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Mini Review (Neuroscience Series)

Ischemia induces short- and long-term remodeling of synaptic activity in the hippocampus

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Abstract

One of the most vulnerable areas to ischemia or hypoglycemia is CA1 hippocampal region due to pyramidal neurons death. Glutamate receptors are involved together with protein-kinase C and nitric oxide synthase. Long-term potentiation (LTP) is generated in anoxic or hypoglycemic conditions via activation of NMDA while inhibition of these receptors atenuates this response. Protein-kinase C and nitric oxide synthase are involved in anoxic LTP mechanism. Postischemic neurons are hyperexcitable in CA3 area while CA1 pyramidal neurons degenerate and dissapear. Changes of glutamate receptors triggered by ischemia and hypoglycemia are discussed in this review.

Keywords: NMDA - AMPA - LTP - ischemia - hypoglycemia - pyramidal neurons - CA1 - CA3

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Introduction

In humans and animals, the CA1 region of the hippocampus is one of the most vulnerable area of the brain to ischemia [1]. In this region, a global ischemic episode induces selective neuronal death of pyramidal neurons, whereas most of interneurons survive even several months after the ischemic insult [2]. The death of CA1 pyramidal neurons leads to the loss of anterograde memory and results in a major impairment of cognitive processes [3]. The cell death of CA1 pyramidal neurones is delayed and occurs 2-3 days after the ischemic insult. Several experiments have been conducted to detect abnormal activities induced after a stroke that might explain the delayed cell death of these neurons. Supporting this idea, a lesion of the Schaffer collateral pathway [4] or administration of NMDA and AMPA receptor antagonists [5–9] performed several hours after the ischemic insults have a protective action. Therefore, the toxic effects mediated by glutamatergic receptors may result from a persistent change of glutamatergic excitatory transmission. Several studies have proposed that CA1 hippocampal pyramidal cells degenerate as a consequence of the long-term change of synaptic excitation during the reperfusion period. In keeping with this hypothesis, studies performed *in vivo* and *ex vivo* have shown that excitatory synaptic transmission is enhanced several hours after ischemic insult [10,11,8].

Receptors involved in hippocampic ischemia-induced responses

After the ischemic episode, there is also a persistent increase of NMDA and AMPA receptor-mediated Ca^{2+} influx. The magnitude of the increase in Ca^{2+} influx correlates with the extent of CA1 pyramidal cell death [10, 11].

Therefore, one important and obvious question was to determine whether AMPA and NMDA receptor-mediated responses are modified after the stroke. One possibility is that the subunit stoichiometry of the AMPA receptor-channel complex has been changed in favor of Ca^{2+}-permeable subunits (for example GluRB changed in favor of GluR A, C or D) and this leads to Ca^{2+} accumulation. In keeping with this, it has been shown that there is a reduction of mRNA encoding GluRB subunit (less permeable to Ca^{2+}) 12-72h after the ischemic episode [12,13,14] and a greater AMPA-induced Ca^{2+} signal [15]. An other possibility is that AMPA receptors indirectly contribute to the rise of intracellular Ca^{2+}. In this scenario, activation of AMPA receptors would be required to relieve the Mg^{2+} blockade of the NMDA receptor-channel (permeable to Ca^{2+}; [16,17]. Many studies have reported a long-term change of the NMDA receptor-mediated responses after an ischemic episode [18,19,8](but see [20]). For example, it has been shown that following an ischemic episode the synaptic response includes a late abnormal component (associated with bursting discharges) [8,18,20]. This late response is mediated by NMDA receptors since a major portion of this response is sensitive to NMDA receptor antagonists [8,18]. The mechanisms triggering and underlying this long-lasting increase of the NMDA receptor-mediated response during the reoxygenation period was not obvious. Part of these questions have been clarified by *in vitro* and *ex vivo* experiments. It was demonstrated that the persistent enhancement of NMDA receptor-mediated responses is a long-term potentiation since it is due to an increase in the synaptic efficacy (leftward shift of input/output curves) and it was not associated with a change of cell excitability. This event has been called "anoxic long-term potentiation (LTP)" [21]. The induction of anoxic LTP required: (i) variations of membrane potential (it was not observed when the changes in membrane potential, occurring during and shortly after the AA episode, were prevented by clamping the voltage); (ii) activation of NMDA receptors (it was not observed if the NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-APV) was applied shortly before and during the anoxic episode); (iii) a rise in $[Ca^{2+}]_i$ (it was prevented by intracellular injection of a Ca^{2+} chelator 1,2-bis (2-aminophenoxy) ethane N,N,N',N'-tetra-acetic acid (BAPTA)) [21,22]. Finally, it was shown that the locus of the expression of the anoxic LTP is post-synaptic, since currents evoked by a focal application of NMDA (when synaptic activity has been fully blocked) are also persistently enhanced by an AA episode ([21] for review see [23].

