Antidepressive and Antipsychotic Treatments: Effects on Nerve Growth Factor and Brain-Derived Neurotrophic Factor in Rat Brain

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ABSTRACT

Recently it has been reported that depression and schizophrenia, the two major psychiatric disorders, are characterized by loss of neurons in specific brain regions. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are proteins involved in neuronal survival and plasticity of dopaminergic, cholinergic and serotonergic neurons in the central nervous system (CNS). It has been also hypothesized that these molecules play a role in the pathophysiology of CNS disorders. To test this hypothesis, we measured by ELISA NGF and BDNF in an animal model of depression, the Flinders Sensitive Line (FSL) rats and their controls, the Flinders Resistant Line (FRL). Increased NGF and BDNF concentrations were found in frontal cortex of female and in occipital cortex of male of "depressed" FSL compared to FRL control rats. These observations suggest that NGF and BDNF might be relevant to some of the neurochemical changes in depression. Using the same model of depression, (FSL/FRL rats), we investigated effects of electroconvulsive stimuli (ECS), a model of antidepressant treatment, on brain NGF and BDNF. In the hippocampus ECS increased NGF concentration in FSL, but not FRL. ECS decreased NGF concentration in the frontal cortex of FSL rats. In both FSL and FRL rats ECS increased NGF levels in the striatum. The data obtained support the notion that ECS may alter NGF brain concentration. In contrast, ECS did not change BDNF concentration in hippocampus, frontal cortex and striatum of FSL and FRL rats. Previous studies have reported that BDNF mRNA increases after ECS in hippocampus of Sprague Dawley rats. To further investigate the effect of ECT, we investigated if ECS alter the regional brain protein concentrations of NGF, BDNF and glial cell line-derived neurotrophic factor (GDNF), another trophic factor for dopaminergic neurons, in Sprague Dawley rats from which the two Flinders lines were originally bred. ECS increased the concentrations of NGF in the frontal cortex and concentrations of BDNF in hippocampus, striatum and occipital cortex. In contrast, ECS decreased GDNF concentrations in hippocampus and striatum.

ECS is used as a model of electroconvulsive therapy (ECT) which was originally introduced as a treatment for schizophrenia, and sometimes still used for that purpose. Since recent findings have raised the possibility that NGF and BDNF are also altered in the CNS of animal models of schizophrenia, we also investigated whether treatment with antipsychotic drugs (haloperidol, risperidone and olanzapine) affects the constitutive levels of NGF, BDNF, choline acetyltransferase (ChAT) immunoreactivities, and the receptor TrkB in the CNS. Haloperidol and risperidone elevated NGF concentrations in hypothalamus but decreased NGF in the striatum and hippocampus. They also decreased ChAT-immunoreactivity in large-size neurons in the septum as well as the Meynert's nucleus. Both haloperidol and risperidone decreased BDNF concentrations in frontal cortex, occipital cortex and hippocampus and altered TrkB receptors in selected brain structures. Olanzapine increased NGF in the hippocampus, occipital cortex and hypothalamus but decreased NGF mRNA in hippocampus and occipital cortex. Olanzapine decreased BDNF in the hippocampus and increased BDNF mRNA in the hippocampus and hypothalamus. In the frontal cortex olanzapine decreased both BDNF and BDNF mRNA. Our findings demonstrate that both typical and atypical antipsychotic drugs can alter the regional brain levels of NGF, BDNF, their mRNAs, and expression of ChAT and TrkB. Since NGF and BDNF can act on a variety of CNS neurons, it is reasonable to hypothesize that alteration of brain level of these neurotrophins could constitute part of the biochemical alterations induced by antipsychotic drugs. The neurotrophin hypothesis of schizophrenia proposes that alterations in expression of neurotrophic factors could be responsible for neural maldevelopment and disturbed neural plasticity both in young and adult subjects, thus constituting a potential line of research for understanding the etiopathogenesis of schizophrenia and development of new methods for diagnosis and treatment.

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To my family

TABLE OF CONTENTS

ORIGINAL PAPERS **6**

ABBREVIATIONS **7**

1. INTRODUCTION **8**

1.1 Neurotrophins **8**

1.1.1. Nerve Growth Factor **9**

1.1.2. Brain-Derived Neurotrophic Factor **9**

1.1.3. Neurotrophin receptors **9**

1.2 Neurotrophins and CNS neurons **11**

1.2.1. NGF-Producing Cells **11**

1.2.2. NGF Receptor Expressing Cells **11**

1.2.3. BDNF-Producing Cells **12**

1.2.4. BDNF Receptor Expressing Cells **12**

1.2.5. Cholinergic neurons and neurotrophins **13**

1.2.6. Dopaminergic neurons and neurotrophins **13**

1.2.7. Serotonergic neurons and neurotrophins **14**

1.3 Neurotrophins and depression? **17**

1.4 Animal models of depression **17**

1.4.1. Flinders Sensitive Line **18**

1.5 Antidepressive treatments **18**

1.5.1. Electroconvulsive treatment **18**

1.6 Neurotrophins and schizophrenia? **18**

1.7 Antipsychotic treatments **19**

1.7.1. Electroconvulsive treatment and schizophrenia **19**

1.7.2. Typical antipsychotic drugs **19**

1.7.3. Atypical antipsychotic drugs **20**

1.8 Peptides and neurotrophic factors **20**
1.8.1. Neuropeptide Y **20**
1.8.2. Glial Cell Line-Derived Neurotrophic Factor **21**

2. AIMS **22**

3. MATERIALS AND METHODS **23**
3.1 Enzyme linked immunosorbent assay (ELISA) **23**
3.2 Immunocytochemistry **24**
3.3 RT-PCR ELISA **25**
3.4 Radioimmunoassay (RIA) **26**

4. RESULTS **27**

5. DISCUSSION **30**

6. CONCLUSIONS **34**

7. ACKNOWLEDGEMENTS **36**

8. REFERENCES **38**

ORIGINAL PAPERS

This thesis consists of six papers that will be referred to by their roman numerals in the text:

I. **Angelucci F.,** Aloe L., Jimenez-Vasquez P., and Mathé A.A. Mapping the differences in the brain concentrations of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in an animal model of depression. Neuroreport 11: 1369-1373, 2000.

II. **Angelucci F.,** Aloe L., Jimenez-Vasquez P., and Mathé A.A. Electroconvulsive stimuli alter nerve growth factor but not brain-derived neurotrophic factor concentrations in brains of a rat model of depression. (submitted)

III. **Angelucci F.,** Aloe L., Jimenez-Vasquez P., and Mathé A.A. Electroconvulsive stimuli alter the regional concentrations of nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in adult rat brain. (J ECT, in press)

IV. **Angelucci F.,** Aloe L., Gruber S.H.M., Fiore M., and Mathé A.A. 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 Neuropsychopharmacology 3: 13-25, 2000.

V. **Angelucci F.,** Mathé A.A., and Aloe L. Brain-derived neurotrophic factor and tyrosine kinase receptor TrkB in rat brain are significantly altered following haloperidol and risperidone administration. J Neurosci Res 60: 783-794, 2000.

VI. **Angelucci F.,** Aloe L., Gruber S.H.M., and Mathé A.A. Effect of chronic olanzapine treatment on nerve growth factor and brain-derived neurotrophic factor and the mRNAs in the rat brain. (Manuscript)

ABBREVIATIONS

BDNF	Brain-derived neurotrophic factor
ChAT	Choline acetyltransferase
CNS	Central nervous system
ECS	Electroconvulsive stimuli
ECT	Electroconvulsive treatment
FRL	Flinders Resistant Line
FSL	Flinders Sensitive Line
GDNF	Glial cell line-derived neurotrophic factor
MRI	Magnetic resonance imaging
NGF	Nerve growth factor
NPY	Neuropeptide Y
NTs	Neurotrophins
PET	Positron emission tomography
PNS	Peripheral nervous system
TrkA	Tyrosine kinase receptor A
TrkB	Tyrosine kinase receptor B

1. INTRODUCTION

1.1 Neurotrophins

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) are derived from a common ancestral gene and are therefore collectively named neurotrophins (Hallbook, 1999). Neurotrophins (NTs) regulate development, maintenance, and function of peripheral (PNS) and central (CNS) nervous systems. These proteins also regulate many aspects of neural function. In the mature nervous system, they control synaptic function and synaptic plasticity, while continuing to modulate neuronal survival. All NTs have a similar overall structure, with a small number of amino acid differences between them, determining the specificity of receptor binding. The mature active forms of NTs are very stable non-covalently associated homodimers, with molecular weights around 28 kDa. Dimerization seems to be an essential requisite for NT receptor activation, and is a feature characterizing other growth factors as well (reviewed by Ibanez, 1998).

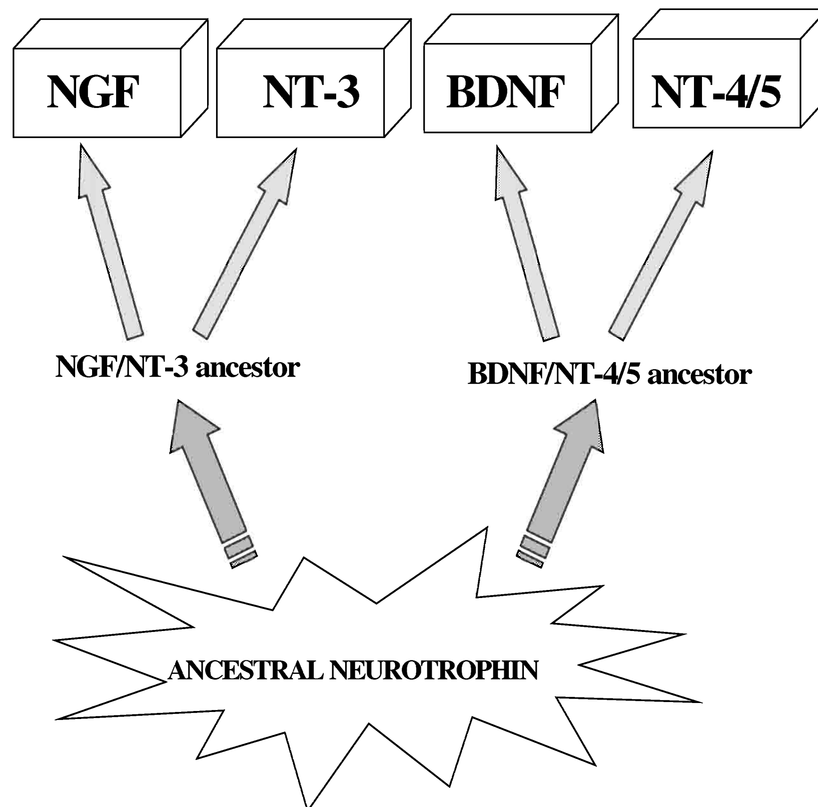


Fig. 1. Putative phylogenetic tree of the four neurotrophins found in humans. The four neurotrophins could have evolved from a common neurotrophin ancestor after two subsequent duplications that occurred at early vertebrate stages.

1.1.1. Nerve Growth Factor

Nerve growth factor (NGF), the first one to be characterized, was discovered during a search for such survival factors (cf review Levi-Montalcini, 1987). This molecule exists as a dimer of two identical polypeptide chains, each of 118 amino acid residues (McDonald and Blundell, 1991). NGF was purified as a factor able to support survival of sympathetic and sensory spinal neurons in culture (Levi-Montalcini, 1987).

