Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses

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Abstract

Disturbed neural development has been postulated as a crucial factor in the pathophysiology of schizophrenic psychoses. The neurobiochemical basis for such changes of cytoarchitecture and changed neural plasticity could involve an alteration in the regulation of neurotrophic factors. In order to test this hypothesis, BDNF and NT-3 levels in post-mortem brain tissue from schizophrenic patients were determined by ELISA. There was a significant increase in BDNF concentrations in cortical areas and a significant decrease of this neurotrophin in hippocampus of patients when compared with controls. NT-3 concentrations of frontal and parietal cortical areas were significantly lower in patients than in controls. These findings lend further evidence to the neurotrophin hypothesis of schizophrenic psychoses which proposes that alterations in expression of neurotrophic factors could be responsible for neural maldevelopment and disturbed neural plasticity, thus being an important event in the etiopathogenesis of schizophrenic psychoses. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: BDNF; ELISA; NT-3; Plasticity; Post-mortem; Schizophrenia

1. Introduction

The maldevelopmental hypothesis of schizophrenic psychoses postulates that an altered cytoarchitectural structure of certain brain areas, dysconnections and changes in neural plasticity are the pathophysiological basis of this devastating group of diseases, and underlie the clinical symptomatology which is characterized by disturbed information processing and psychotic symptoms such as hallucinations, delusions and disorganized speech (Scheibel and Kovelman, 1981; Weinberger and Lipska, 1995; DeLisi, 1997; Raedler et al., 1998).

The inappropriate connectivity and altered biochemical functioning at the neuronal level are manifested clinically in the form of altered cognitive, emotional and intentional functioning (Weinberger, 1995; Weinberger and Lipska, 1995; Bullmore et al., 1997). This hypothesis is supported by a number of histopathological, morphological and clinical studies (Arnold et al., 1991, 1995; Benes et al., 1991; Shenton et al., 1992; Akbarian et al., 1993; Weinberger and Lipska, 1995).
It is likely that these developmental deficits are relevant not only during prenatal embryo- and organogenesis, but persist throughout life, thus activating further structural changes during adolescent pruning of neurons and aging processes (DeLisi 1997).

On molecular and neurobiochemical levels, neurotrophic factors are likely to be involved in these developmental alterations. This group of molecules is responsible for prenatal neuronal differentiation and development, as well as for postnatal neural plasticity. Such considerations lead to the ‘neurotrophin hypothesis’ of schizophrenic psychoses (Thome et al., 1998). There are probably different pathomechanisms which can cause alterations of the neurotrophin system such as genetic, infectious and traumatic factors. Additionally, these factors may vary among different subtypes of schizophrenia. Changes at the level of the neurotransmitter system (dopamine, glutamate) can be interpreted as epiphenomena, since neurotrophic factors strongly interact with the neurotransmitter system and are even responsible for the neurotransmitter phenotype during embryo- and organogenesis.

The neurotrophins are a family of small (approx. 13 kDa), highly basic (pI 9–10.5) proteins which include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), neurotrophin 4 (NT-4) and neurotrophin 5 (NT-5) (Barde, 1989; Lindsay et al., 1994; Barbacid, 1995). The neurotrophins bind with high affinity to receptors of the tyrosine kinase family (TrkA, B, C): NGF binds TrkA, BDNF and NT-4/5 bind TrkB, NT-3 binds TrkC and, to a lesser extent, TrkA (Rodríguez-Tébar et al., 1992; Dechant et al., 1994; Lindsay et al., 1994; Barbacid, 1995). In addition, there is a low-affinity neurotrophin receptor (p75 NTR) (Cheng and Mattson, 1994; Barbacid, 1995). The topographic distributions of individual neurotrophic factors (NTFs) and their receptors are quite distinct and are subject to considerable changes during the course of neural development (Altar et al., 1993).

NTFs are directly involved in cellular proliferation, migration, differentiation and the survival of neurons in the human central nervous system (Barde, 1989; Alderson et al., 1990; Maisonpierre et al., 1990; Hyman et al., 1991; Widmer and Hefti, 1994; Staecker et al., 1995). The NTFs are active not only during embryogenesis and organogenesis, they are also involved in nerve regeneration (Ip et al., 1993) and the maintenance of neural plasticity in adults (Knipper et al., 1994; Prakash et al., 1996), in that they regulate synaptic activity and neurotransmitter synthesis (Altar et al., 1997; Bartrup et al., 1997). A pathological alteration of the neurotrophic factor system may thus lead not only to neural maldevelopment, migrational deficits and dysconnections, proposed to be the characteristic pathogenetic features of the maldevelopmental hypothesis, but also to reduced neural plasticity, which would impair the individual’s ability to adapt to crisis situations.

