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Bradykinin postconditioning ameliorates focal cerebral ischemia in the rat

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ABSTRACT

The goal of this study is to investigate the effects of bradykinin (BR) postconditioning on cerebral ischemic injury. Transient focal cerebral ischemia was induced in rats by 60 min of middle cerebral artery occlusion (MCAO), followed by 3 days of reperfusion. BR as a postconditioner at a dose of 150 μg/kg was applied intraperitoneally 3, 6, 24 and 48 h after MCAO. BR postconditioning significantly reduced total infarct volumes if applied 3 h after MCAO by 95%, 6 h after MCAO by 80% and 24 h after MCAO by 70% in versus vehicle group. Neurological functions were a marked improvement in the BR groups compared to the ischemia group. The number of degenerated neurons in the hippocampal CA1 region was also significantly lower in BR-treated ischemic groups compared to vehicle group. BR postconditioning prevented the release of MnSOD from the mitochondria and reduced the activity of the total SOD and CAT if it is administrated short time after stroke. Our data proves the ischemic tolerance in the brain induced by BR postconditioning resulted as effective agent against as strong an attack as 60 min MCAO even when used many hours after ischemia.

1. Introduction

Bradykinin (BK) is considered an important mediator of the inflammatory response in both the peripheral and central nervous system, and has attracted recent interest as a potential mediator of brain injury following stroke (Sobey, 2003; Zausinger et al., 2002). These characteristics suggest that bradykinin can be effectively used as a stressor inducing ischemic tolerance after parenteral application. Numerous reports have confirmed the effect of bradykinin as pre-conditioner and postconditioner in protection of the heart (Lim et al., 2007; Penna et al., 2007) as well as the brain (Danielisova et al., 2008, 2012, 2009; Lehotsky et al., 2009).

Bradykinin is a physiologically active nonapeptide with a lot of functions in the body, synthesized by kallikreins acting on kinogen precursor molecules. The examinations of cerebral ischemia demonstrated that an activation of B2 receptor resulted in activation of protein kinase C associated with release of Ca^{2+} from intracellular stores, stimulation of other inflammatory mediators, and release of excitatory amino acids which led to brain damage after cerebral ischemia (Zausinger et al., 2002). Bradykinin combined with B2 receptor, subsequently activated protein kinase, accelerated production of NO, and induced mitochondria ATP-sensitive K+ channel opening to protect the ischemic myocardium. It seems plausible that bradykinin acts as a primary trigger of delayed preconditioning, and that this effect is mediated by generation of NO as a signalling intermediate (Baxter and Ebrahim, 2002). Also bradykinin induces ROS, especially superoxide anion generated as a result of mitochondrial uncoupling through a pathway that involves activation of protein kinase C isoenzymes, tyrosine kinases and mitogen-activated protein kinases (Baxter and Ebrahim, 2002).

Ischemic tolerance (preconditioning/postconditioning) is the strongest endogenous neuroprotectant against brain injury after cerebral ischemia (Gidday, 2006; Perez-Pinzon, 2007). Our previous results show that delayed neuronal death in a model of transient forebrain ischemia simulating cardiac arrest as well as kainate intoxication can be prevented by postconditioning 2 days after ischemia. As postconditioners, we used short ischemia, 3-nitropropionic acid, norepinephrine and bradykinin (Burda et al., 2005, 2009, 2006; Danielisova et al., 2006). It was recently

Abbreviations: BR, bradykinin; DG, dentate gyrus; CA1, cornu Ammonis 1 layer of hippocampus; CAT, catalase; FJ B, Fluoro-Jade B; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; SOD, superoxide dismutase; CuZnSOD, copper–zinc dependent superoxide dismutase; MnSOD, manganese dependent superoxide dismutase; NeuN, neuronal nuclear protein; LCBF, local cerebral blood flow.

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demonstrated that ischemic postconditioning protects against transient focal ischemia simulating stroke (Gao et al., 2008a,b; Ren et al., 2008, 2009; Zhao et al., 2006), in which ischemic postconditioning is conducted by a series of brief occlusion and release of the bilateral common carotid arteries after reperfusion. Ideally, the clinical application of this method of ischemic postconditioning requires that the treatment be applied after the onset of stroke (Zhao, 2009). Our most recent results documented the efficacy of a combination of both of these methods of postconditioning (immediate applied just on the onset of reperfusion and delayed applied 2 days after ischemia) in a four-vessel occlusion model of ischemia (Danielisova et al., 2012).

Transient forebrain ischemia induces delayed neuronal death in the hippocampal CA1 region. Oxygen free radicals have been suggested to play a significant role in this neuronal death (Kim et al., 1999; Song et al., 2007). In particular, superoxide generated during cerebral ischemia–reperfusion is implicated. Overexpression of copper/zinc superoxide dismutase (CuZn-SOD) minimises superoxide toxicity, resulting in a reduction of cell death (Sugawara et al., 2002). Thus, understanding radical generation and the subsequent toxicity can lead to therapeutic tools to prevent brain injury including ischemic insult (Choi et al., 2007).

In this study we examined bradykinin's ability to induce ischemic tolerance against neuronal injury in a model of transient focal ischemia. Specially we investigated efficacy of postconditioning if delayed several hours up to one day. We decided to determine whether the application of BR postconditioning reduces infarct volume, improvements neurological score and also reduces the activity of total SOD and CAT in the infarct area and penumbra, as well as in two regions of the hippocampus, the selective vulnerable CA1 region and the relative resistant dentate gyrus in the ipsilateral hemispheres.

