

Middle cerebral artery occlusion produces secondary, remote impairment in hippocampal plasticity of rats – involvement of *N*-methyl-D-aspartate receptors?

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Received 2 December 1999; received in revised form 10 January 2000; accepted 12 January 2000

Abstract

There is increasing evidence that focal cerebral ischaemia produces remote functional alterations that may substantially contribute to the post-stroke neurological outcome. Changes initially limited to peri-infarct areas may evolve and spread via transneuronal connections to other structures. In the present study we investigated whether focal ischaemia produced by 2-h occlusion of the middle cerebral artery (MCAo) in SD rats may influence the physiological function of the hippocampus. Three days later *in vitro* long-term potentiation (LTP) was studied in hippocampal slices from ipsi- and contralateral hemispheres. In rats with MCAo LTP was not-inducible in the ipsilateral hippocampus, while the contralateral side expressed stable potentiation (6.6 ± 4.1 vs. $35.0 \pm 8.0\%$, respectively). Treatment with 6-h *i.v.* infusion of an uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist MRZ 2/579 starting at reperfusion not only preserved but additionally enhanced ipsilateral LTP, while a slight insignificant decrease was observed in the contralateral side (77.0 ± 18.4 vs. $20.8 \pm 6.5\%$). The study demonstrates post-stroke functional changes in the hippocampus that can be modulated by NMDA receptor antagonists. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Middle cerebral artery occlusion; Hippocampus; Long-term-potentiation; *N*-Methyl-D-aspartate receptor antagonist; Rat

There are indications that focal ischaemia in the territory of the middle cerebral artery (MCA) induces widespread neuropathological changes both in the ischaemic region and in areas remote from the original infarct. Shrinkage of thalamus was reported clinically in some post-stroke patients [19]. Following permanent MCAo in rats atrophy of the ipsilateral substantia nigra [17], thalamus [7] and neuronal loss combined with microglial activation in the spinal cord [20] have been described. Since these structures lie outside the ischaemic region, the observed degeneration represents secondary neuronal damage that may involve trans-synaptic retrograde and/or anterograde degeneration [18]. Previous studies concentrated on histopathological analysis of neuronal damage, however recently, electrophysiological changes in function of neurones in the vicinity [9] and remote from the primary stroke area were demonstrated [15] and may be attributed to an imbalance between excitatory and inhibitory neurotransmission [13].

In animal models of focal ischaemia neuroprotection is

typically determined by measurements of infarct volume combined with behavioural somatosensory tests [1,3], however cognitive tests or electrophysiological studies are employed less frequently. Evidence exists showing learning/memory impairment in rats with transient or permanent occlusion of MCA [12,16]. Since the hippocampus is believed to participate in spatial learning one can attribute the observed deficits to its dysfunction. In a suture model of MCA occlusion, piriform and insular cortices connecting with the entorhinal cortex that sends neuronal input to the hippocampal formation, frequently belong to the infarct zone. Previously Okada et al. [12] reported that the magnitude of long-term potentiation (LTP) in ipsilateral hippocampal slices tended to be lower when tested 4 months post permanent MCAo. Therefore, we decided to investigate if MCAo-induced, primarily striatal and cortical insult has any influence on hippocampal plasticity expressed as CA1 LTP assessed 3 days after transient insult and, if this is the case, whether it involves *N*-methyl-D-aspartate (NMDA) receptors.

Male Sprague–Dawley rats (270–300 g) were used for the experiments. Animals were kept on a 12:12 h light-dark

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cycle and had free access to food and water. Three experimental groups were studied: Group A ($N = 7$) - non-treated animals subjected to 2 h MCAo alone, Group B ($N = 6$) - treated with an uncompetitive NMDA receptor antagonist MRZ 2/579 (loading dose 10 mg/kg as i.p. injection 60 min post MCAo, followed upon reperfusion by an i.v. infusion of 6 mg/kg per h over 6 h.). Group C ($N = 5$) - intact, non-treated age-matched controls (for LTP studies).

