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Oxaloacetate restores the long-term potentiation impaired in rat hippocampus CA1 region by 2-vessel occlusion

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ABSTRACT

Various acute brain pathological conditions are characterized by the presence of elevated glutamate concentrations in the brain interstitial fluids. It has been established that a decrease in the blood glutamate level enhances the brain-to-blood efflux of glutamate, removal of which from the brain may prevent glutamate excitotoxicity and its contribution to the long-lasting neurological deficits seen in stroke. A decrease in blood glutamate level can be achieved by exploiting the glutamate-scavenging properties of the blood-resident enzyme glutamate-oxaloacetate transaminase, which transforms glutamate into 2-ketoglutarate in the presence of the glutamate co-substrate oxaloacetate. The present study had the aim of an evaluation of the effects of the blood glutamate scavenger oxaloacetate on the impaired long-term potentiation (LTP) induced in the 2-vessel occlusion ischaemic model in rat. Transient (30-min) incomplete forebrain ischaemia was produced 72 h before LTP induction. Although the short transient brain hypoperfusion did not induce histologically identifiable injuries in the CA1 region (Fluoro-Jade B, S-100 and cresyl violet), it resulted in an impaired LTP function in the hippocampal CA1 region without damaging the basal synaptic transmission between the Schaffer collaterals and the pyramidal neurons. This impairment could be fended off in a dose-dependent manner by the intravenous administration of oxaloacetate in saline (at doses between 1.5 mmol and 0.1 μmol) immediately after the transient hypoperfusion. Our results suggest that oxaloacetate-mediated blood and brain glutamate scavenging contributes to the restoration of the LTP after its impairment by brain ischaemia.

1. Introduction

Neurodegenerative conditions such as stroke and traumatic brain injury are characterized by the presence of extremely high glutamate (Glu) levels in the brain fluids (Castillo et al., 1996; Johnston et al., 2001). The excess Glu which causes neuronal death via excitotoxicity is normally controlled by members of a family of Na^+-dependent Glu transporters (Danbolt, 2001) located on nerve terminals and astrocytes. By pumping Glu, they guarantee the presence of Glu in brain fluids at levels at which it exerts neither excitotoxic nor unsolicited excitatory effects (Sattler and Tymianski, 2001). Glu transporters located on the brain vasculature (O'Kane et al., 1999) may also play an important role in controlling extracellular Glu levels via a brain-to-blood Glu efflux (Berl et al., 1961; Gottlieb et al., 2003; Teichberg et al., 2008). The precise localization and properties of the Glu transporters on the brain microvasculature have not yet been completely established. Light and electron microscopy studies have clearly shown that the Glu transporters EAAT1 and EAAT2 are located on the astrocytic endfeet rather than on the capillary endothelium (Chaudhry et al., 1995; Lehre et al., 1995; Danbolt, 2001). Nonetheless, O'Kane et al. (1999) have presented evidence of the presence of EAAT1, EAAT2 and EAAT3 on the abluminal membrane of bovine brain capillary endothelial cells. In agreement with these findings, by means of western blotting Teichberg et al. (2008) demonstrated the presence of these Glu transporters in porcine brain capillary endothelial cells. At any event, the brain-to-blood Glu efflux mediated by these transporters is fast and greatly enhanced by the blood Glu scavengers oxaloacetate and pyruvate (Gottlieb et al., 2003) which, upon intravenous administration activate the blood-resident glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase, respectively, causing Glu deamination into α-ketoglutarate. The scavenging of blood Glu increases the driving force for a brain-to-blood Glu efflux and leads to a decrease in the excess Glu present in the brain extracellular fluids (Gottlieb et al., 2003; Teichberg et al., 2008).

The hippocampus, where cognitive processes are believed to depend on changes in synaptic efficiency, is a brain region extremely rich in glutamatergic synapses and sensitive to Glu excitotoxicity (Bliss

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and Collingridge, 1993). These glutamatergic synapses underlie the process of long-term potentiation (LTP), which is a leading candidate mechanism in learning and memory (Martin et al., 2000).

