Inverse correlation of brain and blood BDNF levels in a genetic rat model of depression

Betina Elfving¹, Pia Høgh Plougmann¹, Heidi Kaastrup Müller¹, Aleksander A. Mathé², Raben Rosenberg¹ and Gregers Wegener¹

¹ Centre for Psychiatric Research, Aarhus University Hospital, Risskov, Denmark
² Karolinska Institutet – Clinical Neuroscience, Psychiatry M56, Karolinska University Hospital Huddinge, Stockholm, Sweden

Abstract

There is accumulating evidence that brain-derived neurotrophic factor (BDNF) plays a critical role in the pathophysiology of depression. Decreased serum levels have been reported in major depression, and a correlation between BDNF reduction and the severity of the disease was found. Moreover, in post-mortem hippocampal tissue, increased levels of BDNF immunoreactivity have been reported in subjects treated with antidepressants compared to untreated subjects. These findings indicate parallel changes in brain and serum BDNF levels during depression. BDNF has been measured in selected brain areas in several animal models. In investigations between Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) rats, a genetic rat model of depression, no differences were found in BDNF levels in the frontal cortex and hippocampus, areas believed to be core brain regions in depression. However, to our knowledge brain and serum BDNF levels have never been reported in parallel for any psychiatric disease model. Therefore, we examined the levels of BDNF in whole blood, serum, cerebrospinal fluid (CSF), hippocampus, and frontal cortex in male FSL and FRL rats. BDNF levels in serum and whole blood of FSL rats were significantly increased compared to FRL rats. In contrast, in the hippocampus the BDNF level was significantly decreased in FSL compared to FRL rats while no differences were found in the frontal cortex and CSF. The differential regulation of the BDNF levels in hippocampus, serum, and whole blood in FSL/FRL rats adds to the hypothesis that neurotrophic factors are related to the pathophysiology of depression.

Received 7 July 2009; Reviewed 14 August 2009; Revised 21 August 2009; Accepted 24 August 2009; First published online 2 October 2009

Key words: BDNF, blood, correlation, frontal cortex, hippocampus.

Introduction

Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophin family, abundant in the brain and the periphery. BDNF is a key mediator of neuronal plasticity in the adult nervous system and is involved in the dynamic regulation of synaptic composition, neurotransmitter release and excitability (Huang & Reichardt, 2001; Schinder & Poo, 2000). Recent reports have suggested a pathophysiological role for BDNF in major depressive disorder, as decreased serum and plasma levels have been reported (Aydemir et al. 2006; Karege et al. 2002a; Lee et al. 2007; Shimizu et al. 2003). Moreover, BDNF levels in serum were found to correlate with the severity of depression (Karege et al. 2005) and importantly, after antidepressant treatment serum BDNF levels of depressed patients have been reported to increase to the levels of healthy controls (Aydemir et al. 2005; Gonul et al. 2005; Shimizu et al. 2003).

To date only a few studies investigating the expression of BDNF in human brain tissue have been conducted. Increased levels of BDNF immunoreactivity in post-mortem hippocampal tissue were reported in subjects treated with antidepressants at the time of death compared to those of antidepressant untreated subjects (Chen et al. 2001). Another study showed a tendency towards reduced BDNF mRNA expression in dorsolateral prefrontal cortex of depressed patients compared to controls (Molnar et al. 2003). Interestingly, BDNF has been reported to readily cross the blood–brain barrier (Pan et al. 1998) and there is evidence that peripheral blood BDNF levels...
correlate with BDNF concentrations in the central nervous system (CNS), in that brain and serum BDNF levels underwent similar changes during maturation and ageing processes in rats (Karege et al. 2002b). Based on these findings, parallel changes in brain and serum BDNF levels during depression were hypothesized.

BDNF levels have been measured in selected brain areas in a genetic animal model of depression, the Flinders Sensitive Line (FSL) and the Flinders Resistant Line (FRL) rats (Angelucci et al. 2000, 2003; Overstreet, 1993), and diverging results from two different studies have been reported (Angelucci et al. 2000, 2003).