Therefore, alterations in the expression of these molecules could be responsible for morphological anomalies seen in the brains of schizophrenic patients. Thus, the neurotrophin hypothesis represents a possible neurobiochemical explanation for well-known maldevelopmental theories of schizophrenia which propose that disturbances in cell migration and neural development constitute the pathophysiological basis of schizophrenia.

The aim of the present study was to determine the concentrations of two members of the neurotrophin family (BDNF and NT-3) in different regions of post-mortem brain tissue of patients suffering from schizophrenic psychoses in comparison with the values of control subjects. This procedure allows a determination of whether there are alterations in the protein level of BDNF and NT-3 contents in schizophrenic patients and, if so, in which brain areas.

2. Methods

2.1. Subjects

First, 23 brains from non-psychotic individuals were examined, in order to determine the age-dependency of the neurotrophic factor levels. Then, for the comparison between controls and schizophrenia patients, the post-mortem brain tissue from 11 schizophrenic patients (8 females, 3 males) with a mean age (±S.E.M.) of 77.6 ± 4.0 years (range 51–91) was carefully pairwise matched with that from 11 controls [7 females, 4 males; mean age (±S.E.M.): 77.2 ± 2.5 years, range 62–90]. The brain areas investigated were neocortex (frontal, parietal, temporal and occipital), hippocampus, cingulate gyrus and thalamus. Table 1 shows the demographic data of the control and patient groups. Diagnosis of schizophrenia was made according to the ICD-10.
demographic data of control and patient groups\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Schizophrenia ((n = 11))</th>
<th>Control ((n = 11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>77.6 ± 4.0</td>
<td>77.2 ± 2.5</td>
</tr>
<tr>
<td>Range</td>
<td>(51–91)</td>
<td>(62–90)</td>
</tr>
<tr>
<td>Post-mortem delay (h)</td>
<td>28.7 ± 8.8</td>
<td>24.6 ± 5.1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\) There were no significant differences in age, gender and post-mortem delay between the two groups.

criteria (ICD-10 F20; World Health Organization, 1992).

The control group consisted of healthy individuals who were determined to be free of psychosis in a psychiatric assessment. None of them received any neuroleptics. The daily chlorpromazine equivalent (haloperidol up to 8 mg or thioridazine up to 100 mg daily) administered to the patients was up to 400 mg. There was no correlation between medication exposure and BDNF or NT-3 content. In five cases, no information about the medication could be obtained. All patients were actively ill at death. Cognitive impairment was reported only in two diseased cases. Most subjects died of heart attack, stroke, bronchopneumonia or pulmonary embolism. Patients and controls were unrelated, and resided in the same geographical area. Neuropathological examination of brain material was conducted in all control and schizophrenic cases. Data concerning brain material were updated with clinical information gained by standardized procedures as described elsewhere (Gsell et al., 1993).

2.2. Tissue preparation

Brain extracts were prepared by homogenization in 20 volumes (w/v) of cold 100 mM Tris–HCl buffer (pH 7.0), containing 400 mM NaCl, 0.05% sodium azide, 4 mM EDTA, 2% BSA, 2% gelatine, 1 mM PMSF and 80 \(\mu\)l aprotinin, with a Polytron homogenizer (1000 rpm, 30 s), followed by sonification (power 2, 40 pulsations, 20 s). Cellular debris was removed by centrifugation at room temperature (15 min, 13,000 rpm) and the supernatants were used for assessing BDNF and NT-3 concentrations.

2.3. BDNF ELISA

For the determination of BDNF levels in post-mortem tissue, a commercially available ELISA kit of the sandwich type was used (Promega, USA). The method showed less than 3% cross-reactivity to NGF, NT-3 and NT-4.