Bilateral common carotid artery clamping (2-vessel occlusion) in the rat reduces the blood flow in the brain to one-third of its normal value (Farkas et al., 2007). As the hypoperfusion also affects the hippocampus (Todd et al., 1984), it may exert effects on various neuronal properties, including the neuronal cell viability or its electrophysiological behaviour. Thus, an impairment of LTP may be expected to occur in the rat hippocampal Schaffer collateral-CA1 synapses. Indeed, LTP was substantially attenuated after a 10-min 2-vessel occlusion but, surprisingly this was not accompanied by any overt histological damage (Mori et al., 1998). In a similar model in the gerbil, a significant decrease of the Glu transporter GLT-1 was observed without any neuronal damage in the hippocampal CA1 region (Raghavendra Rao et al., 2000). Thus, though the 2-vessel occlusion brain hypoperfusion model does not involve overt neuronal cell loss in the hippocampus CA1 region (Farkas et al., 2007), it nevertheless produces functional alterations that could possibly result from the presence of excess Glu, the level of which increases immediately after the ischaemic insult and subsides after 60 min (Mitani et al., 1992). The excess Glu might either produce an abnormal and sustained activation of the Glu receptors or cause cell death to below the level of detection. Accordingly, this raises the questions of how the LTP is affected by the transient ischaemia and whether elimination of the post-ischaemic increase in extracellular Glu level can restore the LTP.

We have therefore now tested the hypothesis that the intravenous administration of a blood glutamate scavenger, oxaloacetate, administered immediately after a 30-min period of ischaemia, helps the brain retain its synaptic plasticity. In detail, we tested whether a protocol of 2-vessel occlusion based on a 30-min period of ischaemia and 3 days of reperfusion causes changes in the hippocampal CA1 region histology, in the basal synaptic transmission and/or in the LTP machinery, and if so, whether an impaired LTP can be rescued by intravenous administration of oxaloacetate.

2. Materials and methods

2.1. Animals and housing conditions

Male Charles-River (N=58) rats of the Wistar strain were used. Animals weighing 180–230 g were housed individually in standard plastic cages in a light-controlled room (a 12:12-h cycle starting at 09:00 h) at room temperature (23 °C) and maintained on food and water *ad libitum*. Every effort was made to minimize animal suffering and the number of sacrificed animals. The protocol for animal care approved by the European Communities Council Directive of 24 November 1986 (86/609/EEC) was followed.

2.2. Preparation of an ischaemia model and drug administration in the experimental groups

Before the induction of transient cerebral ischaemia, the rats were anaesthetized with 4% chloral-hydrate (intraperitoneal) and the body temperature was maintained at 37±0.5 °C throughout the procedure with a feedback-regulated heating lamp. The common carotid artery were isolated and clamped with non-traumatic aneurysm clips (Aesculap, B. Braun Medical Ltd, Hungary). The sham-operated control rats underwent the same procedure, but without common carotid artery occlusion. The surgery was accompanied by a 100% survival rate following 2-vessel occlusion. The carotid artery blood flow was reperfused by releasing the clips following the 30-min occlusion. Oxaloacetate (Sigma, Germany) was dissolved in 1 M sodium hydroxide diluted with physiological saline and adjusted with 10 M sodium hydroxide to pH 7.4. Oxaloacetate was injected at the various doses tested in a total volume of 1.5 ml. The higher doses of oxaloacetate were selected as they had been shown to reduce blood Glu levels effectively (Gottlieb et al., 2003; Teichberg et al., 2008). The lower doses were selected in order to determine a threshold of efficacy. The drug was injected intravenously immediately after the end of the 30-min common carotid artery occlusion, when the reperfusion started (post-treatment). The drug administration lasted for 30 min (0.05 ml×min^{-1}). Control rats were injected with an equal volume of saline.

2.3. Histological staining: Fluoro-Jade B, S-100 and cresyl violet

The treated and control animals were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer 3 days after 2-vessel occlusion. The brains were removed and postfixed overnight in the same fixative. Coronal sections (36 μm) were cut with a freezing microtome (Frigomobil Model 1206, Reichert-Jung, Nußloch, Germany).

Fluoro-Jade B (FJ-B, Chemicon, Millipore Ltd, Hungary) is a fluorochrome that stains degenerating neurons with high selectivity. The sections were observed under a microscope (BX51, Olympus, Tokyo, Japan) in fluorescent light at an excitation wavelength of 470–490 nm and an emission wavelength of 520 nm.

