Biomarkers, 2012; 17(6): 524–531
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ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2012.694476

RESEARCH ARTICLE

Robust changes in expression of brain-derived neurotrophic factor (BDNF) mRNA and protein across the brain do not translate to detectable changes in BDNF levels in CSF or plasma

Thomas A. Lanz, Susan E. Bove, Catherine D. Pilsmaker, Abigail Mariga, Elena M. Drummond, Gregory W. Cadelina, Wendy O. Adamowicz, Brentt J. Swetter, Sharon Carmel, Jo Ann Dumin, and Robin J. Kleiman

Pfizer Global Research and Development, Groton, CT, USA

Abstract
Adult rats were treated acutely with peripheral kainic acid (KA), and changes in brain-derived neurotrophic factor (BDNF) mRNA and protein were tracked over time across multiple brain regions. Despite robust elevation in both mRNA and protein in multiple brain regions, plasma BDNF was unchanged and cerebrospinal fluid (CSF) BDNF levels remained undetectable. Primary neurons were then treated with KA. BDNF was similarly elevated within neurons, but was undetectable in neuronal media. Thus, while deficits in BDNF signaling have been implicated in a number of diseases, these data suggest that extracellular concentrations of BDNF may not be a facile biomarker for changes in neurons.

Keywords: BDNF, kainic acid, mRNA expression, protein expression, rat

Brain-derived neurotrophic factor (BDNF) is critical to normal neuronal development and differentiation, and continues to play important roles in synaptic plasticity and neuronal survival in the adult brain. The breadth of critical functions regulated by BDNF highlight its potential as a therapeutic target and/or biomarker for a variety of psychiatric and neurodegenerative diseases (Pezet & Malcangio 2004, Woo & Lu 2006) as well as eating disorders and obesity (Rosas-Vargas et al. 2011). Recombinant BDNF has been evaluated in a clinical trial for amyotrophic lateral sclerosis with mixed results (Ochs et al. 2000). Other proposed therapeutic approaches to BDNF modulation include stimulation of BDNF production or release, and measurement of BDNF in cerebrospinal fluid (CSF) and plasma could serve as useful biomarker if changes in these compartments reflect changes in the brain. Alterations in plasma or serum BDNF in multiple disease states, including schizophrenia (Pillai et al. 2010), bipolar disorder (Barbosa et al. 2010), Alzheimer's disease (Angelucci et al. 2010), and Parkinson's disease (Scalzo et al. 2010) have been described. Since BDNF is expressed in multiple tissues throughout the body, peripherally circulating BDNF may not reflect expression levels in the brain. Several groups have reported measurement of BDNF in human CSF, though reported concentrations range over two orders of magnitude between studies (Laske et al. 2007; Capelle et al. 2009; Li et al. 2009; Salehi & Mashayekhi 2009; Pillai et al. 2010; Leverenz et al. 2011). Technical challenges have been reported in the ability to reliably detect BDNF in CSF, (Laske et al. 2007), and with 100–10,000-fold greater BDNF levels in plasma or serum (Salehi & Mashayekhi 2009; Pillai et al. 2010; Yoshimura et al. 2010), even the smallest amount of blood contamination could contribute to a CSF BDNF signal.

A primary goal of the present study was to produce a robust induction of central BDNF in a rodent model to examine the pharmacodynamic relationship of BDNF

Address for Correspondence: Thomas A. Lanz, Neuroscience Research, Pfizer MS# 8220-4243, Eastern Point Rd, Groton, CT 06340, USA. Tel: 860-686-0546. E-mail: thomas.a.lanz@pfizer.com

(Received 13 April 2012; revised 09 May 2012; accepted 14 May 2012)

between brain, CSF and plasma. BDNF production and release is subject to regulation by epigenetic mechanisms and neuronal activity (Cowansage et al. 2010). Robust elevations in BDNF mRNA and protein have been observed in models inducing pronounced increases in neural activity, such as seizure-inducing kainic acid (KA) treatment, both *in vivo* (Dugich-Djordjevic et al. 1992; Rudge et al. 1998; Katoh-Semba et al. 1999; Dong et al. 2006) and in primary neuronal cultures (Dong et al. 2006; Katoh-Semba et al. 1999). To fully characterize the central BDNF response to KA, rats were treated with a single dose of KA, monitored for seizure activity and then multiple brain regions were harvested over a 24-h period for assessment of BDNF mRNA and protein levels. CSF and plasma were collected at each time point to provide biomarker information to correlate with brain BDNF changes. Further evaluation of BDNF protein production and secretion was performed in primary cultures of rat hippocampal neurons and human choroid plexus cells.