In light of these findings further studies are warranted and the present study investigated the possible correlation of peripheral and central BDNF levels using a genetic animal model of depression.

Materials and methods

Animals

Male Flinders Line rats (FSL and FRL, age 20 wk), from the colony maintained at the University of Aarhus, weighing 280–350 g were used. They were housed in pairs (Cage 1291H Eurostandard Type III H, 425 × 266 × 185 mm; Techniplast, Italy) at 20 ± 2 °C on a 12-h light/dark cycle (lights on 07:00 hours). Tap water and chow pellets were available ad libitum. All animal procedures were approved by the Danish National Committee for Ethics in Animal Experimentation (2007/561-1378). The behaviour of FSL and FRL rats was tested with the forced swim test (FST) compared to FRL rats. The increased immobility is a depression related behaviour. The FST was performed using a transparent cylinder (diameter 24 cm, height 80 cm, filled with 40 cm of water (25 ± 0.5 °C) (Porsolt et al. 1977). On the first of two test days, the rats were placed in the cylinder for 15 min. The following day, the rats were gently placed in the cylinder for 5 min. The behaviour was video-recorded, and the immobility time (in seconds) was assessed using Noldus Ethovision XT (The Netherlands).

Measurements of BDNF mRNA level with quantitative real-time polymerase chain reaction (real-time qPCR)

The rats were decapitated and the brains were quickly removed, dissected, and frozen on dry ice powder. Frontal cortex and hippocampus were weighed and stored at −80 °C until required.

Tissue homogenization, RNA extraction, RNA characterization, cDNA synthesis, and real-time qPCR were carried out as previously described (Elfving et al. 2008). Briefly, frontal cortex and hippocampus were homogenized in lysis buffer (Applied Biosystems, USA) with mixer-mill (Retsch, Germany) 2 × 1 min (30 Hz/s). Total RNA was isolated using the ABI Prism™ 6100 Nucleic Acid Prepsystem (Applied Biosystems, USA) following the manufacturer’s instructions. Aliquots of the RNA solution were taken for both RNA quantification and qualification. The integrity of RNA and the RNA concentration was determined with RNA StdSens microfluidic chips using the Experion Automated Electrophoresis System (Bio-Rad, USA). The RNA purity and the RNA concentration were determined by spectrophotometer (UV1650PC Shimadzu, Japan). Data on quality and purity of the extracted RNA was evaluated with Student’s t test. Afterwards RNA was reversely transcribed using random primers and Superscript II Reverse Transcriptase (Invitrogen, USA) following the manufacturer’s instructions and with a RNA concentration per reaction of 27 ng/μl. The cDNA samples were diluted 1:30 with DEPC water before being used as a qPCR template.

Real-time qPCR

The real-time qPCR reactions were carried out in 96-well PCR plates using the Mx3000P (Stratagene, USA) and SYBR Green. Each SYBR Green reaction (20 μl total volume) contained 1x SYBR Green master mix (Bio-Rad), 0.5 μm primer pairs, and 6 μl of diluted cDNA. The gene expression of BDNF and eight different reference genes [18s subunit ribosomal RNA (18s rRNA), β-actin (Actb), cyclophilin A (CycA), glyceraldehyde-3-phosphate dehydrogenase (Gapdh), hydroxy-methylbilane synthase (Hmbs), hypoxanthine guanine phosphoribosyl transferase 1 (Hprt1), ribosomal protein L13A (Rpl13A), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (Ywhaz)] was investigated. The reference genes were selected as described by Bonefeld et al. (2008). The primers were designed and tested as described by Elfving et al. (2008).

