Chronic antipsychotic treatment selectively alters nerve growth factor and neuropeptide Y immunoreactivity and the distribution of choline acetyl transferase in rat brain regions

Francesco Angelucci1,2, Luigi Aloe3, Susanne H. M. Gruber1, Marco Fiore2,3 and Aleksander A. Mathe1

1 Karolinska Institutet, Institution of Clinical Neuroscience, St Göran’s Hospital, SE-112 81, Stockholm, Sweden
2 Institute of Neurobiology, CNR, Viale C. Marx, 15/43, 00137, Rome, Italy
3 Department of Biological Psychiatry, University of Groningen, Hanzeplein 1, 9713 EZ Groningen, The Netherlands

Abstract

Neuropeptides and neurotrophins play a number of roles in the central nervous system (CNS). Nerve growth factor (NGF), the first characterized member of the family of neurotrophins, influences the synthesis of some neuropeptides, including neuropeptide Y (NPY), a peptide amply expressed in the CNS, interacting with catecholamines and modifying behaviour. In this study we investigated whether antipsychotic treatment affects the constitutive levels of NGF-, NPY- and choline acetyl transferase (ChAT)-like immunoreactivities (-LI) in the CNS. Rats were fed food supplemented with haloperidol (1.15 mg/100 g food), risperidone (1.15 or 2.3 mg/100 g food), or vehicle. After 29 d treatment animals were sacrificed with focused high-energy microwave irradiation for radioimmunoassay (RIA) of NPY-LI, by decapitation for analysis of NGF, and by perfusion for immunocytochemistry. Haloperidol and risperidone elevated NGF-LI concentrations in the hypothalamus but decreased NGF-LI in the striatum and hippocampus. In contrast, antipsychotics did not alter NPY-LI in the striatum. Haloperidol increased NPY-LI concentration in the occipital cortex, while risperidone increased NPY-LI in the occipital cortex, hippocampus, and hypothalamus. Significant decreases in ChAT immunoreactivity in large-size neurons following both haloperidol and risperidone treatments in the septum as well as Meynert’s nucleus were observed. Our findings demonstrate that antipsychotic drugs alter the regional brain levels of NGF-LI, NPY-LI and ChAT-LI and raise the possibility that these effects are implicated in their pharmacological and therapeutic properties.

Received 2 May 1999; Reviewed 12 July 1999; Revised 13 September 1999; Accepted 15 September 1999

Key words: Nerve growth factor, neuropeptide Y, choline acetyl transferase, antipsychotics, rat brain.

Introduction

The key mechanism of therapeutic action of antipsychotic drugs is attributed to the blockade of dopamine D2 receptors (Reynold, 1997), a hypothesis dominating this field of research for several decades. However, findings in recent years indicate that the neuronal circuitry of other neurotransmitters, e.g. excitatory amino acids as well as their receptors, may play a role in psychotic processes and the therapeutic action of antipsychotics (Joyce et al., 1997). In addition, neuropeptides such as neotensin, calcitonin gene-related peptide (CGRP) and growth factors have been implicated. A significant change of neuropeptide expression and release in mesolimbic and/or mesocortical pathways has been observed in animal models of psychosis and following acute antipsychotic treatment (Hertel et al., 1996; Mathé et al., 1996, 1997). This is of interest since neuropeptides can act as neurotransmitters and are co-localized and released with dopamine (DA), noradrenaline (NA), acetylcholine (ACh), or GABA (Hökfelt et al., 1995).

