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BRIEF COMMUNICATION

Preconditioning with volatile anaesthetic sevoflurane in ischemic retinal lesion in rats

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Abstract Volatile anaesthetic agents have been recognized for their neuroprotective properties since the 1960s. However, little is known regarding the potential retinoprotective effects of preconditioning by anaesthetic drugs. Retinal ischemia can be modeled by permanent bilateral common carotid artery occlusion (BCCAO). Here we studied the degree of ischemic injury with preconditioning by sevoflurane in the rat retina. During the BCCAO operation and preconditioning Wistar rats were anaesthetized with 1 MAC of sevoflurane. The oxygen, carbon dioxide, and anaesthetic vapor concentration in the anaesthetizing box was monitored with a gas analyzer. We examined 4 groups: non- and preconditioning groups in control and BCCAO animals. The duration of preconditioning period was 1 h and it was performed 1 day before BCCAO. The retinas were processed for histological evaluation after 2 weeks survival to determine the cell number in the ganglion cell layer and the thickness of the whole retina and that of all retinal layers. BCCAO-induced retinal ischemic injury was ameliorated by sevoflurane preconditioning. Retinal thickness and the cell number in the ganglion cell layer were more retained in preconditioned animals after BCCAO compared to non-preconditioned group. These results suggest that preconditioning using sevoflurane could provide a new perspective in retinoprotective strategies.

Keywords Retina · Sevoflurane preconditioning · Ischemia · Retinoprotection

Introduction

Preconditioning is a process through which a prior exposure to certain stimuli can induce protection against the damaging effects of a subsequent insult. In the nervous system, multiple stimuli exert neuroprotection in ischemic conditions (Xu et al. 2008) like short episodes of ischemia or hypoxia, and chemical agents including volatile anaesthetics (Codaccioni et al. 2009; Adamcyk et al. 2010; Wang et al. 2010a, b; Zhu et al. 2010). Isoflurane, sevoflurane and halothane have been shown to induce neuroprotection in cerebral ischemia (Codaccioni et al. 2009; Adamcyk et al. 2010; Wang et al. 2010a, b; Zhu et al. 2010). This phenomenon is referred to as anaesthetic preconditioning and has been demonstrated in various tissues and organs (Kersten et al. 1997; Kitano et al. 2007; Beck-Schimmer et al. 2008; Frässdorf et al. 2009; Kersten 2011). This protection involves two phases, the early and late preconditioning. The early phase begins immediately following the stimulus and lasts for up to 3 h (Kapinya et al.,

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2002). The late phase develops 18–24 h after the stimulus and can last for 3 days (Kaneko et al. 2005).

Similarly to cerebral pathologies, several methods of preconditioning have been shown to exert protection in the retina. For example, low levels of calcium (Brandt et al. 2011), inhalative carbon monoxide (Biermann et al. 2010), hyperbaric oxygen (Wang et al. 2010a) or deferroxamine preconditioning leads to retinal ischemic tolerance (Zhu et al. 2008). Retinal ischemia is a pathology involving mechanisms similar to cerebral ischemia. It is therefore hypothesized that preconditioning with sevoflurane may also lead to protection in retinal ischemia. Retinal ischemia is a factor in many retinal pathologies and experimentally can be produced by various methods (Osborne et al. 2004). Permanent bilateral common carotid artery occlusion (BCCAO) leads to chronic retinal hypoperfusion. We have previously demonstrated that BCCAO leads to degeneration of all retinal layers and causes decrease in the ganglion cell number. Several chemical agents proved to be protective in this model (Atlasz et al. 2007, 2010a, b; Mester et al. 2009; Szabadfi et al. 2010). In this study we evaluated the potential retinoprotective effect of late sevoflurane preconditioning in retinal ischemia in rats.

Materials and methods

Two-month-old adult male rats from the local Wistar colony (n = 7/each examined group) were kept under standardized temperature and light conditions (12 h/12 h light/dark cycle). Animal housing, care, and application of experimental procedures were in accordance with institutional guidelines under approved protocols (No. BA02/2000-20/2006, University of Pecs).