Experimental methods

Animals

All procedures used in this study were approved by, and in accordance with, the guidelines of the Pfizer Animal Care and Use Committee. Animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. Rats were kept in a 12:12 h light–dark cycle and housed in solid bottom cages with corncob bedding. Rats were fed standard rat chow with water available ad libitum. Adult male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 175–200 g were allowed to acclimate in-house for 1 week prior to the initiation of the study and were used at 225–265 g. On the day of the study the rats were administered a single subcutaneous (sc) injection of either vehicle (saline) or KA (12 mg/kg; Sigma). The injections were timed so that the rats would all be sacrificed within a 2-h time frame, thereby eliminating the need for a corresponding vehicle-treated group at each time point and thus reducing the total number of animals used for the study. Rats were divided into 6 groups consisting of 7–9 rats per group as follows: 24, 12, 6, 3, and 1.5 h after KA injection and 3 h after vehicle injection. Immediately following KA injection, rats were observed for a period of 1.5–2 h for the following behaviors indicative of onset of the KA-induced seizures: wet dog shakes, facial clonus, rhythmic head nods, forelimb clonus, loss of balance, rearing, and rearing and falling. Symptoms were scored using the Racine scale (Racine 1972); seizure score distribution is shown in Table 1. No spontaneous deaths were observed during this experiment. Rats were euthanized by CO_2 and blood was collected via cardiac puncture into EDTA vacutainer tubes and stored on ice for plasma collection. CSF was collected and snap-frozen prior to removal of the brain for dissection of cerebellum, hippocampus, striatum, amygdala, and frontal cortex. Each brain region was collected into two tubes divided by hemisphere, reserving one tube for protein and the other for RNA. Pilot experiments showed no difference in BDNF protein concentration between hemispheres in naïve rats. All samples were rapidly frozen on dry ice and stored at –80°C until analysis.

Table 1. Distribution of seizure severity scores (Racine scale) over time.

	Number of rats exhibiting given seizure severity					
Time (h)	1	2	3	4	5	*N*
0	0	0	0	0	0	8
1.5	2	0	3	4	0	9
3	0	1	2	5	1	9
6	0	0	0	7	1	8
12	0	0	0	5	2	7
24	0	0	0	4	4	8

All rats exhibited some level of symptoms following subcutaneous injection of 12 mg/kg kainic acid. Seizure scoring is based on the Racine scale (Racine 1972): (1) facial clonus; (2) facial clonus plus rhythmic head nodding; (3) facial and foreleg clonus, rhythmic head nodding; (4) facial and foreleg clonus, rhythmic head nodding, rearing; (5) falling in addition to previous symptoms.

RNA extraction and qRT-PCR

RNA was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA) according to the kit protocol, treated with DNase (RNase-Free DNase Set; Qiagen) to remove any contaminating genomic DNA and quantified using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). A260/280 and A260/230 ratios were also determined for each sample; average A260/280 ratio = 2.08 ± 0.04 (mean for all samples ± standard deviation) and average A260/230 ratio = 2.13 ± 0.13 indicating that the samples were free from protein and salt contamination, respectively. Two microgram of RNA was converted to cDNA using Applied Biosystems High Capacity RNA-to-cDNA Master Mix (Life Technologies, Carlsbad, CA) in a 40 µL reaction volume. The cDNA was then diluted with water to a working concentration of 5 ng/µL (based on RNA input) to use in subsequent qPCR reactions.

A TaqMan assay was custom-designed for rat BDNF transcript IX (pan BDNF) and ordered from Applied Biosystems as a 20× master mix containing forward and reverse primers and probe CTGGATGCCGCAAACATGTC (forward), CTGCCGCTGTGACCCACTC (reverse), and TCACACACGCTCAGCTCCCCACGG (probe). PAGE-Ultramer synthetic DNA oligos (Integrated DNA Technologies, Coralville, IA) were ordered to use for generation of standard curves for qPCR. Validation of the BDNF assays and their respective oligos was conducted as follows. A 12-point standard curve was made with the oligo using 1:5 dilutions starting with 25 pM as the top point of the curve. The efficiency was calculated and compared to a cDNA standard curve in order to validate that the amplification efficiency was within 10% for both the oligo and cDNA and that a good linear dynamic range could be achieved.

Quantitative PCR was performed on an Applied Biosystems 7900HT thermocycler (Life Technologies,

Carlsbad, CA). Relative standard curve qPCR was conducted using the validated oligo for the standard curves. Ribophorin was used as the reference gene (assay ID Rn00565052_m1; ordered from Applied Biosystems) and has been previously validated in our lab to be stably expressed in rat brain tissues under a variety of treatment conditions. PCR reactions were conducted in a 10 µL reaction volume containing 3 µL cDNA (5 ng/µL), 5 µL 20x TaqMan® Gene Expression Master Mix (Applied Biosystems), 0.5 µL 20x TaqMan assay and 1.5 µL water under the following cycling conditions: 50°C hold for 2 min, 95°C hold for 10 min, 45 cycles of 3 s at 95°C and 30 s at 60°C.

Protein extraction and BDNF ELISA

Brain samples were prepared for BDNF protein analysis using the following lysis buffer suggested by the ELISA kit manufacturer (Promega, Madison, WI): 20 mM Tris–HCl (pH 7), 137 mM NaCl, 1% NP40, 10% glycerol, 1 mM PMSF, 10 µg/mL aprotinin, 1 µg/mL leupeptin, and 0.5 mM sodium orthovanadate. Samples were weighed frozen and homogenized in 10 mL/g lysis buffer using tungsten carbide beads in a TissueLyser (Qiagen, Valencia, CA). Homogenates were centrifuged at 14,000*g* for 30 min at 4°C. Supernatants were saved in 96-well deep-well plates and stored at –80°C until assay.