The following forward and reverse primers were used:

- BDNF – forward: GAAAGTCCCGGTATCAAAG, reverse: CGCCAGCCAATCTCTTCTTTTG (187 bp);
- 18s rRNA – forward: ACGGACCGAGGAAACGAT, reverse: TGCTAATCCTGTCCGGTCC (310 bp);
- ActB – forward: TGTCACCAACTGGGACGATA, reverse: GGGGTGTTGAAGGTCTCAAA (165 bp);
CycA – forward: AGCACTGGGGAGAAAGATT, reverse: AGCCACTCAGTCTTGGCAGT (248 bp);
Gapd – forward: TCACCACCATGGAGAAGGC, reverse: GCTAAGCAGTTGGTGGCA (168 bp);
Hmbs – forward: TCCTGGCTTTACCATTGGAG, reverse: TGAATTCCAGGTGAGGGAAC (176 bp);
Hprt 1 – forward: GCAGACTTTGCTTTCCTTGG, reverse: CGAGAGGTCC TTTTCACCAG (81 bp);
Rpl13A – forward: ACAAGAAAAAGCGGATGGTG, reverse: TTCCGGTAATGGATCTTTGC (167 bp);
Ywhaz – forward: TTGAGCAGAAGACGGAAGGT; reverse: GAAGCATTGGGGATCAAGAA (136 bp).
The primers were obtained from DNA Technology A/S (Denmark).

Data analysis
For data normalization, we first measured mRNA levels for the reference genes. Stability comparison of the expression of the reference genes was conducted with Normfinder software (http://www.mdl.dk) (Andersen et al. 2004). Ywhaz and Hmbs were determined to be the best combination in the hippocampus, whereas Ywhaz and Actb were determined to be the best combination in the frontal cortex. Therefore, values for each individual were normalized with the geometric mean of the reference genes Ywhaz and Hmbs in the hippocampus and Ywhaz and Actb in the frontal cortex.

Measurements of BDNF protein levels with enzyme-linked immunosorbenent assay (ELISA) kits

Collection of cerebrospinal fluid (CSF), brain tissue, and blood
Since some studies in rodents show a circadian rhythm of BDNF in certain brain regions (Allen et al. 2005; Schaaf et al. 2000) the rats were housed and sacrificed by cervical dislocation under similar standardized time conditions. The rats were anaesthetized between 10:30 and 11:30 hours with 2 ml pentobarbital/lidocainhydroxy-chlorid and CSF was taken from the suboccipital cavity. CSF was placed on ice for 5 min while the rat was decapitated. Mixed arteriovenous blood was collected from the neck wound. Whole blood was collected in tubes with EDTA (Terumo, Venosafe™, VF-053STK) and blood for serum samples was collected in anticoagulant-free tubes with gel (Terumo, Venosafe™, VF-054SAS). The brain was quickly removed and frontal cortex and hippocampus were dissected on a cold tile. The tissue was frozen on dry-ice powder and stored at −80 °C.

Preparation of CSF
CSF was centrifuged (800 g, 10 min, 4 °C) and the supernatant collected and stored at −80 °C.

Number of platelets
In the blood BDNF is essentially stored in platelets (Fujimura et al. 2002). Therefore the number of platelets was determined with Sysmex 3000 (Sysmex, Denmark) in FSL and FRL rats.

Preparation of serum and whole blood lysate
The blood collected in tubes with/without EDTA was kept at room temperature for between 30 min and 1 h. Blood for serum samples was centrifuged (1500 g, 10 min, 4 °C) and the supernatant was aliquoted and stored at −80 °C.

Whole blood lysates were prepared by mixing 9 volumes of blood with 1 volume of 10% Triton-X100 in water, followed by incubation at 4 °C for 1 h and centrifugation at 15000 g for 5 min (Fujimura et al. 2002). Whole blood lysates were aliquoted and stored at −80 °C.

Preparation of brain tissue
Brain BDNF protein was extracted from left frontal cortex and hippocampus. The brain tissue was homogenized (1:10, w/v) with a polytron for 30 s in ice-cold extraction buffer containing 100 mM Tris–HCl (pH 7.2), 400 mM NaCl, 4 mM EDTA, 0.05% sodium azide, 0.5% gelatin, 0.2% Triton-X 100, 2% BSA, and Complete™ protease inhibitor mixture (Roche Diagnostics A/S, Denmark). The homogenates were centrifuged (11000 g, 20 min, 4 °C). The supernatant was collected and stored at −80 °C.

