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Research Report

Changes in the BDNF-immunopositive cell population of neocortical layers I and II/III after focal cerebral ischemia in rats

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and is widely distributed in the central nervous system, including the cerebral cortex. BDNF plays an important role in normal neural development, survival of existing neurons, and activity-dependent neuroplasticity. BDNF can also be neuroprotective and evoke neurogenesis in certain pathological conditions, such as cerebral ischemia. Neocortical layer I is an important region that can integrate feedforward and feedback information from other cortical areas and subcortical regions. In addition, it has recently been proposed as a possible source of neuronal progenitor cells after ischemia. Therefore, we investigated changes in the BDNF-immunoreactive cell population of neocortical layers I and II/III after middle cerebral artery occlusion (MCAO)-induced cerebral ischemia in rats. In unaffected condition, the number of $BDNF^+$ cells in layer I was significantly less than in layer II/III in the cingulate cortex and in the motor and sensory areas. The increase in the number of $BDNF^+$ cells in layer I 8 days after MCAO was more remarkable than layer II/III, in all regions except the area of cingulate cortex farthest from the infarct core. Only $BDNF^+$–$Ox\text{-}42^+$ cells showed a tendency to increase consistently toward the infarct core in both layers I and II/III, implying a major source of BDNF for response to ischemic injury. The present study suggests that some beneficial effects during recovery from ischemic injury, such as increased supportive microglia/macrophages, occur owing to a sensitive response of BDNF in layer I.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and is widely distributed in the central nervous system, especially in the cerebral cortex, hippocampus, and spinal cord in mammals (Chen et al., 2013). BDNF acts on a variety of neurons through its receptors, TrkB or p75NTR, and plays an important role in normal neural development, supports the survival of existing neurons, and stimulates the growth and differentiation of new neurons and synapses

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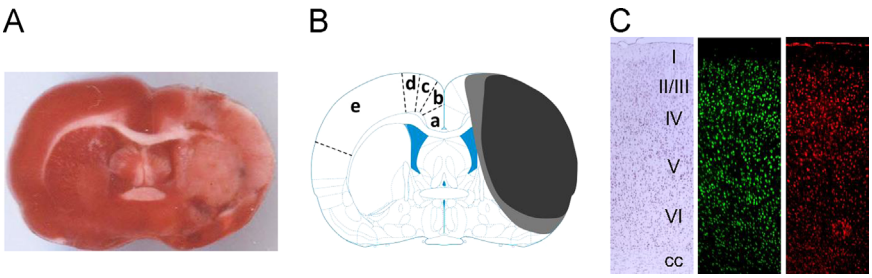

Fig. 1 – Focal cerebral ischemia induced by MCAO and cytoarchitecture in the neocortex. (A) The representative TTC-stained coronal section of rat brain at the level of bregma showing infarct area (pale color) in the right hemisphere 8 days after MCAO. (B) The schematic figure depicting probable infarct core (dark gray) and peri-infarct area (light gray) at the level of bregma (Paxinos and Watson, 2004). (C) Laminar organization of rat neocortex from layer I to VI (left, Nissl-stained) and corresponding immunohistochemistry showing NeuN- (green) and BDNF- (red) positive signals. a, cingulate cortex 2 (Cg2); b, cingulate cortex 1 (Cg1); c, secondary motor cortex (M2); d, primary motor cortex (M1); e, primary somatosensory cortex (S1); cc, corpus callosum.

(Bothwell, 2014). BDNF is also involved in learning, memory, and neuroplasticity (Nakai et al., 2014). Exogenous treatment with BDNF, an enriched environment, or exercise, all of which increase BDNF, facilitates recovery from brain injury (Schabitz et al., 2007; Vaynman et al., 2003). Blocking BDNF by using antisense nucleic acids abolishes exercise-induced cognitive functional recovery after focal ischemia (Ploughman et al., 2009). Finally, BDNF also promotes neurogenesis in the adult brain (Pencea et al., 2001; Scharfman et al., 2005).