BDNF levels were measured using a modified version of the Promega BDNF E_{max}® Immunoassay system (G7611). Half-volume 96-well ELISA plates (Costar®; Corning, Lowell, MA) were coated with 50 µL anti-BDNF mAb at 1:1000 dilution in 0.025 M sodium bicarbonate and 0.025 M sodium carbonate, sealed, and stored at 4°C overnight. Plates were washed 4× with PBS containing 0.05% Tween20, then blocked for 2 h at room temperature (RT) with 130 µL/well Promega blocking buffer (G3311). Samples and standards were prepared in blocking buffer (brain homogenates diluted 1:12; CSF and plasma assayed neat), then loaded onto the plates (50 µL) following a wash step. Plates were sealed and stored overnight at 4°C. On the third day, plates were washed and incubated with 50 µL/well anti-human BDNF pAb at 1:500 dilution in blocking buffer for 2 h at RT. Plates were washed again and incubated with 50 µL anti-IgY HRP conjugate at 1:200 dilution in blocking buffer for 1 h at RT. Following a final wash, 50 µL TMB solution was added to each well. The reaction was stopped after 10 min with 1 N HCl, and 450 nm optical densities were read on a Spectramax plate reader (Molecular Devices). Samples were interpolated off of a standard curve fit by a fourth order polynomial equation using Softmax Pro ($R^2 = 1$). Interpolated BDNF levels were normalized to total protein (DC Protein Assay Kit II, Bio-Rad, Hercules, CA).

Cell culture

Rat primary hippocampal neurons were isolated at embryonic day 18 and cultured at a density of 120,000 cells/well in 24-well plates coated with poly-D-lysine and laminin in serum-free neurobasal media supplemented with NS21 (Chen et al. 2008). Media was changed after 10 days *in vitro* (10 DIV). On 14 DIV, 4–6 wells per condition were treated with vehicle (fresh media), 2 µM bicuculline, or KA (25–100 µM). After 6 or 24 h, media was collected for measurement of LDH (Roche kit #644793001). Neurons were harvested in BDNF lysis buffer and stored at –80°C until assay in the BDNF ELISA. Three replicate experiments were run for each condition.

Frozen human choroid plexus cells were obtained from ScienCell Research Laboratories (Carlsbad, CA). Cells were seeded in a 24-well plate coated with poly-L-lysine at 100,000 cells/well in Epithelial Cell Media (ScienCell). Once cells reached 90% confluency, they were treated with 25 µM KA or vehicle (media). Lysates and media were prepared as described for primary neurons. Three replicate experiments were run for each condition.

Statistical analysis

Differences between groups were compared in GraphPad Prism v5 using a Kruskal-Wallis test followed by Dunn's multiple comparison test.

Results

Following administration of 12 mg/kg KA, all rats exhibited symptoms of a full seizure within 3 h (Table 1). Blood, CSF and brains (frontal cortex, striatum, hippocampus, amygdala, and cerebellum) were harvested at 1.5, 3, 6, 12, and 24 h posttreatment.

Of the regions assessed, hippocampus had the highest levels of BDNF mRNA under both vehicle and stimulated conditions. Hippocampus also showed the most rapid induction of BDNF mRNA dye to KA treatment (Figure 1A). BDNF mRNA was significantly elevated by the first time point assessed (1.5 h, 1162% of vehicle). mRNA expression peaked at 3 h (2785% of vehicle) and then began to decline, but remained significantly elevated 6 h later. Elevations in BDNF protein (Figure 1) were delayed relative to mRNA, with significant elevations detected at 6, 12, and 24 h (270, 424, and 377% of vehicle).

In frontal cortex (Figure 1B), BDNF mRNA was significantly elevated from 1.5 to 12 h. The peak increase (>1600% of vehicle) occurred from 3–6 h, with declining levels by 12 h postdose. As in hippocampus, protein levels were not significantly elevated until 6 h, and remained significantly elevated (>400% of vehicle) through 24 h.

Amygdala showed a slower onset of BDNF mRNA and protein increase relative to other regions (Figure 1C). The peak increase in mRNA was observed at 6 h (2694% of vehicle), and the peak increase in protein levels was not until 24 h (877% of vehicle). In terms of % increase, amygdala showed the highest induction of both mRNA and protein levels of BDNF within the range of the time points assessed.

