**Short Communication**

**Brain-derived neurotrophic factor blood levels in two models of transient brain ischemia in rats**

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**Abstract.** We monitored possible influence of transient focal and global brain ischemia on BDNF blood level. In both models noticeable fluctuation of BDNF concentration mainly in reperfusion was observed. During the first 90 min, BDNF in total blood and in blood cells continuously decreased in both models but plasma BDNF raised at 40 min and peaked at 90 min of reperfusion. Our data confirm the impact of transient brain ischemia on BDNF levels in the circulatory system, suggest blood cells as a possible source of BDNF and demonstrate the interdependence of blood compartments and physiological state of an affected organism.

**Key words:** BDNF — Brain — Global ischemia — Focal ischemia — Blood

Since the purification of brain-derived neurotrophic factor (BDNF) in 1982 (Barde et al. 1982), a great deal of evidence has mounted for its central roles in brain development, physiology, and pathology. Aside from its importance in neural development and cell survival, BDNF appears essential to molecular mechanisms of synaptic plasticity. Basic activity-related changes in the central nervous system are thought to depend on BDNF modification of synaptic transmission, especially in the hippocampus and neocortex. Pathologic levels of BDNF-dependent synaptic plasticity may contribute to conditions such as epilepsy and chronic pain sensitization, whereas utilization of BDNF role in supporting of neuronal survival and encouraging the growth and differentiation of new neurons and synapses may lead to novel therapeutic options in neurodegenerative diseases and perhaps even in neuropsychiatric disorders (Binder and Scharfman 2004; Binder 2007).

At present, it is well known that BDNF has a relatively important neuroprotective role in either global (Beck et al. 1994) or focal brain ischemia (Schabitz et al. 1997, 2000; Yamashita et al. 1997). Transient global forebrain ischemia as well as focal ischemia induces BDNF mRNA upregulation at the site of injury which seems to correlate with resistance to injury in this area (Tsukahara et al. 1994, 1998; Kokaia et al. 1995). Moreover, the concentration of BDNF after an ischemic attack is not homogenous in the whole brain, but is elevated in regions of higher resistance to ischemic condition (Kokaia et al. 1996).

In addition to the finding that ischemia–reperfusion causes changes in tissue levels of BDNF, it was established that BDNF readily crosses the blood brain barrier (BBB). Serum concentrations correlate directly with brain concentrations therefore plasma studies of BDNF accurately reflect CNS concentrations (Pan et al. 1998; Karege et al. 2002; Enstrom et al. 2008). Moreover, several types of blood cells could be a source of this protein (Braun et al. 1999; Kerschensteiner et al. 1999; Fujimura et al. 2002).

Based on this, we decided to study effects of ischemic conditions on blood levels of BDNF. Specifically, we used two models of transient cerebral ischemia to examine changes of BDNF levels in whole blood, blood cells, and plasma during ischemia and short-term reperfusion.

Experiments were approved by the Institutional Ethical Committee, in accordance with current national legislation. All efforts were made to minimize animal suffering and to reduce the number of animals used. Adult male albino *Wistar* rats (*n* = 4 for each ischemia model) weighing 270–330 g were fasted overnight before surgery.

During the surgical procedure the rats were anesthetized with halotan (3.5% initial, 1% to 1.5% maintenance during surgery). Transient focal cerebral ischemia was produced by right middle cerebral artery occlusion (MCAO) (Longa...
et al. 1989) for 90 min followed by 90 min of reperfusion. Neurological deficit was assessed in each animal on a numerical scale of 0–4 at 60 min of MCAO and at 1 hour of postischemic reperfusion: 0, no detectable deficits; 1, turning of torso and contralateral forelimb when lifted by the tail; 2, score 1 and decreased resistance to lateral push, leaning to the contralateral side at rest with prominent circling to the left; 3, score 2 and circling to the contralateral side spontaneously or when rat was held by the tail; 4, no spontaneous walking and a depressed level of consciousness. Rats with a neurological deficit lower than 2 were excluded from the study.

Transient global brain ischemia (10 min) was induced by four vessel occlusion (4VO) model described previously (Pulsinelli and Brierley 1979). Normothermic conditions (approximately 37ºC) were maintained by a feedback-controlled heating lamp and pad. Animals that became unresponsive within 60 s after clip tightening, lost the righting reflex during bilateral carotid artery occlusion, and showed no seizures during and after ischemia were used for the experiment. Rats survived for 7 days for histological analysis of the extent of CA1 hippocampal neurodegeneration using FluoroJade B staining. Only animals with degeneration of at least 70% were included in the experiment.

Samples of whole blood were obtained by permanent cannulation of right external jugular vein. The silicone cannula was routed to the surface of neck skin to eliminate repeated anesthetization of animal during the blood collection and to minimalize influence of anesthesia. Sham control sample (SHC) was collected directly before MCAO and 4VO surgical procedures into heparinized tubes. Test samples were collected 40 and 90 min after ischemia induction in the model of MCAO and at the end of the ischemic insult in the 4VO model, and at 40 and 90 min of restoration of brain blood circulation. Plasma was obtained by centrifugation (10,000 × g, 4°C, 10 min) of 150 μl of the blood. Whole blood samples were prepared by repeated rapid freezing (10 min, –20°C) and defreezing at 37°C and finally were centrifuged at 10,000 × g (4°C, 10 min). Supernatant of whole blood lysates as well as samples of plasma were stored at –80°C until analysis.

