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Kainate postconditioning restores LTP in ischemic hippocampal CA1: Onset-dependent second pathophysiological stress

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ABSTRACT

Postconditioning can be induced by a broad range of stimuli within minutes to days after an ischemic cerebral insult. A special form is elicited by pharmacological intervention called second pathophysiological stress. The present study aimed to evaluate the effects of low-dose (5 mg/kg) kainate postconditioning with onsets 0, 24 and 48 h after the ischemic insult on the hippocampal synaptic plasticity in a 2-vessel occlusion model in rat. The hippocampal function was tested by LTP measurements of Schaffer collateral-CA1 pyramidal cell synapses in acute slices and the changes in density of Golgi-Cox-stained apical dendritic spines. Postconditioning 0 and 24 h after ischemia was not protective, whereas 48-h-onset postconditioning resulted in the reappearance of a normal spine density (>100,000 spines) 3 days after ischemia, in parallel with the long-term restoration of the damaged LTP function. Similar, but somewhat less effects were observed after 10 days. Our data clearly demonstrate the onset dependence of postconditioning elicited by a subconvulsant dose of kainate treatment in global ischemia, with restoration of the structural plasticity and hippocampal function.

1. Introduction

The cerebral consequences of global hypoperfusion can range from slight changes in synaptic plasticity (Marosi et al., 2009), as seen after 2-vessel occlusion (2VO) (Farkas et al., 2007), to extensive (70–75%) cell death in vulnerable brain areas, including the hippocampal CA1 region (Burda et al., 2006; Sas et al., 2008), after 4-vessel occlusion (4VO) (Pulsinelli et al., 1983). The cortex and hippocampus are highly sensitive to hypoperfusion (Pulsinelli et al., 1983). 2VO (only the bilateral common carotid arteries are clamped) reduces the blood flow to one-third in rats (Farkas et al., 2007), with only a slight malfunction in synaptic plasticity (Li et al., 2006; Marosi et al., 2009), and no detectable morphological changes at a cellular level (Fluoro JadeB, S-100 and Nissl staining) (Marosi et al., 2009). Through glutamatergic synapses, long-term potentiation (LTP) involves cellular mechanisms of learning and memory (Bliss and Collingridge, 1993; Martin et al., 2000), expressed mainly in dendritic spines in the hippocampus and cortex. Any LTP malfunction in the ischemic hippocampus is paralleled by changes in dendritic spine density through remodeling of spines. Can appropriate intervention normalize both spine density and LTP function?

Numerous neuroprotectants are tested in ischemic animal models with the aims of infarction reductions and neurological status improvement, but few reach the clinic (Hoyte et al., 2004). Glutamate scavenging by oxaloacetate to reduce excitotoxicity in the ischemic brain appears attractive (Teichberg et al., 2009), preserving neurons (Zlotnik et al., 2008) and synaptic plasticity (Marosi et al., 2009; Nagy et al., 2010). Another endogenous strategy is ischemic postconditioning, which has undergone a renaissance during the last 5–10 years of intensive research (reviewed by Zhao, 2009), after its first description in heart physiology (Sewell et al., 1955). Ischemic postconditioning (Na et al., 1996) in the brain involves several repeated cycles of brief reperfusion and reocclusion of the common carotid artery after the onset of full reperfusion, and is an effective technique (Burda et al., 2006; Wang et al., 2008). Postconditioning can also be elicited by the single post-ischemic administration of pharmaceuticals (second pathophysiological stress), bradykinin, 3-nitropropionic acid and norepinephrine all successfully preserving the principal cells in the hippocampus after 4VO (Burda et al., 2005, 2006; Danielisova et al., 2008, 2009).

Kainic acid (KA), obtained first from the red alga *Digenea simplex*, is a potent agonist of the kainate class of ionotropic glutamatergic receptors, and hence a potent neuroexcitant through

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excitotoxicity (Olney et al., 1974). High doses (at least 10 mg/kg) produce lesions in vulnerable brain areas (Nadler et al., 1978; Sperk et al., 1983), while physiological activation of kainate receptor helps the central nervous system develop normally, building and maintaining synapses and plasticity (Bortolotto et al., 1999).

We have now tested whether KA treatment is suitable for pharmacological postconditioning after incomplete global ischemia. In particular, we determined the optimal application timepoint (onset dependence) for postconditioning during the 72-h post-ischemic period after 2VO, the effectiveness of postconditioning over a longer post-ischemic period (10 days), and the changes in spine density in the hippocampal CA1 region, in the basal glutamatergic synaptic transmission and in the LTP.

2. Methods

2.1. Animals and housing conditions

Male Charles-River rats (200–250 g) of the Wistar strain ($N = 77$) were used. The animals were housed individually in standard plastic cages with free access to food and water. The house of the rats was light-controlled in 12-h cycles and the animals were kept under conditions of constant room temperature (22 ± 1 °C) and humidity. Every effort was made to minimize the number of animals used and their suffering. The principles of animal care (NIH publication No. 85-23), and the protocol for animal care approved by both the Hungarian Health Committee (1998) and the European Communities Council Directive (86/609/EEC) were followed.