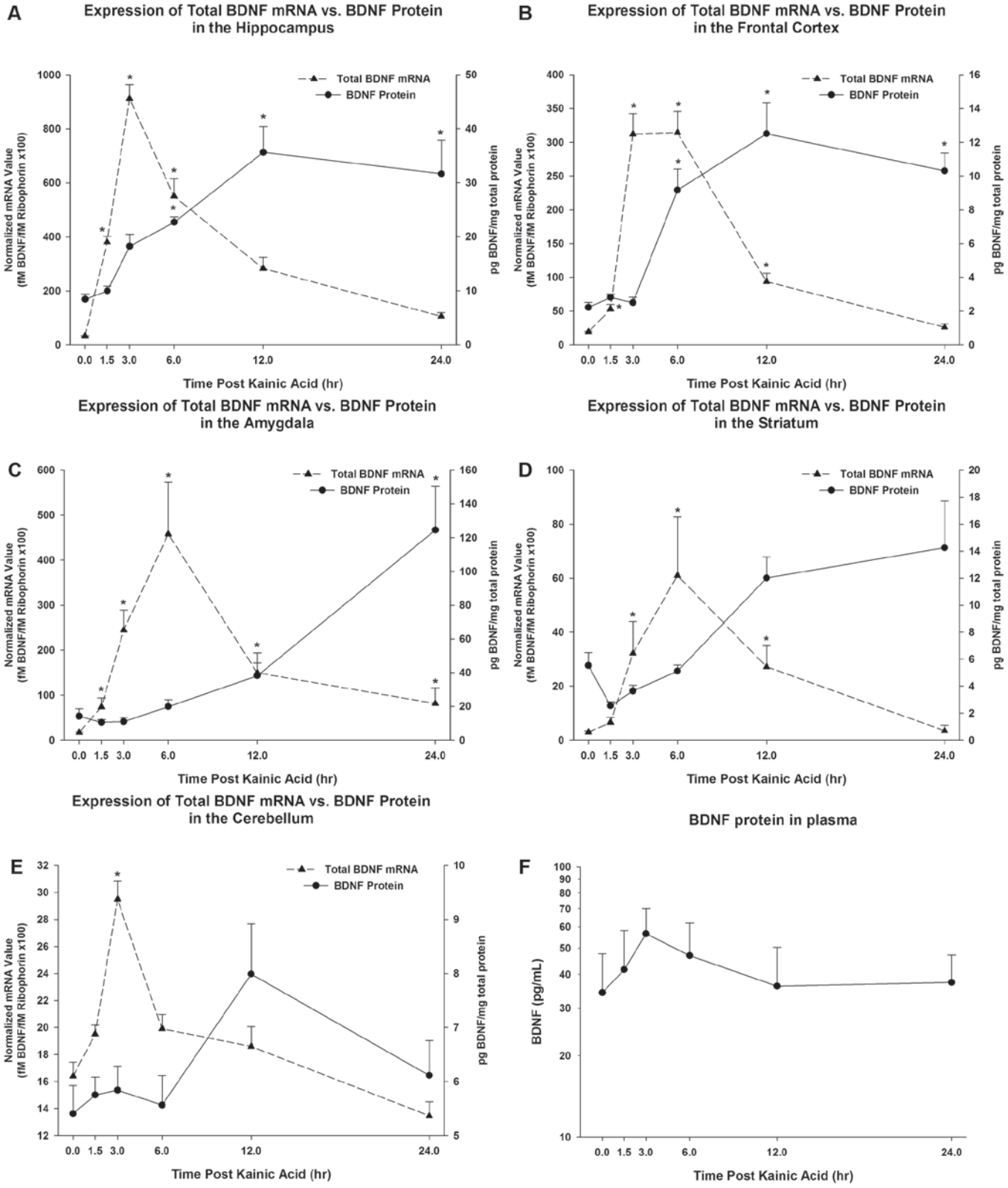

Figure 1. Expression of total BDNF mRNA vs. BDNF protein in the hippocampus (A), frontal cortex (B), amygdala (C), striatum (D), cerebellum (E), or plasma (F) following systemic administration of kainic acid (mean + SEM). The x axis represents time (h) postdose. For brain samples, total BDNF mRNA (left y axis) is represented by triangles connected by dashed lines; values are normalized to ribophorin. BDNF protein (right y axis) is represented by circles connected by solid lines; values are normalized to total protein. *$p < .05$ vs. vehicle control (shown as 0 h on the x axis). No group was significantly different from vehicle in plasma (shown as 0 h).

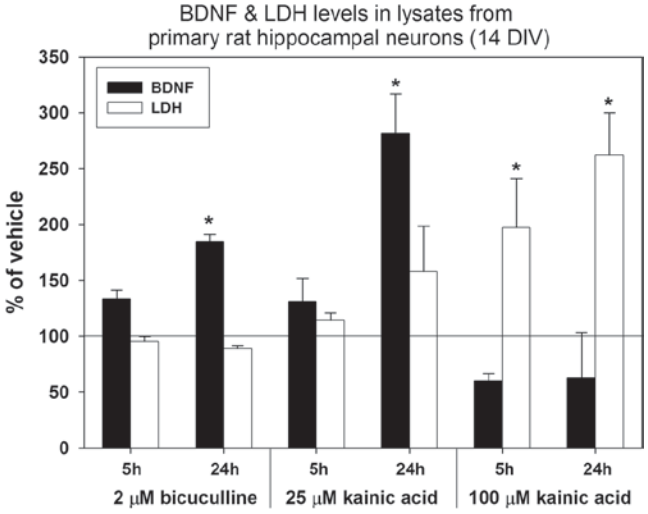

Figure 2. BDNF and LDH measurements in primary cultures of rat hippocampal neurons following administration of bicuculline or kainic acid. BDNF was measured in cell lysates and normalized to total protein. LDH was measured in media. All values are shown as mean + SEM % of vehicle. *$p < .05$ vs vehicle.