Surgeries were performed under halothane anaesthesia (3% for induction, 0.8–1.0% for maintenance in a mixture of 70% N_2O and 30% O_2). Silicone catheters were introduced into the femoral artery and vein for mean arterial blood pressure (MABP) recording, blood sampling and drug infusion. Rectal and head temperatures were maintained at 37–37.5°C. Arterial blood gases and pH were recorded. After surgical preparation a 3-0 nylon filament with its tip rounded by heating near a flame was inserted into the circle of Willis (19–20 mm from the bifurcation of the CCA) to block the origin of the MCA. Two hours later the filament was pulled back. During the time between induction of ischaemia and reperfusion rats were awake. One hour post MCAo somatosensory tests were performed (postural reflex [2] and visual/tactile forelimb placing tests [4]). All animals included in this study scored 10–11 points (normal score = 0). Immediately post testing, rats from the drug treated group were injected with a loading dose of MRZ 2/579. Upon reperfusion a 6 h infusion of MRZ 2/579 was initiated during which the animals could move freely due to a swivel system (the vein catheter was led subcutaneously to exit at the back and was connected to a swivel joined with a syringe). Three days after MCA occlusion animals were sacrificed, their brains dissected, cooled and cut using the rat brain matrix. Four rostral coronal sections were collected for infarct analysis (staining with 2% 2,3,5-triphenyltetrazolium chloride (TTC)). The remaining part of the brain was used for hippocampal slice preparation. As a control, LTP experiments were performed in hippocampi from intact rats of the same age.

Hippocampal slices from both ipsilateral and contralateral hemisphere were prepared as reported previously [6]. Only one slice per hemisphere was used for electrophysiological recordings. Briefly, the slices without the CA3 region were placed in an interface chamber and perfused at a rate of 0.8 ml/min with aCSF at 33°C in an oxygen-enriched (95% O_2 /5% CO_2) humidified atmosphere. After at least 30 min of incubation in the recording chamber, a glass recording electrode (1–3 M Ω) was positioned in the dendritic layer of area CA1 to record extracellular AMPA receptor-mediated field excitatory postsynaptic potentials (fEPSPs). A concentric, stimulating electrode was placed 500–700 μ m away from the recording electrode to activate the Schaffer collateral commissural fibres. Extracellular recordings were made in response to constant voltage (20–25 V, i.e. (200–250 μ A, square pulse for 20 μ s) single shock stimulation once every 15 s. Stimulus intensities were adjusted to evoke fEPSPs of half maximal amplitude. To

induce LTP a single tetanic stimulus (100 Hz, 1 s) was delivered at normal stimulus intensity. The slope of the raising phase of the fEPSP ($mV ms^{-1}$) was measured between 20–80% of the peak amplitude. Averaged fEPSP slopes 31–60 min following the tetanic stimulus were normalised with respect to the 30 min control period prior to tetanizing. The results were analysed by parametric one-way ANOVA, followed by the Student–Newman–Keuls test.

All experimental animals with MCA occlusion developed infarcts both in cortex and striatum. Since the precise measurement of total infarct size was not possible due to the experimental paradigm, the TTC staining was used only to verify the presence of the primary infarction. The neurological deficit of rats treated with MRZ 2/579 was significantly lower ($P < 0.05$ two-way repeated measures ANOVA) (Fig. 1).

In electrophysiological recordings all analyzed slices showed normal excitability. In the ipsilateral hippocampus tetanic stimulation caused only short term potentiation, which decayed to the pre-tetanic level within 1 h ($6.6 \pm 4.1\%$), while slices from the contralateral hemisphere showed stable LTP ($35.0 \pm 8.0\%$; $P = 0.01$ vs. ipsilateral; Fig. 2) with magnitude similar to that found in age-matched, intact rats ($28.1 \pm 3.5\%$; data not shown). In contrast in the group treated with the uncompetitive NMDA receptor antagonist MRZ 2/579, the tetanic stimulus applied to slices from ipsilateral hippocampus produced robust and stable potentiation of fEPSP ($77.0 \pm 18.4\%$, $P < 0.05$ vs. ipsilateral/non-treated control). The magnitude of LTP recorded in slices contralateral to the infarct tended to be lower than in sections from non-treated animals (20.8 ± 6.5 vs. $28.1 \pm 3.5\%$, respectively; non-significant $P = 0.334$) but the potentiation was stable (Fig. 3).