Determination of BDNF concentration with ELISA
Quantification of endogenous BDNF was performed with ELISA kits (Promega, Switzerland) within 2 months from collection. The same batch number was used for the entire experiment. As an inter-plate control, in addition to the standard curves calculated for each ELISA kit, two standards were always included among the other samples. The standard curves and the samples were run in duplicate. Serum and whole blood lysates were diluted 1:20 in Block and Sample buffer (Promega). CSF was not diluted. Hippocampus and frontal cortex were diluted 1:12 and 1:2 in extraction buffer, respectively. The standard curve for determination of BDNF in brain tissue was prepared
in extraction buffer, whereas the standard curve for determination of BDNF in CSF, serum, and whole blood lysate was prepared in Block and Sample buffer. The BDNF standard curves ranged from 7.8 to 300 pg/ml. Briefly, 96-well immunoplates (NUNC, Denmark) were coated with 100 μl/well of monoclonal anti-BDNF mouse antibody and incubated overnight at 4 °C. Non-specific binding was blocked with Block and Sample buffer. Then the samples and standards in duplicate were added to the coated wells (100 μl each) for 2 h at room temperature with shaking. After 2 h, the antigen was incubated with polyclonal anti-human BDNF antibody for 2 h at room temperature with shaking and then incubated with an anti-IgY HRP for 1 h at room temperature. The addition of 3,3′,5,5′-tetramethylbenzidine started the colour reaction. The reaction was stopped 10 min later with 1 M HCl solution, and the absorbency was immediately measured at 450 nm (EL 800 Universal Microplate reader, Bio-Tek instruments Inc., USA). According to the manufacturer the BDNF ELISA kit has <3% cross-reactivity with other related neurotrophic factors, e.g. NGF, NT-3 and NT-4 and the sensitivity is 15.6 pg/ml BDNF.

BDNF in the adult brain is present in two forms: the mature form (mBDNF) and the precursor form (proBDNF) (Martinowich et al. 2007). The above-mentioned protocol was used to measure the amount of free mBDNF in our samples. Total free BDNF was measured as described in the manufacturer’s protocol with acid-treatment of the preparations. Samples were processed with 1 M HCl acidification and subsequent 1 M NaOH neutralization.

Measurements of BDNF protein levels with Western blot

To confirm the BDNF data obtained with the ELISA kit, Western blotting was conducted with serum and brain samples. Serum samples were diluted 1:4 in PBS containing 1% Triton X-100 and 1x Complete™ protease inhibitor mixture (Roche Diagnostics A/S). Samples were mixed with SDS sample buffer [125 mM Tris–HCl (pH 6.8), 20% glycerol, 4% SDS, 0.02% Bromphenol Blue, and 125 mM dithiothreitol] and incubated at 50 °C for 30 min. Brain homogenates were mixed with an additional two volumes of lysis buffer [50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1x Complete™ protease inhibitor mixture] and incubated on ice for 30 min. The samples were centrifuged at 15000 g for 10 min and the supernatants were incubated with SDS sample buffer for 30 min at 50 °C. The serum samples (5 μl) and brain homogenates (10 μl) were analysed by SDS–PAGE using 10% precast NuPAGE gels (Invitrogen, USA) with a MES buffer system. Proteins were transferred onto nitrocellulose membranes using the iBlot dry blotting system (Invitrogen) and membranes were blocked with 5% dry milk in TBS-T [50 mM Tris–HCl (pH 8.0), 150 mM NaCl, and 0.5% Tween-20] for 1 h at room temperature. The membranes were probed overnight at 4 °C with the primary antibodies: rabbit polyclonal anti-BDNF (1:500, sc-546; Santa Cruz) and mouse monoclonal anti-β-actin (1:2000, A 5316; Sigma) followed by incubation with the appropriate peroxidase-conjugated secondary antibody: goat anti-rabbit antibody (1:25,000, no. 1858415; Pierce) and goat anti-mouse antibody (1:2000, no. 1858413; Pierce) for 1 h at room temperature. Immunoreactive bands were visualized using ECL Advance Western Blotting Detection Reagent (GE Healthcare, UK) and the chemiluminescent signals were captured on a Kodak Image Station 440 and relative intensities were quantified by the Kodak 1D3.6 Image Analysis Software.