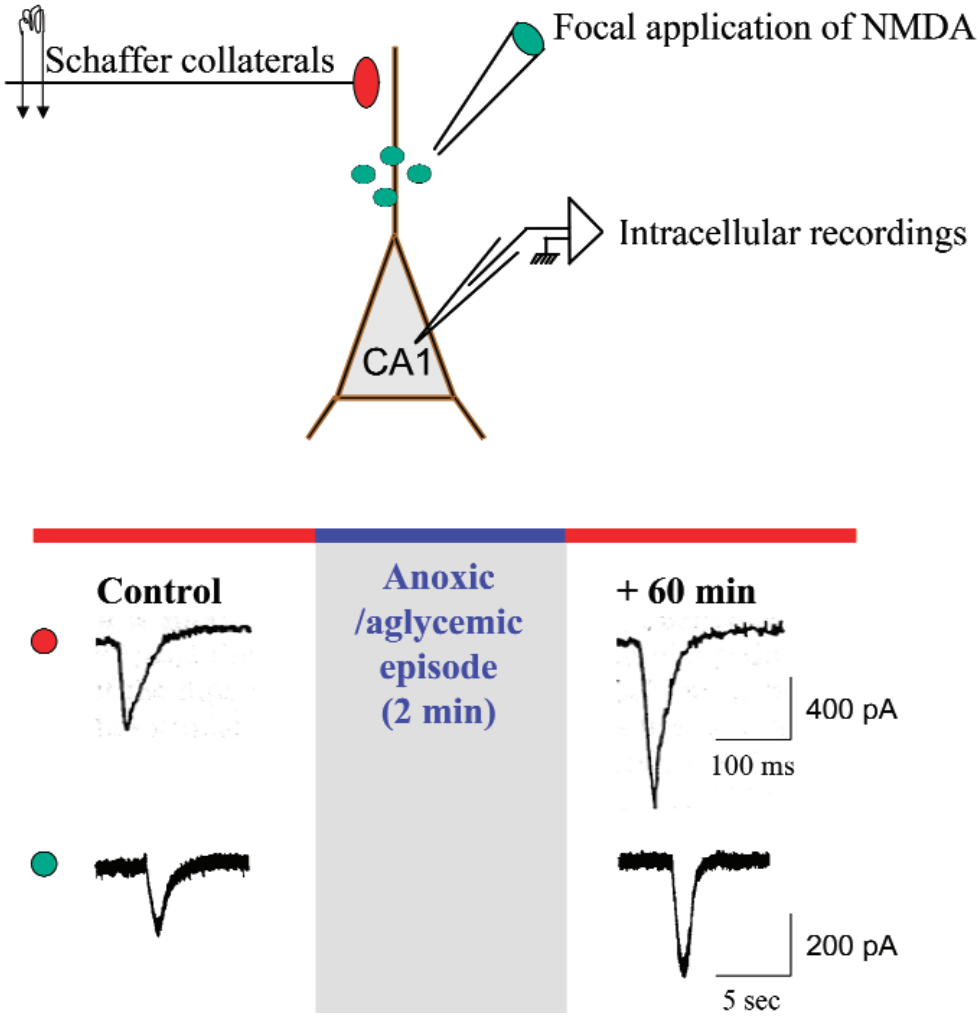

Fig. 1 In CA1 pyramidal cells, anoxic-aglycemic episodes (2 min) induce a long-term potentiation of NMDA receptor-mediated responses evoked either by synaptic stimulations of Schaffer collaterals (red dot ; excitatory post-synaptic currents recorded in SEVC mode at Vh = -65 mV, in the presence of 15 µM CNQX) or by pressures application of NMDA through a micropipette placed in the stratum radiatum (green dot ; 300 µM NMDA, 350 ms duration, 3.5 bar ; current recorded in SEVC mode at Vh = -60 mV, in the presence of 15 µM CNQX, 10 µM bicuculline, and 1 µM TTX).