2.2. Surgical and experimental procedures, drug application

All of the surgical procedures were carried out under deep anesthesia. Before and during the induction of transient cerebral ischemia, the rats were anesthetized with an intraperitoneal (i.p.) injection of 4% chloral-hydrate (Sigma, Germany). The body temperature of the animals was maintained at 37 ± 0.5 °C throughout the procedure with a feedback-regulated heating lamp. The common carotid arteries were exposed via a ventral cervical incision and separated from the vagal nerves. Both arteries were reversibly clamped with non-traumatic aneurysm clips (Aesculap, B. Braun Medical Ltd, Hungary) for a 30-min period (Marosi et al., 2009). The carotid artery blood flow was then reperfused by releasing the clips following the 30-min global hypoperfusion. The surgery was accompanied by a ~70% survival rate following 2VO. The sham-operated control rats underwent the same procedure, but without bilateral common carotid artery occlusion. In the postconditioned groups the reperfusion period was always 72 h (survival time), with the exception of the 10-day-follow-up group (2VO 48 h KA 10 d). For KA treatment (Tocris, UK) the original 8 mg/kg dose (Burda et al., 2009) was lowered to 5 mg/kg i.p. so as to obtain the maximal postcondtioning effects (a result of a personal communication with J. Burda). To gain information concerning the effects of low-dose KA treatment on the normal EEG activity, 3 control and 3 2VO animals were anesthetized (4% chloral-hydrate injection, i.p.) and conventional EEG recordings were made from the frontal and parietal cortices of both hemispheres before and during 5 mg/kg KA treatment, and then for an additional 100 min. No signs of epileptiform activity were observed during these experiments (data not shown). Our observations are in agreement with the experimental data of Hellier et al. (1999). 5 mg/kg KA produced seizure activity only if it was administered repeatedly. In the groups in which KA was applied alone, no deaths occurred and no signs of epileptiform activity were detected in the behavior. In the postconditioned groups, the survival rate was lower, and it was worst (~50%) when 2VO immediately preceded KA. No signs of epileptiform activity were observed in the behavior of the animals in the postconditioned groups. These data are presented in Table 1.

2.3. In vitro electrophysiology

With the exception of the 12 animals sacrificed for Golgi-Cox staining, taken randomly from their experimental groups, all animals in each experimental group underwent electrophysiological recordings after different survival times (see Table 1). Coronal slices (400 μm), prepared from the middle part of their hippocampi with a vibratome (Campden Instruments, UK), were kept in an ice-cold artificial cerebrospinal solution (ACSF) composed of (in mM): 130 NaCl, 3.5 KCl, 1 NaH_2PO_4, 24 $NaHCO_3$, 1 $CaCl_2$, 3 $MgSO_4$ and 10 D-glucose (all from Sigma, Germany), saturated with 95% O_2 + 5% CO_2. The slices then were transferred to a Haas recording chamber and incubated at room temperature for 1 h to allow the slices to recover in the solution used for recording (differing only in that it contained 3 mM $CaCl_2$ and 1.5 mM $MgSO_4$). The flow rate of the recording solution was 1.5–2 ml/min and the experiments were performed at 34 °C. In order to obtain orthodromic stimulation of the Schaffer collateral/commissural pathway, the stimulating electrode (either a micropipette pulled from a theta glass or a bipolar concentric stainless steel electrode developed by Neuronelektrod Ltd, Hungary) was placed in the stratum radiatum between the CA1 and CA2 regions. The stimulus intensity was adjusted to between 30 and 60 μA (constant current, 0.2-ms pulses delivered at 0.05 Hz) to evoke the half-maximal response.

Field excitatory postsynaptic potentials (fEPSPs) were recorded with glass micropipettes with a resistance of 1.5 MΩ filled with ACSF. The recordings were amplified with a neutralized, high-input-impedance preamplifier and filtered (1 Hz–3 kHz). The fEPSPs were digitized (AIF-03, Experimetria Ltd. Hungary), acquired at a sampling rate of 10 kHz, saved to a PC and analyzed off-line with Origin 6.0 software (OriginLab Corporation, USA).

The fEPSPs were monitored for 40–60 min until the amplitudes were generally stable, and their mean value was determined as the 10-min-long baseline. LTP of the Schaffer collateral-CA1 synaptic response was induced by high-frequency stimulation (HFS, 0.2-ms pulses delivered at 100 Hz for 5 s) at 100% intensity of the test stimulus (Marosi et al., 2009; Sas et al., 2008). After the HFS, the fEPSPs were recorded for at least a further 60 min. The basal glutamergic synaptic properties were also tested by means of fEPSP recordings; in IO (input–output) graphs the fEPSP amplitudes are expressed against gradually increasing stimulating currents (0–100 μA, in 5-μA steps) in certain groups. Each slice was subjected to only one measurement (Marosi et al., 2009; Sas et al., 2008); there were 96 recordings for LTP measurements and 18 recordings for IO curves (see Table 1).