Since neurotrophins participate in the regulation of several neurotransmitters, such as NA, ACh, and GABA (Dreyfus et al., 1989), it is reasonable to hypothesize a link between antipsychotic mechanisms and synthesis, and release of neurotrophins. In addition to regulating the expression of specific neurotransmitters and showing survival-promoting activity (Barde, 1989), neurotrophins...
influence the formation of synaptic contacts and modulate synaptic efficacy and activity-dependent plasticity (Garofalo et al., 1992; Prakash et al., 1996). Nerve growth factor (NGF) is the first and best characterized member of the family of neurotrophins, which includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) (Ebendal, 1992; Levi-Montalcini, 1987). NGF is retrogradely transported from the hippocampus and cortex, respectively, to the septal and Meynert basal cholinergic neurons that are involved in cognitive functions (Rylett and Williams, 1994; Seiler and Schwab, 1983). There is considerable evidence that NGF promotes growth and differentiation of cholinergic neurons in the basal forebrain (Shelton and Reinarzdt, 1986), prevents the degeneration of septal neurons after fimbria fornix lesion (Hefti, 1986), and improves cognitive deficits (Mohammed et al., 1990). Moreover, recent studies have shown that neurotrophins, including NGF, localized in diverse brain regions increase significantly during limbic seizures induced by kainic acid (Gall et al., 1991; Lauterborn et al., 1994), epileptiform activity (Akeov et al., 1996; Ernfors et al., 1991), and in forebrain ischaemia (Hashimoto et al., 1992; Lindwall et al., 1992).

Extended animal studies also indicated that NGF influences the synthesis of neuropeptides, including neuropeptide Y (NPY) (Carnahan and Nawu, 1995; Croll et al., 1994; Vedder et al., 1993; Verge et al., 1995). NPY is a peptide widely expressed and exerts a variety of actions in the central nervous system (CNS) (Heilig and Widerlov, 1995; Wahlestedt and Heilig, 1995). Of interest to the field of psychosis research are the findings of interactions between NPY and DA and/or NA. Thus NPY given intracerebroventricularly enhances tissue concentrations of DA and dihydrophenylacetic acid (DOPAC) in rat cortex and striatum (Drumheller et al., 1994; Heilig et al., 1990) and release of DA from brain slices (Ault et al., 1998), as well as in vivo release from striatum (Kerkarian-Le Goff et al., 1992; Mathé et al., 1996). NPY also potentiates potassium-induced NA release or inhibits NA release caused by manual restraint from the hypothalamus (Kyrkouli et al., 1992; Pavia et al., 1995; Shibasaki et al., 1995). Conversely, DA regulates NPY gene expression in rat frontal cortex and striatum (Lindefors et al., 1990). Moreover, chronic cocaine (Wahlestedt et al., 1991) as well as acute and multiple administrations of MK-801 and phencyclidine (PCP) (Midgley et al., 1993, 1994) decrease NPY tissue levels and expression in rat cortex, nucleus accumbens and caudate putamen. On the other hand, blockade of dopamine D<sub>2</sub> and D<sub>1</sub> receptors with chronic haloperidol and repeated intraperitoneal injections of SCH-23390, respectively, elevate NPY expression and levels in the rat frontal cortex (Sakai et al., 1995; Smialowska, 1995) but reduce NPY in striatum. Similarly, in a series of experiments we have shown the effects of acute and chronic dopamine D<sub>1</sub> and D<sub>2</sub> agonists and antagonists and of acute N-methyl-d-aspartate (NMDA) antagonists on both brain levels and release in vivo of several neuropeptides, e.g. NPY. CGRP and neotensin (Gruber et al., 1996; Gruber and Mathé, 1997; Hertel et al., 1996; Mathé et al., 1994, 1996).

A variety of studies also indicates that psychotomimetics and electroconvulsive stimuli significantly increase tissue concentration of neuropeptides and neurotrophins in the central and peripheral nervous systems (Aloe et al., 1997; Bersani et al., 1999; Follesa et al., 1994; Iannitelli et al., 1995; Mathé et al., 1997; Nemeroff et al., 1992; Obuchowicz, 1996; Stenfors et al., 1989). Thus, to further clarify the mechanism(s) of action of antipsychotic drugs, we investigated effects of haloperidol and risperidone on constitutive levels of NGF and NPY in brain structures presumed to be involved in psychosis. We also analysed choline acetyl transferase (ChAT), an enzyme regulated by NGF (Levi-Montalcini, 1987) and presumed to be a marker of basal forebrain cholinergic neurons. These neurons receive trophic support from NGF (Rylett and Williams, 1994; Seiler and Schwab, 1983) and interact with dopaminergic neurons under normal condition and/or during antipsychotic-induced catalepsy (Acquas and Fibiger, 1998; Ushijima et al., 1997).

Materials and methods

Animals

Adult male Wistar rats weighing 200–250 g were used. They were housed 4 to a cage at 21 °C on a 12-h 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 cared for in line with the Karolinska Institute’s ‘Guidelines for Animal Care’.

