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Delayed post-conditioning reduces post-ischemic glutamate level and improves protein synthesis in brain

Petra Bonova *, Jozef Burda, Viera Danielisova, Miroslava Nemethova, Miroslav Gottlieb

Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovak Republic

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ABSTRACT

In the clinic delayed post-conditioning would represent an attractive strategy for the survival of vulnerable neurons after an ischemic event. In this paper we studied the impact of ischemia and delayed post-conditioning on blood and brain tissue concentrations of glutamate and protein synthesis. We designed two groups of animals for analysis of brain tissues and blood after global ischemia and post-conditioning, and one for analysis of blood glutamate after transient focal ischemia.

Our results showed elevated blood glutamate in two models of transient brain ischemia and decreases in blood glutamate to control in the first 20 min of post-conditioning recirculation followed by a consecutive drop of about 20.5% on the first day. Similarly, we recorded reduced protein synthesis in hippocampus and cortex 2 and 3 days after ischemia. However, increased glutamate was registered only in the hippocampus. Post-conditioning improves protein synthesis in CA1 and dentate gyrus and, surprisingly, leads to 50% reduction in glutamate in whole hippocampus and cortex.

In conclusion, ischemia leads to meaningful elevation of blood and tissue glutamate. Post-conditioning activates mechanisms resulting in rapid elimination of glutamate from brain tissue and/or in the circulatory system that could otherwise impede brain-to-blood glutamate efflux mechanisms. Moreover, post-conditioning induces protein synthesis renewing in ischemia affected tissues that could also contribute to elimination of excitotoxicity. In addition, the potential of glutamate for monitoring the progress of ischemia and efficacy of therapy was shown.

1. Introduction

Recently, several reports have appeared concerning post-conditioning as a novel approach to reducing damage from brain ischemia (Zhao et al., 2012). In contrast to ischemic preconditioning, the term post-conditioning underlines the main idea of this process – application of a stressor inducing tolerance of cells after the lethal impact.

The post-conditioning could be divided in accordance with the timing of the second stress application to: (1) rapid post-conditioning induced immediately or within a few minutes of commencing reperfusion (Gao et al., 2008; Pignataro et al., 2008; Zhao et al., 2006) and (2) delayed post-conditioning induced after several hours up to days of post-ischemic reperfusion (Burda et al., 2006; Nagy et al., 2011; Ren et al., 2008; Zhou et al., 2011). From the clinical point of view on models of brain ischemia, delayed post-conditioning is the more attractive strategy for improving neuronal survival.

To determine the positive effects of delayed post-conditioning, selective vulnerable neurons are an appropriate target. Selective vulnerable neurons are characterized by delayed death that appears 2–3 days after a lethal stimuli (Ito et al., 1975; Pulsinelli et al., 1982; Siesjo, 1988) due to the persistent inhibition of protein synthesis (reviewed in DeGracia and Hu, 2007) and exhibit markers identical to apoptotic cell death (Danielisova et al., 2009). Following this, transient global brain ischemia, especially in the CA1 region of hippocampus, was studied. For example, 5 min of ischemia applied 2 days after lethal 8 min ischemia induced by "four vessel occlusion" improved survival of vulnerable CA1 neurons up to 96%. Moreover, application of pharmacological stressors such as 3-nitropropionic acid, norepinephrine or bradykinin applied in the same time frame led to comparable protection of CA1 pyramidal neurons (Burda et al., 2006; Danielisova et al., 2006, 2008).

However, there is no clear evidence about the mechanism of delayed post-conditioning induced tolerance. Experimental results have confirmed links to time dependent protein synthesis activity (Burda et al., 2006), activation of endogenous antioxidant system (Danielisova et al., 2006) or decreases in apoptotic markers (Danielisova et al., 2008; Nemethova et al., 2010). By inference from results in rapid post-conditioning models (reviewed in Zhao et al., 2012) we can suppose that delayed post-conditioning may

* Corresponding author. Address: Institute of Neurobiology SAS, Soltesovej 4/6, Kosice SK-040 01, Slovak Republic. Tel.: +421 55 792 2832; fax: +421 55 678 5074.
E-mail address: kravcukova@saske.sk (P. Bonova).

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also reduce ischemic injury by blocking the activity of reactive oxygen species (ROS) and AKT mediated apoptosis.

One of the key factors connected to ROS generation is glutamate. There is strong evidence for an extracellular elevation of glutamate after global (Andine et al., 1991; Molchanova et al., 2004) as well as focal brain ischemia (Bonova et al., 2013; Yang et al., 2001; Zhang et al., 2008). Increased levels of glutamate in the extracellular spaces of neurons leads to glutamate-mediated neurotoxicity by overproduction of ROS and elevation of oxidative stress (Abou-Sleiman et al., 2006; Arundine and Tymianski, 2004) by way of glutamatergic receptor activation (Yi and Hazell, 2006).

Our previous work focused on development of a pattern in biochemical parameters in rat model of MCAO confirmed the spreading of excitotoxicity as well as meaningful changes in SOD enzymes activity and in protein synthesis in brain tissue during the infarct evolution suggesting the time window at which the process of progressive cell death could be reversible (Bonova et al., 2013). In the present work we decided to estimate whether the application of post-conditioning in model of global ischemia would reduce glutamate concentrations in brain tissues with different sensitivities to ischemic conditions (the CA1 region of hippocampus versus the relatively resistant remainder of the hippocampus and cortex) and would thereby also reduce levels of excitotoxicity. In the same time frame we also measured levels of protein synthesis, serving as a marker of neuronal survival/death in tissues affected by ischemia. As a first, due to previous evidence about fluctuations in glutamate levels in the blood early in post-ischemic reperfusion after global ischemia (Kravcukova et al., 2009, 2010), we decided to measure its changes also following transient focal ischemia and after application of post-conditioning.