Table 1
Summary of animal groups, interventions and morphological and electrophysiological observations.

Groups	*In vitro* electrophysiology		Golgi-Cox staining
	No. of animals/ no. of mortalities	LTP recordings/ IO curve measurements	No. of animals/no. of cells/no. of detected spines
Control	5/1	8/6	3/60/32,774
Postconditioned			
2VO 48 h KA	5/1	8/6	3/60/30,954
2VO 48 h KA 10 d	6/2	8/0	3/60/23,085
2VO 0 h KA	8/4	8/0	0
2VO 24 h KA	6/1	8/0	0
2VO-only			
2VO 24 h	6/2	8/0	0
2VO 48 h	5/1	8/0	0
2VO 72 h	6/2	8/6	3/60/15,036
2VO 96 h	6/2	8/0	0
KA-only			
KA 24 h	4/0	8/0	0
KA 48 h	4/0	8/0	0
KA 72 h	4/0	8/0	0
Total	65/16	96/18	12/240/101,849

In the control and postconditioned groups, the reperfusion period was always 72 h, with the exception of the 2VO 48 h KA 10 d group, where the survival time was 10 d. In the 2VO-only groups, the survival times were 24, 48, 72 and 96 h. In the KA-only groups, the survival times were 24, 48 and 72 h.

2.4. Golgi-Cox staining technique

In order to determine the number of apical dendritic spines in the hippocampal CA1 region in the control, 2VO and KA-postconditioned animals, we used the Golgi techniques (Gibb and Kolb, 1998; Glaser and Van der Loos, 1981). The animals were decapitated, and the brains were rapidly, but carefully removed from the skull. Approximately 3-mm sagittal blocks including the whole hippocampi were placed in Golgi-Cox solution in the dark for 10 days at room temperature (22–23 °C). This solution is prepared from 3 stock solutions: a 5% solution of potassium dichromate in distilled water (DW), a 5% solution of potassium chromate in DW, and a 5% solution of mercuric chloride in DW (all from Sigma, Germany). The components were diluted and mixed as described by Glaser and Van der Loos (1981). The blocks were replaced in new solution every 2–3 days. When the impregnation was complete, the blocks were placed into 30% sucrose at 4 °C for at least 2–3 days until they sank. 100-μm sagittal sections were cut with a vibratome (Leica VT1000S, Leica Biosystems Nussloch GmbH, Germany). Sections were collected in 6% sucrose and mounted on gelatinized (2%) glass slides. The sections were kept wet throughout the sectioning. To ensure that the sections in all certainty stuck on the slides, moist blotting paper was placed onto the sections and downward pressure was exerted via another slide. The blotted slides were stored in a humidity chamber overnight, after which they were placed in a glass staining tray and processed as described by Gibb and Kolb (1998). After the staining and dehydration, the slides were coverslipped with Fluoromount to prevent curling of the sections.

2.5. Quantitative analysis

The clear Golgi sections were studied by light microscopic stereology, using oil-immersion objectives. In this study, a total of 20 cells from each of 3 animals were

used from each of the 4 experimental groups (control, 2VO, 2VO 48 h KA and 2VO 48 h KA 10 d). Pyramidal neurons from the hippocampal CA1 subfield near the subiculum were studied; in this region, the CA3-proceeding Schaffer collaterals synapse with dendritic spines from the dendritic arbor of pyramidal cells, mostly in the stratum radiatum (Amaral and Witter, 1989). The spine density of the proximal apical dendrite area (100–200 μm from the soma) was analyzed. The second-order dendritic ramifications are particularly sensitive to plastic changes (Gould et al., 1990; Pyapali and Turner, 1994). One segment 100 μm in length from a second-order oblique dendrite protruding from its parent apical dendrite was chosen in each examined neuron, for spine density quantification. The dendrites were selected under a 100× oil-immersion lens and the images (600×) of these apical dendrites were captured through a DP70 camera connected to a light microscope (BX51, Olympus, Tokyo, Japan) and a computer. Serial images were made from each dendrite in the whole of the analyzed segment. The captured multiple photomicrographs from one dendrite were then stacked into one file. To stack the images and determine the density of spines, ImageJ 1.44d software (National Institute of Health, Bethesda, USA) was used. The whole process of measurement of the spine number and density was performed separately by three experimenters blind to the analysis. The total numbers of detected spines are presented in Table 1.

2.6. Statistics

For the statistical analysis of IO curves, the Kruskal–Wallis test was chosen (Fig. 2). For LTP measurements, the fEPSP amplitudes were normalized to the means of the 10-min pre-HFS control data. As normal distribution of the data could not be presumed and the Levene test did not demonstrate equality of variances, a nonparametric test on two independent samples was chosen for the statistical analysis of the LTP data (Mann–Whitney *U*-test) in Fig. 4A. A P value of ≤ 0.05 was considered significant. SPSS10.0 for Windows software (SPSS Inc., Chicago, IL, USA) was utilized. In the spine density analysis of the data for the various experimental groups, parametric *t*-tests for two independent samples were used (Fig. 4B).