Rats were subjected to bilateral common carotid artery occlusion (BCCAO), ligating both common carotid arteries with a 3–0 filament through a midline incision. Rats were anaesthetized with 1 MAC (2.3 %) (Crawford et al. 1992) of sevoflurane (Abbott, Hungary) in air during the preconditioning period, while the animals were anaesthetised with 1.5 MAC of sevoflurane during the operation. The rats were breathing spontaneously during both preconditioning and operation (Crawford et al. 1992; Kalenka et al. 2007). Concentrations of oxygen, carbon dioxide and anaesthetic vapor in the anaesthetizing box were monitored with a gas analyzer. The duration of preconditioning period was 1 h, that was the period of exposing animals to sevoflurane and it was performed 24 h before BCCAO. A randomized separated group of animals underwent anaesthesia and all steps of the surgical procedure, except for ligation of the carotid arteries. These subjects served as sham-operated controls.

We examined four groups: sham- and BCCAO-operated animals with no preconditioning and with late-preconditioning.

Standard histological examination was carried out 2 weeks after BCCAO. Rats were decapitated under anaesthesia and eyes were removed. The eyes were immediately dissected in ice-cold phosphate buffered saline and fixed in 4 % paraformaldehyde (Merck, Finland) dissolved in 0.1 M phosphate buffer (Sigma, Hungary). Tissues were embedded in Durcupan ACM resin (Sigma, Hungary), cut at 2 μm, and stained with toluidine blue (Sigma, Hungary). The sections were mounted in DPX medium (Sigma, Hungary) and examined in a Nikon Eclipse 80i microscope. Photographs were taken with a digital CCD camera using the Spot program, from central retinal areas of the same eccentricities. Files were then further processed with Adobe Photoshop 7.0 program. Measurements were taken from the digital photographs with the SPOT Basic program. Samples for measurements derived from at least four tissue blocks/each animals (n = 2–5 measurements from one tissue block). The following parameters were measured: (1) cross-section of the retina from the outer limiting membrane to the inner limiting membrane (OLM-ILM); (2) the width of the outer and inner nuclear and outer and inner plexiform layers (ONL, INL, OPL, and IPL, respectively); (3) the number of cells/100 μm section length in the ganglion cell layer (GCL). For all measurements we have selected the retinal section, from central retinal areas of nearly same eccentricities between 1 and 2 mm from the optic nerve head, on which we randomly measured the thickness of the whole retina and that of each layer. Furthermore, all the cells in 100 μm retina length in the GCL were counted. Results are presented as mean ± SEM. Statistical comparisons were made using the ANOVA test followed by Tukey-B post hoc analysis (*; $\#p < 0.05$) by GraphPadPrism 5.04 software.

Results

All characteristic retinal layers of the mammalian retina were well visible in sham preparations with or without sevoflurane-preconditioning (Fig. 1a, b). The photoreceptor layer (PL) was followed by several rows of photoreceptor cell bodies, forming the outer nuclear layer (ONL). The first synaptic layer, a thin outer plexiform layer (OPL) was followed by the rows from the cell bodies of bipolar, horizontal and amacrine cells form the inner nuclear layer (INL). The inner plexiform layer (IPL) was followed by ganglion and displaced amacrine cells in the ganglion cell layer (GCL). The outer limiting membrane (OLM) marks the border of the PL and ONL layers and the inner limiting