Anti-NGF injections demonstrated that this factor is important in maintaining survival of sympathetic neurons in vivo as well as in vitro. In the PNS, NGF is synthesized and secreted by sympathetic and sensory target organs (cf review Korsching 1993). From these sources, it is captured in nerve terminals by receptor-mediated endocytosis and is transported through axons to neuronal cell bodies where it acts to promote neuronal survival and differentiation. Within the target organs, synthesis of NGF and of other neurotrophins is associated with peripheral tissues such as cutaneous tissues, internal organs and hair follicles, which become innervated by sensory and sympathetic neurons.

In contrast to the periphery, NGF expression in the CNS is much more restricted; NGF mRNA and protein are expressed in a number of brain regions, with the hippocampus providing the single largest source in the entire CNS (Korsching et al, 1985). In the hippocampus, NGF RNA and protein are expressed by the principal excitatory (glutamate) neurons, as well as by a subset of γ-aminobutyric acid (GABA)-containing inhibitory neurons (Rocamora et al, 1996). These hippocampal target cells receive rich innervation from ascending neurons with their cell bodies in the basal forebrain.

1.1.2. Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor (BDNF) was purified from pig brain, due to its survival-promoting action on a subpopulation of dorsal root ganglion neurons (Barde et al, 1982). The amino acid sequence of mature BDNF has a strong homology with that of NGF (Leibrock et al, 1989; Rosenthal et al, 1991). As is the case for NGF, BDNF is necessary for survival of some peripheral sensory neurons, notably those in the vestibular ganglia and nodose-petrosum ganglia. Interestingly, part of the trophic effects of BDNF in the peripheral nervous system seem to depend on autocrine loops and paracrine interactions between adjacent neurons, since sensory neurons can express both BDNF and its high affinity receptor TrkB.

BDNF is more highly expressed and widely distributed than NGF in the CNS, and has survival promoting actions on a variety of CNS neurons including hippocampal and cortical neurons (Ghosh et al, 1994; Lindholm et al, 1996), cholinergic neurons (Alderson et al, 1990), and nigral dopaminergic neurons (Hyman et al, 1991). These facts raised keen interest in BDNF as a potential therapeutic agent for Parkinson's disease and Alzheimer's disease, among other neurodegenerative disorders and non-degenerative pathologies. Recent findings demonstrated that BDNF is anterogradely transported in the CNS, a fact that has considerably expanded the concept of neuronal-derived trophic support, and sustains the hypothesis that BDNF can have actions at the synaptic level (Altar and DiStefano, 1998).

1.1.3. Neurotrophin receptors

The biological actions of NTs are mediated by two classes of membrane receptors (cf reviews Barbacid, 1995; Bothwell, 1995; Chao et al., 1998; Dechant, 2001): the Trk family of receptor tyrosine kinases and a protein nowadays named as NT receptor p75 (p75NTR), a member of the TNF receptor superfamily. All NTs bind with similar affinities (K_d~10^{-9} M) to p75NTR. The mechanisms of transduction mediating the biological effects of p75NTR in neurons are poorly understood. On the one hand, p75NTR can modulate cellular responses to NTs, by interacting with their high-affinity Trk. Modulation of Trk interaction with NTs has been considered as the main p75NTR mechanism of action since the discovery of Trk receptors

(Barbacid, 1995; Bothwell, 1996; Chao and Hempstead, 1995). However, it was clearly established that p75NTR can induce cellular responses in the absence of Trk receptors, such as cell death, when it is activated by NGF (Carter et al., 1996; Frade JM, Barde YA, 1998). Furthermore, NTs bind with distinct selectivities to three highly related receptor protein-tyrosine kinases, known as high-affinity (K_d~10^{-11} M) NT receptors TrkA, TrkB and TrkC. NGF specifically binds to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 binds preferentially to TrkC, but also to TrkB and TrkA with lower efficacy (Kaplan and Miller, 1997; Klein, 1994). Through these receptors, neurotrophins activate many signalling pathways, including those mediated by RAS and members of the cdc-42/ras/rho G protein families, and the MAP kinase, PI-3 kinase, and Jun kinase cascades. Within neural precursors and neurons, the pathways regulated by tyrosine kinases include survival and differentiation, axonal and dendritic growth and remodelling, assembly of the cytoskeleton, membrane trafficking and fusion, and synapse formation and function.

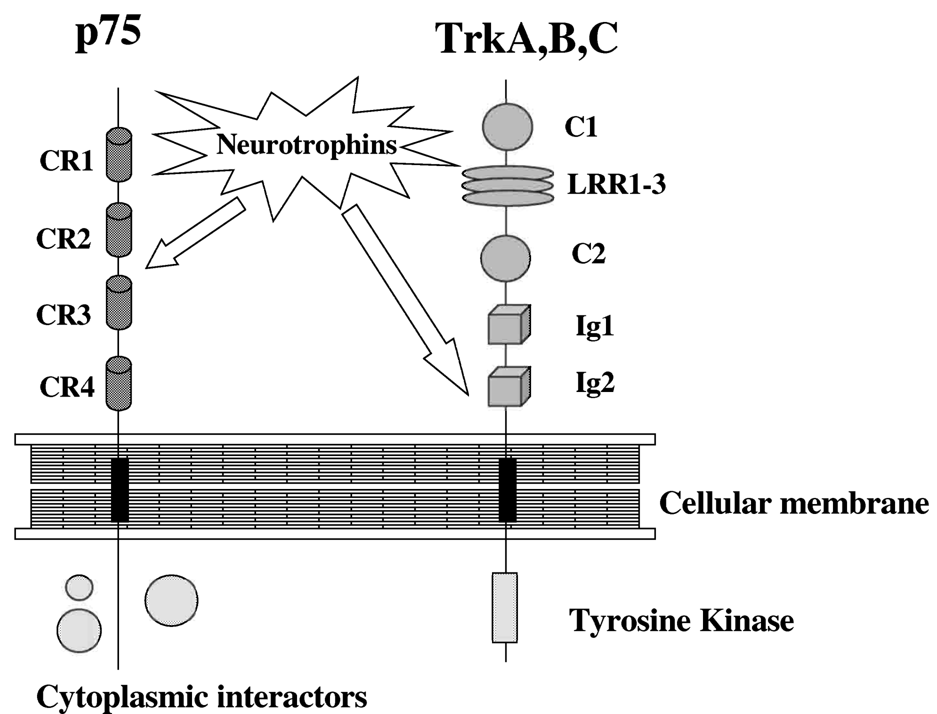


Figure 2. The two receptor classes of the neurotrophins. P75 is a transmembrane glycoprotein receptor of ~75 kD. There are four cysteine repeats (CR1-CR4) in the extracellular domain. Signalling of p75 occurs through cytoplasmic interactors. The Trk receptors are transmembrane glycoproteins of ~140 kD. They are tyrosine kinases with an extracellular ligand-binding domain containing multiple repeats of leucine-rich motifs (LRR1-3), two cysteine clusters (C1, C2), two immunoglobulin-like domains (Ig1, Ig2), and a single transmembrane domain. The second Ig-like domain, whereby each Trk receptor binds the corresponding ligand through a distinct specific sequence, mostly determines binding specificity of the Trk receptors.

1.2 Neurotrophins and CNS neurons

1.2.1. NGF-Producing Cells

NGF is produced in the CNS during development and throughout adult life. Most of NGF-producing cells are neurons, including pyramidal neurons, in the cortical and hippocampal target regions of basal forebrain cholinergic neurons. In the hippocampal formation, pyramidal and dentate granule neurons express NGF, as do subpopulations of GABAergic interneurons (French et al., 1999; Gall and Isackson, 1989; Pascual et al., 1998). NGF expression in hippocampus is regulated by neuronal activity; increases are caused by glutamatergic and cholinergic neurotransmission, and decreases are caused by GABAergic neurotransmission (Berzaghi et al., 1993; Knipper et al., 1994; French et al., 1999). In striatum, NGF is produced by a subpopulation of small interneurons (Bizon et al., 1999).
Among glial cells, NGF is produced throughout the CNS by astrocytes and microglia, and NGF expression in both cell types is markedly upregulated by local tissue injury, inflammation, cytokines, and bacterial lipopolysaccharide (both in vivo and in vitro) (Arendt et al., 1995; Elkabes et al., 1996; Heese et al., 1998, Micera et al., 1998; Yoshida and Gage, 1992). Increased NGF levels in the injured CNS suggest that astrocytes and microglial cells could serve as local sources of NGF for injured neurons and other NGF responsive cell types.

1.2.2. NGF Receptor Expressing Cells

p75NTR gene expression in the CNS is widespread, especially during development. p75NTR expression is more restricted in the adult. Several neuronal populations, including cholinergic neurons of the caudate-putamen and cranial nerve nuclei of the brainstem, show markedly reduced or no expression at the adult stage (Koh and Higgins, 1991). Cerebellar Purkinje neurons, hippocampal pyramidal neurons, and retinal ganglion neurons also downregulate expression to undetectable levels in adults but start again expressing p75NTR after injury (Brann et al., 1999; Eckenstein, 1998; Martinez-Murillo et al., 1998; Yamashita et al., 1999).
The majority of p75NTR-expressing neurons do not express TrkA. However, developing horizontal cells and amacrine cells of the retina express TrkA and potentially p75NTR (Karlsson et al., 1998), whereas cholinergic neurons of the septal-basal forebrain complex express both TrkA and p75NTR during development and throughout adult life (Holtzman et al., 1992). Expression of both TrkA and p75NTR in forebrain neurons is upregulated by NGF (Gage et al., 1989; Holtzman et al., 1992). Adult cholinergic neurons of the extended striatal complex (caudate, putamen, accumbens, etc) express mainly TrkA; however, p75NTR is upregulated to detectable levels, and TrkA expression is increased by local tissue injury or NGF infusions (Gage et al., 1989; Holtzman et al., 1995). Adult neurons that express TrkA, but not p75NTR, are found in the thalamic paraventricular nuclei, rostral and intermediate subnuclei of the interpeduncular nucleus, and various other brain regions (Holtzman et al., 1995; Venero et al., 1994), and also in the spinal cord in regions associated with regulation of the autonomic outflow (Michael et al., 1997). Some hippocampal pyramidal neurons may also express very low levels of TrkA (Cellerino, 1995), and a recent immunocytochemical study points to the presence of TrkA and p75NTR proteins in pyramidal cells of the somatosensory cortex of the mature rat (Pitts and Miller 2000).

Among glial cells, very low levels of p75NTR are present in many mature astrocytes. p75NTR and TrkA are also expressed by astrocytes in vitro, particularly after exposure to NGF or inflammatory cytokines (Hutton and Perez-Polo, 1995; Kumar et al., 1993). Oligodendrocytes express p75NTR (Casaccia-Bonnefil et al., 1996; Kumar et al., 1993). Microglia have the capacity to express p75NTR and TrkA, and expression levels are modulated by inflammatory stimuli, such as cytokines and bacterial lipopolysaccharide (Elkabes et al., 1998).