3. Results

3.1. In vitro electrophysiological recordings

The HFS of Schaffer collaterals resulted in a 143.55% ± 2.60 increase in fEPSP amplitudes in the controls (Fig. 1A), which remained stable throughout the 1-h recording period. A 30-min 2VO, 72 h prior to LTP induction, resulted in a lesser increase (115.75% ± 2.19) in amplitudes (Fig. 1A), with a constant decay in time. These data are in good accordance with our previous results (Marosi et al., 2009). Fig. 1B depicts the changes in LTP inducibility and height as a consequence of postconditioning at 0, 24, 48 h and 10 d post-ischemia. KA postconditioning applied 24 h after ischemia (2VO 24 h KA) had a worsening effect on the LTP function (112.87% ± 5.51). When KA postconditioning was applied immediately after 2VO (2VO 0 h KA), the fEPSP amplitude increase was least, at only 111.84% ± 1.98, and in this group no post-tetanic potentiation was detected. In both these early postconditioned

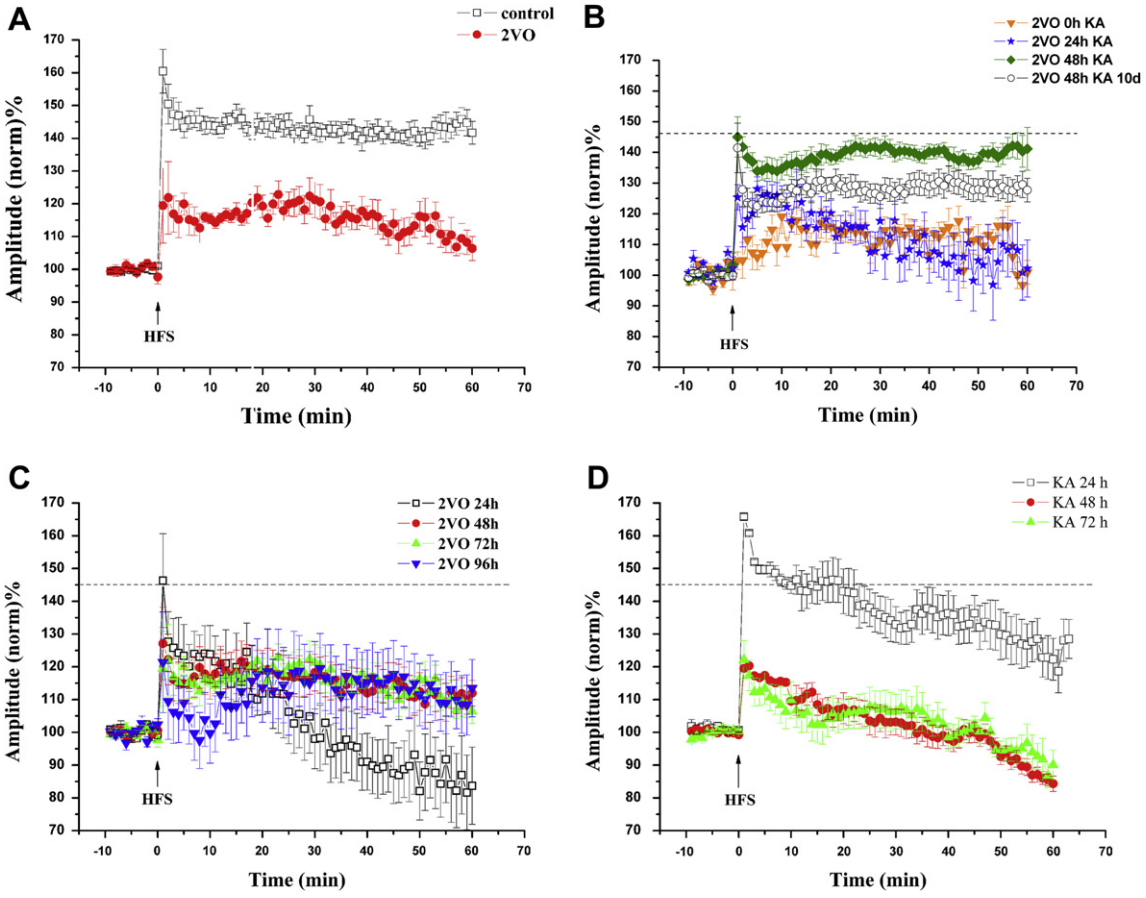

Fig. 1. Post-ischemic kainic acid (KA) administration prevents the global ischemia-induced long-term potentiation (LTP) impairment, but its protective power depends greatly on its application onset. A: In the control group, LTP was induced by high-frequency stimulation (HFS). The 2-vessel occlusion (2VO) group did not show stable LTP. B: As in the 2VO animals, in the early postconditioned groups (0 and 24 h after 2VO) no stable LTP was induced; on postconditioning with 48-h onset after 2VO, the LTP was near the control level. The beneficial effect of 48-h-onset postconditioning persisted 10 days after 2VO (2VO 48 h 10 d). C: LTP impairment caused by 2VO with different reperfusion intervals (24, 48, 72 and 96 h). Even after 96 h of reperfusion, no stable LTP was detected. There were no significant differences in post-HFS amplitudes between the 48, 72 and 96-h groups, but the LTP function was most severely decreased in the 2VO 24 h group. D: LTP impairment caused by a single KA treatment with different survival times (24, 48 and 72 h). In the KA 24 h group, HFS elicited a robust post-tetanic potentiation, and LTP was around the control level during the first third of the registration period, while in the last two-thirds it decayed steadily. In the KA 48 h and KA 72 h groups, no LTP was induced, and the fEPSP values fell even below the pre-HFS levels. Dashed lines (values from A) in B, C and D denote mean levels of LTP in control animals. Data points are means ± S.E.M. of normalized amplitudes of fEPSPs.

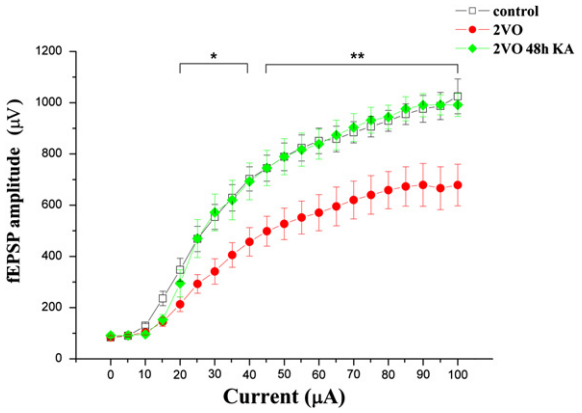

Fig. 2. Input–output curves for fEPSP amplitudes in the control group (□), the 2-vessel occlusion (2VO) group (●) and the group postconditioned with kainic acid (KA ♦), where the protective effect of postconditioning was maximal (2VO 48 h KA). Data points are means ± S.E.M. of amplitudes of fEPSPs; $N = 6$ for all groups presented here. Asterisks ($^*P < 0.05$ and $^{**}P < 0.01$) denote significant differences between the data of the 2VO animals and those of the control and postconditioned (2VO 48 h KA) groups (Kruskal–Wallis test). (For interpretation of the references to colours in this figures legend, the reader is referred to the web version of this article.)