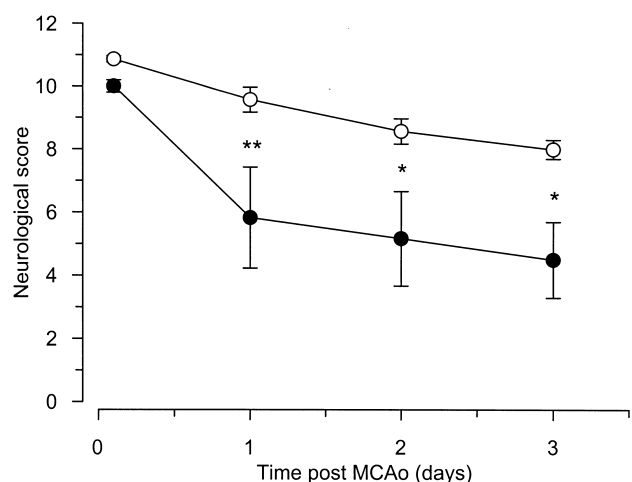


Fig. 1. Neurological deficit (mean \pm SEM) at various times post MCAo in non-treated, MCAo control animals (open circles; $n = 7$) and MRZ 2/579 - treated rats (closed circles; $n = 6$). ** $P < 0.01$; * $P < 0.05$ vs. MCAo control (two - way repeated measures ANOVA). The first score was measured 1 h after induction of ischaemia.

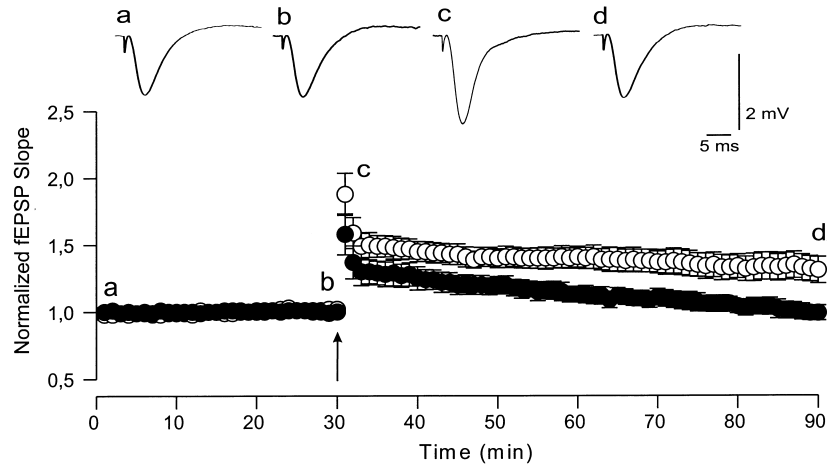


Fig. 2. MCA occlusion blocked the induction of LTP in ipsilateral (closed circles; $n = 7$) but not in contralateral hippocampal slices (open circles; $n = 7$). Traces were averaged in groups of four consecutive responses ($4 \times 15 \text{ s} = 1 \text{ min}$). They were normalized with respect to the grouped average of responses during 30 min prior to tetanic stimulations and plotted as means ($\pm \text{SEM}$) against time. The tetanic stimulus (100 Hz, 1 s) was delivered at a time point indicated by an arrow. The raw data illustrate a representative example of fEPSPs in the hippocampal slice from the ipsilateral hemisphere. Each trace is the average of four consecutive responses. The relation of these responses to the time course of the recording session is shown on the lower graph (a–d).