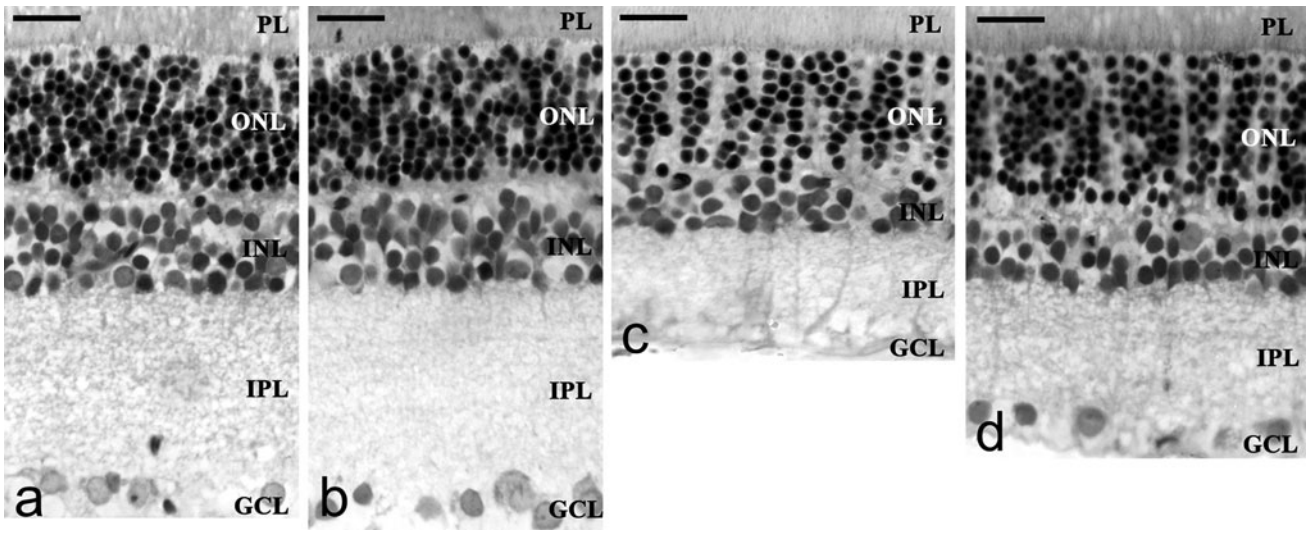

Fig. 1 Microphotographs of representative retinal sections (toluidine blue) from sevoflurane non-preconditioned and preconditioned sham (**a**, **b**) and BCCAO-operated groups (**c**, **d**). There are no significant differences between the two sham-operated control groups. Retinal tissue from BCCAO showed severe degeneration, the total thickness of the retina was significantly reduced. Numerous cells in the ONL, INL and GCL suffered degeneration, shown by the cell body-shaped empty spaces and necrotic cells in these layers. The structure of IPL also displayed signs of degeneration (**c**). In sevoflurane preconditioned BCCAO retinas retained structure was observed (**d**). *Scale bar*: 20 μm. *PL* photoreceptor layer, *ONL* outer nuclear layer, *OPL* outer plexiform layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *GCL* ganglion cell layer

membrane (ILM) was the innermost layer of the retina. No apparent morphological differences could be observed between sevoflurane-preconditioned (Fig. 1b) and non-preconditioned sham-operated animals (Fig. 1a). As previously described (Atlasz et al. 2010a, b) differences between absolute control and sham-operated rats could not be detected in morphological or morphometrical parameters (data not shown).

Bilateral common carotid artery occlusion (BCCAO) in non-preconditioned animals resulted in severe retinal degeneration and reduced thickness of the whole retina and all retinal layers as observed 2 weeks after ligation (Fig. 1c) compared to sham-operated animals (Figs. 1a, b, 2a). Cell body-shaped holes in the nuclear layers and intermingled retina structure were detected (Fig. 1c). All retinal layers suffered marked reduction compared to the sham-operated animals: 66 % in the whole retina thickness (OLM-ILM); 40 % in the OPL; 53.5 % in the INL; 42.5 % in the IPL and 40.5 % in the cell number of 100 μm/GCL (Fig. 2a, b).

In BCCAO-induced ischemic injury sevoflurane preconditioning ameliorated retinal damage as evidenced by morphological and morphometrical analysis (Figs. 1d, 2a, b). Sevoflurane preconditioning led to a nearly intact appearance of the distinct retinal layers (Fig. 1d). This resulted in the clear separation of the nuclear layers in contrast to the non-preconditioned BCCAO rats, where the ONL and INL fused in most animals. This is reflected in the data of the morphometrical analysis: the retinal thickness and the cell number in the ganglion cell layer were more retained in preconditioned animals after BCCAO compared to the non-preconditioned group (Fig. 2a, b). In all retinal layers significant amelioration of the damage is seen as reflected in the percentage of differences, which was significant between sevoflurane non-preconditioned and preconditioned BCCAO animals: the degree of amelioration after preconditioning was 14 % in the whole retina thickness (OLM-ILM); 10 % in the ONL; 23 % in the OPL; 20 % in the INL; 16 % in the IPL and 18 % in the cell number of 100 μm/GCL (Fig. 2a, b).

We noted that the OPL and INL layers are retained better than average, indicative of the importance of the first synaptic layer in this preconditioning phenomenon.