groups, the amplitudes had reached the pre-HFS levels by the end of the 1-h follow-up period. An LTP function recovery was seen only when KA treatment was applied 48 h after ischemia (delayed postconditioning), clearly demonstrating the onset dependence of the postconditioning (Fig. 1B). In the 2VO 48 h KA group, the post-HFS values were close to the control levels (138.95% ± 2.38) throughout the 1-h follow-up period (Fig. 1B). Since KA treatment alone had a worsening effect on the LTP function at 72 h (Fig. 1D), it was important to test whether the protective effect of KA post-conditioning was persistent for a longer time period. In the 2VO 48 h 10 d group, LTP measurements were made 8 days after post-conditioning. As shown in Fig. 1B, the LTP function still existed in the ischemic brain, and the fEPSPs amplitudes were stable throughout the whole of the follow-up period (128.18% ± 3.38).

In order to exclude the possibility of spontaneous recovery in synaptic plasticity over time, a systematic check was carried out on a day-by-day basis from the first (24 h) to the fourth day (96 h) after 2VO. As indicated in Fig. 1C, stable LTP could not be induced at any examined timepoint (here the data of the 2VO 72 h group are taken from Fig. 1A to facilitate comparison). There was no difference in the overall decrease in synaptic plasticity between the 2VO 48 h, 2VO 72 h and 2VO 96 h groups. However, the 2VO 96 h group exhibited a more pronounced decrease in fEPSP amplitudes, which reached the pre-HFS levels during the first 15 min. The reason for this is unclear. The 2VO 24 h group displayed the most severe decrease in synaptic plasticity: the fEPSP amplitudes reached the pre-HFS levels after only 30 min. We also tested the temporal effects of single KA treatments at different survival times (Fig. 1D). In the KA 24 h group, the amplitudes were around the control values (140–145%) during the first 10–15 min after HFS, but they later began to decrease steadily in time, revealing disturbances in synaptic plasticity. No LTP was observed in this group. In the KA 48 h and KA 72 h groups, a more severe malfunction was detected, the fEPSP amplitudes reaching the pre-HFS values by 30 min, with a steady decay throughout the 1-h follow-up period. The electrophysiological data presented in Fig. 1A and B are summarized in Fig. 4A, where the post-HFS fEPSP amplitudes are box-plotted.

The basal synaptic properties were also tested to evaluate how the various experimental interventions impaired the Schaffer collateral-CA1 synaptic transmission. IO curves were established by plotting the fEPSP amplitude against various intensities of the test pulse, ranging from 0 to 100 μA in 5 μA steps. It is clearly seen in Fig. 2 that there was a marked difference between the IO curves of the control and the 2VO groups, implying that the basal functions of the pyramidal cells and synapses were affected by the 2VO. However, the KA-postconditioned group demonstrated normal IO values (Fig. 2).

