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Research Report

Bihemispheric ischemic tolerance induced by a unilateral focal cortical lesion

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ABSTRACT

The purpose of the present study was to determine whether a unilateral photothrombotic brain lesion induces bilateral ischemic tolerance towards a subsequent severe ischemia performed 5 days later. Severe ischemia was induced by transient (1 h; t) or permanent (p) occlusion of the middle cerebral artery (MCAO). Rats were sacrificed 24 h later. Preconditioning reduced the size of subsequent infarcts in both hemispheres. This effect was most prominent with tMCAO, and ipsilateral preconditioning was more effective than contralateral preconditioning (% of hemispheric volume, mean±SD: 31.9±3.7 to 19.0±10.3 with ipsilateral tMCAO; 31.9±3.7 to 22.9±4.9 with contralateral tMCAO; 64.7±4.3% to 47.2±12.5% with ipsilateral pMCAO; 64.7±4.3% to 53.1±8.9% with contralateral pMCAO). Ischemic preconditioning was associated with a successive bilateral up-regulation of superoxide dismutases which may be involved in the development of ischemic tolerance. Our data suggest that a focal ischemic brain lesion induces neuroprotective mechanisms in extensive brain areas and thus cause bilateral ischemic tolerance within a certain time window.

1. Introduction

As for other organs, preconditioning with a sublethal noxious stimulus renders the brain more resistant to a subsequent, otherwise lethal ischemia. This adaptive response is transient and comprises of two time windows, with cerebral tolerance usually following the delayed pattern. Early tolerance occurs within minutes to hours after preconditioning whereas delayed tolerance starts at 24 h and lasts up to 7 days. An ischemia tolerant phenotype can be induced not only by brief

Abbreviations: MCAO, middle cerebral artery occlusion; pMCAO, permanent MCAO; tMCAO, transient MCAO; SOD, superoxide dismutase

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focal or global ischemia itself, but also by a multitude of stimuli (e.g. spreading depression, hypoxia, hypo- and hyperthermia, inflammation, anesthetics; Gidday, 2006; Stetler et al., 2008) that are not necessarily endogenous to the brain (i.e. "remote preconditioning"; Hausenloy and Yellon, 2008; Ren et al., 2008). Several factors have been identified that probably mediate this adaptive response, including heat shock proteins (HSP70, HSP27), hypoxia-inducible factor isoform 1α, adenosin A1 receptor mediated opening of K_{ATP} channels, immediate early genes, protein kinases (MAPK, Akt, PKC), NMDA receptor activation, anti-apoptotic proteins (e.g. Bcl-2), inflammatory cytokines (e.g. TNFα, IL-1β, IL-6), growth factors (e.g. BDNF) as well as antioxidant enzymes (superoxide dismutases, metallothioneins; Gidday, 2006; Stetler et al., 2008). For focal preconditioning, such mediators are activated almost exclusively in the affected ipsilateral hemisphere (Glazier et al., 1994; Welsh et al., 1992). Interestingly, we previously observed a transient bilateral induction of both intracellular isoforms of superoxide dismutases (SODs), the constitutive Cu/Zn–SOD and the inducible Mn–SOD, after unilateral cortical ischemic lesions (Bidmon et al., 1997; Bidmon et al., 1998). These enzymes effectively detoxify reactive oxygen species (ROS) which are one of the key mediators of cellular damage following ischemia and reperfusion (Chan, 2001; Chen et al., 2011). Consequently, studies applying transgenic or pharmacological approaches to interfere with SOD activity have provided compelling evidence for a role of SOD in neuroprotection against ischemic injury (Chen et al., 2011; Huang et al., 2012; Liu et al., 1989).

In the present study we investigated whether a unilateral focal ischemic lesion also protects the contralateral hemisphere from a subsequent severe ischemic injury. We induced a small cortical photothrombosis as preconditioning stimulus and evaluated the infarct volume resulting from a subsequent middle cerebral artery occlusion (MCAO; transient, permanent) inflicted either ipsi- or contralaterally to the photothrombosis. In parallel, we analyzed SOD immunoreactivity in response to focal ischemic preconditioning, both before and after induction of the severe insult, which may help to understand the involvement of these enzymes in the development of ischemic tolerance.