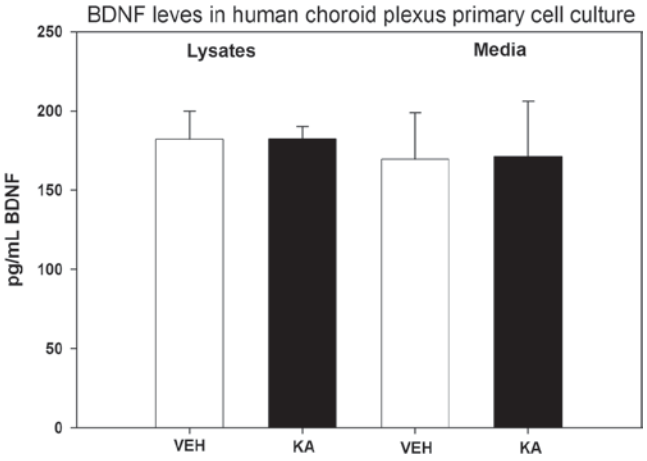

Figure 3. BDNF measurements in lysates and conditioned media from primary cultures of human choroid plexus cells following administration of 25 μM kainic acid. All values are shown as mean + SEM % of vehicle. *$p < .05$ vs vehicle.

In striatum (Figure 1D), BDNF mRNA was significantly elevated by 3 h, peaked at 6 h (2054% of vehicle) and then declined, but was still significantly increased at 12 h. The absolute magnitude of mRNA induction was considerably smaller in striatum as compared to hippocampus, frontal cortex or amygdala. Protein levels of BDNF in striatum showed a trend toward elevation at later time points (12–24 h) but did not achieve statistical significance.

Cerebellum (Figure 1E) was the least responsive region to KA treatment. BDNF mRNA was significantly elevated only at the 3-h time point, and its peak effect was modest relative to all other regions assessed (180% of vehicle). BDNF protein exhibited a nonsignificant increase at the 12-h time point.

Despite robust and long-lasting elevations in BDNF protein in multiple brain regions, BDNF was undetectable in CSF in any treatment group (not shown; assay detection limit was 2–4 pg/mL). Plasma BDNF was measurable, though did not significantly change with treatment (Figure 1F). As a control, recombinant BDNF was spiked into rat CSF, stored at 37°C, and assayed at multiple time points up to 24 h. The BDNF signal was measurable by ELISA at all time points, and exhibited only a 40% decline in signal by 24 h (data not shown).

To evaluate whether changes in neuronal BDNF might translate into detectable changes in secreted BDNF *in vitro* under conditions of enhanced neuronal activity, primary rat hippocampal neurons were treated with the GABA A receptor antagonist bicuculline (2 μM) or KA to enhance neuronal activity (25–100 μM) (Evans 1986; Barker et al. 1987). Bicuculline (2 μM) and KA (25 μM) produced significant elevations in intracellular BDNF by 24 h in the absence of LDH induction (Figure 2). High concentrations of KA (100 μM) were toxic, elevating LDH but not BDNF. BDNF was not detected in the collected media under any condition.

To assess whether choroid plexus cells, responsible for the production of CSF, are capable of producing and secreting BDNF, a primary culture of human choroid plexus cells was treated with KA (25 μM) for 24 h. BDNF was easily measurable in cell lysates before and after KA treatment, though levels were not significantly altered by treatment in this cell type (Figure 3). In contrast to the primary neurons, BDNF levels could be robustly measured in the media regardless of treatment.

Discussion

The present study demonstrated increases in BDNF mRNA and protein in response to KA in multiple brain regions. For each region assessed, we have compared the time course of mRNA induction on one hemisphere to the time course of protein induction in the other hemisphere. Hippocampus showed the most rapid and robust increases in BDNF mRNA (1.5–3 h) and protein production (6 h onward), followed by frontal cortex. In cerebellum, there was a transient increase in BDNF mRNA (3 h) without any significant elevations in protein. Striatum and amygdala both had delayed elevations in BDNF mRNA (3–6 h) and protein (24 h) relative to hippocampus. The initiation of seizure activity in hippocampus before spreading to other brain regions has previously been shown by electrographic endpoints and 2-DG uptake (Ben-Ari et al. 1981). Delayed effects in amygdala including elevated 2-DG uptake and rhythmic spiking have been reported to persist days after a single injection of KA (Ben-Ari et al. 1981).