3.2. Golgi-Cox impregnation

The Golgi-Cox staining method labeled a subset of neurons in all parts of the hippocampus. There were no differences in overall staining between the 4 studied groups: usually 20–25 fully impregnated CA1 pyramidal cells could be detected per slice. No signs of irregular dendritic swellings were detected in the histologically examined ischemic groups (Fig. 3). Although different types of spines (e.g. thin, stubby, mushroom, wide, double and branched) (Gonzalez-Burgos et al., 2007) were always clearly seen, our study was restricted merely to the determination of spine density (Fig. 3). In the control group, the spine density was 182.07 ± 3.25/100 μm (Figs. 3 and 4B), a value similar to that detected by others (116.7 spines/50 μm) (Gonzalez-Burgos et al., 2007); the difference is probably due to slight variations in staining technique. In the 2VO animals, the mild, incomplete ischemia resulted in a marked decrease in spine density (84.95 ± 2.23/100 μm) (Fig. 4B). With the aid of prior electrophysiological experiments (LTP measurements), the ideal timepoint for KA postconditioning was determined, which proved to be 48 h after 2VO. For examination of the consequences of postconditioning as concerns the spine density, measurements were subsequently made only at this single timepoint. As a result of KA post-conditioning, by 48 h after ischemia the spine density had virtually recovered to the control level (171.96 ± 2.58/100 μm vs 182.07 ± 3.25/100 μm) (Fig. 4B) in parallel with restoration of the synaptic plasticity. The elevated spine density level persisted 10 days after 2VO (128.25 ± 1.41/100 μm, Fig. 4B).

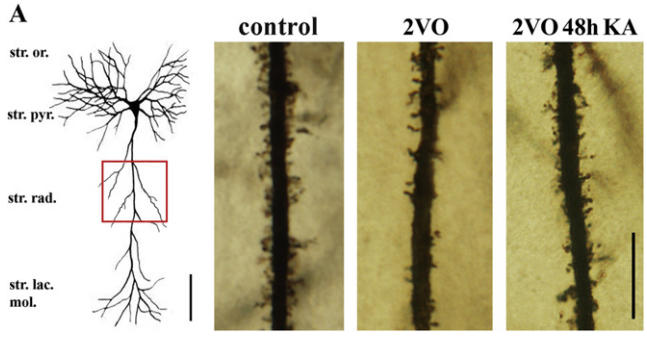

Fig. 3. Golgi-Cox staining revealing changes in spine density. A: A schematic drawing of a CA1 subfield pyramidal neuron, with a small box showing the site of spine density measurement. B: Photomicrographs of oblique dendritic segments from hippocampal CA1 pyramidal neurons of control, 2VO and 2VO-postconditioned animals treated with kainic acid (KA) at 48 h post-ischemia (2VO 48 h KA). Note the fewer spines on the dendrites in the 2VO than in the control and the 2VO-postconditioned animals. Scale bar for A: 100 μm; for B: 20 μm.

4. Discussion

A great wealth of experimental data strengthens the assumption that remodeling and stabilization of the spine synapses of the pyramidal cells are features of the cellular mechanism by which LTP and memory processes occur (Kandel, 2004; Kasai et al., 2003). Pyramidal cells receive almost all their glutamatergic excitatory input through their spine synapses, and changes in spine density