2. Results

All animals subjected to the photothrombotic brain lesion or the corresponding sham procedure recovered quickly without suffering weight loss or any apparent gross impairment. Four of the animals treated by pMCAO died within the first 24 h. Additional 14 animals showing one or more of the following deviations were excluded from the study: hypo- or hyperthermia (defined as $<36.0\,^{\circ}C$ or $<39.4\,^{\circ}C$ rectal temperature) 50 min after MCAO ($n=4$); bleeding during MCAO procedure ($n=1$); lack of arterial backflow during filament removal ($n=2$); lack of a photothrombotic lesion ($n=2$) or lack of infarction after MCAO ($n=4$); subarachnoid or epidural bleeding ($n=3$; probably as a consequence of an incorrectly advanced filament).

Preconditioning significantly reduced the size of a subsequent infarct independent of whether it was induced ipsi- or contralaterally to the lethal ischemia. With tMCAO, ipsilateral preconditioning reduced the size of the infarct by 40.5% and contralateral preconditioning reduced it by 28.0%. With pMCAO, the relative infarct volume was 27.0% smaller after ipsilateral preconditioning and 17.9% smaller after contralateral preconditioning. For statistical comparison of the volumetric values see Fig. 1 and Table 1. Both tMCAO and pMCAO caused severe ischemic damage of the subcortical caudoputamen and parts of the cerebral cortex (including parietal and occipital areas). Cortical tissue "salvaged" by the preconditioning lesion included mostly dorsolateral and lateral portions of the neocortex (Fig. 1A–C).

We next analyzed the expression of Mn–SOD and Cu/Zn–SOD at different time points after the preconditioning stimulus. One day after photothrombosis Mn–SOD immunoreactivity preferentially involved the lesion rim (Fig. 2A). Within the next 24 hours staining expanded to the contralateral area homotopic to the lesion (Fig. 2B), as has been previously described (Bidmon et al., 1998). Five days after photothrombosis, Mn–SOD staining was massively enhanced and almost homogenously distributed throughout the entire ipsilateral and contralateral cortex (Fig. 2C). A similar pattern of increased immunoreactivity was observed for Cu/Zn–SOD (Fig. S1; Bidmon et al., 1997). TMCAO subsequent to an ipsi- or contralateral photothrombosis additionally increased Mn–SOD and Cu/Zn–SOD immunoreactivity throughout the ischemic hemisphere (as compared to photothrombosis alone; Fig. 2D and E, Table 2, Fig. S1C and D). Sham MCAO rats showed a similar pattern of SOD-immunoreactivity as native animals (Table 2). SOD-immunoreactivity after pMCAO has not been quantified. The semiquantitative results of Mn–SOD and Cu/Zn–SOD immunoreactivity are summarized in Table 2.

We furthermore analyzed whether preconditioning affects the MCAO related body weight loss. Animals that underwent pMCAO revealed a mean weight loss of $12.6\pm0.3\%$ ($n=25$) relative to their weight just prior to the onset of pMCAO (i.e. 24 h earlier), independent of whether rats were preconditioned or not (Table 1). Following tMCAO, weight loss was affected by preconditioning. In particular, rats with ipsilateral preconditioning lost significantly less body weight after tMCAO compared to non-preconditioned animals (Table 1). In rats with contralateral preconditioning, tMCAO-induced weight loss was reduced, though this reduction was not significant (Table 1).

Neurological deficit of the animals was assessed prior to sacrifice. TMCAO resulted in a neurological deficit of 1.4 ± 0.8 on the Bederson scale ($n=23$). Subgroup analysis revealed that ipsilateral preconditioning had a tendency to reduce this deficit (transient control 1.6 ± 0.8 vs. transient ipsi 1.0 ± 0.6). Following pMCAO, we found no difference between preconditioned and control groups; the mean Bederson score of all animals was 2.4 ± 0.5 ($n=25$). Outcome data are summarized in Table 1.

3. Discussion

The present study demonstrates that a unilateral focal brain lesion induces a bihemispheric partial tolerance against a subsequent focal ischemia. Prior studies applying unilateral focal ischemia to induce ischemic tolerance mainly focused on