Despite robust increases in BDNF detectable at the level of both mRNA and protein in multiple regions across the brain, plasma BDNF remained unchanged, and BDNF was not detected in CSF in any group of animals. Published studies of BDNF measured in human CSF (Laske et al. 2007; Capelle et al. 2009; Li et al. 2009; Salehi & Mashayekhi 2009; Pillai et al. 2010; Leverenz

et al. 2011), and two reports measuring BDNF in rat CSF (Xia et al. 2000; Mannari et al. 2008) have utilized similar ELISA based measures of BDNF as reported in the present study. Pilot experiments with rat and human CSF using alternative ELISA kits, protease inhibitors, acidification, or solid-phase extraction failed to yield a reliable BDNF signal in CSF (data not shown). The relatively few studies that have characterized CSF BDNF levels do not provide consistent estimates of the absolute quantity measured and range from less than 2 pg/mL (Laske et al. 2007) to greater than 200 pg/mL (Li et al. 2009; Leverenz et al. 2011). Technical challenges in detecting a signal could be a contributing factor. One published report analyzing BDNF in CSF and plasma acknowledged the difficulty in getting a reliable BDNF signal in human CSF, and were only able to obtain a BDNF signal in 50% of their control samples (Laske et al. 2007). Differences in sample handling or integrity, slight differences in assay sensitivity, or trace amounts of blood contamination could have sizable impact on variability against a backdrop of low baseline levels of CSF expression.

If BDNF released from neuronal cells is into interstitial fluid and moved to the CSF via a bulk flow path, one would expect the magnitude of BDNF elevation produced by kainate treatment in brain tissue in the present study should have generated a detectable change in CSF BDNF. Failure to detect a robust BDNF signal in CSF despite widespread induction of mRNA and protein could be due to strict control of BDNF secretion and re-uptake. Given the plethora of critical roles known for BDNF, such control might be expected. To test this hypothesis, we induced BDNF production in primary rat hippocampal neurons. If activity-induced BDNF is secreted from stimulated neurons without immediate re-uptake by other neurons, then we expected to detect BDNF in the media collected from the neurons. Similar to what is observed *in vivo*, robust increases could be measured in neuronal lysates, but a detectable signal from conditioned media remained elusive. Since CSF is produced in the choroid plexus, it is reasonable to expect that BDNF could be secreted from these cells under the right conditions *in vivo*. We tested this possibility and showed that choroid plexus cells were not only able to produce BDNF intracellularly, but a robust BDNF signal could be detected in media collected from these cells, suggesting that BDNF can be secreted from nonneuronal cells proximal to the CSF compartment. Exogenously added BDNF has previously been shown to be endocytosed by primary rat hippocampal neurons following binding to TrkB (Santi et al. 2006). Others have shown that BDNF levels in primary neuronal media are at the limit of detection unless media samples are concentrated (Chen et al. 2006) or BDNF-specific antibodies are present in the wells to sequester BDNF immediately upon secretion (Balkowiec & Katz 2000). Thus, neurons producing BDNF could be secreting it locally, but the majority of the protein may be quickly internalized, likely via the TrkB receptor. While neurons express predominantly full-length TrkB, choroid plexus cells have been shown to express only a truncated form of TrkB (T1), which lacks the catalytic domain (Klein et al. 1990). Thus even if BDNF is being secreted from choroid plexus cells *in vivo*, the rapid uptake by neurons observed *in vitro* could be responsible for the absence of a measurable signal in CSF.

The ability to monitor levels of circulating BDNF in the brain could provide the basis for viable biomarkers to monitor a number of neurodegenerative and psychiatric disorders in which alterations in BDNF expression and signaling have been reported. Reduced levels of BDNF mRNA and protein in pathologically affected brain regions have been reported in Alzheimer's disease (Connor et al. 1997; Hock et al. 2000), Parkinson's disease (Parain et al. 1999), Huntington's disease (Ferrer et al. 2000), depression (Dwivedi et al. 2003), and schizophrenia (Weickert et al. 2005; Wong et al. 2010). The val66met polymorphism of BDNF, which results in reduced activity-dependent secretion (Rybakowski 2008), is associated with increased risk of schizophrenia (Alonso et al. 2008), reduced episodic memory (Egan et al. 2003), reduced hippocampal volume (Pezawas et al. 2004; Szeszko et al. 2005), and other structural and functional changes in the brain. BDNF-based therapies have been successful in providing neuroprotection from disease-relevant insults in multiple animal models of neurodegeneration (Tsukahara et al. 1995; Ferrer et al. 2001; Canals et al. 2004; Nagahara et al. 2009) and depression (Schmidt & Duman 2010). Given the breadth of diseases in which BDNF has been studied, however, very few reports describe changes in CSF BDNF levels.

Collectively, the results herein suggest that CSF BDNF does not reflect the status of BDNF expression in brain, and may not be well suited as a biomarker or pharmacological endpoint. It remains possible that a reduction in the capacity of neurons to bind and endocytose BDNF or altered CSF flow or volume in a disease state might contribute to abnormally high levels of circulating BDNF. Under normal conditions, however, our data are consistent with the hypothesis that neurons sequester most circulating BDNF, even when intracellular concentrations are already significantly elevated, as induced by KA. Development of therapeutic approaches that focus on enhancing BDNF production or release in brain are therefore not likely to be easily monitored in the clinic by measuring CSF or plasma BDNF levels. Yet given the number of diseases that have described deficient BDNF signaling in the brain and preclinical therapeutic benefits ascribed to increased BDNF function in the central nervous system, the need for biomarkers to assess the state of BDNF signaling in the brain would provide tremendous value for clinical development of novel therapeutic approaches to neurodegenerative and psychiatric disorders.