Protein S-100 is one of a large family of calcium-binding proteins. In human cerebrovascular diseases, a significant correlation has been reported between the plasma concentration of the S100 protein and the volume of the cerebral infarct (Aurell et al., 1991). There is evidence that S-100 regulates the calcium-dependent cellular signalling in neuronal differentiation, outgrowth and apoptosis. The astroglial overexpression of the S100 protein is considered to play a key role in infarct expansion by causing alterations in the activities of multiple intracellular signalling pathways and the expression of various downstream proteins (Matsui et al., 2002; Asano et al., 2005). S-100 was recently found to be a promising marker for central nervous system injury (Martinez et al., 1998; Ishiuchi et al., 2007). Polyclonal rabbit antibodies to the cow S-100 protein (anti-S-100) were used to investigate the distributions of glial cells associated with S-100 after 2-vessel occlusion. The sections were incubated overnight at room temperature with the primary antibody directed against S-100 (1:10000 rabbit anti-cow S-100; Dako, Glostrup, Denmark) and were treated with the secondary antibody (1:1000 Cy™3-conjugated donkey anti-rabbit IgG; Jackson ImmunoResearch Europe Ltd, UK) for 3 h at room temperature. Finally, the labelling was observed under a fluorescence microscope at an excitation wavelength of 530–550 nm and an emission wavelength of 590 nm.

The procedure of *cresyl violet* (Sigma, Germany) staining was performed as usual with a 1% filtered solution: the slide racks passed through the sequence of baths for the times indicated: 95% ethanol–15 min, 70% ethanol–1 min, 50% ethanol–1 min, distilled water–2 min, distilled water–1 min, cresyl violet stain–5 min, distilled water–1 min, 50% ethanol–1 min, 70% ethanol–2 min, 95% ethanol–2 min, 95% ethanol–a few dips, 100% ethanol–1 min, respectively.

2.4. In vitro electrophysiology

The electrophysiological recordings were conducted 3 days after the termination of the bilateral common carotid artery occlusion. The rats were decapitated, and vibratome-cut (Campden Instruments, UK) coronal slices (400 μm) were prepared from the middle part of their hippocampi 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 and 5% CO_2. The slices were then transferred to a Haas-type 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 was 1.5–2 ml×min^{-1} and the experiments were performed at 34 °C. The stimulating electrode (a bipolar glass electrode pulled from a theta capillary) was placed in the stratum radiatum near the border between the CA1 and CA2 regions to allow orthodromic stimulation of the