2.4. NT-3 ELISA

For the measurement of the NT-3 levels in post-mortem tissue, an enzyme-linked immunoassay of the sandwich type was developed. Brain homogenates were incubated in duplicate in a 96-well microplate, which had previously been coated with an anti-NT-3 monoclonal antibody (R&D Systems, England). The samples were applied, followed in turn by the second polyclonal antibody (Chemicon, USA), and then a biotinylated anti-rabbit IgG antibody (Boehringer, Germany). After addition of streptavidine and colorimetric visualization with chlorphenol red-\(\beta\)-d-galactopyranoside, NT-3 concentration was determined by measurement of the absorbance at 570 nm with an automatic microplate reader (Dynatech MR4000; Dynatech Laboratories, USA). The method showed less than 1% cross-reactivity to NGF, BDNF and NT-4.

2.5. Protein quantification

Protein quantification, based on the method of Lowry et al. (1951), was carried out using a protein assay kit (Sigma Diagnostics, USA).

2.6. Statistical analysis

The NT-3 and BDNF concentrations in different brain areas of the patient group were compared with those of the control group. Statistical analyses were carried out using the two-tailed \(t\)-test for paired samples. The level of significance was set at \(P = 0.05\). For the correlation analysis of BDNF and NT-3 concentration and possible confounding parameters, such as age, Pearson product–moment correlation was used. All statistical tests were performed with the statistical program PrismPad.
Table 2
Correlation with age of BDNF and NT-3 concentrations in various regions of the normal brain

<table>
<thead>
<tr>
<th>Brain region</th>
<th>BDNF concentration (ng/mg protein)</th>
<th>NT-3 concentration (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex (n = 22)</td>
<td>−0.54</td>
<td>−0.39</td>
</tr>
<tr>
<td>Parietal cortex (n = 23)</td>
<td>−0.03</td>
<td>−0.50</td>
</tr>
<tr>
<td>Temporal cortex (n = 20)</td>
<td>−0.21</td>
<td>−0.24</td>
</tr>
<tr>
<td>Occipital cortex (n = 16)</td>
<td>0.12</td>
<td>−0.01</td>
</tr>
<tr>
<td>Hippocampus (n = 14)</td>
<td>0.19</td>
<td>−0.83</td>
</tr>
<tr>
<td>Cingulate gyrus (n = 18)</td>
<td>0.02</td>
<td>−0.13</td>
</tr>
<tr>
<td>Thalamus (n = 10)</td>
<td>0.30</td>
<td>−0.09</td>
</tr>
</tbody>
</table>

* Data represent the r values (Pearson product–moment correlation). n = number of control samples examined. Mean post-mortem time: 26.33 ± 5 h. Statistical analysis — BDNF: frontal cortex, P = 0.009; NT-3: hippocampus, P = 0.004.

3. Results

3.1. Influence of age, gender and post-mortem time on BDNF and NT-3 levels

BDNF and NT-3 concentrations in the control and patient groups were tested for dependence on post-mortem delay and age. There was no correlation with post-mortem time, indicating a high post-mortem stability of these neurotrophic factors. However, a statistically significant negative correlation with age was found (see Table 2) for BDNF concentration in frontal cortex (Fig. 1) and NT-3 concentration in hippocampus (Fig. 2). Neurotrophic factor levels in tissue were expressed as a function of the total amount of extracted protein, thus avoiding the possibility that changes in BDNF and NT-3 levels could be masked by changes due to the total amount of extractable protein.

3.1. BDNF and NT-3 in schizophrenia

The BDNF content in cortical areas of the brain of schizophrenic patients was significantly higher than in controls (frontal: 2.70 ± 0.40 vs. 1.68 ± 0.21, parietal: 2.93 ± 0.53 vs. 1.59 ± 0.22, temporal: 2.80 ± 0.40 vs. 1.39 ± 0.18, occipital: 2.91 ± 0.60 vs. 1.34 ± 0.16 ng/mg protein). In the hippocampus, the opposite effect was observed: the BDNF concentrations were significantly lower in schizophrenia cases than in controls (2.70 ± 0.42 vs. 4.84 ± 0.61 ng/mg protein). In the control samples, the hippocampus was the area with the highest BDNF content (more than three times higher than in cortical areas). But in schizophrenic patients the hippocampal BDNF concentration was not higher than the cortical concentration. The cingulate gyrus, however, showed relatively high immunoreactivity for BDNF in the patient group when compared with controls (4.80 ± 0.90 vs. 2.30 ± 0.60 ng/mg protein), without reaching statistical significance. Also, in the thalamus no significant differences were seen. Table 3 summarizes these results.