1.2.3. BDNF-Producing Cells

BDNF mRNA is widely expressed by neurons. High levels of BDNF mRNA are present in the rodent (Hofer et al., 1990; Enfors et al., 1990ab; Wetmore et al., 1990) and human brain (Phillips et al., 1990). BDNF and TrkB are co-expressed by neurons in the rat cerebral cortex and hippocampal formation (Kokaia et al., 1993) suggesting the existence of autocrine/paracrine functional interactions. In addition, cortical and hippocampal BDNF can function as target-derived neurotrophic factor for local circuit neurons, cortical, and hippocampal afferent neurons. BDNF protein is present in striatal fibers but not in striatal neuronal cells bodies (Dugich et al., 1995; Kawamoto et al., 1996; Conner et al., 1997; Yan et al., 1997) and levels of BDNF mRNA are very low in the rodent striatum (Wetmore et al., 1990; Conner et al., 1997). To our knowledge, there are no reports demonstrating the presence of BDNF mRNA in basal forebrain human cholinergic neurons. Rat studies show that a minority of cells in the basal forebrain express BDNF mRNA, but many cells contain BDNF mRNA in the medial septum (Conner et al., 1997; Yan et al., 1997). It seems that BDNF is synthesized by a small number of cholinergic neurons projecting to the cerebral cortex and hippocampal formation. BDNF-immunoreactivity (Dugich et al., 1995; Kawamoto et al., 1996; Conner et al., 1997; Yan et al., 1997), and BDNF mRNA expression (Wetmore et al., 1990; Conner et al., 1997) have also been observed in rat and monkey nigral neurons. Seroogy et al. (1994) verified the dopaminergic nature of BDNF-containing nigral neurons in double labelling experiments, demonstrating the co-localization of BDNF mRNA and tyrosine hydroxylase in the rat mesencephalon. In the rat, BDNF mRNA is found in granule cells, but not Purkinje cells (Wetmore et al., 1990; Rocamora et al., 1993). However, in rodents, locus coeruleus neurons have BDNF mRNA (Castrén et al., 1995; Conner et al., 1997; Yan et al., 1997), and BDNF-immunoreactive cell bodies (Kawamoto et al., 1996).

BDNF mRNA expression in rodent cultured glial cells has been demonstrated. Acheson et al. (1991) reported BDNF expression by Schwann cells in culture, whereas Rudge et al. (1992) reported low levels of BDNF mRNA in primary rat astroglial cultures. Elkabes et al. (1996) demonstrated that rodent microglia express BDNF mRNA in vitro. Similar to NGF, BDNF expression and release by glial cells can be induced by injury (Batchelor et al., 1999).

1.2.4. BDNF Receptor Expressing Cells

Many neurons co-express both BDNF and TrkB (Kokaia et al., 1993). Consequently, BDNF can potentially be involved in: (1) a kind of neurotransmitter-like axo-dendritic communication; (2) in autocrine loops and paracrine interactions between neighbouring cells; (3) in a more classic mechanism as a trophic factor, that is, retrograde communication from dendrites to axon terminals. In rodents, cortical and hippocampal interneurons express full-length TrkB but not BDNF, (Gorba et al., 1999). The mRNA for the catalytic form of TrkB is expressed in neurons of the human cerebral cortex, and pyramidal and granule cell layers of the hippocampus (Benisty et al., 1998).

In the rat, most nigral dopaminergic neurons express TrkB mRNA and protein (Merlio et al., 1993; Altar et al., 1994; Numan and Seroogy, 1999). In the rodent cerebellum, TrkB-immunoreactivity (Schwartz et al., 1997), and TrkB mRNA (Gao et al. 1998), was found both in granule and Purkinje cells.

In humans TrkB is expressed by nigral dopaminergic neurons and striatal neurons (Allen et al., 1994; Benisty et al., 1998; Nishio et al., 1998). Cholinergic neurons of the nucleus basalis of Meynert express TrkB mRNA and protein in the human brain (Benisty et al., 1998; Boisiére et al., 1997).

Cultured microglia express p75NTR and all Trks receptors for NTs and also respond to BDNF (Elkabes et al., 1996; Nakajima et al., 1998). Glial cells were found to express the

truncated and the catalytic forms of TrkB (Nakajima et al., 1998). Rubio (1997) demonstrated that cultured mouse astrocytes express the non-catalytic truncated form of TrkB, which mediates exogenous BDNF internalization and storage.

1.2.5. Cholinergic neurons and neurotrophins

The distribution of NGF and its receptors suggests an intimate interrelationship between cholinergic neurons and their NGF-producing targets. Cholinergic neurons account for nearly all of the NGF-responsive neurons in the CNS, although there are groups of noncholinergic neurons that express TrkA, including some of the principal cells of the hippocampus (Cellerino et al., 1996).

To what extent NGF is necessary as a survival factor for adult cholinergic neurons remains controversial. It promotes the survival of cholinergic neurons after axotomy (Hefti, 1986). In the adult, NGF influences the messenger RNA levels, structural plasticity, response to injury, and maintenance of basal forebrain cholinergic neurons. Administration of NGF to the uninjured CNS causes a number of effects on cholinergic neurons, including hypertrophy, sprouting, upregulation of NGF receptors, increased levels of choline acetyltransferase (ChAT), and increased choline uptake (Heisenberg et al., 1994; Higgins et al., 1989; Lapchak et al., 1994). These results are all consistent with a role of NGF to maintain the cholinergic phenotype through retrograde transport of the NGF/TrkA signalling complex from cholinergic nerve terminals in the hippocampus to the cell bodies in the basal forebrain (Seiler and Schawb, 1984).

NGF can cause rapid activation of calcium-dependent potassium channels (Holm et al., 1997) and voltage-sensitive calcium channels (Jia et al., 1999), actions that may underlie NGF effects on cholinergic neuronal excitability and acetylcholine release. These findings fit well with the emerging evidence that neurotrophins can behave as neurotransmitter-like molecules. In fact, in a similar manner to a neurotransmitter, NGF can be released by neurons upon depolarization (Blöchl and Thoenen, 1995). Finally, NGF administration prevents cholinergic neuron atrophy caused by lesions of the septohippocampal and basal forebrain-cortical systems ((Hefti, 1999). Although basal forebrain cholinergic neurons are responsive to NGF during their development, other trophic factors are required for their survival. The actual survival factors are unknown, but it is clear that developing cholinergic neurons are responsive to a number of trophic factors, particularly BDNF (Ward and Hagg, 2000) and maintain this responsiveness in adult life (Koliatsos et al., 1994). Shortly after BDNF discovery, Alderson et al. (1990) showed that BDNF promotes survival of rat embryonic septal cholinergic neurons in culture. BDNF increased the activity of the enzymes involved in acetylcholine synthesis and degradation, ChAT and acetylcholinesterase. Most studies show that BDNF administration provides partial protection to basal forebrain cholinergic neurons against damage induced by axotomy or target cell destruction. It must be mentioned, however, that all studies comparing the protective effects of BDNF and NGF on forebrain cholinergic neurons, found that the former is not as effective as NGF (Koliatsos et al., 1994; Venero et al., 1994).

1.2.6. Dopaminergic neurons and neurotrophins

NGF influences dopaminergic neurons following neurotoxin injury (Garcia 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).

Early reports by Hyman et al. (1994) and Knüsel et al. (1997) showed that BDNF prevents the spontaneous death of dopaminergic neurons in rat primary mesencephalic cultures. Hyman et al. (1991) further demonstrated that BDNF administration protects TH-immunoreactive neurons from the selective toxin MPP^+ (1-methyl-4-phenylpyridinium).

These results raised interest in BDNF as a putative novel therapeutic agent for Parkinson's disease. Cultured dopaminergic neurons treated with BDNF show increased TH activity, dopamine uptake and dopamine content, release more dopamine upon depolarization, and display an increased cell body size and higher neuritic complexity (Beck et al., 1993; Hyman et al., 1994). The protective effect of BDNF in vitro against specific toxins like MPP^+ and 6-OHDA, has likewise been confirmed (Beck et al., 1992)

Shultz et al. (1995) studied the effect of repeated intrastriatal injections of BDNF on damage induced by intrastriatal administration of 6-OHDA, and found evidence of behavioral improvement, and moderate sparing of striatal dopaminergic nerve endings around the injection site. Hung and Lee (1996) further reported a protective effect of direct nigral infusion of BDNF against reductions in striatal dopamine content induced by MPTP in mice. In summary, there is a body of work suggesting that exogenous BDNF administration can increase survival and/or promote recovery of injured mesencephalic dopaminergic neurons at least in some experimental conditions.

1.2.7. Serotonergic neurons and neurotrophins

It has been reported that 45% of the serotonergic raphe neurons also express both TrkA (Gibbs and Pfaff, 1994; Sobreviela et al., 1994) and p75NTR (Koh et al., 1989). However, a functional relationship between NGF and serotonergic neurons has not yet been shown. Serotonergic lesion of the median raphe nucleus results in biphasic changes of NGF protein content and in a delayed increase in the vulnerability of septohippocampal cholinergic neurons (Hellweg et al, 2001).

Neuroprotection by the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) has been demonstrated against excitotoxic neuronal damage in cholinergic neurons of the rat magnocellular nucleus basalis (Oosterink et al., 1998). Stimulation of 5-HT_{1A} receptors inhibits apoptosis initiated by serum deprivation in cultured neurons from chick embryo, possibly by induction of nerve growth factor (NGF) synthesis (Ahlemeyer and Krieglstein, 1997), and mediates the neurotrophic effect of 5-HT in rat septal cholinergic neurons in culture and on developing dentate granule cells (Riad et al., 1994; Yan et al., 1997) BDNF has been demonstrated to have potent neurotrophic effects on 5-HT neurons when infused into midbrain (Siuciak et al., 1994). In addition, infusion of BDNF into forebrain results in a dramatic elevation of 5-HT neuronal fiber density, and protection of neurons from neurotoxic damage (Mamounas et al., 1995). BDNF expression can also be regulated by serotonin. For example, BDNF mRNA is decreased in the rat hippocampus and increased in the frontal cortex by treatment with serotonin agonists suggesting that 5-HT modulates BDNF mRNA levels in rat brain (Nibuya et al., 1996; Vaidya et al., 1997; Zetterström et al., 1999).