Antipsychotic drugs

Since one of the aims of the study 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 food 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 (Gruber and Mathé, 1997; Gruber et al., 1998) and resulted 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**

One hundred rats were randomly divided into 4 groups receiving 1.15 mg haloperidol/100 g food, 1.15 mg risperidone/100 g food, 2.30 mg risperidone/100 g food, or vehicle. After 29 d treatment, animals were sacrificed with focused high-energy microwave irradiation for 2 s (since this procedure increases peptide recovery; Mathé et al., 1990b; Theodorsson et al., 1990) for analysis of peptides (n = 10/group). by decapitation for analysis of neurotrophins (n = 10/group), or by perfusion for immunocytochemistry (n = 5/group).

**Tissue preparation**

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 −70 °C until neurochemical analysis. For peptide extraction the brain tissues were homogenized with ultrasonication and twice extracted in 1 M acetic acid and water. The homogenates were centrifuged at 3000 g for 20 min and the supernatants lyophilized and stored at −70 °C until analysis. The supernatants were dissolved in assay buffer before RIA. For neurotrophin extraction the brain tissues were homogenized with ultrasonication in extraction buffer with 0.2% Triton X-100. The homogenates were centrifuged at 10000 g for 20 min, the supernatants were collected, diluted 1:1 with extraction buffer containing 0.1% Triton X-100 and processed for quantification of endogenous NGF by a sensitive and specific two-site enzyme immunoassay described in detail previously (Weskamp and Otten, 1986).

**NPY-like immunoreactivity determination by radioimmunoassay**

All samples were analysed twice, using two different anti-NPY antibodies. Aliquots of 100 µl sample and standard [synthetic NPY, no. 7180, neuropeptide Y (human, rat) from Peninsula Laboratories, England] were mixed with 100 µl antiserum [rabbit neuropeptide Y, no. RAS 7180 (human, rat) antiserum, also from Peninsula] or with 100 µl ‘Brown’ antiserum [anti-NPY, previously described by Heilig and Ekman (1995)], and incubated for 48 h at 4 °C. Thereafter, 100 µl 125I-Bolton Hunter-labelled NPY (Amersham International, England) was added and the solution was incubated for an additional 24 h. Free and antibody-bound NPY were separated using 50 µl Sac-Cel (anti-rabbit solid-phase second antibody-coated cellulose suspension; IDS, Bolton, England). Samples were left for 30 min at room temperature. The reaction was then blocked with 1 ml distilled H2O. Samples were centrifuged at 3000 g for 20 min at 4 °C and the supernatants decanted. Pellets were counted in a gamma counter for 3 min. The results obtained using anti-NPY antiserum (Peninsula) are presented in Figure 2. The sensitivity of the assay was 11 pmol/l and the intra- and inter-assay coefficients of variation were 7 and 12%, respectively.

**NGF measurement by enzyme-linked immunosorbent assay (ELISA)**

Ninety-six-well immunoplates (NUNC, Denmark) were coated with 50 µl/well of 0.4 µg/ml monoclonal anti-mouse-NGF antibody 27/21 (Boehringer, Germany). Parallel wells were coated with mouse IgG for evaluation of non-specific signals. After an overnight incubation at 20 °C the plates were washed 3 times with buffer and the samples were incubated in the coated wells (50 µl each) overnight at 20 °C. After an additional 3 washes the immobilized antigen was incubated with 50 µl/well of the monoclonal antibody 27/21 conjugated with β-d-galactosidase for 2 h at 37 °C (enzyme activity 0.5 µM/well). The plates were washed again with buffer, and then incubated with chlorophenol red-β-d-galactopyranoside (Boehringer, Germany) in substrate buffer for another 2 h at 37 °C. The colorimetric reaction product was measured at 570 nm using a microplate reader (Dynatech MR 5000, Germany). NGF concentrations were determined from the regression line for the purified mouse NGF standard (ranging from 1.56 to 50.0 pg/well) incubated under similar conditions in each assay. The limit of sensitivity of NGF ELISA was 5 pg/assay. Under these conditions the sensitivity was 3 pg/ml and the recovery of NGF in our assay ranged from 80 to 90%. The recovery was estimated by adding a known amount of purified NGF to the hippocampus and cortex extracts, and the yield of the exogenous NGF was calculated by subtracting the amount of this NGF from the endogenous NGF. Data were represented as pg/g wet weight and all assays were performed in triplicate (Bracci-Laudiero et al., 1992; Weskamp and Otten, 1986).