Discussion

In the present study we showed that late-preconditioning with the volatile anaesthetic sevoflurane induced retinal protection in carotid artery occlusion-induced ischemic lesion. Sevoflurane, along with other volatile anaesthetics, has already been shown to provide protection in conditions of cerebral ischemia. This is the first time to demonstrate similar protective potential of sevoflurane in retinal ischemia. The protective effect of this preconditioning was long-lasting, observable also when the morphological changes induced by different injuries reach final degree in the retina (2 weeks).

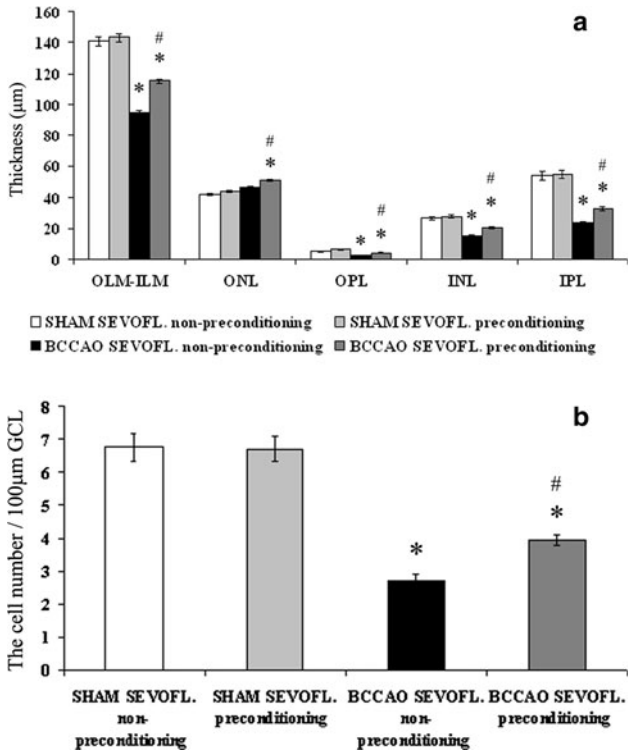

Fig. 2 Statistical and morphometrical analysis of different retinas. Quantification of the thickness in retinal layers (**a**) and the cell number of 100 μm GCL length (**b**). The studied groups were: sevoflurane non-preconditioned and preconditioned sham-operated control rats; rats with non-preconditioning and preconditioning after BCCAO. $*p < 0.05$ compared to sham-operated retinas; $\#p < 0.05$ compared to non-preconditioned BCCAO retinas. *OLM* outer limiting membrane, *ILM* inner limiting membrane, *ONL* outer nuclear layer, *OPL* outer plexiform layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *GCL* ganglion cell layer

Postoperative visual loss is a rare but serious consequence of both ophthalmic and non-ophthalmic surgery. Regarding non-ophthalmic procedures it affects most frequently patients after cardiac and spine operations. The prevalence following cardiac surgery may be as high as 0.08 %. In most of the cases ischemic retinal damage is responsible for the visual loss (Roth 2009; Shen et al. 2009).

Sevoflurane preconditioning has been shown to provide neuroprotection in the brain, the exact mechanism of which is not known, however. Recently, several pieces of the puzzle have been elucidated and a complex neuroprotective mechanism is suggested. For example, sevoflurane preconditioning has been demonstrated to upregulate antioxidant enzyme activity before ischemic injury in a model of focal cerebral ischemia (Yang et al. 2011). Anti-inflammatory effects have also been shown: sevoflurane preconditioned animals have displayed suppressed expression of inflammatory cytokines, NK-kappa B and p38 MAPK in a stroke model (Wang et al. 2011). The involvement of other MAP kinases has also been suggested (Wang et al. 2010b). It has been reported that sevoflurane pre- and postconditioning protect the brain via the mitochondrial K ATP channel (Adamcyk et al. 2010). A further possible mechanism could be the hyperpolarizing effect of sevoflurane anesthesia, which has been described in hippocampal slices after hypoxia (Wang et al. 2006). Even anti-apoptotic pathways have been shown to be activated upon sevoflurane exposure (Codaccioni et al. 2009). Although the mechanism of retinal protection can only be hypothesized at the moment, based on the similar pathways involved in retinal and cerebral ischemic degeneration, similar mechanisms can be proposed in the sevoflurane-induced retinal protection.

Conclusions

The human implications of retinal protection by anaesthetic preconditioning in the rat needs further investigations, but our study provides the basis for future experiments by demonstrating retinoprotective efficacy of sevoflurane anaesthetic preconditioning.

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