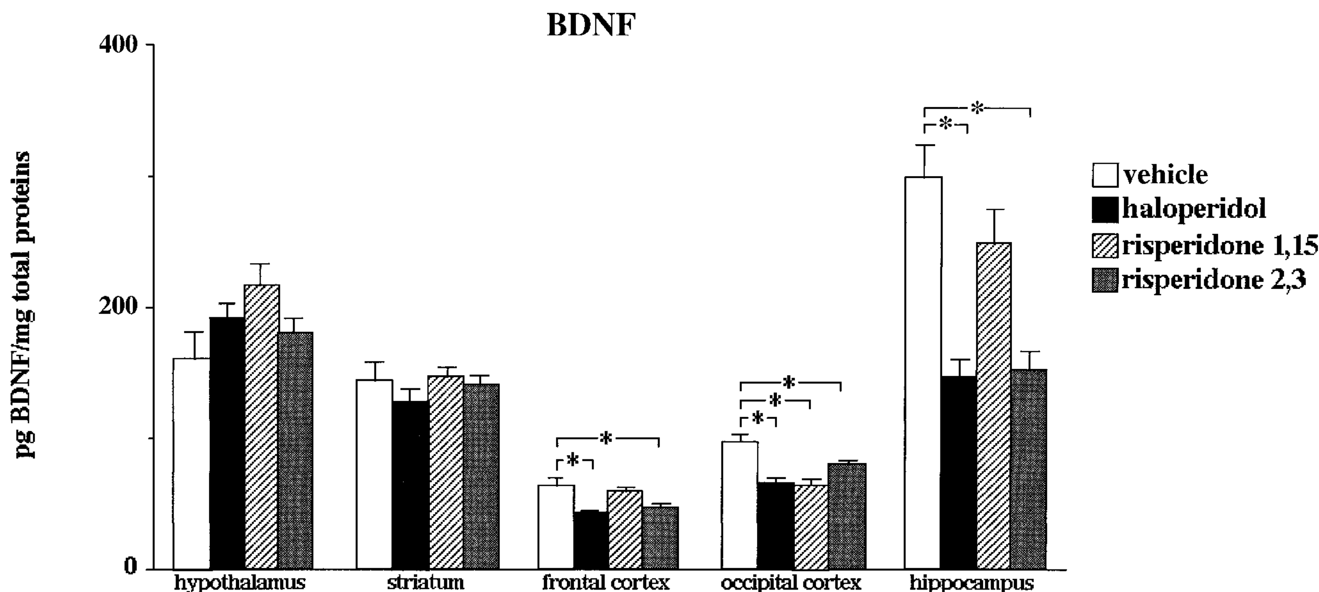

Fig. 2. BDNF concentration (expressed as pg of BDNF/mg of total proteins) in the brain regions of haloperidol, risperidone and vehicle-treated rats. Results presented as mean ± SEM (n = 10 animals/group). Asterisks indicate significant between-group differences (*$P < 0.05$).

weight and rats treated with risperidone showed body weight gain similar to that of control rats (Uguru-Okorie, 1981; Marder and Meibach, 1994; Aravagiri et al., 1998).

Food Intake

To determine whether drug treatment influences food behavior, we monitored the amount of food intake in all four groups. Daily examination indicated that the average of food intake of the four experimental groups was between 23 and 27 mg/day (mean 25 mg/day) and no statistic significance in food intake between groups was found.

Plasma and Brain Concentrations of Haloperidol and Risperidone

The results are presented in Table I. No risperidone was detectable in animals receiving vehicle or haloperidol, and vice versa, no haloperidol was detectable in animals receiving risperidone or vehicle. The plasma and brain tissue drug concentrations were comparable to those attained in other experiments and deemed to achieve a high degree of receptor occupancy.

Effect of Chronic Haloperidol and Risperidone on Brain BDNF Levels

Figure 2 shows brain BDNF levels in 5 rat brain regions. In the hypothalamus, BDNF levels were not significantly changed after treatment with antipsychotic drugs although the means were increased. The BDNF content in the striatum was also not affected by the treatment. In the frontal cortex, however, haloperidol and the high dose of risperidone (risperidone 2.30) induced a decrease in BDNF concentration compared to controls ($P < 0.01$ for the effect of the treatment, $P < 0.05$ in post-hoc comparison). The decrease in BDNF levels was more pronounced in the haloperidol-treated rats (Fig. 2). In the occipital cortex, both haloperidol and the two risperidone doses decreased BDNF concentration ($P < 0.01$ for the effect of the treatment). Post-hoc comparisons showed that both haloperidol and risperidone, 1.15 and 2.30 mg/100 g food doses, decreased BDNF content in the occipital cortex ($P < 0.05$ in post-hoc). In the hippocampus, haloperidol and risperidone, given at 2.30 mg/100 g food pellet, also decreased BDNF content ($P < 0.01$

for the effect of the treatment, $P < 0.05$ in post-hoc comparison).

Trkb Immunoreactivity in the Hippocampal Region After Haloperidol or Risperidone Treatment

In the dentate gyrus of hippocampal region an increase in the number of TrkB-immunoreactive cells/brain section of haloperidol-treated rats was observed ($P = 0.01$ for the effect of treatment, $P < 0.05$ in post-hoc comparison). In risperidone-treated rats the number of immunopositive neurons was not statistically different from controls (Figs. 3,4,5). In the hippocampus (CA1, CA2, and CA3) the number of TrkB-positive cells counted in each section was reduced in haloperidol-treated rats ($P < 0.01$ for the effect of the treatment, $P < 0.05$ in post-hoc comparison) when compared to controls. No differences were noted after treatment with risperidone (Fig. 3).

Trkb Immunoreactivity in the Ventral Mesencephalic Areas

In the substantia nigra pars reticulata we observed an increase in the number of TrkB-immunoreactive neurons/section after haloperidol treatment ($P < 0.01$ for the effect of the treatment, $P < 0.05$ in post-hoc comparison). Risperidone, however, did not change the number of immunopositive cells (Figs. 4, 6). In the ventral tegmental area, haloperidol also induced an increment in the number of TrkB-positive cells per mm^2 ($P < 0.05$ for the effect of the treatment, $P < 0.05$ in post-hoc comparison), whereas Risperidone had no effect (Figs. 4, 7).