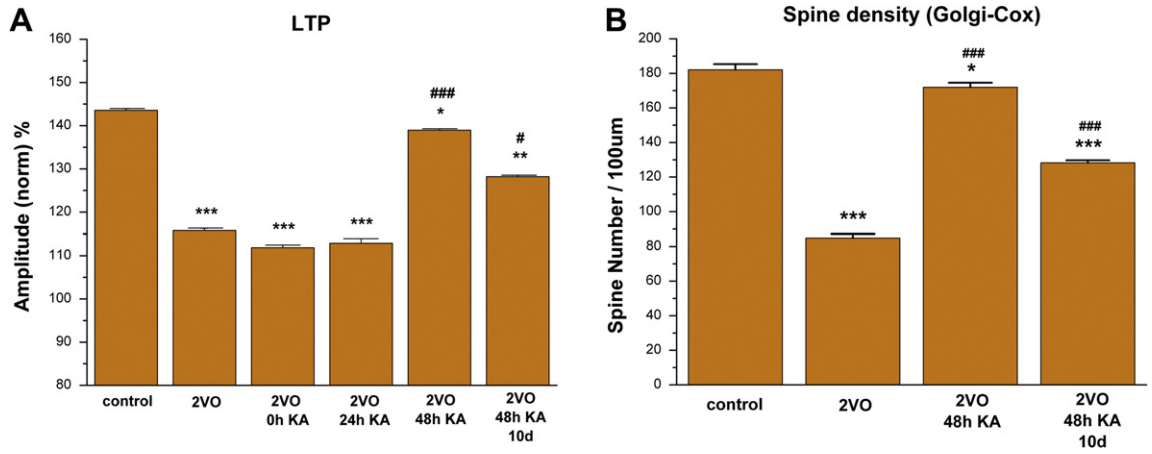

Fig. 4. Changes in LTP and spine density. A: Post-ischemic administration of kainic acid (KA) prevents the long-term potentiation (LTP) impairment only within its therapeutic window. Box-plots demonstrate the means ± S.E.M. of all amplitudes measured throughout the 60-min recording period following HFS. In the intact animals (control), HFS resulted in LTP with amplitudes remaining stable up to the end of the test period. In the 2-vessel occlusion (2VO) animals, however, after HFS the potentiation level was low during the 1-h follow-up period. KA postconditioning prevented the LTP impairment in an onset-dependent manner in the ischemic animals: in the 2VO 0 h KA and 2VO 24 h KA groups, the fEPSP amplitudes were around or even below the level of the 2VO group. Full protection was seen only when postconditioning was performed on the second post-ischemic day (2VO 48 h KA). When the effect of 48-h postconditioning was measured 8 days after KA treatment (2VO 48 h KA 10 d), a stable LTP could still be induced, though with a somewhat smaller amplitude. Asterisks denote significant differences from the control, and $^{\#}$ denotes significant differences from the 2VO groups ($^{\#}P < 0.05$, $^{**}P < 0.001$, ***, $^{\#\#\#}P < 0.001$ Mann–Whitney *U*-test). B: Apical dendritic spine density analysis revealed that the transient global ischemia induced a reduction in spine density 72 h after the ischemic insult in the 2VO group relative to the controls. In the postconditioned group (treated with KA) 48 h after the ischemic insult, (2VO 48 h KA), the spine density was close to the control level. 10 days after the ischemic insult, the spine number elevation due to KA postconditioning was still detected (2VO 48 h KA 10 d group). In each experimental group, 60 dendrite shafts of 3 animals were analyzed. $^{*}P < 0.05$, $^{***}P < 0.001$ significant differences from the control group; $^{\#\#\#}P < 0.001$ significant differences from the 2VO group (parametric *t*-tests for two independent samples).

and spine synapses can therefore greatly influence the excitability and responsiveness of these cells. During the development of LTP, profound morphological changes in spine morphology occur (Matsuzaki et al., 2004). As a consequence of the changes in dendritic spine morphology, the conduction of postsynaptic potentials (Araya et al., 2006) and the diffusion signal proteins (Hugel et al., 2009) between spines and dendritic shafts undergo alteration. Under pathological circumstances, as in the ischemic brain, the longer dendritic spines (Brown et al., 2008) at the network level can isolate the soma biochemically and electrically from the local excitotoxic effects, preventing the initiation of apoptotic processes. Spines can buffer the elevated Ca^{2+} levels during excitotoxicity (Majewska et al., 2000). The dendritic plasticity in the post-ischemic brain is the early adaptive response by which affected neurons can fend off pathological processes, such as excitotoxicity, increased Ca^{2+} influx (Bano and Nicotera, 2007), and spontaneous spreading depolarizations (Risher et al., 2010). However, incomplete forebrain ischemia, e.g. that caused by 2VO, does not lead to cell death (Marosi et al., 2009); thus, the change in dendritic plasticity alone may be responsible for the reduction of LTP in the CA1 region.

KA is a neuroexcitatory and neurotoxic substance (Olney et al., 1974); its administration locally or systemically induces widespread neurodegeneration in the brain, the most intense being in the hippocampus (Sperk et al., 1985), accompanied by the loss of the principal cells in the CA1, CA3 and hilar regions (Nadler et al., 1978; Sperk et al., 1983). However, the administration of KA also triggers neurogenesis in the dentate gyrus (Gray and Sundstrom, 1998; Jaako et al., 2009), resulting in aberrant synaptic contacts and neural networks, affecting the hippocampal functioning (Scharfman et al., 2000). Rats receiving KA display a status epilepticus, with recurrent seizures and severe spatial memory and learning impairments (Mikati et al., 1994). Administration of KA at a convulsant dose (e.g. 10 or 50 mg/kg) also induces the expression of proto-oncoproteins, such as Fra, c-Fos and Jun in the rat hippocampus (Le Gal La Salle, 1988; Popovici et al., 1990). These changes in local gene expressions have been suggested to be related to hippocampal neural death and survival after KA-induced seizure activity (Xia et al., 1995). KA also induces the activities of mitogen-activated kinases (MAPKs): extracellular signal-regulated protein kinase (ERK), c-Jun N terminal kinase (JNK) and p38 MAPK (p38) (DeCoster et al., 1998). JNK targets several apoptosis-related proteins, including c-Jun, p53 and Bcl-2, and can activate the caspase cascade response (Haydar et al., 1999). Whether or not a cell survives or undergoes apoptosis is dependent on the activation of these MAPKs (Xia et al., 1995). Calcium/calmodulin-dependent kinase II (CaMKII) regulates a number of cellular functions related to elevated intracellular Ca^{2+} concentrations (Hudmon and Schulman, 2002). CaMKII plays a key role in mediating cell death following hypoxia/hypoglycemia (Hajimohammadreza et al., 1995), and displays an elevated phosphorylated level following KA treatment (Lee et al., 2003). The activation of MAPKs and CaMKII after KA treatment can be blocked by cycloheximide pretreatment, suggesting the need for newly synthesized proteins (Lee et al., 2003). It should be noted that one or more brief, non-harmful seizure episodes (seizure preconditioning) evoked by either low-dose KA (Tanaka et al., 2010) or domoic acid (a KA analog (Hesp et al., 2007)) can dramatically reduce hippocampal damage when given prior to status epilepticus, via a G-protein coupled pathway (PKA and CaMKII). In our experiments, the applied KA concentration was too low to induce epileptiform activity; thus, our results appear to be related not to the pathological over activation, but rather to the physiological activation of kainate receptors.