Statistics

Student’s t test was used to compare the FSL and FRL groups in the FST, at the mRNA, and protein level; p < 0.05 was considered statistically significantly different. Correlation between measurements of BDNF in blood and brain preparations was determined by calculation of the Spearman coefficient, r. Correlations were considered significant when p < 0.05. To achieve a sufficient number of animals for correlation the FSL and FRL groups were combined.

Results

The FSL rats from our breeding colony displayed increased immobility in the FST compared to FRL rats (83% vs. 47%, p < 0.001) (Fig. 1).

Determination of BDNF mRNA levels with qPCR

No differences between FSL and FRL rats were found with respect to the 18s/28s rRNA ratio or RNA purity in the frontal cortex or in the hippocampus (data not shown).

The normalized mRNA levels of BDNF in hippocampus and frontal cortex are given in Fig. 2. The hippocampal mRNA level was significantly lower in FSL rats compared to FRL rats, whereas the mRNA level in frontal cortex was similar in FSL and FRL rats.
Determination of free mBDNF levels in CSF, serum, whole blood and in extracts from hippocampus and frontal cortex

The free mBDNF concentrations in CSF, serum, whole blood, hippocampus, and frontal cortex from FSL and FRL rats are shown in Table 1. The BDNF level in CSF was low and in some samples they were actually below the sensitivity level of the assay (15.6 pg/ml); therefore these values were excluded.

As shown in Table 1 and Fig. 3 the concentrations of free mBDNF in serum and whole blood were significantly higher in FSL rats compared to FRL rats, whereas the concentration of free mBDNF in hippocampus was significantly lower in FSL rats compared to FRL rats ($t$ test, $p < 0.05$).

Serum-to-blood ratios were determined to 89 ± 3% in FSL rats ($n = 8$) and to 89 ± 11% in FRL rats ($n = 6$).

The significant decrease of free mBDNF in hippocampus in FSL rats compared to FRL rats obtained with the ELISA kit (65% of FRL) (Fig. 3) was confirmed with Western blotting (53% of FRL) (Fig. 4).

We were unable to detect BDNF in serum using Western blotting. Therefore, in order to confirm the interesting BDNF results (Fig. 3 and Table 1), we collected serum from a new rat cohort. The newly obtained serum BDNF levels (mean ± S.D.) in FRL rats ($n = 8$): 2985 ± 372 pg/ml (100 ± 12% of FRL) and FSL rats ($n = 6$): 4737 ± 980 pg/ml (159 ± 33% of FRL) were identical with the results from the first colony and the BDNF levels were statistically significant in FSL and FRL rats ($t$ test, $p < 0.05$).

In blood, BDNF is essentially stored in platelets (Fujimura et al. 2002). Therefore, we determined the number of platelets in FSL and FRL rats. No statistically significant difference in the number of platelets between FSL rats ($1039 ± 74 \times 10^9/l$) and FRL rats ($991 ± 41 \times 10^9/l$) was found.

**Correlation of the free mBDNF level in blood and brain**

The free mBDNF levels in serum and whole blood show negative correlation with the free mBDNF concentration in hippocampus (Fig. 5) ($p < 0.05$). Serum/hippocampus: $r = -0.65$, $p = 0.0044$; $n = 17$; whole blood/hippocampus: $r = -0.61$, $p = 0.0113$; $n = 16$; serum/frontal cortex: $r = -0.16$, $p = 0.57$; $n = 15$; whole blood/frontal cortex: $r = -0.38$, $p = 0.19$; $n = 14$; whole blood/serum: $r = 0.82$, $p < 0.0001$; $n = 16$.