2. Material and methods

The experiments were approved by the Institutional Ethical Committee in accordance with current national legislation. Every effort was made to minimize animal suffering and reduce the number of animals used. Adult male albino Wistar rats weighing 250–300 g were maintained on a 12 h light/dark cycle and given food and water ad libitum. Food was withdrawn one day before surgery.

2.1. Design of experiments

Three independent experiments were designed. The first was ($n = 8$) was dedicated to analysing blood glutamate after transient focal ischemia (Fig. 1A). Blood was collected at intervals 30 min (I30), 60 min (I60) and 90 min (I90) during the transient focal ischemia and at intervals from 30 to 240 min (from I90 R30 to I90 R240) and at first (I90 R1d), second (I90 R2d) and third day (I90 R3d) of post-ischemic reperfusion. The second group ($n = 8$) was intended to measuring glutamate levels in blood after transient global ischemia (I8) and post-conditioning (I5) at time intervals 1 day (I8 R1d) or 2 days (I8 R2d) of reperfusion after ischemia and at time intervals from 20 min up to 1 day after post-conditioning (from I8 R2d I5 R20 to I8 R2d I5 R1d). Sham control samples (SHC) were collected directly before ischemia. The third group of rats was dedicated to analysing glutamate levels and protein

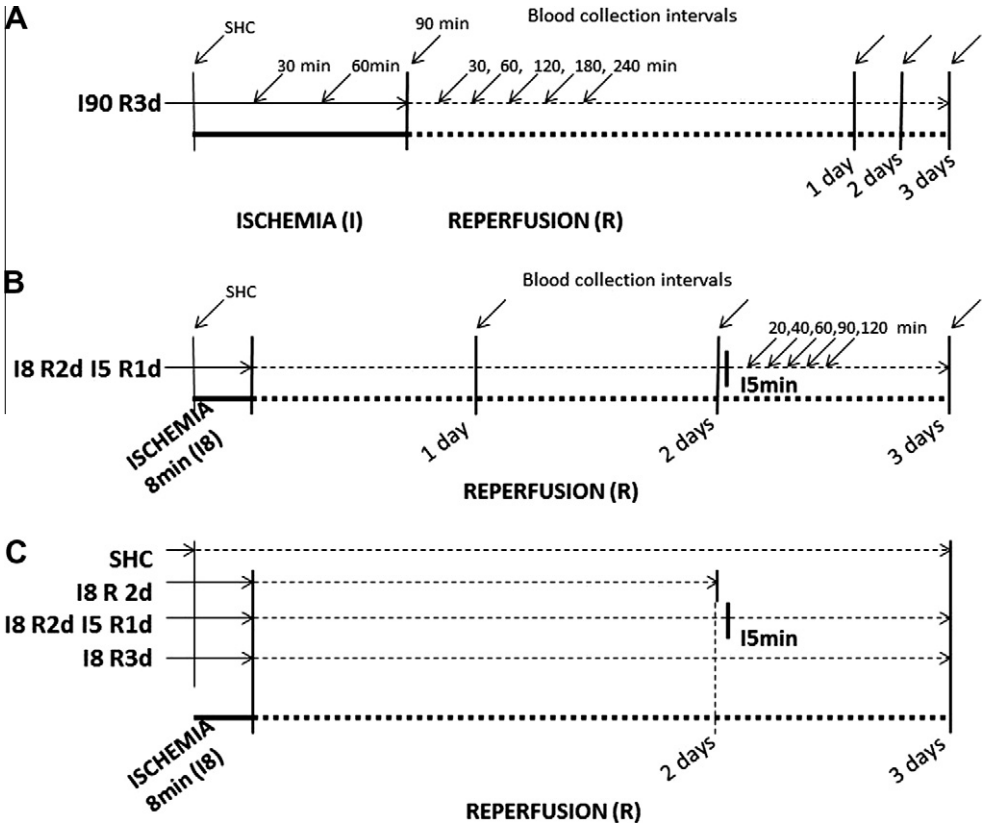

Fig. 1. Schematic drawing of experimental design. (A) The animals were subjected to transient focal brain ischemia for 90 min followed by 3 days of reperfusion (I90 R3d). Blood samples were collected during ischemia and reperfusion over the time intervals marked with an arrow. Control samples were collected just prior to induction of ischemia. (B) The animals were subjected to transient global brain ischemia for 8 min followed by 3 days of reperfusion with application of post-conditioning in the form of 5 min episodes of forebrain ischemia on the second day of reperfusion (I8 R2d I5 R1d). Blood samples were collected at the time intervals marked with an arrow. Control samples were collected immediately prior to induction of ischemia. (C) The animals were subjected to transient global brain ischemia for a duration of 8 min followed by reperfusion for 3 days with (I8 R2d I5 R1d) or without (I8 R3d) application of post-conditioning in the form of 5 min episodes of forebrain ischemia on the second day of reperfusion. Tissue samples were collected on the second and third day of reperfusion. SHC – sham control, I – ischemia, R – reperfusion, d – day.