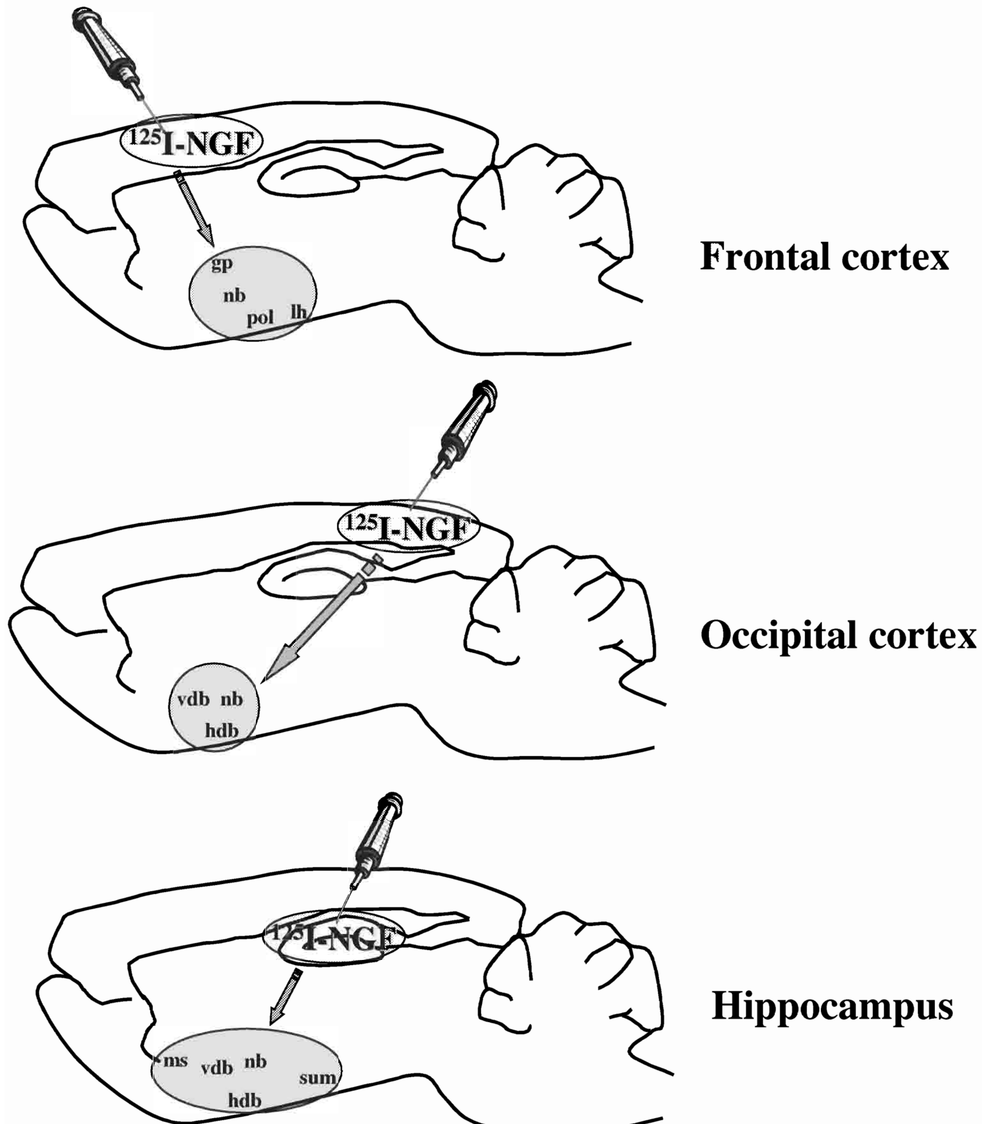
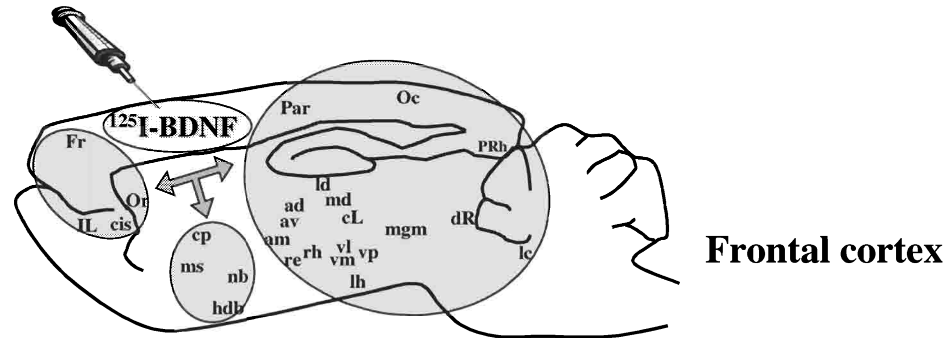
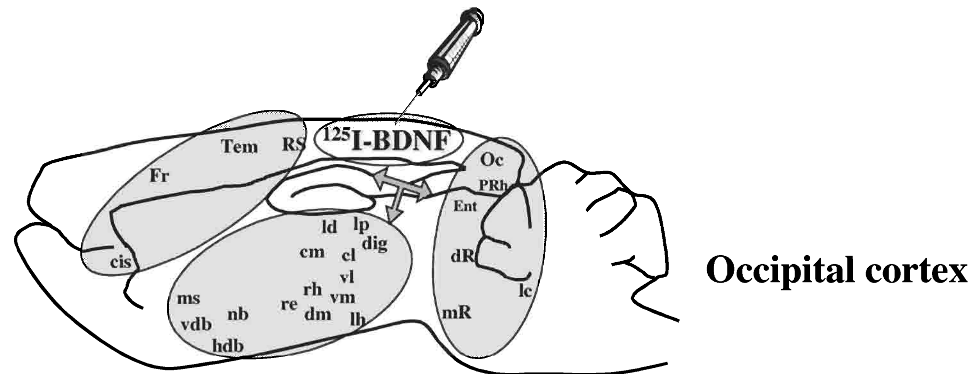


Fig. 3. Retrograde transport of iodinated NGF following injection into the frontal cortex, occipital cortex and hippocampus from the sites of injection. gp: globus pallidus; hdb: nucleus of the orizontal limb of the diagonal band; lh: lateral hypothalamic area; ms: medial septal nucleus; nb: nucleus basalis; pol: lateral preoptic nucleus; sum: supramammilary nucleus; vdb: nucleus of the vertical limb of the diagonal band (compiled from Mufson et al., 1999).

BDNF injections





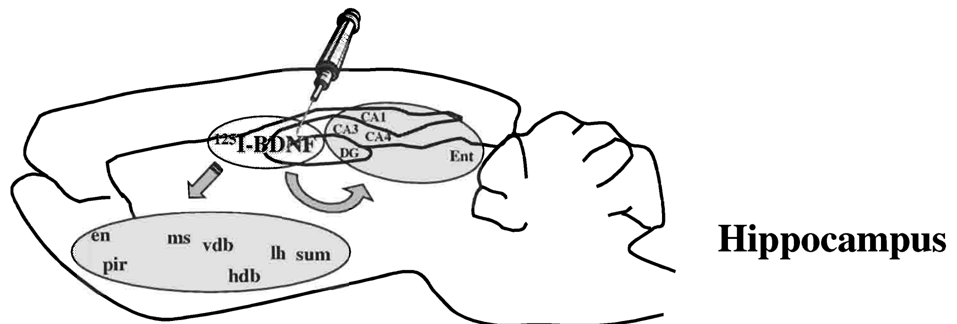


Fig. 4. Retrograde transport of iodinated BDNF following injection into the frontal cortex, occipital cortex and hippocampus from the sites of injections. ad, am, av: anterior thalamic nuclei; CA 1-4: subfields of Ammon`s horn; cp: caudate putamen; DG: dentate gyrus; dR: dorsal raphe nucleus; en: endopiriform nucleus; Ent: entorhinal cortex; Fr: fasciculus retroflexus; gp: globus pallidus; hdb: nucleus of the orizontal limb of the diagonal band; lc: locus coeruleus; ld: laterodorsal thalamic nucleus; lh: lateral hypothalamic area; md: mediodorsal thalamic nucleus; mgm: medial geniculate nucleus; ms: medial septal nucleus; nb: nucleus basalis; Oc: occipital cortex; Or: orbital cortex; Par: parietal cortex; pir: piriform cortex; PRh: perirhinal cortex; pol: lateral preoptic nucleus; re, rh: reuniens and rhomboid thalamic nuclei; sum: supramammilary nucleus; vdb: nucleus of the vertical limb of the diagonal band; vl, vm, vp: ventral thalamic nuclei (compiled from Mufson et al., 1999).

1.3 Neurotrophins and depression?

Recent findings suggest that neurotrophin function might be altered in depression. For example, decreased serum levels of BDNF in patients with major depression (Karege et al., 2002) and an increased BDNF immunoreactivity in hippocampal BDNF in postmortem brain of patients treated with antidepressant drugs (Chen et al., 2001b) have been observed. Moreover, chronic infusion of BDNF is reported to have antidepressant effects in an animal behavioral model of depression (the learned helplessness) and in the forced swim test (Siuciak et al., 1997). In addition, stress, a precipitating factor in depression, has been shown to cause atrophy and, in severe cases, death of vulnerable CA3 neurons in the rat hippocampus (Sapolsky et al., 1990; McEwen BS and Magarinos, 1997) and to decrease the expression of BDNF in CA3 pyramidal and dentate gyrus granule cell layers in rats (Smith et al 1995). Thus, atrophy and decreased functions of the hippocampus could explain the decrease, observed in some depressed patients, of negative feedback control that this brain region exerts on the hypothalamic-pituitary-adrenal axis (Young et al., 1990).

In addition to monoamines, several neuropeptides, including neuropeptide Y (Husum et al., 2001; Jimenez-Vasquez et al., 2000; Mathé et al., 1998; Stenfors et al., 1995), are thought to play a role in depression or learned helplessness behavior in rats. Several recent papers have examined the effects of central administration of BDNF in rats on neuropeptide distribution (Croll et al., 1994; Nawa et al., 1994). Therefore, the modulation of neuropeptide systems by BDNF, whether a direct or indirect effect, may contribute to the antidepressant-like effects of this protein.

Although these pilot studies have demonstrated an antidepressant-like effect of BDNF, the anatomical sites capable of mediating this effect remain to be determined. Furthermore, the possibility of BDNF-induced changes in monoamine and/or peptide receptors has not yet been addressed.

1.4 Animal models of depression

To date, several animal models of depression have been developed. They include models in which "depressive behavior" is the result of genetic selection or manipulation, environmental stressors during development or in adulthood. Genetic models of depression include the Fawn-Hooded (FH/Wjd) rat, that is an inbred strain of rat reported to exhibit high immobility in the forced swim test (Rezvani et al., 2002) and the Wistar Kyoto (WKY) rat, characterized by hyperreactivity to stress and depressive-like behavior in several standard behavioral tests (Solberg et al., 2001). During postnatal development, maternal separation induces a response characterized by reduced activity and other depressive-related behaviors (Hennessy et al., 2001; Husum et al., 2002; Jimenez-Vasquez et al., 2001). A regimen of repeated, mild, unpredictable stressors is used to induce anhedonia and depressive-like behavior in rats (Moreau et al., 1995). In addition, learned helplessness, in which exposure to inescapable stress produces deficits in escape testing, has been proposed as a valid animal model of stress-induced behavioral depression (Jenkins et al., 2001). The assessment of these animal models is based either on behavioral tests measuring traits that are homologous to symptoms of the human disorder, or behavioral tests responsive to appropriate pharmacological treatments. Three general criteria for the validity of a model of disease, in this case depression, are: (1) face validity (similarity between the behavior observed in the model and the symptoms of depression), (2) predictive validity (manipulation known to influence the pathological state should have similar effects in the model), and (3) construct validity (implies that human and animal responses are homologous so that the response has a clinical significance for the disorder). In this study we have used the Flinders Sensitive Line (FSL) rats, a genetic animal model of depression described below.

1.4.1. Flinders Sensitive Line

The Flinders Sensitive Line (FSL) rats and their corresponding controls, the Flinders Resistant Line (FRL) rats, were established by selective breeding for high and low sensitivity, respectively, to the anticholinesterase agent, diispopropyl fluorophosphate (Overstreet et al., 1979). This rat line was developed following findings of Janowsky et al. (1972) that an overactive cholinergic system seems to be associated with depression-like behavior while depressed patients displayed increased sensitivity to cholinergic agonists (Janowsky et al., 1980; Risch et al., 1981). These rats show an exaggerated immobility in the forced swim test compared to their control Flinders Resistant Line (FRL) rats (Overstreet et al., 1986). Immobility in the swim test may be used for evaluating the efficacy of antidepressant drugs (Lucki, 1997). Indeed, the exaggerated immobility in the forced swim test in the FSL rats can be counteracted by the administration of antidepressant drugs that are used clinically (Overstreet et al., 1995; Schiller et al., 1992). Thus, the FSL rats seem to meet the criteria of face, construct, and predictive validity for an animal model of depression (Overstreet, 1993) for studying the neurochemical mechanisms involved in depressive-like behavior and in the mode of action of antidepressants.