To determine whether dopaminergic neurons express BDNF receptors, brain sections containing the ventral mesencephalic regions were immunostained with TrkB and dopamine D_2 receptors. As shown in Figure 8, ventral mesencephalic neurons stain positively for the TrkB and dopamine D_2 receptors suggesting that these two markers are co-localized in the soma neurons (Fig. 8).

DISCUSSION

We have previously reported that the basal levels of brain NGF are significantly affected by haloperidol and risperidone (Angelucci et al., 2000), suggesting that these treatments induce changes in synthesis or release of endogenous growth factors. Because BDNF is involved in growth and function of brain DA neurons, the question arose whether antipsychotic drugs might also affect this neurotrophin. Consequently, we carried out appropriate experiments and results obtained can be summarized as follows: (1) haloperidol and risperidone decrease the basal levels of BDNF in frontal and occipital cortex and hippocampus, (2) consistent with the decrease in BDNF levels, haloperidol also significantly decreased TrkB immunoreactivity in neurons of the hippocampus, substantia nigra and ventral tegmental area, whereas the effects of risperidone were in the same direction but not statistically significant. (3) TrkB receptors are co-localized with dopamine D_2 receptors in the neurons of the ventral mesencephalon. Analysis of serial brain sections also revealed no cell death, or gliosis in these brain regions.

The findings that there were significant differences between controls and haloperidol or risperidone-treated rats both in terms of BDNF levels and receptors expression suggest that the effect is due to a direct action of the two drugs. The evidence that untreated rats display no differences in body weight or brain weight compared with rats treated with drugs also supports this hypothesis.

Although the functional significance of these effects and of the differential effects of haloperidol and risperidone is not known, the results demonstrate a novel action of these drugs. The observation that only haloperidol exerts a significant effect both on BDNF and BDNF high affinity receptor, however, suggests a closer correlation between haloperidol and BDNF as compared to risperidone and BDNF. Haloperidol is a potent antagonist of D_2 receptors. The fact that BDNF is a specific survival factor for the dopaminergic neurons indicates that haloperidol can induce BDNF changes as an adaptive response via the TrkB receptor. Indeed there is considerable experimental evidence indicating that the trophic actions of BDNF on ventral mesencephalic dopamine neurons in vitro and in vivo are mediated by the TrkB receptor (Numan and Seroogy, 1999). The TrkB receptor could be synthesized essentially by all mesencephalic dopaminergic neurons (Numan and Seroogy, 1999). Moreover BDNF is also produced locally by subpopulations of dopaminergic neurons (Numan and Seroogy, 1999). Thus the neurotrophins can influence the dopaminergic neurons through autocrine or paracrine mechanisms. The fact that in ventral mesencephalic neurons we observed an increase in TrkB-positive neurons supports the hypothesis of an activation of defense mechanism against D_2 antagonist action. It has also been reported that haloperidol and other D_2 antagonists induce NGF mRNA expression in the hippocampus, piriform cortex and striatum (Ozaki et al., 1999). The opposite direction of neurotrophin and its receptor changes in some areas and parallel changes in others (like hippocampus) are also consistent with such a notion. These results also raise the possibility that haloperidol and risperidone can act on different target cells, or alternatively that these drugs influence in a different manner BDNF-producing or responsive cells. Speculatively, this could be due to the risperidone lower D_2 antagonist potency compared to haloperidol, combined with $5HT_2$ receptor-blocking properties.

Several lines of evidence indicate that neurotrophins can modulate the DA and 5HT metabolism in rat mesencephalic neurons (Martin-Iverson et al., 1994; Nishio et al., 1998). For example, DA neurons in substantia nigra and the ventral tegmental area express high levels of BDNF mRNA, indicating that these cells are responsive to this neurotrophin (Seroogy and Gall, 1993). Furthermore, haloperidol and risperidone modify the firing rate of substantia nigra reticulata neurons as evidenced by extracellular recordings of neuronal activity (Bruggeman et al., 1997). In our study, haloperidol treatment increased TrkB-immunoreactive neurons in both ventral tegmental area and substantia nigra suggesting that TrkB may have a role in antipsychotic action. This is also supported by the finding that BDNF can stimulate DA release via the TrkB receptor (Blochl and Sirrenberg, 1996). Whether the in-

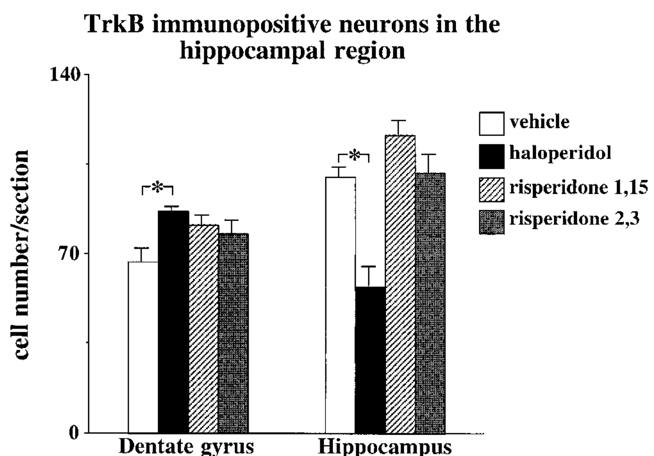

Fig. 3. Number of TrkB-immunopositive cells/section in the dentate gyrus and hippocampus after antipsychotic or vehicle treatment. Results presented as mean ± SEM (n = 5 animals/group). Asterisks indicate significant between-group differences (*$P < 0.05$).