Declaration of interest

All authors were employees of Pfizer at the time these experiments were performed.

References

Alonso P, Gratacòs M, Menchón JM, Saiz-Ruiz J, Segalàs C, Baca-García E, Labad J, Fernández-Piqueras J, Real E, Vaquero C, Pérez M, Dolengevich H, González JR, Bayés M, de Cid R, Vallejo J, Estivill X. (2008). Extensive genotyping of the BDNF and NTRK2 genes define protective haplotypes against obsessive-compulsive disorder. *Biol Psychiatry* 63:619–628.

Angelucci F, Spalletta G, di Iulio F, Ciaramella A, Salani F, Colantoni L, Varsi AE, Gianni W, Sancesario G, Caltagirone C, Bossù P. (2010). Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI) patients are characterized by increased BDNF serum levels. *Curr Alzheimer Res* 7:15–20.

Balkowiec A, Katz DM. (2000). Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. *J Neurosci* 20:7417–7423.

Barbosa IG, Huguet RB, Mendonça VA, Neves FS, Reis HJ, Bauer ME, Janka Z, Palotás A, Teixeira AL. (2010). Increased plasma levels of brain-derived neurotrophic factor in patients with long-term bipolar disorder. *Neurosci Lett* 475:95–98.

Barker JL, Dufy B, Harrison NL, Owen DG, MacDonald JF. (1987). Signal transduction mechanisms in cultured CNS neurons and clonal pituitary cells. *Neuropharmacology* 26:941–955.

Ben-Ari Y, Tremblay E, Riche D, Ghilini G, Naquet R. (1981). Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline or pentetrazole: Metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy. *Neuroscience* 6:1361–1391.

Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martín-Ibañez R, Muñoz MT, Mengod G, Ernfors P, Alberch J. (2004). Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. *J Neurosci* 24:7727–7739.

Capelle HH, Weigel R, Schmelz M, Krauss JK. (2009). Neurotrophins in the cerebrospinal fluid of patient cohorts with neuropathic pain, nociceptive pain, or normal pressure hydrocephalus. *Clin J Pain* 25:729–733.

Chen Y, Stevens B, Chang J, Milbrandt J, Barres BA, Hell JW. (2008). NS21: Re-defined and modified supplement B27 for neuronal cultures. *J Neurosci Methods* 171:239–247.

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* 314:140–143.

Connor B, Young D, Yan Q, Faull RL, Synek B, Dragunow M. (1997). Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res Mol Brain Res* 49:71–81.

Cowansage KK, LeDoux JE, Monfils MH. (2010). Brain-derived neurotrophic factor: A dynamic gatekeeper of neural plasticity. *Curr Mol Pharmacol* 3:12–29.

Dong M, Wu Y, Fan Y, Xu M, Zhang J. (2006). c-fos modulates brain-derived neurotrophic factor mRNA expression in mouse hippocampal CA3 and dentate gyrus neurons. *Neurosci Lett* 400:177–180.

Dugich-Djordjevic MM, Tocco G, Lapchak PA, Pasinetti GM, Najm I, Baudry M, Hefti F. (1992). Regionally specific and rapid increases in brain-derived neurotrophic factor messenger RNA in the adult rat brain following seizures induced by systemic administration of kainic acid. *Neuroscience* 47:303–315.

Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN. (2003). Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch Gen Psychiatry* 60:804–815.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112:257–269.

Evans RH. (1986). Pharmacology of amino acid receptors on vertebrate primary afferent nerve fibres. *Gen Pharmacol* 17:5–11.

Ferrer I, Goutan E, Marín C, Rey MJ, Ribalta T. (2000). Brain-derived neurotrophic factor in Huntington disease. *Brain Res* 866:257–261.

Ferrer I, Krupinski J, Goutan E, Martí E, Ambrosio S, Arenas E. (2001). Brain-derived neurotrophic factor reduces cortical cell death by ischemia after middle cerebral artery occlusion in the rat. *Acta Neuropathol* 101:229–238.

Hock C, Heese K, Hulette C, Rosenberg C, Otten U. (2000). Region-specific neurotrophin imbalances in Alzheimer disease: Decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch Neurol* 57:846–851.

Katoh-Semba R, Takeuchi IK, Inaguma Y, Ito H, Kato K. (1999). Brain-derived neurotrophic factor, nerve growth and neurotrophin-3 selected regions of the rat brain following kainic acid-induced seizure activity. *Neurosci Res* 35:19–29.

Klein R, Conway D, Parada LF, Barbacid M. (1990). The trkB tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain. *Cell* 61:647–656.

Laske C, Stransky E, Leyhe T, Eschweiler GW, Maetzler W, Wittorf A, Soekadar S, Richartz E, Koehler N, Bartels M, Buchkremer G, Schott K. (2007). BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. *J Psychiatr Res* 41:387–394.