Proteinkinase C and NOS involvement in anoxic long-term potentiation

The generation of anoxic LTP in the CA1 area has been confirmed by Huang and Hsu [24]. These authors have also shown that activation of NMDA receptors and a rise of $[Ca^{2+}]_i$ are critical steps in the induction of this phenomenon. They have found, however, that the brief anoxic episode potentiates both the AMPA- and the NMDA receptor-mediated component of the excitatory postsynaptic potential (EPSP), suggesting that the generation of the anoxia-induced LTP in the CA1 region probably involves both presynaptic and postsynaptic loci. The same authors have also reported that either the superfusion of the hippocampal slices or the intracellular application of protein kinase C (PKC) inhibitors prevent the induction of anoxic LTP, indicating that this form of synaptic plasticity requires the activation of postsynaptic PKC [25]. Conversely, protein kinase A (PKA) inhibitors have no effect on the anoxic LTP, suggesting that, at least in the CA1 hippocampal area, this second messen-

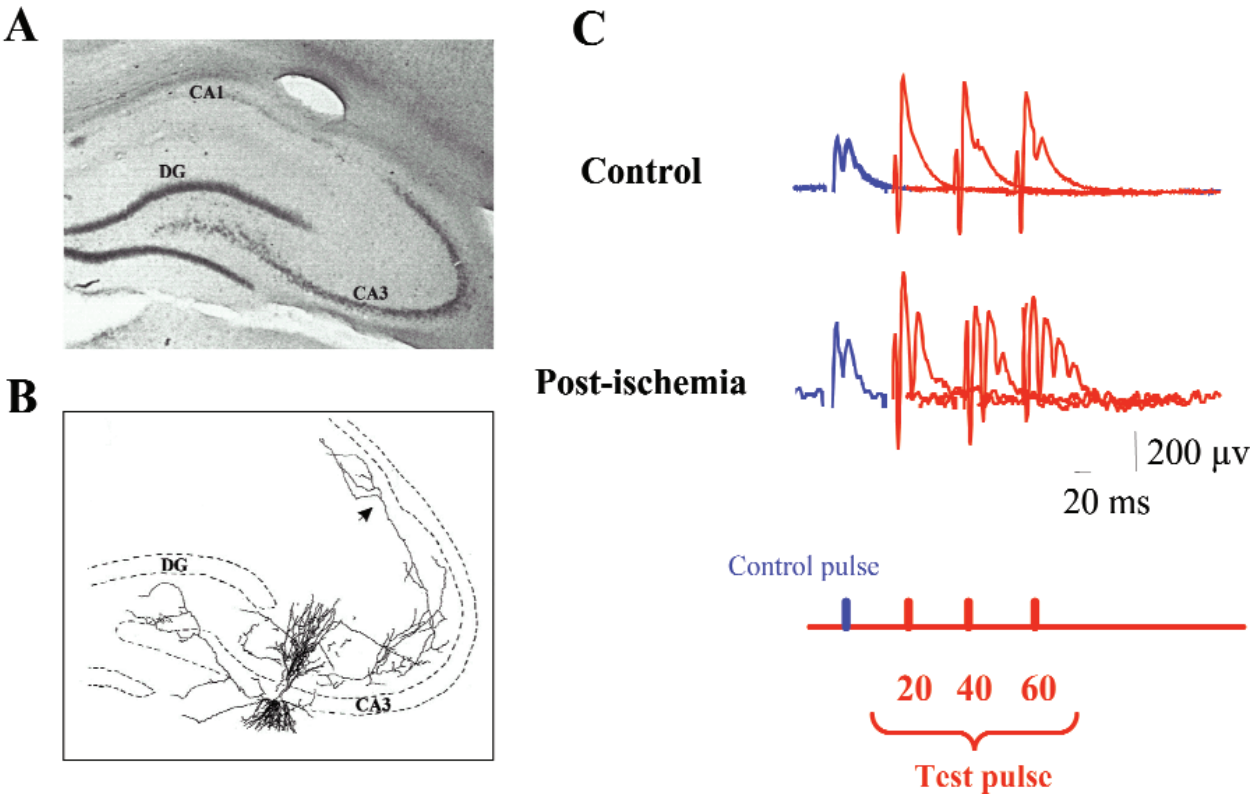

Fig. 2 Post-ischemic CA3 pyramidal neurons are hyper-excitable. *A*: cresyl violet staining of a coronal section of rat hippocampus 3 months after ischemia. Note that the CA1 pyramidal layer disappears, whereas the CA3 region and the dentate gyrus is preserved. *B*: camera lucida drawing of a 3 months post-ischemic CA3 pyramidal neuron labeled intracellularly with biocytin. Arrow: Schaffer collaterals projecting to the CA2 area. Note that pyramidal features of the post-ischemic cell are preserved. *C*: field potentials evoked by paired-stimulations with interpulses intervals ranging from 20 to 60 ms in control and post-ischemic cells. The first stimulation evokes the conditioning response and the second stimulation evokes the test response. Note that paired-pulse protocols evoke synchronized burst discharges in post-ischemic neurons in contrast to control cells.