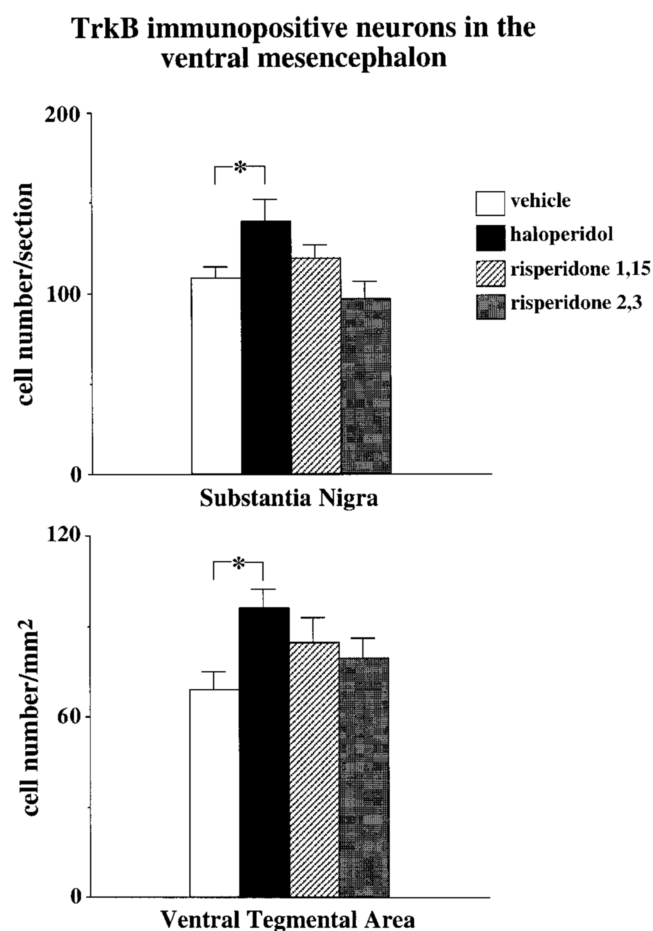

Fig. 4. Number of TrkB-immunopositive cells/section in the substantia nigra and cells/mm^2 in the ventral tegmental area after antipsychotic or vehicle treatment. Results presented as mean ± SEM (n = 5 animals/group). Asterisks indicate significant between-group differences (*$P < 0.05$).

creased TrkB-immunoreactivity constitutes an adaptive response to the DA antagonist action is not clear. The observation that infusion of BDNF in these areas modifies spontaneous behavior (Martin-Iverson and Altar, 1996) and reduces MPTP toxicity in rat (Hung and Lee, 1996), however, indicates that the BDNF-TrkB system is involved in haloperidol mechanism(s) of action. This is of interest because TrkB is a signaling molecule required for maturation and synaptogenesis of hippocampal connections (Martinez et al., 1998). High expression of TrkB was found in the hippocampal formation (Yan et al., 1997). The results that haloperidol induced a decrease in the number of TrkB-positive neurons in the hippocampus suggest that it can alter the neuronal plasticity. Indeed it has been shown that, in addition to the classical neurotrophic role, BDNF/TrkB signaling regulates neuronal differentiation and connectivity and the synaptic transmission in the hippocampus (Kang and Schuman, 1995; McAllister et al., 1996), a brain structure crucially involved in cognitive junctions (Friedman et al., 1999; Weinberger, 1999). Whether the reduction of TrkB expression is linked to a reduced BDNF synthesis, however, remains to be seen.

The finding that in the dentate gyrus, a hippocampal subregion, haloperidol and also risperidone (though not significantly) enhance the expression of TrkB raises the possibility that this effect constitutes a stress-related response associated with drug administration. In this context it might be relevant that insults in the entorhinal cortex can result in a bilateral increase in TrkB mRNA expression in the dentate granule cell layer and the pyramidal cell layer of the hippocampus (Falkenberg et al., 1992), an area that is implicated in stress related responses (McEwen and Magarinos, 1997). This is consistent with the observation that BDNF and TrkB mRNA increase in this brain region after repeated electroconvulsive seizures and antidepressant drug treatments (Nibuya et al., 1995).

Functional and anatomical evidence indicates that hippocampal regions are affected in schizophrenia (Falkai and Bogerts, 1986; Weinberger, 1999). Because hippocampus synthesizes the largest amount of NGF and BDNF, the decrease in BDNF/NGF hippocampal levels observed after treatment with antipsychotic drugs raises the possibility of a functional correlation between low availability of neurotrophins and cognitive deficits occurring in schizophrenia. Moreover, there are several recent structural and biochemical studies indicating that schizophrenia or treatment with neuroleptic drugs may reduce dendritic spines in brain regions (Kelley et al., 1997), alter brain cholinergic activity (Mahadik et al., 1988), and impair cognitive abilities (Gallhofer et al., 1996). Because neurotrophic factors, such as NGF and BDNF, are known to play an important role in these events (Lo, 1995; Thoenen 1995), a heuristic hypothesis is that changes in synthesis and release of brain neurotrophins may significantly influence the course of cholinergic-related deficits associated with schizophrenia. Thus, prolonged treatment with haloperidol and risperidone might negatively in-