2. Methods

2.1. Animals

Seventy adult male albino Wistar rats weighing 270–300 g were group-housed and maintained on a 12 h light/dark cycle, with ad libitum access to water and rodent chow. The experiments were carried out in accordance with the protocol for animal care approved by both the Slovak Health Committee (1998) and the European Communities Council Directive (86/609/EEC). The animals were randomly subdivided as follows: 4 controls, 6 sham-operated and 60 ischemic.

2.2. Surgical procedures

Transient focal ischemia was induced by middle cerebral artery occlusion (MCAO) using the intraluminal filament technique (Longa et al., 1989). Rats were anaesthetised with 4% halothane in an anaesthetic chamber and maintained during surgery at 1.5% halothane using a rodent mask. Body temperature was maintained at 37 °C with a heat pad. MCAO was carried out for 60 min by inserting a 4–0 nylon monofilament (Lorca Marin, Spain) via the right external carotid artery into the internal carotid artery to block the origin of the MCA. Sham-operated controls were treated similarly to the ischemic group, but the middle cerebral artery was not occluded.

The Severity of MCA occlusion was confirmed by measuring of local cerebral blood flow (LCBF) using a laser-Doppler flowmeter (PeriFlux System 5000, Perimed AB, Sweden). A 407 probe with an adequate holder was situated on the skull over the MCA location (5 mm lateral and 1 mm posterior to bregma). Only rats with blood flow reduced by more than 80% were used for experiments (data not shown).

In the ischemic group, MCA occluded rats ($n = 6$) received an injection (1 ml/kg i.p.) of normal saline as a vehicle. The treated groups ($n = 6$ each) were MCA-occluded rats receiving an i.p. injection of 150 μg/kg bradykinin (Sigma–Aldrich Chemie Gmbh, Germany) administered 3, 6, 24 or 48 h after MCA occlusion. Animals were euthanised for immunochemical studies 3 days after ischemia.

2.3. Evaluation of neurological deficits

Neurological deficit was assessed in each animal on a numerical scale of 0–4 at 60 min after MCAO termination and every 24 h afterwards. The scoring system of Bederson (Bederson et al., 1986b) was used: 0, no detectable deficits; 1, forelimb flexion and turning of torso to the contralateral side when lifted by the tail; 2, same behaviour as grade 1 and decreased resistance to lateral push; 3, same behaviour as grade 2 with unilateral circling; and 4, no spontaneous walking and a depressed level of consciousness. Rats with a neurological deficit lower than 2 were excluded from the study.

2.4. Determination of infarct volume

The animals were decapitated under chloral hydrate anaesthesia after 3 days of reperfusion. The brains were rapidly dissected out and the forebrains were cut into five coronal sections, 2 mm thick, using a rat brain matrix (Activational Systems, MI, USA). Analysis of cerebral ischemic damage was carried out by using 2,3,5-Triphenyltetrazolium chloride (TTC, Sigma) (Bederson et al., 1986a). The sections were stained by incubating them in a solution of 1% TTC at 37 °C for 15 min and fixed in 10% formalin. For imaging, the sections were scanned by a high-resolution scanner (Hewlett Packard Scanjet). The non-ischemic hemisphere, ischemic hemisphere and infarct area of each brain section was measured in a blinded manner, using Image J software (National Institutes of Health, Bethesda, Maryland, USA). The average infarct area (mm^2) was calculated by the formula: (infarct area on the anterior surface + infarct area on the posterior surface):2. The corrected infarct area in a slice was calculated to compensate for brain edema (Swanson et al., 1990). Corrected infarct volumes (mm^3) were calculated by multiplying the corrected area by the slice thickness and summing the volume.

2.5. Fluoro-Jade B staining

Two millimetre coronal sections after TTC staining were transferred to 20% sucrose (w/v) in 0.1 M phosphate buffer (PB) until equilibrated. The tissue was then frozen in Tissue-Tek OCT mounting medium and 10 μm coronal sections were cut and subsequently mounted on gelatinised microscope slides; these were allowed to air dry, and were then placed in 70% ethanol and ultrapure water for 3 min. The sections were oxidised by soaking in a solution of 0.06% $KMnO_4$ for 15 min then washed 3 times in ultrapure water for 1 min each and stained in 0.001% Fluoro-Jade B (Histo-Chem Inc., USA) in 0.1% acetic acid for 20 min. Two millimetre coronal sections after TTC staining were transferred to 20% sucrose (w/v) in 0.1 M phosphate buffer (PB) until equilibrated. 10 μm coronal sections were stained in 0.001% Fluoro-Jade B (Histo-Chem Inc., USA) by method Schmued and Hopkins (Schmued and Hopkins, 2000). The slides were subsequently washed 3 times in ultrapure water for 1 min each wash and dried overnight at room temperature. The dried sections were cleared by xylol and cover-slipped with DPX Mountant for histology (Fluka Chemie AG, Switzerland). The slides were examined using an Olympus BX 51 microscope with a digital camera DP 50 (Olympus Optical CO. LTD, Japan). Fluoro-Jade B (FJ-B) positive count was