Differences in NT-3 concentrations were less
pronounced (Table 4). However, there was a significantly lower NT-3 content in the frontal cortex of schizophrenic patients than in the respective cortical area of controls (0.39 ± 0.08 vs. 0.62 ± 0.05 ng/mg protein). In both groups, NT-3 was more abundant in cortex than in the other brain regions investigated. The hippocampus showed only a weak NT-3 immunoreactivity in both groups.

4. Discussion

The results of this study demonstrate that alterations in the level of neurotrophic factor expression can be detected in post-mortem brain

Table 4

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Schizophrenia (ng/mg protein)</th>
<th>Control (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex (n = 6)</td>
<td>0.39 ± 0.08</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>Parietal cortex (n = 7)</td>
<td>0.45 ± 0.08</td>
<td>0.48 ± 0.07</td>
</tr>
<tr>
<td>Temporal cortex (n = 7)</td>
<td>0.58 ± 0.12</td>
<td>0.57 ± 0.14</td>
</tr>
<tr>
<td>Occipital cortex (n = 7)</td>
<td>0.47 ± 0.06</td>
<td>0.63 ± 0.11</td>
</tr>
<tr>
<td>Hippocampus (n = 5)</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Cingulate gyrus (n = 10)</td>
<td>0.24 ± 0.07</td>
<td>0.21 ± 0.08</td>
</tr>
<tr>
<td>Thalamus (n = 8)</td>
<td>0.33 ± 0.09</td>
<td>0.27 ± 0.07</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± S.E.M. n = number of brain pairs (schizophrenic and matched control) examined. Statistical analysis — schizophrenia vs. control: frontal cortex, P = 0.02; parietal cortex, P = 0.02; temporal cortex, P = 0.01; occipital cortex, P = 0.01; hippocampus, P = 0.001.

originating from schizophrenic patients. These alterations exhibit a neuroanatomically differentiated pattern. Whereas BDNF levels were increased in the cerebral cortex and decreased in hippocampus of patients when compared with non-psychotic controls, NT-3 levels were reduced in the cortex of patients.

The alterations of the neurotrophic factor level in schizophrenia seems to follow a neuroanatomically differentiated pattern which could be characteristic for these disorders. Such a differential regulation of the different neurotrophic factors which is increased in some brain regions and reduced in others could also explain why no alterations of NT-3 can be detected in the CSF of schizophrenic patients. In the CSF, the concentrations of this neurotrophic factor are below the detection threshold, whereas some neurological diseases show a significant increase of NT-3 in the CSF (Gilmore et al., 1997).

In accordance with the maldevelopmental hypothesis, neurohistological post-mortem studies have shown disoriented, heterotopic neurons and reduced neuronal size in hippocampal and cortical brain regions (Arnold et al., 1991, 1995; Benes et al., 1991; Akbarian et al., 1993; Bernstein et al., 1998; Luts et al., 1998). These findings are supported by in vivo neuroimaging studies demonstrating structural deficits in the form of reduced gray matter and increased corpus callosum volumes in schizophrenic patients (Jacobsen et al., 1997; Buchanan et al., 1998). It was possible to show that anatomic anomalies in mesotemporal structure such as temporolimbic volume reduction are associated with the psychopathology in chronic schizophrenia, i.e. positive psychotic symptoms (Bogerts et al., 1993). PET studies have identified ‘hypofrontality’ dynamically depending on the acuity of the episodes, and reduced hippocampal activation during episodic memory retrieval in schizophrenic patients (Heckers et al., 1998; Spence et al., 1998). Additionally, in a recent functional MRI study, diminished activation of sensorimotor cortex and supplementary motor area has been found in schizophrenic patients during motor performance (Schröder et al., 1999).