**Determination of total BDNF in hippocampus and serum**

Total BDNF was determined by acid treatment of the serum and hippocampus preparations. The total free serum BDNF values (data not shown) were lower than the free mBDNF values (Table 1). According to the manufacturer’s instructions acid treatment is a species- and tissue-specific phenomenon and can, as in our experiment, lead to a decrease in BDNF levels. In hippocampus the total BDNF levels were statistically significant in FSL rats compared to FRL rats ($t$ test, $p < 0.05$). The total BDNF levels were 29642 ± 2705 pg/g wet weight (100 ± 9% of FRL) and 24647 ± 4885 pg/g wet weight (83 ± 16% of FRL) in the FRL and FSL rats, respectively (Fig. 6).
Discussion

To our knowledge this is the first report of BDNF determinations in parallel in the CSF, brain, and blood in an animal model of depression. The BDNF levels were measured in two brain regions, the frontal cortex and hippocampus as they are considered to be core brain regions involved in depression (Drevets, 2007; Maletic et al. 2007). The most salient findings were: (1) BDNF was decreased at both mRNA and protein level in FSL rats compared to FRL rats in the hippocampus, whereas no significant differences were found in the frontal cortex, and (2) in the CSF the BDNF levels were similar in the two strains, while they were increased in the serum and whole blood in FSL rats compared to FRL rats.

Previous studies with the FSL rat: a selectively bred putative animal model of depression

The FSL rat model of depression exhibits some behavioural, neurochemical, and pharmacological features that have been reported in depressed individuals, and have good construct, face and predictive validities (Overstreet et al. 2005). In two previous studies, the levels of BDNF have been measured in selected brain areas in female and male FSL/FRL rats, showing no difference in hippocampal BDNF levels in FSL rats compared to FRL rats (Angelucci et al. 2000, 2003). These findings are in contrast with the present study, where we report a decreased hippocampal BDNF level in male FSL vs. FRL rats. However, some important differences exist between the two studies, which may partly explain the discrepancies. First, the age of the animals in the present study was around 140 d vs. the 70 d in the previous reports. In another study, it was reported that the levels of brain and serum BDNF levels undergo changes during maturation and ageing processes (Karege et al. 2002b). Therefore, our study and the previous study can be regarded as two distinct measures in different time-slots. Second, the animals in the present study were assessed in the FST 3 wk before euthanization, which was not the case in the previous studies. Although it may be considered unlikely, it cannot be excluded that this stressful

Table 1. Mature BDNF levels in serum, whole blood, hippocampus, frontal cortex, and cerebrospinal fluid (CSF) from FRL and FSL rats

<table>
<thead>
<tr>
<th>BDNF levels</th>
<th>FRL (n = 8)</th>
<th>FSL (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (pg/ml)</td>
<td>2259 ± 267</td>
<td>3692 ± 114*</td>
</tr>
<tr>
<td>Whole blood (pg/ml)</td>
<td>2925 ± 186</td>
<td>4168 ± 101*</td>
</tr>
<tr>
<td>Hippocampus (pg/g wet weight)</td>
<td>19418 ± 1021</td>
<td>12576 ± 1046*</td>
</tr>
<tr>
<td>Frontal cortex (pg/g wet weight)</td>
<td>768 ± 110</td>
<td>611 ± 69</td>
</tr>
<tr>
<td>CSF (pg/ml)</td>
<td>42 ± 12 (n = 4)</td>
<td>39 ± 7 (n = 5)</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. * Indicate significant between-group differences (p < 0.0005).

Fig. 3. Free mature BDNF expressed as % of FRL in serum, whole blood, hippocampus, and frontal cortex. Values are mean ± s.e.m. FRL (■; n = 8), FSL (□; n = 9). * Indicates significant between-group differences (p < 0.0005).