The kainate type of glutamate receptors also plays pivotal roles in the modulation of glutamatergic synaptic transmission and plasticity in the brain (Bortolotto et al., 1999). Kainate receptors have a specific role in synaptic development (Marchal and Mulle, 2004; Tashiro et al., 2003), and can regulate the motility of the filopodia at hippocampal mossy fibers (Tashiro et al., 2003), an early step during synapse formation. The CA1 interneurons containing kainate receptors depolarize the cell soma through ionotropic action (Cossart et al., 1998; Rodriguez-Moreno et al., 2000), while another population of kainate receptors depress evoked inhibitory postsynaptic currents and GABA release through a G-protein-dependent mechanism

(Christensen et al., 2004; Clarke et al., 1997; Mulle et al., 2000; Rodriguez-Moreno et al., 2000). In the CA1, the pharmacological activation of Glu5R-containing kainate receptors strongly depresses glutamatergic transmission (Clarke and Collingridge, 2002; Vignes et al., 1998) through the action of the presynaptic receptors via G-protein coupled signaling (Frerking et al., 2001; Lauri et al., 2006). Moreover, the sustained activation of GluR5 receptors causes a specific and lasting increase in the number of glutamatergic synapses in area CA1, dependent on PKC activation (Vesikansa et al., 2007). It is possible that a similar G-protein-dependent signaling mechanism underlies the kainate receptor-dependent synaptic stabilization in both the CA3 and CA1 areas. However, these receptors are endogenously activated in the developing, but not in the adult hippocampus (Lauri et al., 2006). Our results demonstrate that, while KA treatment hampers synaptic plasticity in the adult hippocampus under normal circumstances, in the ischemic hippocampus KA is able to restore the injured synaptic plasticity within its therapeutic window through restoration of the control level of spine density and normal-like glutamatergic synaptic transmission.

Ischemic postconditioning *per se* cannot be studied separately from the ischemic event, and its underlying mechanisms, at least in part, are common with those seen in connection with kainate receptor activation. Postconditioning enhances Akt phosphorylation and Akt activity (Gao et al., 2008). The MAPK pathways (ERK1/2, p38 and JNK) are also closely related to ischemic injury and neuronal survival (Sawe et al., 2008). The ERK1/2 phosphorylation level, transiently elevated after reperfusion, is reduced during postconditioning (Gao et al., 2008) in the penumbra. Postconditioning also involves PKC pathways: δPKC activity usually leads to cell death (Shimohata et al., 2007b), whereas εPKC promotes neuronal survival (Shimohata et al., 2007a). Postconditioning inhibits the increase in level of the cleaved form of δPKC and improves εPKC phosphorylation (Gao et al., 2008). *De novo* synthesized proteins, e.g. heat shock proteins (Liu et al., 1993), Bcl-2 (Shimazaki et al., 1994), superoxide dismutase (MnSOD) (Bordet et al., 2000) and pro-survival inhibitor-of-apoptosis (Tanaka et al., 2004), contribute to the acquisition of delayed ischemic tolerance. Delayed postconditioning is based on the synthesis of proteins and is able to reverse the ischemia-induced apoptosis-like process of delayed neuronal death 48 h after ischemia.

Our experiments have demonstrated that KA treatment induces reappearance of the normal spine density in the adult hippocampal CA1 subfield after incomplete global ischemia, which results in a marked restoration of experimental LTP induction. The beneficial effects of KA postconditioning on the hippocampal function are long-lasting and can be detected as late as 8 days after KA treatment. However, KA proved most effective on the second post-ischemic day, revealing a strict onset-dependent feature of its application. This is in agreement with previous studies, which involved either ischemic or pharmacological postconditioning with therapeutic windows ranging from 6 h (Ren et al., 2008) up to 2 days (Burda et al., 2005, 2006; Danielisova et al., 2008, 2009). Future experiments are needed to clarify the exact mechanism of the KA-mediated protection reported here.

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