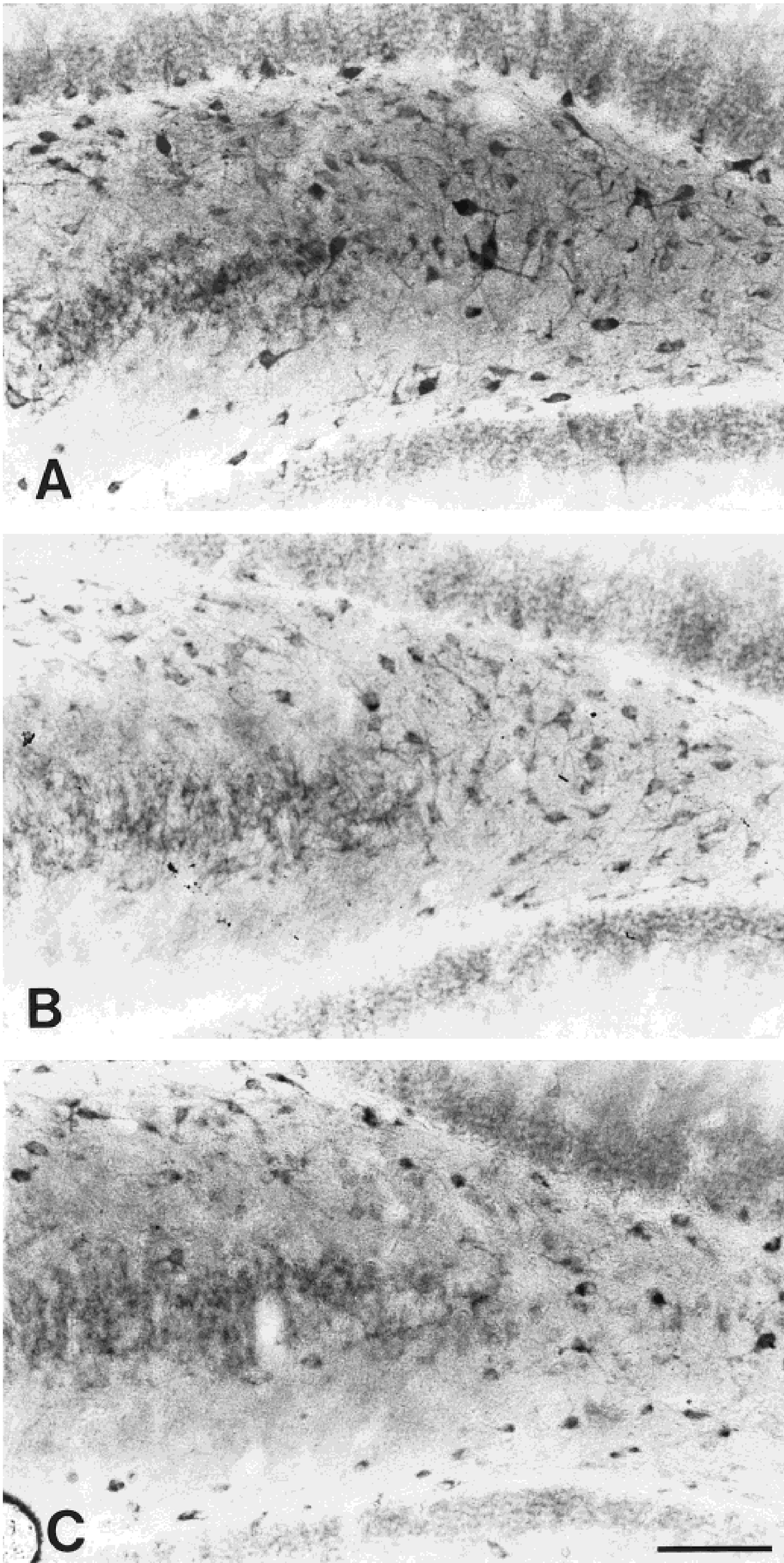

Fig. 5. TrkB-immunoreactivity in the dentate gyrus of rats treated with haloperidol (**A**), risperidone at dose of 2.3 mg/100 g food (**B**), and vehicle (**C**). Note the increased neuronal immunoreactivity in haloperidol-treated rats. Scale bar = 170 μm.