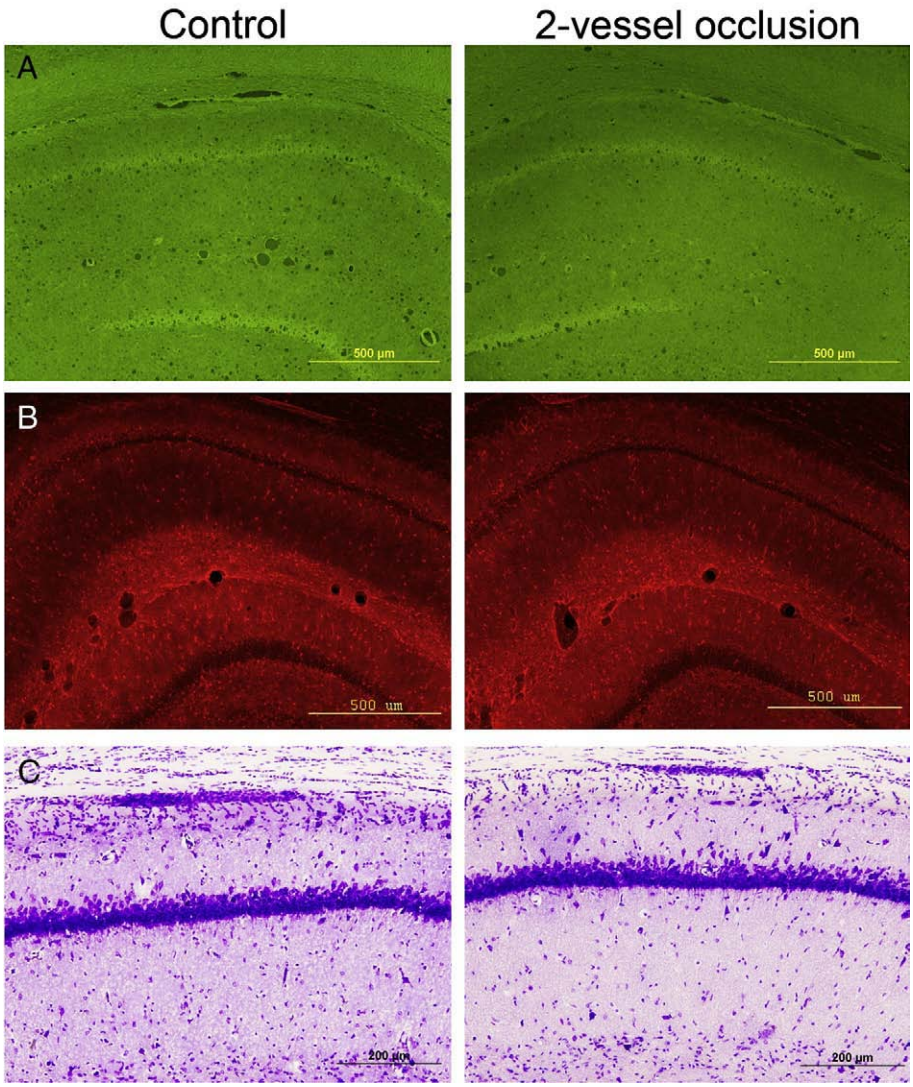

Fig. 1. Representative photomicrographs of the hippocampal CA1 region in the control (left) and the 2-vessel occlusion groups (right), visualized by Fluoro-Jade B (A), S-100 (B) and cresyl-violet (C).

Schaffer collateral/commissural pathway (constant current, 0.2 ms pulses delivered at 0.05 Hz) as described previuosly (Sas et al., 2008).

Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum and stratum pyramidale with a 1–2-MOhm resistance glass microelectrode that was filled with aCSF and connected to a neutralized, high input-impedance preamplifier (100x gain) with a high-pass filter set at 5 kHz. The test stimulus intensity was adjusted to between 30 and 60 µA to induce ~50% of the minimum stimulus intensity that evoked a saturated EPSP (maximum fEPSP response) in the control rats. The fEPSPs were digitized, saved with a PC equipped with a Digidata 1200 interface and an Axoscope10.0 recording system (Molecular Devices Corporation, Sunnyvale, CA, USA) and analysed off-line with Origin6.0 software (OriginLab Corporation, Northampton, MA, USA).

LTP of the Schaffer collateral-CA1 synaptic response was induced by high-frequency stimulation (0.2-ms pulses delivered at 100 Hz for 5 s) at 100% intensity of the test stimulus, and after the high-frequency stimulation the fEPSPs were recorded for at least a further 60 min. The fEPSPs were monitored for 40–60 min before conditioning stimulation until the amplitudes were generally stable, and their mean value was determined as the 10-min-long baseline. Paired-pulse facilitation was measured by using the intensity of the test stimulus with an interpulse interval of 25, 50 or 100 ms, and input-output curves were created to

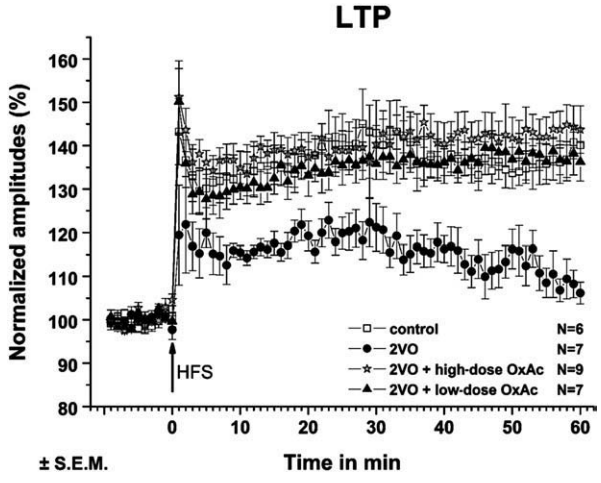

Fig. 2. Post-ischaemic administration of oxaloacetate (OxAc) prevents the long-term potentiation (LTP) impairment caused by global ischaemia. In the control group, LTP was induced by high-frequency stimulation (HFS) of the Schaffer collaterals (500 pulses at 100 Hz). The 2-vessel occlusion (2VO) group received only the 30-min 2-vessel occlusion 3 days earlier. The 2-vessel occlusion high-dose oxaloacetate and 2-vessel occlusion low-dose oxaloacetate groups received the same ischaemic insult, followed immediately by an intravenous injection of either 1.5 mmol or 2.7 µmol oxaloacetate. Datapoints are means ±S.E.M. of normalized amplitudes of fEPSPs.

measure the basal glutamatergic synaptic function. Slices from the same animal were generally used for several tests, including LTP, input-output curves or paired-pulse facilitation. Each slice was subjected to only one particular test.

2.5. Statistics

The fEPSP amplitude is expressed as a percentage of the 10-min baseline value before high-frequency stimulation. The maximum LTP was expressed as a percentage of the baseline. A P value of ≤0.05 was considered significant. As a normal distribution of the data could not be presumed and the Levene test did not demonstrate an equality of variances, a non-parametric test on two independent samples was chosen for statistical analysis of the electrophysiological data (Mann-Whitney U-test) in Fig. 2. In the course of the data analysis presented in Fig. 3, one-way ANOVA with the Bonferroni *post hoc* test was used. SPSS9.0 for Windows (SPSS Inc., Chicago, IL, USA) software was utilized.