BDNF concentration was measured spectrophotometrically in samples of plasma and whole blood using ChemiKine™ BDNF Sandwich ELISA Kit (Chemicon International, Inc., USA) according instruction manual. Each sample was measured in duplicate. Results were expressed as mean pg BDNF/mg of protein ± SEM. BDNF concentration in blood cells was calculated as the difference between whole blood and plasma BDNF concentration. Statistical analysis was performed with one-way ANOVA followed by Dunnett posttest. The value of p less than 0.05 was considered to be statistically significant.

Figure 1. BDNF concentration in whole blood, plasma and blood cells during and after MCAO (A) and after transient global brain ischemia (B). Data are expressed as mean ± SEM of 4 animals in each group. * p < 0.05, ** p < 0.01. SHC, sham control; I10, I40, I90 – ischemia in minutes; R40, R90 – reperfusion in minutes.
On the contrary, plasma level of BDNF started to decrease during ischemia with a minimum at the end of insult (910) when it was at 44% (34.9 pg/mg of protein) of sham control concentration (78.55 pg/mg of protein). Reperfusion period led to a subsequent elevation of BDNF approximately to the baseline level before ischemia (Fig. 1A).

In the model of 10 min global ischemia we did not observe any changes in BDNF blood levels immediately after the ischemic insult. However, notable changes of its concentration during the reperfusion were reported. In whole blood we observed significant decrease of this protein at 40 min (by about 28%) up to 90 min of blood supply restoration. In blood cells, BDNF concentration was reduced to about 75% of control value 40 min after the beginning of reperfusion period and, in contrast to MCAO animals, continuously decreased to 50% of control by 90 min or reperfusion. Plasma level was not changed during 40 min of recirculation, but reached a 155% elevation at 90 min when compared to control (Fig. 1B).

Essential role of BDNF in brain development, physiology and pathology is unquestioned. Moreover, changes in plasma level of BDNF seem to be related to several neurological, mainly neuropsychiatric disorders, such as depression (Shimizu et al. 2003) or bipolar disorder (Machado-Vieira et al. 2007). Literal data confirm variation of brain tissue BDNF also in models of the most common cause of neurological death, brain ischemia (Tsukahara et al. 1994, 1998; Kokaia et al. 1995, 1996). However, there is no clear evidence about its variations in blood.

In our previous work we observed changes of some biochemical parameters in blood in model of global (Kravcukova et al. 2009, 2010) as well as focal ischemia (unpublished data) that are clearly expressed mainly in early period of posts ischemic recirculation. Based on this we decided to study ischemia impact on blood BDNF during blood supply restriction and early reperfusion and examine its effect on whole blood, plasma and blood cells.

Exogenous additional administration of BDNF seems to be a potential neuroprotector after ischemic attack. It was shown that a direct injection of BDNF into the infarcted brain region (Sakane and Pardridge 1997) or into cerebrospinal fluid by intraventricular administration (Menzies et al. 1993) was neuroprotective. Although BDNF readily crosses the BBB (Pan et al. 1998), there are several reports of insufficient crossing of BDNF molecule from blood to brain tissue after intravenous administration (Zhang and Pardridge 2001, 2006). However, several studies provide an additional important piece of information: blood cells could be a source of BDNF protein.

Fujimura and coworkers (2002) reported that platelets appear to bind, store and release BDNF upon activation at the site of traumatic injury to facilitate the repair of peripheral nerves or other tissues. Moreover, immune cells serve as a source of BDNF. It was observed, that peripheral blood leukocytes isolated from autistic and non-autistic children produce an increased level of this trophic factor after stimulation with lipopolysacharide or phytohemaglutinin ex vivo (Enstrom et al. 2008). Furthermore, an analysis of BDNF production by isolated lymphocyte subsets revealed T cells as a cellular source of BDNF in a mouse model of allergic inflammation (Braun et al. 1999). BDNF secreted by immune cells (T cells, B cells, and monocytes) upon antigen stimulation is bioactive, as it supports neuronal survival in vitro. Using an anti-BDNF monoclonal antibody and a polyclonal antisemur, BDNF immunoreactivity was demonstrated in inflammatory infiltrates in the brain of patients with acute disseminated encephalitis and multiple sclerosis (Kerschensteiner et al. 1999). Changes of BDNF blood levels in our experiments not only show blood cells as a possible source of BDNF protein, but also illustrate the interdependence of blood compartments and their relationship to physiological state of an organism. The best examples of this interdependence are: a) a reduction of BDNF level in whole blood and blood cells, but an unchanged or elevated concentration of this growth factor in plasma during focal ischemia, and b) detected opposite changes of BDNF concentration in blood cells vs. plasma at 90 min of reperfusion in both experimental models of transient brain ischemia.

Our data on BDNF blood concentrations in two different models of brain ischemia demonstrate an impact of this injury on circulatory system, causing changes in blood, plasma and cellular levels of BDNF similar to changes described in several other, mainly neuropsychiatric disorders. The results of our experiments contribute to the investigation of the role of BDNF in the pathology of ischemic injury and confirm the importance of blood as a source of this neurotrophic factor.

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References