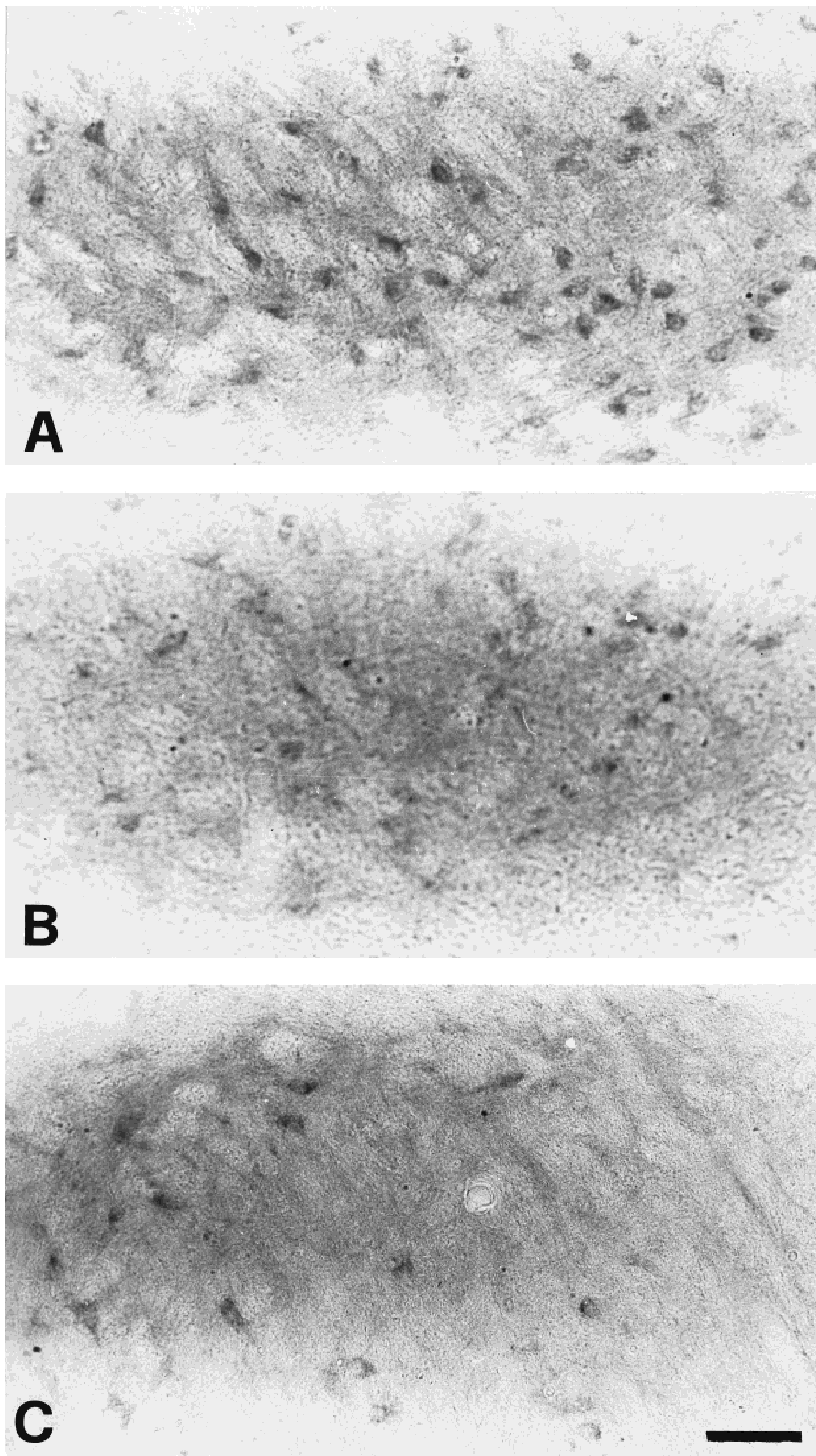

Fig. 6. TrkB-immunoreactivity in the substantia nigra of rats treated with haloperidol (**A**), risperidone at dose of 2.3 mg/100 g food (**B**), and vehicle (**C**). Note the increased number of immunoreactive neurons in haloperidol-treated rats. Scale bar = 120 μm.

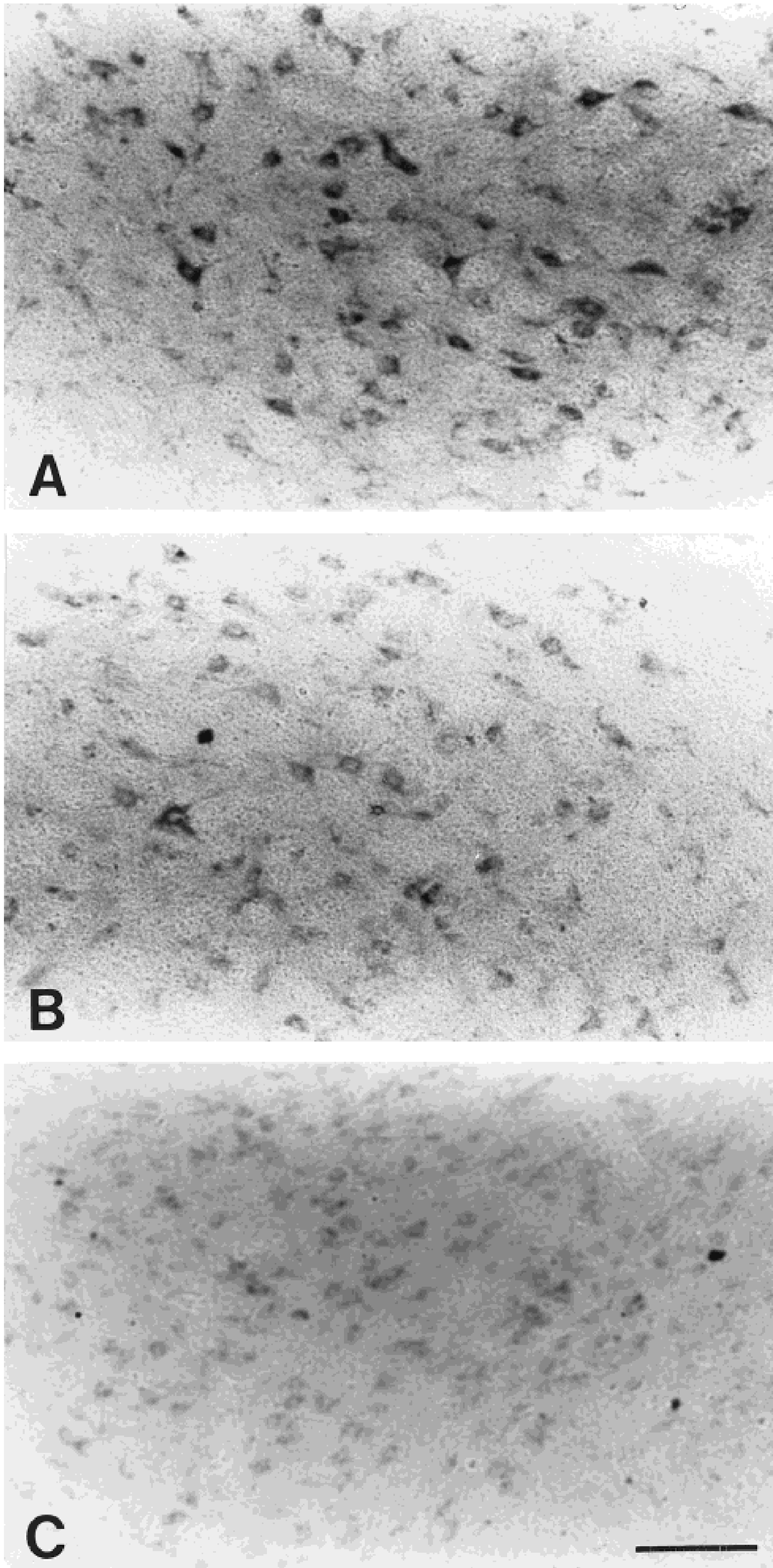

Fig. 7. TrkB-immunoreactivity in the ventral tegmental area of rats treated with haloperidol (**A**), risperidone at dose of 2.3 mg/100 g food (**B**), and vehicle (**C**). Note the increased number of immunoreactive neurons in haloperidol-treated rats. Scale bar = 120 μm.