3. Results

The histological analysis of the brain sections carried out 3 days after the transient brain hypoperfusion did not detect any changes in the CA1 region of the hippocampus. Neither Fluoro Jade B staining, nor S-100 immunohistochemistry nor cresyl violet staining revealed injuries/changes after the transient 2-vessel occlusion-induced hypoperfusion of the CA1 region (Fig. 1).

In the sham group (control in Fig. 2), the high-frequency stimulation caused a robust increase (35–40%) in the amplitude of the fEPSPs (Fig. 2, Table 1), which remained at this elevated level throughout the 1-h registration period.

The same conditioning protocol induced a significantly smaller increase in the amplitude of the fEPSPs in the 2-vessel occlusion group, observed 3 days after the start of the reperfusion. In this group, the high-frequency stimulation resulted in only a ~20% increase in amplitude, which remained at this level for about 30 min, and then gradually decreased. At the end of the registration period, the amplitudes were near the baseline (107–108%, Fig. 2, Table 1).

Table 1
Potentiation of the amplitudes after high-frequency stimulation in the different groups in comparison with the controls

Facilitation in post-tetanic minutes (1–60 min) as a percentage of the control period level														
Time	Control 20th min.	1	5	10	15	20	25	30	35	40	45	50	55	60
SHAM	100.00±0.4	143.2±7.6	130.1±4.4	134.19±4.6**	135.6±4.5**	138.3±4.7*	141.8±5.6*	142.9±7.5*	140.8±6.9**	135.6±4.3*	133.3±3.6*	133.7±3.5**	135.8±4.6**	140.1±4.0**
2VO	100.3±0.2	119.4±11.5	119.9±3.3	115.5±1.6	117.6±2.7	119.3±3.4	119.9±3.6	121.2±5.5	115.1±4.2	116.3±3.1	113.9±4.4	116.2±4.5	108.5±3.4	106.2±2.5
2VO+high (1.5 mmol) OxAc	100.3±0.7	151.2±8.3	136.2±2.7**	134.9±3.7**	139.0±4.1**	137.4±3.5*	138.9±4.5*	140.4±4.3*	143.4±3.3**	140.4±4.9**	141.8±3.9**	141.7±4.8**	142.1±4.5**	143.7±5.5**
2VO+low (2.7 mmol) OxAc	99.9±0.5	150.2±7.6	127.7±4.7	130.5±2.9**	132.5±3.6*	133.2±4.8	135.5±4.5*	135.8±3.6*	137.9±2.9**	136.2±3.1**	136.1±4.2**	136.8±2.8**	136.3±4.0**	136.3±4.4**

*$P<0.05$, **$P<0.01$ significant differences from the 2-vessel occlusion (2VO) group; ±S.E.M.

Fig. 3. Post-ischaemic administration of oxaloacetate (OxAc) prevents the long-term potentiation (LTP) impairment in a concentration-dependent manner. The columns demonstrate the potentiation of amplitudes recorded during the last 10 min (between 50 and 60 min) of the 1 h-long testing following the high-frequency stimulation (HFS). In the intact animals (control), HFS resulted in LTP with amplitudes remaining at around 136% at the end of the tested period. In the 2-vessel occlusion (2VO) animals, however the effectiveness of the HFS was much weaker: the potentiation level reached only around 110% at the end of 1 h period. Oxaloacetate (OxAc) administration prevented the LTP impairment in a dose-dependent manner in the 2VO animals. *$P<0.05$, ***$P<0.001$ significant differences from the control group; ###$P<0.001$ significant differences from the 2-vessel occlusion (2VO) group; ±S.E.M. (one-way ANOVA, Bonferroni *post hoc* test).

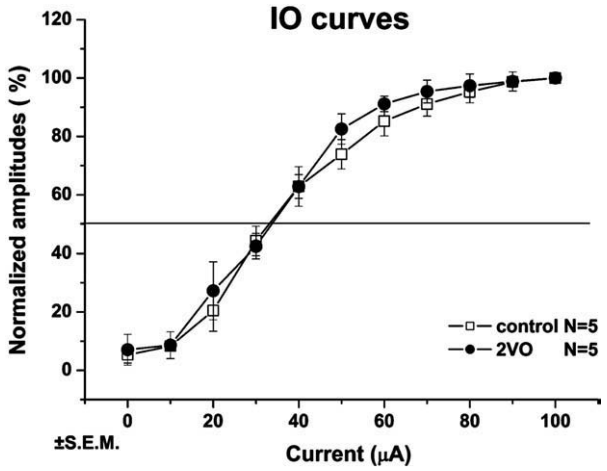

Fig. 4. Input/output (I/O) curves for EPSP amplitudes in the control group (□) and 2-vessel occlusion (2VO) group (●).