1.5 Antidepressive treatments

A variety of antidepressant drugs primarily affect serotonin and norepinephrine, including tricyclic antidepressants, selective serotonin (SSRIs) and norepinephrine (SNRIs) reuptake inhibitors, monoamine oxidase inhibitors (MAOIs), and newer, more receptor-specific agents such as venlafaxine and mirtazapine. Lithium and antiepileptic drugs as well as electroconvulsive therapy (ECT) are used in treatment and prophylaxis of mood disorders. In this thesis we focused on electroconvulsive stimuli (ECS), used as a model of ECT in animals.

1.5.1. Electroconvulsive treatment

The introduction of effective means of administration reducing the adverse secondary effects associated with the procedure has made electroconvulsive therapy (ECT) a safe treatment in psychiatry (American Psychiatric Association, 1990). ECT is used to treat psychiatric disorders, particularly major depressive disorder. In some cases, such as psychotic or melancholic depressive disorders, the technique has proved to be more effective than pharmacological treatments, particularly with regard to response latency (Bernardo et al., 1996). ECS cause massive release of noradrenaline, serotonin, GABA and dopamine (DA) (McGarvey et al., 1993; Sackeim, 1999; Tortella and Long, 1988; Zis et al., 1992). Moreover, ECT induces release of hormones, neuropeptides, and fatty acids (Madsen et al., 2000; Mathé, 1999).

1.6 Neurotrophins and schizophrenia?

Although a number of hypotheses (genetic alterations, complication during pregancy and birth, anatomical abnormalities and neurochemical deficits) have been proposed in an attempt to explain the pathophysiology of schizophrenia no single theory seems to account for all symptoms of the disease. Each hypothesis explains some of the phenomena associated with schizophrenia and it is probable that many variables described in these hypotheses interact to produce the disorder. Recent studies on animal models and in human lead to the hypothesis that schizophrenia might be associated with neurodegenerative processes (Ashe et al., 2001). The neurotrophic factor hypothesis (Thome et al., 1998) proposes that these changes are the

result of alterations involving the neurotrophic factors. Neurotransmitter deficits are thereby considered as epiphenomena of underlying neurotrophic factor deficiency. In neonatal lesion models of schizophrenia (Weinberger, 1987) altered expression of BDNF mRNA have been reported in the rat hippocampus and frontal cortex (Lipska et al., 2001). In addition, NGF and BDNF are abnormally regulated in the CNS of an animal model (exposure to agents interfering with prenatal cell proliferation) of schizophrenia (Aloe et al., 2000; Fiore et al., 2000). In human neuronal development of embryonic brain tissue derived from pregnant schizophrenic women shows neurite-growth deficits (Freedman et al., 1992), and the polymorphism gene of neurotrophin-3 is associated with schizophrenia (Nanko et al., 1994). Studies on post-mortem tissues have also shown that BDNF protein is increased in the anterior cingulate cortex and hippocampus of schizophrenic patients (Takahashi et al 2000).

1.7 Antipsychotic treatments

1.7.1. Electroconvulsive treatment and schizophrenia

There is evidence that ECT either alone or in combination with antipsychotic drugs (Friedel, 1986; Tharyan, 2000), provides benefit for treatment-resistant schizophrenic patients. However, ECT is less effective than antipsychotic drug treatment (Tharyan, 2000). ECT may be used as an adjunct to antipsychotic medication for those patients with schizophrenia who show a limited response to medication alone. In spite of clinical use, the administration of ECT to patients with schizophrenia lacks a strong research base.

1.7.2. Typical antipsychotic drugs

Conventional antipsychotic drugs are generally considered first-line therapy for schizophrenia. Blockade of limbic D_2 receptors with haloperidol, as well as the other typical neuroleptics, improves positive symptoms (delusions, hallucinations, etc.) (Brunello et al., 1995; Kapur et al., 2000). However, a population of patients (10-25%) remains refractory to treatment (McGrath and Emmerson, 1999). Furthermore, negative (alogia, flattened affect, social withdrawal) and cognitive (working and verbal memory, etc.) symptoms are little improved, probably because they reflect, at least partially, diminished function of mesocortical dopaminergic projections (Knable and Weinberger, 1997). Possible side effects of concomitant antagonism of striatal D_2 receptors are extrapyramidal motor side (EPS) effects as well as tardive dyskinesia.

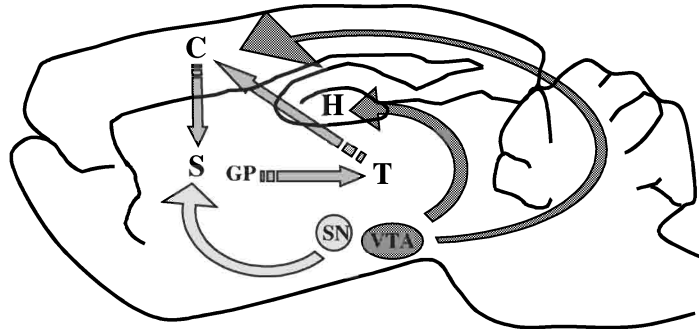


Figure 5. Dopaminergic neuronal circuits implicated in schizophrenia and in the mechanism of action of antipsychotic drugs. Substantia nigra (SN) neurons project to GABAergic neurons in the striatum (S), which receive input from the cortex (C) and send projections back to the cortex via the globus pallidus (GP) and thalamus (T). Ventral tegmental area (VTA) neurons project to the cortex and limbic regions such as the hippocampus (H). (Picture modified from Konradi and Heckers, 2001)

1.7.3. Atypical antipsychotic drugs

Clozapine in therapeutically relevant dosages was the first antipsychotic that did not produce EPS or tardive dyskinesia; this dissociation was the basis for the term "atypical" antipsychotic (Meltzer, 1995). Atypical antipsychotics differ from typical psychotics in their "limbic-specific" dopamine type 2 (D_2)-receptor binding and high ratio of serotonin type 2 (5-HT_2)-receptor binding to D2 binding (Kapur et al., 1998; 1999). However, it is unclear whether the superiority of atypicals in avoiding extrapyramidal side effects and effects on prolactin (Kapur et al., 1999; 2000) should be accorded to their 5-HT_2 blockade. Alternative possibilities may include differences in occupancy of other neurotransmitter receptors or effects on other molecules such as neuropeptides and neurotrophins.

Risperidone

Risperidone has been approved for use in schizophrenia since 1994 and is indicated for the treatment of positive and negative symptoms. Clinical trials show that risperidone is an effective treatment for positive psychotic symptoms (Chouinard and Anott, 1993; Keck et al., 1995; Marder et al., 1997).

Olanzapine

Olanzapine was approved in 1996 for the treatment of schizophrenia. It has a receptor-binding profile that is similar to that of clozapine, leading to expectation that it would show similar efficacy. There are several studies showing a possible efficacy with olanzapine in treatment-resistant patients (Dossenbach et al., 2000; Thomas and Labbate, 1998; Tollefson and Kuntz, 1999).

1.8 Peptides and neurotrophic factors

1.8.1. Neuropeptide Y

NGF and BDNF have also been shown to regulate neuropeptide expression in the CNS (Sofroniew et al., 2001) and in the PNS (Bracci-Laudiero et al., 2002) and immune system

(Levi-Montalcini, 1987). Whether the effects of NGF and BDNF on neuropeptides have a functional role in pathological conditions is unknown. A peptide of significance in this context is neuropeptide Y (NPY). NGF and BDNF can induce the synthesis of NPY in the rat brain (Croll et al., 1994). Another finding is that NPY expression is altered in rat frontal cortex and striatum by chronic haloperidol (Gruber and Mathé; 2000; Sakai et al., 1995), suggesting that this peptide may be involved, though so far not clear in which way, in the action of antipsychotic drugs. An association between decreased NPY in frontal cortex and anhedonia, and perhaps other negative symptoms of schizophrenia, has also been hypothesized (Caberlotto and Hurd, 1999; Mathé et al., 1997; 1998).

1.8.2. Glial Cell Line-Derived Neurotrophic Factor

Members of other families of proteins, most notably the glial cell–derived neurotrophic factor (GDNF) family and the neuropoietic cytokines, have been shown to also regulate survival, development, and maintenance in the nervous system (Baloh et al., 2000). GDNF exerts a specific trophic action on dopaminergic neurons; for instance it is effective in ameliorating neurodegeneration in animal models of Parkinson's disease (Bohn et al., 2000). Recently it has been demonstrated that acute and chronic ECS enhance the expression of GDNF receptor GFRα-1 and GFRα-2 mRNA in the dentate gyrus of the rat hippocampus (Chen et al., 2001a). Moreover, BDNF and GDNF and their receptors are present in the same ventral mesencephalic dopaminergic neurons (Engele, 1998), although the two factors may have different signalling mechanisms (Feng et al., 1999a) and mediate different functions in dopaminergic neurons (Feng et al., 1999b). Thus GDNF is of interest in studies of depression and antidepressive treatments.

2. AIMS

The overall aims of this project are to investigate the neurotrophin system in psychiatric disorders.

The specific aims are:

(1) to determine whether brain levels of neurotrophins are impaired in an animal model of depression and if there is a regional specificity to any of such impairments,

(2) to determine whether electroconvulsive antidepressant treatment affects the levels of neurotrophins in specific brain regions of an animal model of depression and normal rats,

(3) to study the effects of antipsychotic drugs, typical (e.g. haloperidol) and atypical (e.g. risperidone and olanzapine), on NGF and BDNF in distinct brain regions and the possible implication in mechanism(s) associated with antipsychotic action.

3. MATERIALS AND METHODS

A brief description of the materials and methods used is presented in this section. Further details as well as the different treatment modalities are in the papers.

3.1 Enzyme linked immunosorbent assay (ELISA)

NGF measurement by ELISA (papers I-IV, VI)

96-well immunoplates (NUNC, Denmark) are coated with 50 µl/well of 0.4 µg/ml monoclonal anti-mouse-NGF antibody 27/21 (Boehringer, Germany). After an overnight incubation at 4°C the plates are washed three times with buffer and the samples were incubated in the coated wells (50 µl each) overnight at 4°C. After additional three washes the antigen is 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 mU/well). The plates are 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 is measured at 570 nm using a microplate reader (Dynatech MR 5000, Germany). NGF concentrations are determined from the regression line for the purified mouse NGF standard (ranging from 1.56 to 1000 pg/ml) incubated under similar conditions in each assay. Under these conditions the sensitivity is 3 pg/ml. Data are presented as pg/g wet weight and all assays are performed in triplicate. Intra- and interassay coefficients of variation were 7% and 12%, respectively.