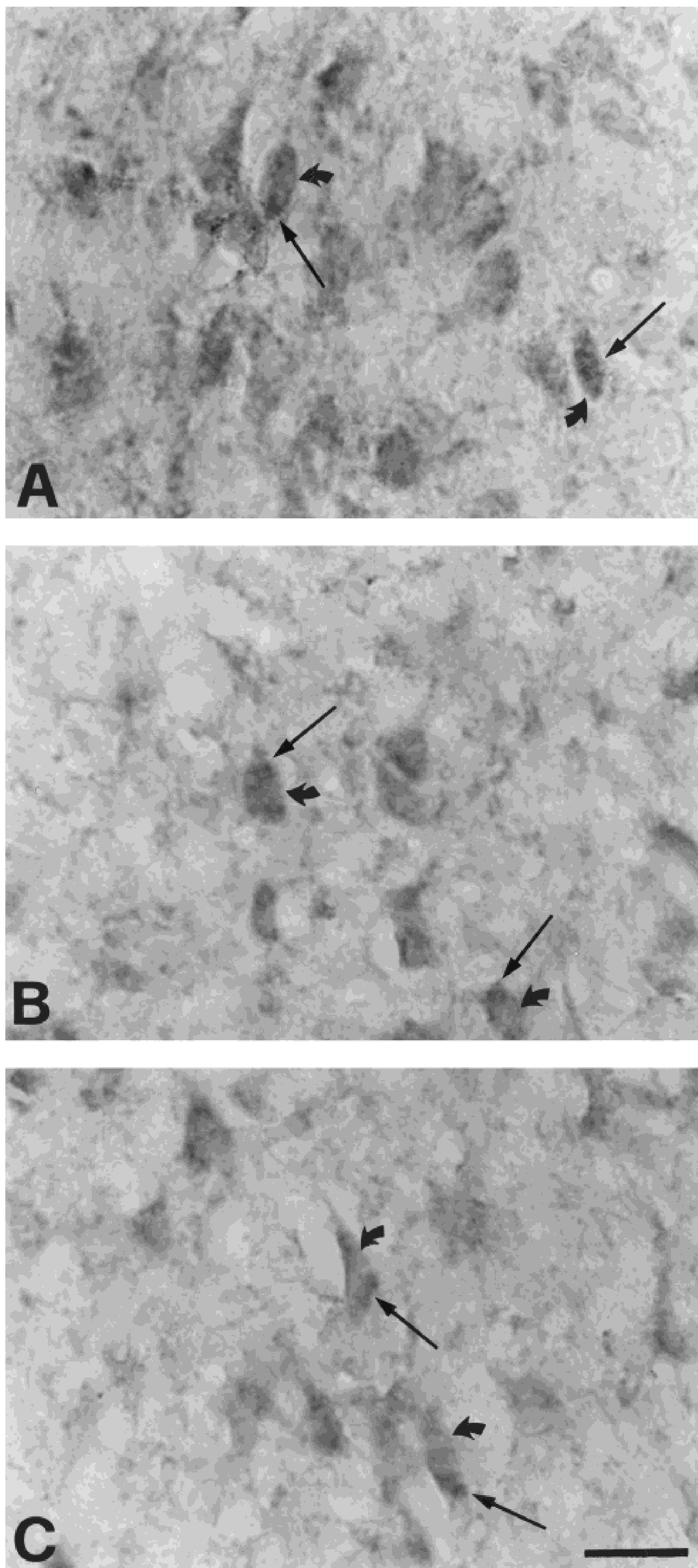

Fig. 8. Double staining of TrkB- and D_2-receptors immunoreactivity on neurons of the ventral tegmental area of rats treated with haloperidol (**A**), risperidone at dose of 2.3 mg/100 g food (**B**), and vehicle (**C**). Co-localization of D_2-receptors with TrkB is indicated by orange-brown color (thin arrows) TrkB-receptors are indicated by light gray color (thick arrows). Scale bar = 45 μm.

fluence cognitive processes. The observation that decreased distribution of TrkB is associated with memory deficits supports this hypothesis (Croll et al., 1998). This hypothesis is consistent with studies showing that risperidone treatment compared to haloperidol causes less severe deficit in patient's ability to perceive emotion, (Kee et al., 1998; Kern et al., 1998). Whether the effect of risperidone on cognitive processes is linked to a lower efficacy of risperidone compared with haloperidol in reducing the brain level of neurotrophins remains to be demonstrated. In this context it is relevant that infusion of BDNF was found to improve neurochemical and behavioral deficits associated with nigrostriatal DA lesions (Altar et al., 1994) and activate striatal DA and 5-HT metabolism and related behaviors (Martin-Iverson et al., 1994). Additionally, the findings that schizophrenic patients have low circulating levels of NGF (Bersani et al., 1999), that neuronal development of embryonic brain tissue derived from schizophrenic women shows neurite-growth deficits (Freedman et al., 1992) and that the polymorphism gene of neurotrophin-3 is associated with schizophrenia (Nanko et al., 1994), are in line with findings that schizophrenia affects cognitive processes and that a prolonged haloperidol and risperidone treatment could influence these events. The involvement of neurotrophins in schizophrenia is also suggested by observations that experimentally induced neurodeficits in the entorhinal cortex are characterized by significant changes in NGF levels (Angelucci et al., 1999). Moreover, haloperidol markedly alters NGF plasma levels in human (Aloe et al., 1997) and NGF brain levels in mice (Alleva et al., 1996). These and our observations further support the hypothesis that haloperidol and risperidone treatment in human might influence brain tissue and circulating levels of neurotrophins.

As haloperidol seems to have a more pronounced effect on BDNF and TrkB expression than risperidone, mechanisms regulating these differences remain to be elucidated and could perhaps be associated with risperidone's atypical properties.

Further animal studies are necessary to evaluate whether the chronic administration of antipsychotic drugs causes impairments of the constitutive neurotrophins, such as NGF and BDNF levels.

ACKNOWLEDGMENTS

We thank Dr. W. Coussement, Janssen Pharmaceutica, Belgium and B. Eriksson, Janssen Pharmaceutica, Sweden for support and measurements of haloperidol and risperidone concentrations in serum and brain tissue.

REFERENCES

Alberch J, Brito B, Notario V, Castro R. 1991. Prenatal haloperidol treatment decreases nerve growth factor receptor and mRNA in neonate rat forebrain. Neurosci Lett 131:228–232.

Alderson RF, Alterman AL, Barde YA, Lindsay RM. 1990. Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. Neuron 5:297–306.

Alleva E, Della Seta D, Cirulli F, Aloe L. 1996. Haloperidol treatment decreases nerve growth factor levels in the hypothalamus of adult mice. Prog Psychopharmacol Biol Psychiatry 20:483–489.

Aloe L, Bracci-Laudiero L, Alleva E, Lambiase A, Micera A, Tirassa P. 1994. Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. PNAS 91:10440–10444.