The effect of oxaloacetate on the 2-vessel occlusion-induced LTP impairment was tested on hippocampal slices from animals exposed to intravenous oxaloacetate administered at doses of 1.5 mmol or 2.7 μmol. These doses were equally effective in protecting the LTP from the 2-vessel occlusion-induced impairment. The post-hypoperfusion administration of oxaloacetate resulted in a high-frequency stimulation-induced LTP comparable to that observed in the hippocampi of the control animals. Just after the high-frequency stimulation, a transient and statistically insignificant decrease in amplitudes occurred. The amplitudes then returned to a plateau at about 135–140%, similarly to the LTP observed in the hippocampi from the control rats (Fig. 2, Table 1). As compared with the 2-vessel occlusion group, the level of significance was reached by the control group at 7 min, by the 2-vessel occlusion high-dose oxaloacetate group at 7 min, and by the 2-vessel occlusion low-dose oxaloacetate group at the 33 min post-high-frequency stimulation.

Fig. 3 shows that the protective effects of oxaloacetate on the LTP were dose-dependent. While maximum protective effects were observed for the doses of 1.5 mmol and 2.7 μmol, 0.1 μmol oxaloacetate was ineffective in preventing the hypoperfusion-induced LTP impairment.

The basal synaptic properties were also tested to evaluate whether the 2-vessel occlusion impaired the Schaffer collateral-CA1 synaptic transmission. Input-output curves were established by plotting the fEPSP amplitude against various intensities of the test pulse ranging from 10 to 100 μA. It is clearly seen in Fig. 4 that there is no distinguishable difference between the input-output curves of the control and the 2-vessel occlusion groups, implying that the basal functions of the pyramidal cells and synapses were not affected by the 2-vessel occlusion-induced hippocampal hypoperfusion.

The paired-pulse ratio was also measured with interpulse intervals of 25, 50, 75 and 100 ms in both groups. The results strongly suggested that the transient incomplete ischaemia did not impair the probability of presynaptic Glu release, since there was no significant difference between the groups ($P > 0.05$, Fig. 5).

4. Discussion

In the present study, we tested the hypothesis that the elevated brain-to-blood Glu efflux achieved by intravenous administration of the Glu scavenger oxaloacetate exerts a restoring effect on the reduced synaptic plasticity produced in the hippocampus CA1 region by 2-vessel occlusion-induced brain hypoperfusion. This ischaemia model was chosen because it modifies some of the properties of the synapses between the Schaffer collaterals and pyramidal cells in the CA1 region, possibly by affecting the N-methyl-D-aspartate (NMDA) receptor function (Farkas et al., 2007). Although its mechanism is not known in detail, it has recently been shown that conditions of hypoperfusion lead to the activation of NMDA receptors (Wahl et al., 2008) while 2-vessel occlusion, has been observed to cause an increase in the NMDA receptor density in the hippocampus (Farkas et al., 2007). One of the possible mechanisms is that the transient ischaemia affects the phosphorylation of the NMDA receptor 2A and 2B subunits via the non-receptor tyrosine kinases FAK, PYK2 and Src (Zalewska et al., 2005). It has been demonstrated that, in the CA1 region of the hippocampus, the combined activation of Src family tyrosine kinases with other kinases results in the phosphorylation of glutamate-receptor-gated ion channels and enhancement of the subsequent postsynaptic current (Soderling and Derkach, 2000; Zalewska et al., 2005). Depending on the severity of the ischaemia, consequences ranging from a slight physiologically detectable decrease in plasticity up to a severe hippocampal neuronal cell loss can be observed (Farkas et al., 2007). The negative outcome of brain ischaemia correlates positively with the rise in the Glu level in the brain interstitial fluid (Davalos et al., 2000). This suggests that a decrease of this excess Glu may improve the consequences and outcome of ischaemic conditions (Gottlieb et al., 2003). Oxaloacetate was chosen as a blood Glu scavenger because it has proved to be effective in lowering the blood Glu concentration and hence causing an increased brain-to-blood Glu efflux (Gottlieb et al., 2003; Teichberg et al., 2008) and in furnishing marked neuroprotection after traumatic brain injury (Zlotnik et al., 2007). In the latter study, the beneficial effects observed after oxaloacetate administration were found to be linearly correlated with the degree of decrease of the blood Glu level (Zlotnik et al., 2007). Using dual probe brain microdialysis, Teichberg et al. (2008) obtained direct evidence that oxaloacetate-mediated blood Glu scavenging decreases the amount of excess Glu present in the brain parenchyma extracellular fluid.