**Immunocytochemistry**

In order to remove circulating blood elements animals were perfused via the aorta with 4% paraformaldehyde in 0.1 M PBS. To quench endogenous peroxidase activity, sucrose post-equilibrated coronal brain sections (20 µm in
Plasma and brain concentrations of haloperidol and risperidone

Plasma concentrations of antipsychotic drugs were: 1.61 ± 1.09 ng/ml haloperidol; 13.02 ± 2.26 ng/ml risperidone 1.15; 19.37 ± 2.58 ng/ml risperidone 2.3.

Brain concentrations were: (a) 78.75 ± 17.47 ng/g haloperidol; 4.59 ± 0.35 ng/g risperidone 1.15; 3.02 ± 0.50 ng/g risperidone 2.3; (b) 92.20 ± 25.2 ng/g haloperidol; 2.49 ± 0.35 ng/g risperidone 1.15; 3.67 ± 0.15 ng/g risperidone 2.3; (c) 86.0 ± 29.7 ng/g haloperidol; 3.09 ± 1.46 ng/g risperidone 1.15; 3.02 ± 0.50 ng/g risperidone 2.3.

**Effects of haloperidol and risperidone on NPY-like immunoreactivity**

Figure 2 shows the concentrations of NPY-LI in 5 brain regions. In the hypothalamus, haloperidol and risperidone increased NPY levels (p < 0.01 for the effect of the treatment). Post-hoc comparisons showed that both haloperidol and risperidone, 2.3 mg/100 g food, significantly elevated NGF. In contrast, in the striatum, haloperidol induced a decrease in NPY-LI content (p = 0.05 for the effect of the treatment, p < 0.05 in post-hoc comparison), whereas risperidone did not significantly affect NPY-LI. In the frontal and occipital cortex neither haloperidol nor risperidone produced changes in NPY-LI levels compared to controls. Parallel to effects in the striatum, haloperidol and risperidone, 2.3 mg/100 g food, also decreased the NGF content in the hippocampus (p < 0.01 for the effect of the treatment; p < 0.05 in post-hoc comparison). Lower risperidone dose, 1.15 mg/100 g food, had no effect.
Antispychotic treatment and brain NGF, NPY and ChAT

Lastly, in the hippocampus, risperidone given at both doses caused a significant increase in NPY-LI ($p < 0.01$ for the effect of the treatment, $p < 0.05$ in post-hoc comparison). The results obtained with the two NPY antibodies were parallel, the Peninsula antibody giving about 20–25% higher immunoreactivity than the Brown antibody. The correlation coefficient between the two sets of results was $R = 0.823$, $p < 0.0001$. Consequently, only the set of results obtained with Peninsula antibody is presented.

**Effects of haloperidol and risperidone on ChAT immunoreactivity**

The effects of haloperidol and risperidone on cholinergic neurons of medial septum and Meynert basal nucleus are shown in Figures 3–6.

Although no marked differences in the total number of neurons were noted between the groups, a significant decrease in number of ChAT-immunoreactive large-size neurons following both haloperidol and risperidone treatments in the septum (Figure 3) and Meynert’s nucleus (Figure 4) was found. In the septum there was a significant effect of the treatment for the neurons with area ranging 500–600 µm$^2$ and 600–700 µm$^2$ ($p < 0.05$) and in Meynert’s nucleus for the neurons with area ranging 300–400 µm$^2$ and 400–500 µm$^2$ ($p < 0.01$).

Post-hoc comparisons showed that in the medial septum (Figure 3), haloperidol decreased ChAT-immunoreactivity in neurons with area ranging 500–600 µm$^2$ ($p < 0.01$) and 600–700 µm$^2$ ($p < 0.05$). Both risperidone doses decreased ChAT-immunoreactivity in the neurons with an area between 500 and 600 µm$^2$ and 600–700 µm$^2$ ($p < 0.05$). Figure 5 shows that ChAT-immunoreactivity

---

**Figure 1.** Mean ± s.e.m. concentration of endogenous NGF (expressed as pg/g of fresh tissue) in the brain regions of control, haloperidol- and risperidone-treated rats. Asterisks indicate significant between-group differences (*$p < 0.05$, **$p < 0.01$).