Several studies have reported the presence of neurogenesis in the adult mammalian neocortex under pathological conditions such as brain injury and stroke (Jin et al., 2006; Leker et al., 2007; Moraga et al., 2014). Interestingly, a recent study showed that there are neural progenitor cells in neocortical layer I of adult rats, which can differentiate to GABAergic interneurons under ischemic conditions (Ohira et al., 2010). Neocortical layer I is an important region in which the feedforward and feedback information from other cortical areas and subcortical regions can be integrated (Barbas et al., 2013; Jiang et al., 2013), but the role of BDNF expressed in layer I in recovery from ischemia is still not well understood.

According to several studies, the expression of neuronal survival- or growth-associated proteins including BDNF peaks at 7 days after cerebral ischemia (Batchelor et al., 1999; Miyake et al., 2002). These protein changes correlate well with findings that the critical period of stroke recovery ranges from 1 to 2 weeks after injury (Murphy and Corbett, 2009).

Therefore, in the present study we examined quantitative changes in BDNF immunopositive cells in neocortical layers I and II/III of several neocortical regions at 8 days following ischemic injury, during the period of maximal BDNF levels. We also evaluated the type of cells primarily involved in the change in BDNF following an ischemic event to elucidate which cells play a greater role in the recovery progress.

2. Results

Reperfusion after 1 h of occlusion produced an infarct around 25% of the total area of the brain slice at bregma (according to the atlas of Paxinos and Watson, 2004), consisting of a large portion of the cortex and striatum at day 8 after MCAO (Fig. 1A). There was a peri-infarct area, the so-called penumbra, between the intact area and infarct core, although the border was difficult to define (Fig. 1A and B). From the 2,3,5-triphenyltetrazolium chloride (TTC) staining results, regions Cg1 and Cg2 of the brain slice at the level of bregma were mostly unaffected by the ischemia, but M2 and M1 were predictably affected. More specifically, in some rats of the MCAO group, these regions were in the peri-infarct area, but in others, they were partially within the infarct core. The S1 region was mostly found to be in the infarct core after ischemic injury.

To examine differences related to ischemic injury, we examined the number of BDNF-positive ($BDNF^+$) cells of layers I and II/III in several cortical regions that showed the amount of infarct damage. $BDNF^+$ cells in a fixed sized area (100 μm × 600 μm) were counted. The number of $BDNF^+$ cells was represented as a percentage of total cell count by DAPI. Generally, the number of $BDNF^+$ cells in layer I is significantly less than in the corresponding layer II/III of a given region in sham animals (Fig. 1C and Fig. 2B).

MCAO significantly increased the number of $BDNF^+$ cells in layer I as well as in layer II/III of all regions except Cg2 and the increase in layer I was larger than that in layer II/III, although total cell counts by DAPI were not increased in both layers of all regions except S1. (Fig. 2A and B). When an increase was represented as a ratio of the number of $BDNF^+$ cells in the MCAO to that in the sham condition (MCAO/sham ratio), the ratio was greater in layer I than in layer II/III of all regions except Cg2, such that the differences of the ratios between layer I and layer II/III were statistically significant (Fig. 2C).

To investigate the type of cells that are BDNF immunoreactive and responsible for generating the change in the BDNF-positive cell population in layers I and II/III after MCAO, the numbers of neurons (double-labeled with BDNF/NeuN), astrocytes (BDNF/GFAP-labeled), and microglia/macrophages (BDNF/Ox-42-labeled) were determined in the intact area, peri-infarct area, and infarct core (Fig. 3). In infarct core, total cell counts were significantly higher than intact or peri-infarct area in layer I and II/III (Fig. 3C). In layer I, the number of $BDNF^+/NeuN^+$ cells of the infarct core was significantly lower than in the other two areas. The numbers of $BDNF^+/GFAP^+$ cells tended to be reduced toward the core. In layer II/III, the numbers of $BDNF^+/NeuN^+$ and $BDNF^+/GFAP^+$