The oxaloacetate-stimulated brain-to-blood Glu efflux is mediated by Glu transporters present on the brain vasculature. Immunoreactivity against Glu transporters has been localized to the astrocytic endfeet encasing the blood capillaries (Danbolt, 2001), while evidence has been presented of the presence of Na^+-dependent Glu transporters (EAAT1, EAAT2 and EAAT3) on the abluminal membrane of the brain capillary endothelial cells, in line with the role of the latter in the regulation of the extracellular Glu level in the brain (O'Kane et al., 1999). The activity of the Glu transporters in transient ischaemic and hypoxic conditions is crucial, when neurons and astrocytes are depolarized, the Glu transporters function in reverse mode (Nicholls and Attwell, 1990; Szatkowski et al., 1990), and release intracellular Glu into the extracellular fluid. In brain hypoperfusion, the capillary endothelial cells and possibly the astrocytic endfeet are likely to be less

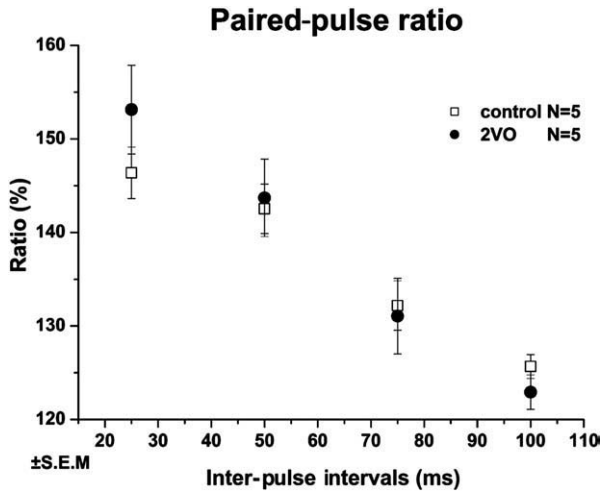

Fig. 5. Percentage paired-pulse ratio (see Section 2.4) as a function of inter-pulse intervals. Paired-pulse stimuli with inter-pulse intervals of from 25 to 100 ms were applied to the Schaffer collateral-CA1 path.

influenced than the astrocyte cell bodies or neurons by the decreases in oxygen and glucose, since it is likely that their closer proximity to the blood and its components will allow them to monopolize the meagre energy supply exclusively for their own needs. Thus, the Glu transporters present on the blood-brain barrier may be the only ones in a position to function in their normal pumping mode and remove the elevated Glu from the extracellular fluid. Under normal conditions (Oldendorf and Szabo, 1976; Drewes et al., 1977; Gottlieb et al., 2003;), these transporters mediate Glu transport out of the brain and may provide a possible innate protective mechanism against Glu neurotoxicity, causing reduced synaptic plasticity and/or neuronal injury. The fact that the increase in Glu in the brain extracellular fluids following ischaemia or traumatic brain injury is transient and self-limiting, rarely lasting more than 60 min (Faden et al., 1989; Guyot et al., 2001), may be due to the contribution of the brain Glu transporters to the brain-to-blood Glu efflux.

The hippocampus (and particularly its CA1 subfield) is one of the brain regions most sensitive to ischaemia (Farkas et al., 2007). The reasons for the selective vulnerability in ischaemia in the CA1 region are likely to be multifactorial. The region-specific vascularization is probably relevant since the CA1 region is characterized by a low capillary density as compared with the neighbouring CA3 region. Furthermore, the CA1 vessels display a more extensive blood-brain barrier leakage than that of the CA3 vessels after an ischaemic insult (Cavaglia et al., 2001) and thus the CA1 neurons may be exposed to an additional and excitotoxic excess of Glu originating from the blood plasma.