**Figure 2.** Mean ± s.e.m. NPY-like immunoreactivity (-LI) (expressed as fmol/mg of fresh tissue), measured with Peninsula antibody in the brain regions of control, haloperidol- and risperidone-treated rats. Asterisks indicate significant between-group differences (*$p < 0.05$).
is reduced in the septum of rats treated with haloperidol or risperidone, 2.3 mg/100 g food (similar results were seen with risperidone, 1.15 mg/100 g food). As shown in Figure 6, in Meynert’s basal nucleus, post-hoc comparisons showed that haloperidol and risperidone, 2.3 mg/100 g food, were effective in producing a decrease ($p < 0.01$, $p < 0.05$, respectively) in ChAT-immunopositive neurons with an area between 300 and 400 $\mu$m$^2$ and 400–500 $\mu$m$^2$ (Figure 4).

**Discussion**

In this series of experiments we explored effects of haloperidol and risperidone on NGF, a neurotrophin...
which plays a crucial role in differentiation and function of
specific brain neurons (Thoenen et al., 1987) and on NPY,
implicated in a variety of CNS functions and also, as
previously demonstrated, affected by several treatment
modalities, such as ECT and lithium (Mathé et al., 1990a,
1997).

The results show that haloperidol causes a consistent
upregulation of NGF in the hypothalamus and a decrease
in the hippocampus. In addition, haloperidol lowers the
basal NGF level in the striatum, the brain region intimately
involved in dopaminergic-related behaviours. Parallel to
haloperidol, risperidone also raised NGF levels in the
hypothalamus and reduced them in the hippocampus. Our
findings also demonstrate that haloperidol induces a
significant increase in NPY only in the occipital cortex,
whereas risperidone causes a significant increase of NPY
in the hypothalamus, occipital cortex, hippocampus, and
in a non-significant manner in the frontal cortex. In the
medial septum and in Meynert basal nucleus haloperidol
and risperidone, at the two doses used, caused a reduction
in the number of large-size ChAT-immunopositive
neurons. The reduction of ChAT-immunopositive
neurons in haloperidol-treated rats reached a higher level
of significance as compared to risperidone-treated rats.

NGF is mainly produced and stored in the hippocampus
and cortex (Chronwall et al., 1985) but other brain regions
are known to synthesize NGF both under normal
conditions and following neuronal damage (Springer et
al., 1994). For example, chronic neurotoxic injury increases
the expression of the NGF gene in a number of brain areas
including the basal forebrain nuclei and their cortical
target regions (Strauss et al., 1994). The mechanism
through which haloperidol and risperidone alter the levels
of NGF and the functional significance of these changes
are not known. However, since NGF influences dopa-
minergic neurons following neurotoxin injury (García et
al., 1992) and in Parkinson’s disease (Lorigrados et al.,
1996), and these effects appear to be mediated by
dopaminergic neurons expressing NGF receptors (Nishio
et al., 1998), it is possible that haloperidol and risperidone
might interfere with or enhance, depending on the
circumstances, the synthesis and uptake of brain NGF (Liu
and Alreja, 1997). Thus, the changes observed following
haloperidol and risperidone might alter the ability of
specific sets of neurons to synthesize and release NGF or
the response of NGF-responsive cells. In this context it is
worth mentioning that DA receptor antagonists have also
been shown to block NGF-induced hyperactivity caused
by intracerebroventricular NGF administration (Kobay-
ashi et al., 1997) and that haloperidol and other D₂
antagonists induce NGF mRNA expression in the hippo-
campus, piriform cortex and striatum (Ozaki et al., 1999).
These observations therefore raise the possibility that
chronic intake of antipsychotic drugs can influence
endogenous molecules such as neurotrophic factors,
which are known to play an important role in macro- and
micro-plasticity of brain neurons and in neurotransmitter
regulation (Garofalo et al., 1992; Prakash et al., 1996).
Hippocampal regions are directly affected in schizo-
phrenia (Falkai and Bogerts, 1986; Weinberger, 1999).
Since the hippocampus synthesizes and stores the largest
amount of NGF, the decrease in NGF hippocampal levels
observed after treatment with antipsychotic drugs raises
the possibility of a functional correlation between low