Fig. 4. Western blot analysis of BDNF in homogenates of hippocampus from FSL (□) and FRL (■) rats.
(a) Representative immunobots probed with antibodies for BDNF and β-actin as an internal loading control.
(b) Quantification of signals based on densitometry measurements of the immunobLOTS. Within each blot the values were converted to percent of the mean in control FRL rats and combined to express data as percentage of FRL + s.e.m. * Indicates significant between-group differences (p < 0.05).
experience may affect brain BDNF levels. Finally, all animals in the present study were anaesthetized with pentobarbital at euthanization, as CSF samples were also collected. In the previous studies, no anaesthesia was used. In an animal model of epilepsy it has been shown that a mixture of pentobarbital and diazepam together had a positive effect on hippocampal BDNF expression (Biagini et al. 2001). Further studies are warranted to elucidate the contribution of these possible factors.

Serum and whole blood BDNF levels are different in FSL and FRL rats

BDNF levels were increased significantly in serum and whole blood from FSL rats. This is in contrast to human studies where decreased levels of serum and plasma BDNF have been reported in drug-free patients diagnosed with major depressive disorder compared to control subjects (Karege et al. 2005; Lee et al. 2007). Whole blood BDNF levels were similar in depressed patients and control subjects (Karege et al. 2005). In humans the alterations in serum and plasma

---

**Fig. 5.** Correlation of free mature BDNF concentrations in FSL and FRL rats. The correlations of serum/hippocampus, whole blood/hippocampus, and whole blood/serum are considered significant ($p < 0.05$). Fr ctx, Frontal cortex; Hip, hippocampus.

**Fig. 6.** The total BDNF levels expressed as % of FRL in hippocampus. Values are mean $\pm$ s.e.m. FRL (■; $n = 8$), FSL (□; $n = 9$). * Indicates significant between-group differences ($p < 0.05$).
BDNF have been related to mechanisms of BDNF release (Karege et al. 2005); our results could indicate different BDNF release mechanisms in rats and humans. The diverging results obtained with serum from FRL/FSL rats compared to human studies have been confirmed with two different colonies of FRL/FSL rats. In both cases the free serum mBDNF level in FSL rats was increased to 163% and 159% of FRL rats, respectively. It has been demonstrated that the BDNF serum concentration is nearly identical to that found in washed platelet lysates, as platelets appear to be a unique BDNF transportation system in the human body (Fujimura et al. 2002). Since the number of platelets was similar in FSL and FRL rats this is not likely to be a contributing factor to the observed group difference. To further explore these results, the serum samples were acid-treated to determine the total amount of free BDNF. We did not obtain useful data as the serum BDNF level was lower after the acid treatment. This phenomenon has been described previously with NGF in serum samples from goat, sheep, horse, and cow. A possible explanation is species specificity (Okragly & Haak-Frendscho, 1997).

In this study, in both strains, BDNF serum levels were 89% of those measured in whole blood. In drug-free major-depressed patients the BDNF serum levels were 64% of those found in whole blood and 74% of control subjects (Karege et al. 2005). No possible explanation regarding the low serum-to-blood ratio was reported in that study. However, it has recently been published that in humans BDNF can be more accurately assessed in whole blood than in serum, particularly when the samples are stored for > 12 months. When human serum samples stored at −20°C for < 12 months were compared to their corresponding whole blood sample no significant differences in BDNF concentration were found (Trajkovska et al. 2007). Our samples were stored at −80°C and the BDNF serum and whole blood protein measurements were carried out within 2 months. Consequently, the small difference between the serum and blood BDNF levels is probably due to the blood collection and processing procedures. Additionally, in serum there will always be an unreleasable pool of BDNF.

In summary, a possible explanation for the increased BDNF protein levels in serum and whole blood in FSL rats compared to FRL rats is that it reflects one feature of the FSL depressive endophenotype. Further studies regarding the fate of BDNF as well as thrombocyte properties in FSL and FRL rats as well as in other rodent models of depression are needed to suggest a plausible explanation.