Aloe L, Iannitelli A, Bersani G, Alleva E, Angelucci F, Maselli P, Manni L. 1997. Haloperidol administration in human lowers plasma nerve growth factor level: evidence that sedation induces opposite effects to arousal. Neuropsychobiology 36:65–68.

Altar CA, Boylan CB, Fritsche M, Jones BE, Jackson C, Wiegand SJ, Lindsay RM, Hyman C. 1994. Efficacy of brain-derived neurotrophic factor and neurotrophin-3 on neurochemical and behavioral deficits associated with partial nigrostriatal dopamine lesions. J Neurochem 63:1021–1032.

Angelucci F, Aloe L, Gruber S, Fiore M, Mathé AA. 2000. Chronic antipsychotic treatment selectively alters nerve growth factor and neuropeptide y immunoreactivity and the distribution of choline acetyl transferase in rat brain regions. Int J Neuropsychopharmacol.

Angelucci F, Fiore M, Cozzari C, Aloe L. 1999. Prenatal ethanol effects on NGF level, NPY-, and ChAT-immunoreactivity in mouse entorhinal cortex: a preliminary study. Neurotoxicol Teratol 21:415–425.

Aravagiri M, Yuwiler A, Marder SR. 1998. Distribution after repeated oral administration of different dose levels of risperidone and 9-hydroxy-risperidone in the brain and other tissues of rat. Psychopharmacology 139:356–363.

Benisty S, Boissiere F, Faucheux YA, Hirsch EC. 1998. TrkB messenger RNA expression in normal human brain and in the substantia nigra of Parkinsonian patients: an in situ hybridization study. Neuroscience 86:813–826.

Bersani G, Aloe L, Iannitelli A, Maselli P, Alleva E, Angelucci F, Pancheri P. 1999. Low nerve growth factor plasma levels in schizophrenic patients: a preliminary study. Schizophr Res 37:203–205.

Blochl A, Sirrenberg C. 1996. Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and p75Lntr receptors. J Biol Chem 271:21100–21107.

Bruggeman R, Westerink BH, Timmerman W.1997. Effects of risperidone, clozapine and haloperidol on extracellular recordings of substantia nigra reticulata neurons of the rat brain. Eur J Pharmacol 324:49–56.

Croll SD, Ip NY, Lindsay RM, Wiegand SJ. 1998. Expression of BDNF and TrkB as a function of age and cognitive performance. Brain Res 812:200–208.

Duman RS, Vaidya VA, Nibuya M, Morinobu S, Rydelek Fitzgerald L. 1995. Stress, antidepressant treatment, and neurotrophic factors: molecular and cellular mechanisms. Neuroscientist 1:351–360.

Ebendal T. 1992. Function and evolution in the NGF family and its receptors. J Neurosci Res 32:461–470.

Ernfors P, Bengzon J, Kokaia Z, Persson H, Lindwall O. 1991. Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron 7:165–176.

Falkai P, Bogerts B. 1986. Cell loss in the hippocampus of schizophrenics. Eur Arch Psychiatry Neurol Sci 236:154–161.

Falkenberg T, Ernfors P, Persson H, Lindefors N. 1992. Cortical transsynaptic activation of tyrosine kinase receptor trkB messenger RNA expression in rat hippocampus. Neuroscience 51:883–889.

Freedman R, Stromberg I, Seiger A, Olson L, Nordstrom AL, Wiesel FA, Bygdeman M, Wetmore C, Palmer MR, Hoffer BJ. 1992. Initial studies of embryonic transplants of human hippocampus and cerebral cortex derived from schizophrenic women. Biol Psychiatry 32:148–163.

Friedman J, Temporini H, Davis K. 1999. Pharmacological strategies for augmenting cognitive performance in schizophrenia. Biol Psychiat 45:1–16.

Gallhofer B, Bauer U, Lis S, Krieger S, Gruppe H. 1996. Cognitive dysfunction in schizophrenia: comparison of treatment with atypical antipsychotic agents and conventional neuroleptic drugs. Eur Neuropsychopharmacol 6(Suppl 2):S13–S20.

Gerlach J. 1991. New antipsychotics: classification, efficacy, and adverse effects. Schizophr Bull 17:289–309.

Ghohs A, Carnahan J, Greenberg ME. 1994. Requirement for BDNF in activity-dependent survival of cortical neurons. Science 263:1618–1623.

Glowinsky J, Iversen LL. 1965. Regional studies of catecholamines in the rat brain: the disposition of (^{3}H) norepinephrine, (^{3}H) dopamine and (^{3}H) dopa in various regions of the brain. J Neurochemistry 13:655–669.

Goutan E, Marti E, Ferrer I. 1998. BDNF, and full length and truncated TrkB expression in the hippocampus of the rat following kainic acid excitotoxic damage. Evidence of complex time-dependent and cell-specific responses. Brain Res Mol Brain Res 59:154–164.

Hohon A, Leibrock J, Bailey K, Barde YA. 1990. Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. Nature 344:339–341.

Hung HC, Lee EH. 1996. The mesolimbic dopaminergic pathway is more resistant than the nigrostriatal dopaminergic pathway to MPTP and MPP+ toxicity: role of BDNF gene expression. Brain Res Mol Brain Res 41:14–26.

Hyman C, Hofer M, Barde YA, Juhasz M, Yancopulos G, Squinto SP, Lindsay RM. 1991. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350:230–232.

Joyce JN, Goldsmith SG, Gurevich EV. 1997. Limbic circuits and monoamine receptors: dissecting the effects of antipsychotics from disease processes. J Psychiat Res 31:197–217.

Kang H, Schuman EM. 1995. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267:1658–1662.

Kee KS, Kern RS, Marshall BD, Green MF. 1998. Risperidone versus haloperidol for perception of emotion in treatment-resistant schizophrenia: preliminary findings. Schizophr Res 31:159–165.

Kelley JJ, Gao XM, Tamminga CA, Roberts RC. 1997. The effect of chronic haloperidol treatment on dendritic spines in the striatum. Exp Neurol 146:471–478.

Kern RS, Green MF, Marshall BD, Wirshing WC, Wirshing D, McGurk S, Marder SR, Mintz J. 1998. Risperidone versus haloperidol on reaction time, manual dexterity, and motor learning in treatment-resistant schizophrenia patients. Biol Psychiatry 44:726–732.