It is striking that the changes in the neurotrophin levels were evident in exactly those areas in which neurodevelopmental alterations have been shown in histological and brain imaging studies of schizophrenic patients, i.e. hippocampus and cerebral

Table 3

BDNF concentrations in various brain regions of schizophrenic patients and controls

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Schizophrenia (ng/mg protein)</th>
<th>Control (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex (n = 10)</td>
<td>2.70 ± 0.40</td>
<td>1.68 ± 0.21</td>
</tr>
<tr>
<td>Parietal cortex (n = 11)</td>
<td>2.93 ± 0.53</td>
<td>1.59 ± 0.22</td>
</tr>
<tr>
<td>Temporal cortex (n = 10)</td>
<td>2.80 ± 0.40</td>
<td>1.39 ± 0.18</td>
</tr>
<tr>
<td>Occipital cortex (n = 10)</td>
<td>2.91 ± 0.60</td>
<td>1.34 ± 0.16</td>
</tr>
<tr>
<td>Hippocampus (n = 5)</td>
<td>2.70 ± 0.42</td>
<td>4.84 ± 0.61</td>
</tr>
<tr>
<td>Cingulate gyrus (n = 5)</td>
<td>4.80 ± 0.90</td>
<td>2.30 ± 0.60</td>
</tr>
<tr>
<td>Thalamus (n = 7)</td>
<td>2.46 ± 0.68</td>
<td>2.39 ± 0.62</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± S.E.M. n = number of brain pairs (schizophrenic and matched control) examined. Statistical analysis — schizophrenia vs. control: frontal cortex, P = 0.02; parietal cortex, P = 0.02; temporal cortex, P = 0.01; occipital cortex, P = 0.01; hippocampus, P = 0.001.
cortex. The question of whether these alterations represent primary-causal or secondary-reactive changes, or whether these alterations are supportive of a disturbance of aging rather than of development, remains open. Association studies trying to link different variants in genes coding for neurotrophic factors have not been conclusive (Hattori and Nanko, 1995; Thome et al., 1996, 1997a, 1997b; Tanaka et al., 1998). It is known that changes in the neurotrophic factor system can be induced by other primary pathomechanisms, such as ischemia, hypothermia, opiate treatment and withdrawal, administration of methylmercury, traumatic brain injury and seizures or convulsions (Andersson et al., 1997; Boris-Moller et al., 1998; Numan et al., 1998; Oyesiku et al., 1999; Vezzani et al., 1999).

However, the findings of this study suggest that an alteration in the neurotrophic factor system is one of the factors which have to be considered in the etiological cascade of schizophrenic psychoses. The theory that alterations of the neurotrophic factor metabolism is a pathophysiological event in schizophrenia and may be related to maldevelopmental phenomena which have been postulated for this group of psychotic disorders is supported by studies describing alterations in the level of receptors for neurotrophic factors in schizophrenia (Schramm et al., 1998). In the same study, the reduction of trkB mRNA in the frontal cortex of schizophrenic patients seemed to be medication-independent, whereas there was a correlation of the trkC mRNA level in the cerebellum with fluphenazine lifetime equivalents (Schramm et al., 1998).

Nevertheless, it cannot be excluded that alterations of the neurotrophic levels in the brain are induced by antipsychotic drugs rather than by the psychosis itself. It is known that antidepressive drugs and electroconvulsive seizures regulate BDNF and trkB mRNA in rat brain (Nibuuya et al., 1995). Dopaminergic stimulation up-regulates the in vivo expression of BDNF, an effect which can at least partially be inhibited by the application of haloperidol (Okazawa et al., 1992). On the other hand, MK-801, a highly psychotomimetic substance which has been used in order to study ‘model psychoses’, has a differential effect on BDNF-mRNA levels in the rat brain: it decreases BDNF mRNA in hippocampus and the superficial layers of the cerebral cortex, but increases it in single cells of the middle cortical layer and the midline thalamic nuclei (Castren et al., 1993). Alterations of the neurotrophic factor metabolism could be a pathophysiological event in psychotic disorders, as well as being a trans-synaptic mechanism of action of antipsychotic drugs.

Presently, we are establishing cell-culture systems in order to reveal the interaction between antipsychotic agents and neurotrophic factors; in parallel, we are continuing to investigate the role of neurotrophins in the etiopathogenesis of schizophrenic psychoses using post-mortal material. This pilot study shows that there are likely to be subtle alterations in the neurotrophic factor system of schizophrenic patients which could explain, at least partially, some of the morphological, cytoarchitectural and neurobiochemical anomalies that have been found in this major group of psychotic diseases.

Acknowledgements

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References