**Correlation of blood and brain mBDNF protein levels**

Considering the reports regarding BDNF passage from blood to CNS and vice versa (Pan et al. 1998) and the positive correlation observed between cortical and serum BDNF contents in newborn rats (serum BDNF < 3000 pg/ml) (Karege et al. 2002b), we hypothesized an interaction between blood and brain BDNF in the rat depression model. In our study, the free mBDNF levels in serum and whole blood did correlate negatively with the free mBDNF level in hippocampus but not in the frontal cortex in FSL/FRL rats. This could be a peculiarity of FSL/FRL rats and it is uncertain whether these findings can be extrapolated to humans. Therefore additional studies are called for to ascertain whether BDNF could be used as a possible biomarker of depression and/or successfully in antidepressant treatment.

**Differential regulation of the BDNF level in CNS**

In order to identify a possible link between changes occurring in the brain and blood, we determined the free mBDNF level in CSF. The concentration was relatively low (~ 40 pg/ml), but similar in FSL and FRL rats. These low values seem reasonable as a total BDNF concentration of 200 pg/ml has been found in CSF from adult male Wistar rats (Mannari et al. 2008). The similar mBDNF CSF levels in FSL and FRL rats are in accord with a human study where the mBDNF CSF levels were identical in depressive patients and healthy controls (~ 15 pg/ml) (Blasko et al. 2006).

In frontal cortex the BDNF mRNA expression and the protein level did not differ between FSL and FRL rats. However, in the hippocampus a significant down-regulation of mRNA expression (80% of FRL), mBDNF level (65% of FRL), and total BDNF protein level (83% of FRL) were found. This is in good accord with a study by Roceri et al. (2002) where the modulation of BDNF has been examined following a 24 h maternal separation (MS) on postnatal day 9 in Wistar rats. MS had no effect on mRNA levels in the frontal cortex in the adult rat whereas in hippocampus both BDNF mRNA and protein level were decreased to ~ 65% of control (Roceri et al. 2002). It was not reported whether it was free mBDNF or total BDNF that was determined. In our study BDNF levels were determined with and without acid treatment of hippocampus preparations. Acid treatment caused a substantial increase in BDNF levels. At present the mechanism of action is not clear but it may be related to the cleavage of proBDNF. BDNF is first synthesized as a precursor proBDNF, which is proteolytically cleaved to generate mBDNF. Alternatively, the acid
treatment may dissociate BDNF from its receptor, the tyrosine kinase receptor B (TrkB) (Martinowich et al. 2007; Okragly & Haak-Frendscho, 1997). Both explanations seem plausible and more studies are warranted to understand possible differences in proBDNF vs. mBDNF in FSL/FRL rats. In conclusion, we have found differential regulation of mBDNF levels in hippocampus, serum, and whole blood in FSL and FRL rat strains. In the hippocampus the BDNF mRNA level, mBDNF level, and total BDNF protein level were down-regulated in FSL rats compared to FRL rats. In addition, correlations of the mBDNF levels in hippocampus/serum and hippocampus/whole blood were found.

We believe that our study with parallel optimized analyses of the BDNF levels in the CSF, brain, and blood in FSL/FRL rats contributes significantly to the understanding of BDNF regulation in the genetic rat model of depression.

Acknowledgements

B. Elfving was supported by the Danish Research Council, Ministry of Science, Technology and Innovation. The study was supported by the Augustinus Foundation and The Biomedical Laboratory Scientist Education and Research Fund. We thank David H. Overstreet for providing us with the initial FSL/FRL breeding pairs.

Statement of Interest

None.

References


Karege F, Schwald M, Cisse M (2002b). Postnatal developmental profile of brain-derived neurotrophic


Molnar M, Potkin SG, Bunney WE, Jones EG (2003). MRNA expression patterns and distribution of white matter neurons in dorsolateral prefrontal cortex of depressed patients differ from those in schizophrenia patients. *Biological Psychiatry* 53, 39–47.