BDNF measurement by ELISA (papers I-III, V, and VI)

A two-site enzyme immunoassay kit (Promega, USA) performs quantification of endogenous BDNF. 96-well immunoplates (NUNC) are coated with 100 µl per well of monoclonal anti-mouse-BDNF antibody and incubated overnight at 4°C. Then the plates are washed three times with wash buffer and the samples are incubated in the coated wells (100 µl each) for 2 h at room temperature with shaking. After additional washes, the antigen is incubated with an anti-human BDNF antibody for 2 h at room temperature with shaking. The plates are washed again with wash buffer and then incubated with an anti-IgY HRP for 1 h at room temperature. After another wash the plates are incubated with a TMB/Peroxidase substrate solution for 15 min. and phosphoric acid 1M (100 µl/well) was added to the wells. The colorimetric reaction product is measured at 450 nm using a microplate reader (Dynatech MR 5000, Germany). BDNF concentrations are determined from the regression line for the BDNF standard (ranging from 7.8 to 500 pg/ml-purified mouse BDNF) incubated under similar conditions in each assay. The sensitivity of the assay is 15 pg/ml of BDNF and the cross-reactivity with other related neurotrophic factors, e.g. NGF, NT-3 and NT-4 is less than 3%. BDNF concentration is expressed as pg/g wet weight and all assays are performed in triplicate. Intra- and interassay coefficients of variation were 8% and 14%, respectively.

GDNF measurement by ELISA (paper III)

Quantification of GDNF is performed by a two-site enzyme immunoassay kit from Promega (USA). 96-well immunoplates (NUNC) are coated with 100 µl per well of monoclonal anti-GDNF antibody. After an overnight incubation at 4°C, the antibody is removed from the plates and the samples are incubated in the coated wells (100 µl each) with shaking for 6 h at room temperature. The plates are then washed five times with buffer and the antigen incubated overnight with a polyclonal anti-human GDNF antibody at 4°C. The plates are washed again with buffer, and then incubated with an anti-chicken IgY HRP conjugate for 2 h

at room temperature with shaking. The plates are incubated with a TMB/Peroxidase substrate solution for 15 min. and phosphoric acid 1M (100 μl/well) is added to the wells. The colorimetric reaction product is measured at 450 nm. GDNF concentrations are determined from the regression line for the GDNF standard (ranging from 15.6 to 1000 pg/ml-purified human GDNF). The sensitivity of the assay is 30 pg/ml and the cross-reactivity with other related neurotrophic factors, e.g. TGF β1, rhTGFα and NGF, is less than 5%. GDNF concentration is expressed as pg/g wet weight and all assays were performed in triplicate. Intra- and interassay coefficients of variation were 9% and 14%, respectively.

3.2 Immunocytochemistry (papers IV and V)

Animals are deeply anaesthetized with Nembutal and perfused via aorta first with 0.1M PBS to remove circulating blood elements, and then with paraformaldehyde 4% in 0.1M PBS to fix the brains. Brains are left in paraformaldehyde, cryoprotected in 0.1M PBS with 20% sucrose, and then coronally cut on a cryostat (20 μm in thickness) at -18± 2°C. To quench the endogenous peroxidase activity, the brain sections are washed in 1 ml of PBS 0.1% triton X-100, 2% phenylhydrazine, 10% of normal horse (ChAT) or goat (TrkB) serum for 45 minutes.

ChAT immunocytochemistry (paper IV)
Prepared coronal brain sections are incubated overnight at 4°C with a mouse monoclonal anti-ChAT specific {ChAT mAB17 (Cozzari et al., 1990)}. The sections are then washed 3 times with 0.1% triton X-100 PBS, followed by incubation for 2 h with a secondary antibody solution. This solution is prepared by adding one drop of biotinylated horse anti-mouse IgG (Vectastain Kit, Vector Laboratories, California) to 1 ml of PBS with 0.1% triton X-100 and 5% of normal horse serum. The sections are then washed 3 times in PBS with 0.1% triton X-100 and incubated for 2 h in an avidin horseradish peroxidase solution prepared according to the manufacturer instructions. Sections are again washed 3 times in PBS with 0.1% triton X-100 and reacted with a mixture of 15 mg 3,3'-diaminobenzidine (DAB) (Sigma Chemical Company, Missouri) 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 are washed several times in PBS, mounted, dehydrated and cover slipped. Immunocytochemical control sections are processed identically except for the exposure to the primary antibodies. Coded sections are evaluated with a Zeiss Axiophot microscope equipped with a Vidas Kontron images analysis system following methods previously described (Cimino et al., 1996; Angelucci et al., 1997).

TrkB immunocytochemistry (paper V)
Coronal brain sections are incubated overnight at 4°C with a rabbit anti-TrkB (Santa Cruz, California), specific for BDNF high affinity receptor. The sections are then washed 3 times in PBS 0.1% triton X-100, followed by incubation for 2 h with a secondary antibody solution. This solution is prepared by adding one drop of biotinylated goat anti-rabbit IgG (Vectastain Kit, Vector Laboratories, California), to 1 ml of PBS 0.1% triton X-100, 5% of goat serum. The sections are then washed 3 times in PBS 0.1% triton X-100, and incubated for 2 h in an avidin horseradish peroxydase solution (Vectastain). 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, USA) 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 (paper V)
To assess whether dopaminergic neurons express BDNF receptors, sections of the ventral mesencephalon are double stained with the rabbit anti-TrkB (Santa Cruz, California; see previous section, page 25) and a polyclonal goat anti-Dopamine D_2 receptor (Santa Cruz, California). The first procedure is identical to that used for localization of TrkB immunopositive cells alone (see before). Between the two immunostaining we use an avidin-biotin blocking step (SP-2006, Vector Lab.) to avoid aspecific cross-reactions. The second procedure is identical to the first regarding the sequence of the steps and time of incubations, but it differs for the composition of the secondary antibody solution [biotinylated rabbit anti-goat IgG (Vectastain Kit, Vector Laboratories, California)]. As first chromogen we use DAB-nickel ammonium sulphate for a black precipitate, and as the second chromogen DAB alone, which gives an orange-brown precipitate. Controls for double labelling include 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).

3.3 RT-PCR ELISA

NGF AND BDNF mRNAs determination by RT-PCR ELISA (paper VI)
RT-PCR ELISA for NGF and BDNF is carried out following methods recently described (Tirassa et al., 2001). Briefly, total RNA is extracted from the brain regions by using the method of Chomczynsky and Sacchi (1987) as modified in the TRIzol Kit (Gibco). RNA is analyzed by gel electrophoresis and its concentration measured by spectrophotometer reading at 260 and 280 nm (1 A_{260} absorbance unit equal to 40 mg/ml).
Complementary DNA (cDNA) is synthesized from 1µg of total RNA using 200 Units of M-MLV reverse transcriptase (Promega Italia, Milano, Italy) in 20µl of total volume reaction. To optimize reproducibility of cDNA synthesis a master mix solution containing 250 ng Oligo $(dT)_{12-18}$ primer, 0.5 Units RNasin ribonuclease inhibitor and 0.5mM dNTP in 5X Reaction Buffer (250 mM Tris-Cl pH 8.3; 375 mM KCl; 15 mM $MGCl_2$; 50mM DTT) was used. The mixture is incubated at 42°C for 1h and the reaction was terminated with a further incubation at 95°C for 5 min. One to ten dilutions in H_2O DEPC of the synthesized cDNAs are aliquoted and stored at -20°C until use.
PCR amplification is carried out using 5' biotinylated primers to generate biotinylated PCR products detectable by digoxygenin labeled probes in an immunoenzymatic assay (ELISA). Briefly, an aliquot of cDNA is mixed with 5µl 10x buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 2.5 Units of Taq DNA polymerase (Promega), 12.5 pmol of NGF ($^{5'}$TCCACCCACCCAGTCTTCCA$^{3'}$; $^{5'}$GCCTTCCTGCTGAGCACACA$^{3'}$), 7.5 pmol of BDNF ($^{5'}$AGCTGAGCGTGTGTGACAGT$^{3'}$ $^{5'}$TCCATAGTAAGGGCCCGAAC$^{3'}$) and 3.12 pmol of GAPDH ($^{5'}$CACCACCATGGAGAAGGCC$^{3'}$; $^{5'}$CACCACCATGGAGAAGGCC$^{3'}$) primers in a final volume of 50 µl. A sample containing all reaction reagents except cDNA is used as PCR negative control in all amplification. Ten microlitres of 1:10 RT mixture without enzyme is used as further PCR negative control. The mixes are incubated for the indicated cycles (denaturation 1 min at 95°C; annealing 1 min at 55°C; extension 2min at 72°C) in a GeneAmp PCR System 9600 (Perkin-Elmer). The correct size of all PCR products is confirmed by comparing with a DNA standard on agarose gel and the identity is confirmed by Southern blotting (data not shown).
Biotinylated PCR products diluted in PBS containing 3% Bovine Serum Albumine (PBSB) are distributed in triplicates (100 µl/well) onto avidin-coated microplates and incubated 1 hr at room temperature. After incubation the microplates are washed three times with PBS

containing 0.02% Tween 20 (washing buffer). DNA is denatured using 0.25 M NaOH at room temperature for 10 min. Following the washing, 100 µl/well of 4 pmol/ml digoxygenin-labelled probes (for NGF $^{5'}$TCCTGTTGAGAGTGGTGCCGGGGCATCGA$^{3'}$; for BDNF $^{5'}$TAACCCATGGGATTACACTTG-GTCACGTAG$^{3'}$ and for GAPDH $^{5'}$ACAATCTTGAGTGAGTTGTCATATTTCTCG$^{3'}$) in DIG Easy Hybridization buffer (Boehringer Mannheim) are added and incubated at 42°C for two hours. After washes, anti-digoxygenin POD-coupled antibody (Boehringer Mannheim) is added (1:1000 in PBS) and incubated 1hr at 37°C. The reaction is developed by TMB (3,3',5,5'-tetramethylbenzidine; 0.6mg in citrate buffer, pH 5.0) and blocked after 30 min with 2 M HCl. The amount of amplified products is measured as optical density at 450/690 nm ($OD_{450/690}$) using a Dynatech ELISA Reader 5000. GAPDH $OD_{450/690}$ level is used to normalize for the relative differences in sample size, integrity of the individual RNA and variations in reverse transcription efficiency.

3.4 Radioimmunoassay (RIA)

NPY-like immunoreactivity (-LI) determination by radioimmunoassay (paper IV)
All samples are analyzed twice, using two different anti-NPY antibodies. 100 µl aliquots of 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 48h at 4°C. Thereafter, 100 µl ^{125}I-Bolton Hunter labelled NPY (Amersham International, England) is added and the solution incubated for additional 24h. Free and antibody-bound NPY are separated using 50 µl Sac-Cel (Anti-Rabbit Solid Phase Second Antibody Coated Cellulose Suspension; IDS, Bolton, England). Samples are left for 30 min at room temperature. The reaction is then blocked with 1 ml distilled H_2O. Samples are centrifuged at 3000 x g for 20 min at 4°C and the supernatants decanted. Pellets are counted in a gamma counter for 3 min. The sensitivity of the assay is 11 pmol/l and the intra- and inter-assay coefficients of variation are 7 and 12%, respectively.

4. RESULTS

Altered brain baseline levels of neurotrophins in a rat model of depression compared to controls (paper I).

Using an established animal model of depression, the Flinders Sensitive Line (FSL) rats and their controls, the Flinders Resistant Line (FRL), we demonstrated that brain regions of FSL rats express higher concentrations of BDNF and NGF as compared to brain regions of FRL controls. Specifically, in the frontal cortex, the female FSL (but not the male) showed significantly higher concentrations of both BDNF and NGF. In contrast, in the occipital cortex, the male FSL (but not the female) had significantly higher concentration of BDNF and NGF. In the hypothalamus, higher levels of BDNF were found in both male and female FSL rats. Moreover, in the frontal cortex, higher NGF concentrations were found in the male compared to female rats in both strains. Cumulatively these observations suggest that altered BDNF and NGF levels may be relevant to depression phenotype and, hypothetically, different brain regional concentrations of BDNF and NGF in male and female animals may be relevant to gender differences in vulnerability to depression.