Kerns JM, Sierens DK, Kao HL, Klawans HL, Carvey PM. 1992. Synaptic plasticity in the rat striatum following chronic haloperidol treatment. Clin Neuropharmacol 15:488–500.

Levi-Montalcini R. 1987. The nerve growth factor 35 years later. Science 237:1154–1162.

Lindwall O, Ernfors P, Bengzon J, Kokaia Z, Smith M, Siesjo B, Persson H. 1992. Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 in the adult rat brain following cerebral ischemia and hypoglycemic coma. PNAS 89:648–652.

Lindwall O, Kokaia Z, Bengzon J, Elmer E, Kokaia M. 1994. Neurotrophins and brain insults. Trends Neurosci 17:490–496.

Lo DC. 1995. Neurotrophic factors and synaptic plasticity. Neuron 15: 979–981.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–75.

Mahadik SP, Laev H, Korenovsky A, Karpiak SE. 1988. Haloperidol alters rat CNS cholinergic system: enzymatic and morphological analyses. Biol Psychiatry 24:199–217.

Mamounas LA, Blue M, Siuciak JA, Altar CA. 1995. Brain-derived neurotrophic factor promotes the survival and sprouting of serotoninergic axons in rat brain. J Neurosci 15:7929–7939.

Marder SR, Meibach RC. 1994. Risperidone in the treatment of schizophrenia. Am J Psychiat 151:825–835.

Martin-Iverson MT, Altar CA. 1996. Spontaneous behaviors of rats are differentially affected by substantia nigra infusions of brain-derived neurotrophic factor and neurotrophin-3. Eur J Neurosci 8:1696–1706.

Martin-Iverson MT, Todd KG, Altar CA. 1994. Brain-derived neurotrophic factor and neurotrophin-3 activate striatal dopamine and serotonin metabolism and related behaviors: interactions with amphetamine. J Neurosci 14:1262–1270.

Martinez A, Alcántara S, Borrell V, Del Rio JA, Blasi J, Otal R, Campos N, Boronat A, Babacid M, Silos-Santiago I, Soriano E. 1998. TrkB and TrkC signaling are required for maturation and synaptogenesis of hippocampal connections. J Neurosci 18:7336–7350.

McAllister AK, Katz LC, Lo DC. 1996. Neurotrophin regulation of cortical dendritic growth requires activity. Neuron 17:1057–1064.

McEwen BS, Magarinos AM. 1997. Stress effects on morphology and function of the hippocampus. Ann NY Acad Sci 821:271–284.

Nanko S, Hattori M, Kuwata S, Sasaki T, Fukuda R, Dai XY, Yamaguchi K, Shibata Y, Kazamatsuri H. 1994. Neurotrophin-3 gene polymorphism associated with schizophrenia. Acta Psychiat Scand 89:390–392.

Nawa H, Carnahan J, Gall C. 1995. BDNF protein measured by a novel enzyme immunoassay in normal brain and after seizure: partial disagreement with mRNA levels. Eur J Neurosci 7:1527–1535.

Nibuya M, Morinobu S, Duman R. 1995. Regulation of BDNF and TrkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15:7539–7547.

Nishio T, Furukawa S, Akiguchi I, Sunohara N. 1998. Medial nigral dopamine neurons have rich neurotrophin support in humans. NeuroReport 9:2847–2851.

Numan S, Seroogy KB. 1999. Expression of TrkB and TrkC mRNA by adult midbrain dopamine neurons: a double-label in situ hybridization study. J Comp Neurol 403:295–308.

Ozaki T, Mui K, Yamagami S. 1999. Comparison of the effects of dopamine D_1 and D_2 receptor antagonists on nerve growth factor mRNA expression. Eur J Pharmacol 369:133–143.

Paxinos G, Watson C. 1986. The rat brain in stereotaxic coordinates. London: Academy Press plates: 33–39.

Reynold G. 1997. What is an atypical antipsychotic? J Psychopharmacol 11:195–199.

Seroogy KB, Gall CM. 1993. Expression of neurotrophins by midbrain dopaminergic neurons. Exp Neurol 124:119–128.

Shimohama S, Tamura Y, Akaike A, Tsukahara T, Ohara O, Watanabe S, Kimura J. 1993. Brain-derived neurotrophic factor pretreatment exerts a partially protective effect against glutamate-induced neurotoxicity in cultured rat cortical neurons. Neurosci Lett 164:55–58.

Spenger C, Hyman C, Studer L, Egli M, Evtouchenko L, Jackson C, Dahl-Jorgensen A, Lindsay RM, Seyler RW. 1995. Effects of BDNF on dopaminergic, serotoninergic and GABA-ergic neurons in cultures of human fetal ventral mesencephalon. Exp Neurol 133:50–63.

Thoenen H. 1995. Neurotrophins and neuronal plasticity. Science 270: 593–598.

Uguru-Okorie DC. 1981. Effects of chronic haloperidol treatment on ingestive behavior and body weight regulation in the rat. Psychopharmacology 73:384–387.

Ventimiglia R, Mather PE, Jones BE, Lindsay RM. 1995. The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur J Neurosci 7:213–222.

Weinberger DR. 1999. Cell biology of the hippocampal formation in schizophrenia. Biol Psychiatry 45:395–402.

Weinberger DR, Lipska BK. 1995. Cortical maldevelopment, antipsychotic drugs, and schizophrenia: a search for common ground. Schizophr Res 16:87–110.

Yan Q, Matheson C, Lopez OT, Miller JA. 1994. The biological responses of axotomized adult motorneurons to brain-derived neurotrophic factor. J Neurosci 14:5191–5201.

Yan Q, Radeke MJ, Matheson CR, Talvenheimo J. Welcher AA, Feinstein SC. 1997. Immunocytochemical localization of TrkB in the central nervous system of the adult rat. J Comp Neurol 378:135–157.

Yntema OP, Korf J. 1987. Transient suppression by stress of haloperidol induced catalepsy by the activation of the adrenal medulla. Psychopharmacology 91:131–134.