Figure 6. ChAT-immunoreactivity in the Meynert basal
nucleus of rats treated with (A) vehicle, (B) risperidone,
2.3 mg/100 g food, and (C) haloperidol. Note the decreased
 immunoreactivity in the cholinergic neurons. Magnification,
×300; scale bar, 50 µm.
availability of neurotrophins and cognitive deficits occurring in schizophrenic disease. Recent studies indicating that schizophrenia and/or treatment with neuroleptic drugs reduces dendritic spines in brain regions (Kelley et al., 1997), alters brain cholinergic activity (Mahadik et al., 1988), and impacts cognitive abilities (Gallohofer et al., 1996), are consistent with this hypothesis. Since NGF is known to play an important role in these events (Lo, 1995; Theoen, 1995), it is possible that prolonged treatment with haloperidol and risperidone might negatively influence cognitive processes. This hypothesis is in line with studies showing that risperidone treatment causes less severe deficit in the patient's ability to perceive emotion, compared to haloperidol (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, however, to be demonstrated. 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 NT-3 is associated with schizophrenia (Nanko et al., 1994), are consistent 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) and that haloperidol markedly alters NGF plasma levels in humans (Aloe et al., 1997) and NGF brain levels in mice (Alleva et al., 1996). These observations further support the hypothesis that haloperidol and risperidone treatment in humans might influence brain tissue and circulating levels of neurotrophins.

Our present data also showed an increase in NGF level in the hypothalamus with both haloperidol and risperidone. Classical antipsychotics elevate levels of circulating hormones, like prolactin, acting on pituitary gland and causing side-effects, like galactorrhoea (Goebel et al., 1992; Van Putten et al., 1991). Other authors showed that transient suppression by stress of haloperidol-induced catalepsy is mediated by activation of adrenal medulla (Yntema and Korf, 1987). These collateral effects are reduced by using atypical substances like risperidone (Kleinberg et al., 1999). It is possible, therefore, that an activation of adrenal axis is mediated by the hypothalamic increase in NGF levels. The increase in NGF is most probably linked to long-term treatment associated with behavioural tolerance. In fact in a previous study in mice we observed a decrease of NGF in the hypothalamus after acute intraperitoneal haloperidol administration, a result more in line with an anti-stress immediate action of the compound (Alleva et al., 1996). Based on these studies, a possible explanation regarding these different results is that haloperidol was administered at high doses with a single intraperitoneal administration and most probably the effect on NGF was transient, whereas in our present chronic treatment the effect was long lasting. Despite these differences, the observations that stress-related events such as aggressive and defensive behaviour (Aloe et al., 1986), alcohol withdrawal syndrome (Aloe et al., 1996), and administration of antipsychotics (Aloe et al., 1997, Bersani et al., 1999) significantly alter the level of NGF and NGF receptor expression in specific brain regions, further indicate that the antipsychotic drugs used in our studies influence the constitutive level of brain NGF and most probably the neurotransmitter's synthesis and release and/or synaptic plasticity.

NPY is one the most abundant neuropeptides in the mammalian brain (Chronwall et al., 1985) and plays a role in several central regulatory functions (Heilig and Widerlöv, 1995; Wahlestedt and Heilig, 1995). In our experiment the brain concentrations of NPY were increased after both haloperidol and risperidone treatment in selected brain regions. These findings are in line with previous experiments where we have demonstrated that dopamine D1 and D2 agonists and antagonists and NMDA antagonists affect both brain levels and release in vivo of several neuropeptides, e.g. NPY, CGRP and neurotensin (Gruber et al., 1996, Gruber and Mathé, 1997; Hertel et al., 1996; Mathé et al., 1994, 1996). Similarly, haloperidol and SCH-23390, another dopamine D1 antagonist, increase NPY levels in rat cortex (Sakai et al., 1995; Smialowska, 1995). Moreover, compounds increasing DA availability like cocaine (Wahlestedt et al., 1991), MK-801 and PCP (Midgley et al., 1993, 1994) decrease NPY tissue levels and expression in rat cortex, nucleus accumbens and caudate putamen. Since DA interacts with NPY in the brain it is conceivable that haloperidol and risperidone affect NPY either via their antidopaminergic action or by some, unknown, direct mechanism. In either case, these findings are in line with the previously reported NPY–DA interactions and indicate a novel mechanism of action of antipsychotic drugs.