Electroconvulsive stimuli (ECS) alter neurotrophin concentrations in brains of a rat model of depression and in Sprague Dawley rats (papers II, III).

Several lines of evidence suggest that neurotrophic factors serve as mediators of activity-dependent structural plasticity. One implication of this hypothesis is that one of the long-term targets of electroconvulsive stimuli (ECS), used as a model of electroconvulsive treatment (ECT), may be the regulation of neurotrophic factors. To explore the validity of this hypothesis, we used the FSL rat model of depression and the Sprague Dawley rats and investigated the effects of repeated ECS on the concentration of neurotrophic factors known to be implicated in neuronal brain plasticity.

The results revealed that in the hippocampus ECS increased NGF concentration in FSL, but not FRL. ECS decreased NGF concentration in the frontal cortex of FSL rats. In both FSL and FRL rats ECS increased NGF levels in the striatum. In contrast, ECS did not change BDNF concentration in hippocampus, frontal cortex and striatum of FSL and FRL rats.

In Sprague Dawley rats, ECS increased the concentrations of NGF in the frontal cortex and concentrations of BDNF in hippocampus, striatum and occipital cortex. In contrast, ECS decreased GDNF concentrations in hippocampus and striatum. Our data indicate that neurotrophic factors are altered by ECS treatment in a region-specific manner.

Table 1. Effects of electroconvulsive stimuli (ECS) on NGF, BDNF and GDNF in FSL, FRL and Sprague Dawley rats.

FSL		NGF	BDNF
	Striatum	↑	NC
	Frontal cortex	↓	NC
	Occipital cortex	NC	NC
	Hippocampus	↑	NC

FRL		NGF	BDNF
	Striatum	↑	NC
	Frontal cortex	NC	NC
	Occipital cortex	NC	NC
	Hippocampus	NC	NC

SPRAGUE DAWLEY		NGF	BDNF	GDNF
	Striatum	NC	↑	↓
	Frontal cortex	↑	NC	NC
	Occipital cortex	NC	↑	NC
	Hippocampus	NC	↑	↓

NC = no changes
↑ = up
↓ = down

Administration of haloperidol and risperidone alters NGF, BDNF, tyrosine kinase receptor TrkB, ChAT and NPY in rat brain regions (papers IV, V).
Haloperidol mainly acts on the dopamine (DA) D_2 receptor while risperidone binds to both DA and serotonin (5-HT) receptors. Observations that neurotrophins participate in the regulation of certain brain neurotransmitters, such as DA, ACh, glutamate and GABA, leads to the hypothesis of a link between antipsychotic mechanisms and the synthesis and release of neurotrophins. Consequently, the aim of these experiments were to investigate whether antipsychotic treatment affects the rat brain levels of NGF, BDNF, TrkB, choline acetyltransferase (ChAT) and NPY immunoreactivities (-LI). It was found that haloperidol and risperidone elevated NGF-LI concentrations in hypothalamus but decreased NGF-LI in the striatum and hippocampus. Moreover, we found a significant decrease in ChAT-immunoreactivity in large-size neurons following both haloperidol and risperidone treatments in the septum as well as in the Meynert's nucleus. These drugs also decreased the basal levels of BDNF in the frontal cortex and occipital cortex and hippocampus. Haloperidol significantly reduced the number of TrkB immunoreactive neurons of the hippocampus, substantia nigra and ventral tegmental area, while risperidone also reduced BDNF and its receptor, but not significantly.

We also analyzed the effect of haloperidol and risperidone on brain NPY-LI. This is of interest to the field of psychosis in view of interactions between NPY and dopamine. NPY can enhance tissue concentrations of dopamine in rat cortex and striatum Conversely, dopamine can regulate NPY gene expression in rat frontal cortex and striatum. Moreover NGF and BDNF can induce NPY expression in the CNS. Administration of haloperidol increased NPY-LI concentration in the occipital cortex, while risperidone increased NPY-LI in the occipital cortex, hippocampus, and hypothalamus.

Taken together, our findings seem to suggest that one effect of antipsychotic drugs is to modify the concentrations of neurotrophins in selected brain regions.

Administration of olanzapine alters NGF and BDNF proteins and the mRNAs in the rat brain (paper VI).

The atypical antipsychotic olanzapine is effective in reducing both positive and negative symptoms of schizophrenia with a limited incidence of side effects such as extrapyramidal and anticholinergic symptoms. Since NGF and BDNF are involved in the regulation of cholinergic, dopaminergic and serotonergic neurons the question was asked whether chronic olanzapine treatment influences the concentration of NGF and BDNF and mRNAs in these brain regions. Olanzapine increased NGF in the hippocampus, occipital cortex and hypothalamus but decreased NGF mRNA in hippocampus and occipital cortex. Olanzapine treatment caused also a decrease in BDNF in the hippocampus and an increase in BDNF mRNA in the hippocampus and hypothalamus, while in the frontal cortex olanzapine decreased both BDNF and BDNF mRNA. These observations seem to provide additional evidence for the hypothesis that neurotrophins are altered by antipsychotic drugs.

Table 2. Effects of treatment with haloperidol, risperidone and olanzapine on NGF, BDNF and NPY

		NGF	BDNF	NPY
HALOPERIDOL	Hypothalamus	↑	NC	NC
	Striatum	↓	NC	NC
	Frontal cortex	NC	↓	NC
	Occipital cortex	NC	↓	↑
	Hippocampus	↓	↓	NC
RISPERIDONE	Hypothalamus	↑	NC	↓
	Striatum	NC	NC	NC
	Frontal cortex	NC	↓	NC
	Occipital cortex	NC	↓	↑
	Hippocampus	↓	↓	↑
OLANZAPINE	Hypothalamus	↓	NC	
	Striatum	NC	NC	
	Frontal cortex	NC	↓	
	Occipital cortex	↑	NC	
	Hippocampus	↑	↓	

NC = no changes
↑ = up
↓ = down

5. DISCUSSION

The major aim of this P.h.D. thesis was to investigate whether and to what extent brain neurotrophins are involved in the biochemical alterations induced by some of the treatments used for the therapy of depression and schizophrenia. The pathophysiology of these disorders is not known. However, the finding that depressed patients have reduced hippocampal as well as the prefrontal cortex volume (Bremner et al., 2000) has led to the hypothesis that neuronal loss or decreased neurogenesis occur in brains of subjects with a long history of depression (Duman et al., 1997). The hypothesis that schizophrenia might be associated with alteration in neurotrophins is supported by biochemical, behavioral and molecular studies of animal models (Aloe et al., 2000; Fiore et al., 2000; Lipska et al., 2001) and human tissues (Takahashi et al., 2000), both in the adult and developing brain (Freedman et al., 1992; Weinberger, 1987).

The presented results are consistent with the hypothesis that neurotrophins can play a role in the biochemical alterations associated with the pathogenesis and/or treatment of these disorders. Particularly, the results seem to be relevant in view of recent hypotheses that these disorders are characterized by neurodegenerative processes and by the evidence that neurotrophins have a role in preventing brain neurodegenerative disorders (Murer et al., 2001). The possible implications of these working hypotheses are discussed below.

Involvement of NGF and BDNF in depression (paper I)

The observations that BDNF produces an antidepressant-like effect when injected into the brain (Suciak et al., 1997) and that stress changes the constitutive levels of neurotrophins (Aloe et al., 1994; Ueyama et al., 1997) have raised the question about whether alterations in neurotrophin production and/or function are instrumental in the pathophysiology of depression (Altar, 1999).

To address this question we measured the brain levels of BDNF and NGF in an animal model of depression, the FSL rats, and their controls, the FRL and found that FSL animals had higher levels of both BDNF and NGF in the frontal cortex and occipital cortex, whereas in the hypothalamus only the levels of BDNF were affected. The concentrations of NGF and BDNF were unchanged in the hippocampus (the major area of synthesis of these two neurotrophins). Our results are not consistent with data obtained by others showing decreased levels of neurotrophins in depression (Altar, 1999). An accumulation of neurotrophins could reflect a decreased release/breakdown or a compensatory increase in neurotrophin synthesis in response to primary neurotransmitter changes in the limbic system during depression (Duman et al., 1997). This does not explain the obtained differences. The seeming discrepancy could be due to different animal models and experimental proceeding used.

Since gender differences in many neurotransmitters, e.g. the serotonergic and cathecolaminergic systems, and neuropeptides both in the CNS and periphery have been described (De Vries, 1990), it was of interest to explore whether the same is valid for neurotrophins. Moreover, since women exhibit higher frequency of and greater susceptibility to depression (Maier et al., 1999; Weissman and Olfson, 1995), we investigated whether neurotrophins might be selectively changed in brains of FSL female rats. Our study indicated that the male and female brains, irrespective of the strain, contain different levels of neurotrophins, at least in some brain regions. Though the functional significance of these findings remains to be established, the data revealed clear-cut sex differences and, by inference, indicate that BDNF and NGF may also play a role in susceptibility to depression.

NGF and BDNF are altered by ECS in a rat model of depression (paper II)

To further investigate the role of neurotrophins in depressive disorders during antidepressant treatments, we investigated the effect of repeated electroconvulsive stimuli (ECS) on the brain regional concentrations of NGF and BDNF in FSL rats. ECS increased NGF concentration in the hippocampus of FSL, but no changes were found in FRL or Sprague Dawley rats (paper III). These data suggest that ECS selectively alter NGF in the hippocampus of "depressed" rats. ECS also decreased NGF concentration in the frontal cortex of FSL rats. Whether the decrease in NGF synthesis is relevant to the pathophysiology of depression is not known. However, since a reduced functionality of medial prefrontal cortex has been observed in depressed patients (Drevets, 2000), the possibility exists that neurotrophin synthesis and release could also be altered in depression. Since striatal cholinergic neurons utilize NGF produced in the cortex, the increase in NGF levels observed after ECS in the striatum in both FSL and FRL rats suggests that ECS may alter NGF production and transport in the cortico-striatal pathway.

ECS did not change BDNF concentration in the hippocampus of FSL and FRL rats, but it did increase BDNF concentration in Sprague Dawley rats (paper III). The reason for these different changes is not clear. In Sprague Dawley rats these data are in line with other studies showing that BDNF mRNA is increased by ECS (Duman and Vaidya, 1998) and BDNF can lead to the recovery of behavioral deficits in animal models of depression (Suciak et al., 1997). One possibility regarding the lack of ECS induced neurotrophin hippocampal changes is that more ECS treatment is needed in FSL and FRL rats to induce BDNF changes or alternatively the breakdown/release of neurotrophins is faster in the FSL and FRL strain. More exploration needs to be done on the synthesis of neurotrophins in Sprague Dawley and FSL/FRL rats during ECS treatment.

ECS alter NGF, BDNF and GDNF in Sprague Dawley rats (paper III)

Several lines of evidence suggest that neurotrophic factors serve as mediators of activity-dependent structural plasticity (Schinder and Poo, 2000) suggesting that one of the long-term target effects of ECS may be regulation of neurotrophic factors (Follesa et al., 1994; Nibuya et al., 1995). Among them, glial cell line-derived neurotrophic factor (GDNF), a more recently identified and characterized neurotrophic factor, seems to exert a specific trophic action on dopaminergic neurons; for instance GDNF has been shown to be effective in ameliorating neurodegeneration in animal models of Parkinson's disease (Bohn et al., 2000). Consequently, to further characterize the role of neurotrophic factors in the action of ECS, we investigated the effects of repeated ECS on NGF, BDNF and GDNF brain concentrations in Sprague Dawley rats from which the two Flinders lines were originally bred.