Leverenz JB, Watson GS, Shofer J, Zabetian CP, Zhang J, Montine TJ. (2011). Cerebrospinal fluid biomarkers and cognitive performance in non-demented patients with Parkinson's disease. *Parkinsonism Relat Disord* 17:61–64.

Li G, Peskind ER, Millard SP, Chi P, Sokal I, Yu CE, Bekris LM, Raskind MA, Galasko DR, Montine TJ. (2009). Cerebrospinal fluid concentration of brain-derived neurotrophic factor and cognitive function in non-demented subjects. *PLoS ONE* 4:e5424.

Mannari C, Origlia N, Scatena A, Del Debbio A, Catena M, Dell'agnello G, Barraco A, Giovannini L, Dell'osso L, Domenici L, Piccinni A. (2008). BDNF level in the rat prefrontal cortex increases following chronic but not acute treatment with duloxetine, a dual acting inhibitor of noradrenaline and serotonin re-uptake. *Cell Mol Neurobiol* 28:457–468.

Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, Masliah E, Chiba AA, Tuszynski MH. (2009). Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat Med* 15:331–337.

Ochs G, Penn RD, York M, Giess R, Beck M, Tonn J, Haigh J, Malta E, Traub M, Sendtner M, Toyka KV. (2000). A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 1:201–206.

Parain K, Murer MG, Yan Q, Faucheux B, Agid Y, Hirsch E, Raisman-Vozari R. (1999). Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport* 10:557–561.

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR. (2004). The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *J Neurosci* 24:10099–10102.

Pezet S, Malcangio M. (2004). Brain-derived neurotrophic factor as a drug target for CNS disorders. *Expert Opin Ther Targets* 8:391–399.

Pillai A, Kale A, Joshi S, Naphade N, Raju MS, Nasrallah H, Mahadik SP. (2010). Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: Correlation with plasma BDNF and psychopathology. *Int J Neuropsychopharmacol* 13:535–539.

Racine RJ. (1972). Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281–294.

Rosas-Vargas H, Martínez-Ezquerro JD, Bienvenu T. (2011). Brain-derived neurotrophic factor, food intake regulation, and obesity. *Arch Med Res* 42:482–494.

Rudge JS, Mather PE, Pasnikowski EM, Cai N, Corcoran T, Acheson A, Anderson K, Lindsay RM, Wiegand SJ. (1998). Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. *Exp Neurol* 149:398–410.

Rybakowski JK. (2008). BDNF gene: Functional Val66Met polymorphism in mood disorders and schizophrenia. *Pharmacogenomics* 9:1589–1593.

Salehi Z, Mashayekhi F. (2009). Brain-derived neurotrophic factor concentrations in the cerebrospinal fluid of patients with Parkinson's disease. *J Clin Neurosci* 16:90–93.

Santi S, Cappello S, Riccio M, Bergami M, Aicardi G, Schenk U, Matteoli M, Canossa M. (2006). Hippocampal neurons recycle BDNF for activity-dependent secretion and LTP maintenance. *EMBO J* 25:4372–4380.

Scalzo P, Kümmer A, Bretas TL, Cardoso F, Teixeira AL. (2010). Serum levels of brain-derived neurotrophic factor correlate with motor impairment in Parkinson's disease. *J Neurol* 257:540–545.

Schmidt HD, Duman RS. (2010). Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. *Neuropsychopharmacology* 35:2378–2391.

Szeszko PR, Lipsky R, Mentschel C, Robinson D, Gunduz-Bruce H, Sevy S, Ashtari M, Napolitano B, Bilder RM, Kane JM, Goldman D, Malhotra AK. (2005). Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Mol Psychiatry* 10:631–636.

Tsukahara T, Takeda M, Shimohama S, Ohara O, Hashimoto N. (1995). Effects of brain-derived neurotrophic factor on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in monkeys. *Neurosurgery* 37:733–739; discussion 739–741.

Weickert CS, Ligons DL, Romanczyk T, Ungaro G, Hyde TM, Herman MM, Weinberger DR, Kleinman JE. (2005). Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia. *Mol Psychiatry* 10:637–650.

Wong J, Hyde TM, Cassano HL, Deep-Soboslay A, Kleinman JE, Weickert CS. (2010). Promoter specific alterations of brain-derived neurotrophic factor mRNA in schizophrenia. *Neuroscience* 169:1071–1084.

Woo NH, Lu B. (2006). Regulation of cortical interneurons by neurotrophins: From development to cognitive disorders. *Neuroscientist* 12:43–56.

Xia YX, Ikeda T, Xia XY, Ikenoue T. (2000). Differential neurotrophin levels in cerebrospinal fluid and their changes during development in newborn rat. *Neurosci Lett* 280:220–222.

Yoshimura R, Umene-Nakano W, Hoshuyama T, Ikenouchi-Sugita A, Hori H, Katsuki A, Hayashi K, Atake K, Nakamura J. (2010). Plasma levels of brain-derived neurotrophic factor and interleukin-6 in patients with dysthymic disorder: Comparison with age- and sex-matched major depressed patients and healthy controls. *Hum Psychopharmacol* 25:566–569.