The reduction of ChAT-LI in the medial septum after haloperidol and higher dose of risperidone treatment is positively correlated with the decrease in NGF in the hippocampus. This finding further indicates the NGF trophic action on this type of neuron (Rylett and Williams, 1994) and suggests that these drugs may impair the effect of NGF on the septo-hippocampal pathway (Rylett and
levels change in different brain regions seems to suggest different extent. The observation that NPY and NGF in the hypothalamus, striatum and hippocampus to a part, NPY expression in the periphery. Haloperidol that NGF vicissitudes in the hypothalamus regulate, in the CNS message in the periphery, it is conceivable and the hypothalamus plays a crucial role in mediating since NGF regulates NPY expression in the periphery blockade is possibly not the only mechanism involved.

Our findings revealed that the concentrations of NGF and NPY in the hypothalamus and hippocampus are inversely correlated. The level of NGF is low in the hypothalamus and high in the hippocampus, whereas NPY concentration is high in the former and low in the latter. Whether this type of correlation has a functional significance remains to be clarified. In the CNS, NGF administration increases NPY mRNA production in the adult rat forebrain and NPY can be regulated by NGF in the hippocampus (Carnahan and Nawa, 1995; Croll et al., 1994). As for behaviour, both NGF and NPY participate in the fine tuning of learning and other behavioural processes of vertebrates (Alleva et al., 1993; Bouchard et al., 1997; Richardson et al., 1995) demonstrating further links between these two compounds.

The increase in NPY content in the hypothalamus after antipsychotic treatment was already observed in other experiments (Obuchowicz, 1996). It has been hypothesized that blockade of DA receptors by neuroleptics abolishes the inhibitory dopaminergic control of NPY content (Li and Pelletier, 1986). We found a major effect of risperidone indicating that dopaminergic receptor content (Li and Pelletier, 1986). We found a major effect of risperidone on NGF production in the hippocampus. This finding suggests the possible existence of a dose–responsive effect or other mechanisms of action of risperidone on NGF production in the hippocampus. Likewise, the changes in ChAT immunoreactivity in the basal nucleus of Meynert were not associated with a concomitant decrease in NGF level in the cortex. One possible explanation is that under our experimental conditions the ChAT expression is not regulated by the cortical NGF production. Another possibility is that these antipsychotic treatments could influence cholinergic enzyme activity through or associated with other neurochemical mediators. Indeed, evidence that dopaminergic and cholinergic interaction can affect cateleptic responses to antipsychotics has been reported (Ushijima et al., 1997). Nevertheless, a neuroleptic responsive mouse line showed a significantly higher number of striatal cholinergic neurons suggesting their possible involvement during haloperidol action (Dains et al., 1996).

In summary, our findings indicate that chronic antipsychotic treatment alters the brain levels of both NGF and NPY in selected brain regions and that, in some instances, the changes are in opposite directions. In view of NGF–NPY interactions (Carnahan and Nawa, 1995; Croll et al., 1994) and their interactions with the dopaminergic system (Drumheller et al., 1994; Garcia et al., 1992; Heilig et al., 1990; Lindefors et al., 1990; Lorigrados et al., 1996; Nishio et al., 1998), it is reasonable to assume that one of the mechanisms of action of antipsychotic drugs is exerted via both NPY and neurotrophins.

Acknowledgements

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. Supported by the Swedish Medical Research Council grant no. 10414, the Italian National Research Council (CNR) and the Karolinska Institutet.

References


Mahadik SP, Laev H, Korenovsky A, Karpick SE (1988). Haloperidol alters rat CNS cholinergic system: enzymatic...
and morphological analyses. *Biological Psychiatry* 24, 199–217.


Antispychotic treatment and brain NGF, NPY and ChAT