ECS increased NGF in the frontal cortex and BDNF in the hippocampus, striatum and occipital cortex. Thus, NGF concentrations changed only in the brain region where BDNF was unaffected, indicating no overlapping effect and/or a region specific action of ECS on these neurotrophins. These data are consistent with observations that acute and chronic ECS lead to increased mRNA expression of NGF (Follesa et al., 1994), BDNF and its receptor mRNAs in limbic brain regions (Nibuya et al., 1995).

In addition, we found that ECS decreased GDNF in hippocampus and striatum. This effect is consistent with previous results showing that acute and chronic ECS enhance the expression of GDNF receptor GFRα-1 and GFRα–2 mRNA in the dentate gyrus of the rat hippocampus (Chen et al., 2001a). It is of interest that ECS changed the levels of BDNF and GDNF, two growth factor affecting dopaminergic neurons, in opposite direction in the hippocampus and striatum. One possible explanation is that although BDNF and GDNF and their receptors are present in the same ventral mesencephalic dopaminergic neurons (Engele, 1998), the two

factors may have different signalling mechanisms, as reported by Feng (Feng et al., 1999ab) in cultured ventral mesencephalic neurons.

Neurotrophins are altered following administration of antipsychotic drugs (papers IV, V, VI)

Recent studies have raised the possibility that NGF and BDNF are abnormally expressed in the CNS of animal models of schizophrenia (Aloe et al., 2000; Lipska et al., 2001). These findings and the fact that ECT may be efficient in treatment of schizophrenia suggest that neurotrophins might be altered by antipsychotic drugs. To test this hypothesis, we investigated the effect of chronic treatment with haloperidol, risperidone and olanzapine on NGF and BDNF concentrations in the rat brain.

Haloperidol and Risperidone (papers IV, V)

Haloperidol caused upregulation of NGF in the hypothalamus, a decrease in the hippocampus, and, in addition, lowered the basal NGF level in the striatum. Likewise, risperidone enhanced NGF levels in the hypothalamus and reduced it in the hippocampus. In the medial septum and in Meynert basal nucleus haloperidol and risperidone caused a reduction in the number of large-size ChAT-immunopositive neurons. The effect of haloperidol was more pronounced, as evident in the greater reduction of ChAT-immunopositive neurons in haloperidol-treated rats as compared to risperidone-treated rats.

Since the hippocampus is involved in the regulation of learning and memory processes and synthesizes the largest amount of NGF, the decrease in NGF hippocampal levels after treatment with haloperidol and risperidone raises the possibility of a correlation between low synthesis of neurotrophins and cognitive deficits observed after antipsychotic medication. This hypothesis is supported by the observation that the reduction of ChAT-immunoreactivity in the medial septum after haloperidol and higher dose of risperidone treatment is positively correlated with the decrease in NGF in the hippocampus and striatum. In contrast, the changes in ChAT immunoreactivity in basal nucleus of Meynert were not associated with a concomitant decrease in NGF level in the cortex perhaps indicating that under our experimental conditions the ChAT expression is regulated by the NGF produced in the cortex and in addition by dopaminergic and glutamatergic systems, as reported by others (Kayadjanian et al., 1999; Ushijima et al., 1997).

Our findings also demonstrate that the effect of haloperidol on neurotrophins is associated with an increase in NPY in the occipital cortex, whereas risperidone causes an increase of NPY in the hypothalamus, occipital cortex, hippocampus, and in a non-significant manner in the frontal cortex. As mentioned in the introduction, NPY can be regulated by NGF and interacts with the dopaminergic system in the CNS. However, since NPY and NGF concentrations are altered in different directions by antipsychotic drugs the data seem to suggest that the mechanism by which haloperidol and risperidone alter NPY could be different from mechanisms by which they affect NGF.

Our results also showed that haloperidol and risperidone decreased the basal levels of BDNF in the frontal and occipital cortex and the hippocampus. Moreover, haloperidol decreased TrkB immunoreactivity in neurons of the hippocampus, substantia nigra and ventral tegmental area. Risperidone also induced similar changes, though the differences were not statistically significant. 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. The observation that only haloperidol exerts a significant effect both on BDNF and TrkB high affinity receptor suggests a closer correlation between haloperidol and BDNF response as compared to risperidone. The fact that BDNF is considered a survival factor for the dopaminergic neurons might suggest that haloperidol can induce BDNF changes via the TrkB

receptor. These results, however, do not exclude the possibility that haloperidol and risperidone can act on different target cells, or alternatively that these drugs influence in a different manner BDNF-producing and/or responsive cells. Speculatively, this could be due to the risperidone's lower D_2 antagonist potency compared to haloperidol, combined with 5-HT_2 receptor-blocking properties.

Olanzapine (paper VI)

Olanzapine also altered the brain concentrations of neurotrophins. Chronic treatment decreased BDNF in the hippocampus, a result in line with our previous findings (papers IV, V) and indicating most likely a common effect of antipsychotic drugs on neurotrophins. In addition, in the frontal cortex BDNF protein and mRNA were also reduced by olanzapine. Since olanzapine antagonizes 5-HT_{2A} receptors in the frontal cortex (Bymaster et al., 1999; Kapur et al., 1998), one possibility is that olanzapine could reduce BDNF mRNA via this mechanism. This hypothesis is in line with the findings that activation of 5-HT_{2A} receptor in the cortex results in enhanced presynaptic release of glutamate, stimulation of AMPA receptors and subsequent increase in BDNF-mRNA synthesis (Vaidya et al., 1997).

In contrast with the data obtained with haloperidol and risperidone, olanzapine treatment increased NGF in the hippocampus and occipital cortex, though within the same brain regions NGF mRNA was decreased. Since treatment with olanzapine is associated with an anticholinergic action at the muscarinic receptor (Bymaster et al., 1999; Moore, 1999), it is possible that olanzapine may alter the synthesis and release of NGF in the target regions of basal forebrain cholinergic neurons. This hypothesis is supported by findings that ChAT-immunoreactivity (which is regulated by NGF) immnunoreactivity decreases in the septum and nucleus basalis of Meynert after haloperidol and risperidone administration (paper IV).

6. CONCLUSIONS

1) The presented data suggest that neurotrophins, particularly NGF and BDNF, may play a role in depression and may be altered by antidepressant treatment such as ECS.

Possible significance of ECS-induced alterations of neurotrophic factors

The data presented in this thesis indicate that ECS lead to alteration in the synthesis of brain neurotrophins. It has been reported that ECS can affect neuronal morphology by increasing the sprouting of mossy fiber pathway and the number of granule cells in the hippocampus (Duman and Vaidya, 1998). Thus, ECS can increase neurogenesis in adult rat hippocampus (Malberg et al., 2000) and this could account at least in part, if also true for ECT, for ECTs therapeutic effects. An enhanced availability of neurotrophic factors in specific brain regions might therefore lead to longer neuronal survival, and consequently could be of relevance for the action of ECT (Duman, 1998; Jacobs et al., 2000). Moreover, it can be hypothesized that ECT, as well as other therapeutic approaches, can reverse the atrophy of stress-vulnerable hippocampal neurons (Smith et al., 1995), most probably via regulation of neurotrophic factors (Duman and Vaidya, 1998). In our rat model of depression we observed opposite changes in NGF in frontal cortex and striatum after ECS. Since ECS increased NGF levels in the striatum, and since NGF is transported from the cortex and utilized as trophic support for the cholinergic neurons, it is possible that this increase is secondary to the reduced levels observed in the frontal cortex of FSL rats. One implication of this assumption is that in depressed subjects NGF may not be sufficiently utilized by target neurons in the striatum. Thus these findings suggest that cholinergic neurons might become a new target in future studies on depression. It should be stressed, however, that the functional significance of the brain concentration changes of NGF in our model of depression (paper II) and BDNF in Sprague Dawley rats (paper III) is not yet clear.

2) The reported findings also demonstrate that both typical and atypical antipsychotic drugs can alter the regional brain levels of NGF, BDNF, and their mRNAs. Since NGF and BDNF affect neurons, it is reasonable to hypothesize that alteration of brain level of these neurotrophins plays a role in the action of antipsychotic drugs.

Possible significance of the neurotrophic factor alterations associated with treatment with antipsychotic drugs

Disturbed neural development has been postulated as an important factor in the pathophysiology of schizophrenia. The results of the presented studies suggest in addition to evaluating the role of classical neurotransmitters, such as dopamine and serotonin, the effects on molecules involved in neuronal plasticity and neuroprotection, such as neurotrophins, should be explored. Our results suggest that the alterations of NGF and BDNF brain concentrations could in part explain the brain maldevelopment, which in adult life can lead to the neuropathological manifestation of schizophrenia. This hypothesis is supported by findings indicating that in schizophrenic patients BDNF protein is elevated in the anterior cingulate cortex and hippocampus (Durany et al., 2001; Takahashi et al 2000).

Haloperidol and risperidone reduced NGF and BDNF in the hippocampus, a brain region that displays cell loss in schizophrenia (Falkai and Bogerts, 1986; Weinberger, 1999). Since NGF and BDNF play a role in cognitive processes (Lo, 1995; Thoenen 1995), it is possible that prolonged treatment with antipsychotic drugs on cognitive processes is secondary to neurotrophin changes. Indeed, recent studies indicate that treatment with neuroleptic drugs reduces dendritic spines in brain regions (Kelley et al., 1997), decreases hippocampal and striatal ChAT activity (Mahadik et al., 1988), and impairs cognitive abilities (Gallhofer et al.,

1996). The observation that the number of ChAT immunoreactive neurons is reduced in the basal forebrain is consistent with this hypothesis. Moreover, the fact that haloperidol reduced NGF in the striatum suggests that this drug may impair the effect of NGF on septo-hippocampal pathway (Rylett and William, 1994; Seiler and Schwab, 1983). Previous studies showed that treatment with risperidone causes less severe deficit in 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 in reducing the brain level of neurotrophins remains, however, to be demonstrated. The observation that in the frontal cortex (haloperidol, risperidone and olanzapine) and occipital cortex (haloperidol and risperidone) BDNF production was reduced, further suggests that cognitive processes in these regions might be affected by chronic treatment with antipsychotic drugs.

Our findings provide also evidence that in the hypothalamus NGF level increases after treatment with haloperidol and risperidone and decreases after treatment with olanzapine. Since NGF and prolactin are cosecreted by the mammotroph cells of the anterior pituitary by a dopamine-regulated mechanism (Missale et al., 1996), one possible explanation is that hyperprolactinemia typically observed after antipsychotic medication with haloperidol (Spitzer et al., 1998) and risperidone (Bowden et al., 1992) but not with olanzapine (Beasley et al., 1996; Tollefson et al., 1997) could be associated with increased NGF levels in the hypothalamus.

Cumulatively our data show that chronic antipsychotic treatment alters the levels of NGF and BDNF and their mRNAs in specific brain regions. Thus, even though the functional significance of these changes needs to be explored before drawing definitive conclusion, it is possible that the differences in the action of antipsychotic drugs could be due to different effect of these drugs on neurotrophins in the brain.

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