ger is not involved in pathological plasticity [26]. Nitric oxide (NO) has been involved in the pathophysiology of brain ischemia [27] as well as in the formation of activity-dependent synaptic plasticity [28]. Accordingly, application of nitric oxide synthase (NOS) inhibitors significantly attenuates anoxic LTP in the CA1 hippocampal area without altering the baseline EPSP amplitude. The inhibitory effects of these drugs on the anoxic LTP are blocked by L-arginine, a substrate for NOS [29]. Interestingly, hypoglycemic episodes may also have *per se* long-lasting effects on synaptic function. Temporary suppression of glycolysis by 2-deoxy-D-glucose (2-DG), long enough to abolish CA1 population spikes and reduce field EPSPs, is followed by a sustained increase of EPSP amplitude [30]. This pathological LTP is prevented by NMDA receptors antagonists, and is not pathway-specific. Aglycemic LTP is prevented by exposing slices to dantrolene sodium, a depletor of intracellular Ca^{2+} stores, but not by thapsigargin, a blocker of Ca^{2+} uptake into intracellular stores [31]. More recently, it has been shown that anoxic/aglycemic episodes can also rapidly modify the structure of synapses [32,33]. This structural remodeling that includes the growth of filopodia, enlargements of existing spines, and formation of new spines are Ca^{2+} and NMDA receptor dependent. These dendritic rearrangements following energy deprivation could represent the anatomical substrate of post-ischemic LTP.

In conclusion, a short ischemic / aglycemic episode, through AMPA and NMDA receptor activation and Ca^{2+} influx, induces a profound structural remodeling of synaptic networks, that can lead to abnormal activities and delayed neuronal cell death.

An ischemic episode induces permanent reduction of seizure threshold in resistant CA3 pyramidal neurons

Most of the studies on ischemia have focused on short-term effects (up to 1 wk) (for review see [2], and despite several immunohistological studies [2], long-term modifications and the physiology of the post-ischemic adult hippocampal network are poorly known. Only recently, long-term abnormal activities have been reported in post-ischemic neocortical pyramidal neurons [34,35]. The possibility of persistent changes of synaptic network activity after an ischemic stroke is of major interest as patients surviving this type of insult often express delayed epileptic syndromes months or years after the initial insult [36,37,38]. This raises the possibility that a transient ischemic insult, besides neuronal cell death, may also induce persistent modifications of network activities leading to epileptiform discharges. In keeping with this, it has been shown in animal models that ischemia induces long-term abnormal activities in neocortical pyramidal neurons [34,39]. The CA3 region is a good candidate to subserve these long-term alterations because this region is one of the most susceptible regions in the brain for the generation of seizures [40]. In addition, the CA3 pyramidal cells have lost their principal target cells (CA1 neurons) but still receive most of their excitatory inputs including the granule cells of the fascia dentata [2], and it is known that neurons deprived of their projecting sites or after axotomy are often hyperexcitable [41,42]. Examination of the synaptic transmission in post-ischemic CA3 pyramidal cells show that there is no seizure-like activity in normal conditions [43]. However, we observe that those neurons are more susceptible than the control ones to display evoked and spontaneous synchronized burst discharges when low doses of convulsive agents (as K^+ and kainate) are bath applied. We also show that synchronized burst discharges can be induced in normal ACSF conditions when a paired-pulse protocol is used. We observe that these burst discharges develop when the interpulse interval ranged from 60 to 100 ms. At this interpulse interval the GABA-A receptor-mediated inhibition is reduced by 25-50% [44]. Study of the intrinsic membrane properties of the post-ischemic CA3 pyramidal cells reveal that there is a clear shift of the membrane potential [45]. It is suggested that the positive shift of the resting membrane potential toward the spike threshold may facilitate the generation of the synchronized burst discharges in post-ischemic CA3 pyramidal neurons.

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