The selective vulnerability of the hippocampal CA1 neurons in ischaemia, relative to the CA3 neurons, which are also exposed to an elevated Glu level in ischaemia, has been suggested to result not only from the ischaemia-related Glu increase, but also from the nature of the postsynaptic receptors at which Glu acts (Mitani et al., 1992). The accumulating data suggest that the malfunction of the NMDA receptor due to the reduced level of phosphorylation of its NMDA receptor 2B subunits is the most probable candidate for the impairment of the hippocampal LTP. The question arises as to how ischaemia causes reduced phosphorylation and hence LTP impairment (Mori et al., 1998; Kiprianova et al., 1999). The molecular mechanisms might be explained as follows: The NMDA receptor complex contains several proteins that may be tyrosine-phosphorylated by members of the Src family of kinases: Src, Fyn and Lyn, all present in the postsynaptic density (Yu et al., 1997; Kalia et al., 2004). The Src family of kinases regulates the NMDA receptor function. Short-term ischaemia elevates the tyrosine-phosphorylation of the NMDA receptor 2A and the NMDA receptor 2B subunits and also the postsynaptic density levels of Src and Fyn (Takagi et al., 1999; Cheung et al., 2003), while long-term ischamia (up to 30 min) causes changes in the opposite direction, i.e. decreased phosphorylation of both subunits and their dissociation from postsynaptic density-95 and from the Src family of kinases (Zalewska et al., 2005). The Src associated with NMDA receptor 2B plays a crucial role in the regulation of the NMDA receptor 2B tyrosine phosphorylation level during ischaemia and reperfusion, and the increase in protein-tyrosine kinase activity caused by ischaemia has in fact been attributed to the increase in Src activity. This increase in Src activity is not related to the expression of the Src protein, but it is related to the activation of the NMDA receptor and the L-type voltage-gated calcium channel (Pei et al., 2000). Excitotoxic Glu stimulation activates calpain, a calcium-activated protease, resulting in the cleavage and degradation of NMDA receptor 2B (Simpkins et al., 2003), which can be prevented by tyrosine phosphorylation (Bi et al., 2000; Rong et al., 2001). The Src-mediated tyrosine phosphorylation of NMDA receptor 2B stabilizes the NMDA receptors on the cell surface and hence increases the NMDA responses (Grosshans et al., 2002; Salter and Kalia, 2004). It is known that the tyrosine phosphorylation of the NMDA receptors (Rostas et al., 1996; Yu et al., 1997; Salter and Kalia, 2004) is a crucial step in the induction of LTP (O'Dell et al., 1991; Collingridge and Bliss, 1995).

The time window of effective oxaloacetate treatment is rather narrow: no longer than 2 h in rats (Gottlieb et al., 2003; Zlotnik et al., 2007). This time period is compatible with the duration of the elevated Glu levels in the rat brain after ischaemic stroke or traumatic brain injury (Faden et al., 1989; Guyot et al., 2001). However, in humans, this therapeutic window is likely to be much longer since elevated Glu levels in the brain can be observed for hours or even days after stroke or a traumatic brain injury (Baker et al., 1993; Bullock et al., 1998; Vespa et al., 1998).

In the present study, oxaloacetate was administered in the first 30 min of the reperfusion period and was found to prevent the LTP impairment caused by ischaemia without affecting the basal glutamatergic synaptic functions, as it was concluded from the results of the paired-pulse facilitation and the input-output curves. The most apparent explanation is that intravenous oxaloacetate causes an increased elimination of excess Glu from the hippocampus extracellular space by virtue of its blood Glu-scavenging properties at the higher oxaloacetate doses tested (Zlotnik et al., 2008). A more tenuous explanation would be that oxaloacetate may enter the brain via a dicarboxylate transporter possibly present on brain capillary endothelial cells (Chen et al., 1999). As such a dicarboxylate transporter has not yet been detected, it may be assumed that its density is significantly lower than that of GOT in the blood, and thus that oxaloacetate is likely to be involved in the GOT-mediated transamination of Glu before being transported into the brain. However, if oxaloacetate does succeed in crossing the blood-brain barrier, it might activate the GOT present in the brain interstitial fluids and give rise to a decrease in the excitotoxic Glu level. It could also induce an improvement in the neuronal energetics to correct for the ischaemia-induced mitochondrial dysfunction.

In the future, the administration of oxaloacetate to human might open up new therapeutic possibilities basically different from those involving the administration of Glu receptor antagonists: 1) In contrast which the use of Glu receptor antagonists, the activity of Glu scavengers in stimulating the brain-to blood Glu efflux is self-limiting, since this activity progressively diminishes as the elevated brain Glu level decreases to concentrations below the threshold of activation of the Glu transporters on the brain vasculature (i.e below their Km values). 2) Blood Glu scavengers do not affect ionotropic Glu receptors, whereas Glu receptor antagonists obviously do, and hence they will not block the beneficial effects of Glu in the neurorepair proceeding after brain injury (Biegon et al., 2004).

The present study has provided the first evidence that the blood Glu scavenger oxaloacetate improves the impaired LTP in the hippocampal CA1 region after ischaemia, and furnishes new insight into a novel mechanism for the treatment of ischaemic stroke.

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