The present study demonstrates that transient occlusion of the MCA causes functional impairment in the hippocampus ipsilateral to the side of occlusion and that these functional changes can be modulated/prevented by a NMDA receptor antagonist (Fig. 3). Our results are in line with recent electrophysiological studies that have shown functional post-ischaemic changes extending beyond the infarct areas in ipsi- and contralateral hemispheres. Neumann-Haefelin and colleagues reported a time progressing reduction of field potentials amplitude and paired-pulse inhibition in the peri infarct cortical areas that was detectable even up to 7 mm away from the infarct border [10]. In rats with permanent MCAo, excitability was increased in the neocortex of contralateral hemisphere still one week after the insult

[15]. The hyperexcitability accompanying ischaemic insults most likely results from an imbalance of excitatory and inhibitory neurotransmission (glutamate and GABA) [8,13], which can possibly cause post-stroke epileptiform discharges [9]. Functional impairment of the hippocampus seen in our experiment may arise from an increased excitatory input of the entorhinal cortex which presumably constitutes a disinhibited peri infarct area. This in turn may lead to some pathological and/or adaptive changes altering functional properties of this structure. Rearrangement of NMDA and GABA receptors [13] or changes in their phosphorylation state [14] might be involved but these issues were not addressed in the present study.

One should stress that in humans ischaemia-induced

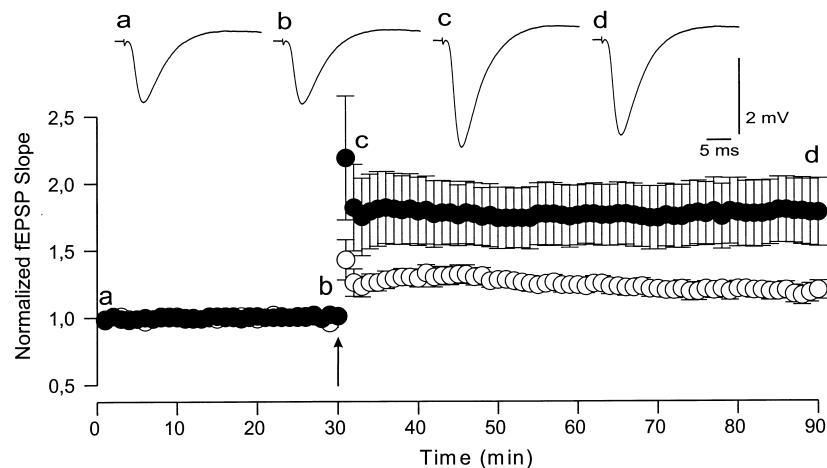


Fig. 3. Treatment with MRZ 2/579 saved and potentiated LTP in hippocampal slices ipsilateral to the infarct (closed circles; $n = 6$) without apparent influence on contralateral hippocampus (open circles; $n = 6$). The raw data illustrate a representative example of the potentiation of fEPSPs by tetanic stimulation in the hippocampal slice from the ipsilateral hemisphere of MRZ 2/579 treated rat. For further details see Fig. 1.

distant changes may strongly participate in late post-stroke neurological outcome and such alterations can possibly be prevented by pharmacological interventions. To support this, delayed transneuronal degeneration in the substantia nigra pars reticulata after permanent MCAo was prevented by long-term application of an alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist YM872 [11]. The observed effect was accompanied by an improved neurological score, however without reduction of primary cortical/striatal infarcts suggesting, that the threshold for protection in remote areas might be lower.

MRZ 2/579 has been found neuroprotective in both in vitro and in vivo models of cerebral ischaemia at concentrations/doses which still allow synaptic plasticity [5], (Ginsberg - personal communication). Here we report that this compound is able to change the pattern of remote functional alterations in the ipsilateral hippocampus induced by a transient MCA occlusion. A question arises whether the observed changes seen after treatment with this NMDA receptor antagonist result from the influence of the primary infarct area or have their origins in the hippocampus and these issues require further investigations. Nevertheless, the present findings bring new insight into the pathomechanism of stroke and open further prospects for NMDA receptor antagonists in stroke therapy.

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