ORIGINAL ARTICLE

Effect of transient ischemia on long-term potentiation of synaptic transmission in rat hippocampal slices

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Submitted: 2008-06-27 Accepted: 2008-09-04

Key words: **hippocampus; ischemia; long-term potentiation; high-frequency stimulation; memory; *in vitro***

Neuroendocrinol Lett 2008; **29**(5):702–705 **PMID:** 18987602 NEL290508A33

Abstract

OBJECTIVES: Long-term potentiation (LTP) of neuronal activity in the hippocampus is thought to be a substrate for learning and memory. The influence of ischemia (IS) (hypoxia/hypoglycemia) on induction of LTP of synaptic transmission (ST) by high frequency stimulation (HFS) was investigated in rat hippocampal slices.
METHODS: Neurons were stimulated via Schäffer collaterals and field excitatory postsynaptic potentials (fEPSP) were recorded extracellularly from the CA1 region. LTP was induced by one single train (100 Hz, 1s).
RESULTS: In controls LTP of ST after HFS was 179.70±12.53%. Short IS of 2.5–4.5 min elicited a transient failure of ST, with return to former value followed by further increase of fEPSP amplitude to 142.28±16.24%, compared to amplitude before IS. HFS was elicited 40 min after exposure to IS and LTP was measured over further 40–60 min. LTP in slices exposed to 2.5–4.5-min IS was 139.94±14.01%. IS of 6–7.5 min elicited a prolonged failure of ST, with almost full recovery (96.69±14.42%). LTP was not activated 40 min after 6–7.5-min IS and the amplitude of fEPSP was even reduced to 80.14±19.19% compared to the former mean value of fEPSP 10 min before HFS.
CONCLUSION: The results revealed that prolonged 6–7.5-min IS influenced induction of LTP of ST in the hippocampus and thus it could have deleterious effects on learning and memory. These findings may have clinical implications in stroke, brain ischemia, sleep apnoe and call for studying the effect of neuroprotectants on the induction of LTP in hippocampus exposed to oxidative stress.

Abbreviations

ACSF	- arteficial cerebrospinal fluid
fEPSP	- field excitatory postsynaptic potential
HFS	- high frequency stimulation
IS	- ischemia
LTP	- long term potentiation
ST	- synaptic transmission

INTRODUCTION

Long-term changes in synaptic transmission (ST) as long-term potentiation (LTP) are thought to be a substrate for learning and memory (Rison & Stanton, 1995; Andersen, 2003; Lomo, 2003). The strength of synapses is not fixed but can be modified by activity, indicating that the brain is plastic. Synaptic plasticity is crucial to the development of the nervous system and thereafter to the ability of an individual to learn and remember new information (Cooke & Bliss, 2006). Synaptic plasticity is also believed to be important in neurological disorders, such as epilepsy and neurodegeneration, and in recovery from neuronal injury (Bortolotto *et al*., 2001). Thus the phenomenon of LTP attracts many researchers during the recent period.

MATERIALS AND METHODS

All procedures involving animals were performed in compliance with the Principles of Laboratory Animal Care issued by the Ethical Committee of the Institute of Experimental Pharmacology, Slovak Academy of Sciences as well as by the State Veterinary and Food Administration of Slovak Republic. Male Wistar rats (190–260 g) were briefly anesthetized by ether and than decapitated. The brain was removed and the hippocampus was quickly dissected in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O_2 + 5% CO_2, at a pH of 7.4. Coronal slices, 400 μm thick, were cut using the McIlwain Tissue Chopper and placed by suction pipette into a gas/liquid interface incubation chamber with ACSF at 34°C. Slices were incubated for at least 80 min under the given conditions before electrophysiological assessment. For synaptic transmission measurement, each slice was placed into a gas/liquid interface recording chamber perfused with gas-saturated ACSF at a rate 0.6 ml/min, the chamber volume being 0.6 ml. The interface chamber was restrained in external chamber and the gas mixture was used both to saturate the ACSF and to ventilate the interface chamber.To produce and retain ischemic (IS) conditions, O_2 in the original gas mixture was substituted by N_2. Simultaneously the perfusion medium was switched to ACSF saturated with 95% N_2 + 5% CO_2, containing 4 mmol/l glucose. Reoxygenation was attained by restoring the former conditions. The rate of oxygen exchange in the recording chamber was monitored by the miniature Clark 733 oxygen electrode (Diamond Micro Sensors) placed approximately 10 mm next to the slice. The O_2 concentration in the recording chamber atmosphere decreased to less than 5% within 2 min of IS. Neurons were stimulated via Schäffer collaterals using bipolar stainless wire electrode and field excitatory postsynaptic potentials (fEPSP) were recorded extracellularly from the CA1 region. Recordings were amplified, visualized on the oscilloscope Tektronix 2230, digitalized by the DigiData 1322A with sampling rate of 10 kHz and stored on personal computer for off-line analysis by the software AxoScope 9.2 and 10.2. The stimulus intensity was adjusted in 50% of fEPSP amplitude where a population spike generation was detected. The stimulus frequency was 0.05 Hz. LTP was induced by one single train of 100 Hz frequency, 1s duration. In control slices after 10–20 min stabilization period, HFS was induced by a single train and then ST was measured for at least 40–60 min. In IS slices preparations were exposed to 2.5–4.5-min or 6–7.5-min IS after 10–20-min stabilization period, then HFS was applied 40 min after IS and ST was monitored for at least further 40–60 min.

Statistical Analyses

All values are given as means of 8–15 experiments ± SEM. Statistical significance of differences between means was established by Student´s t-test and *p* values below 0.05 were considered statistically significant.

RESULTS

We found that a single train of pulses with 100 Hz frequency and duration of 1 s elicited immediate increase of a fEPSP amplitude in the first minutes after HFS, followed by slow decay. However the fEPSP amplitude remained still increased to 179.70±12.53% during the next 40–60 minutes of measurement, compared to the mean baseline fEPSP magnitude obtained 10 min before the train onset, which represented 100% of response (Figure 1a, Figure 2b).Short exposure to IS (2.5–4.5 min) elicited a transient failure of ST, with return to the former value within 5–15 min with further increase of fEPSP amplitude to 142.28±16.80% compared to the amplitude before IS (Figure 1b, Figure 2a). HFS was initiated 40 min after exposure to 2.5–4.5-min IS and LTP was measured over further 40–60 min. HFS-induced LTP of the fEPSP amplitude in slices previously exposed to 2.5–4.5-min IS was reduced to 139.94±14.01% compared to slices not exposed to oxidative stress (Figure 1b, Figure 2b). An exposure to 6–7.5-min IS elicited a prolonged failure of ST, with almost full recovery of ST in reoxygenation (96.69±14.42% of fEPSP amplitude before IS) (Figure 1c, Figure 2a). An exposure to 6–7.5-min IS, 40 min before HFS, elicited a reduction of fEPSP amplitude to 80.14±19.19% compared to the former mean value of fEPSP measured 10 min before HFS (Figure 1c, Figure 2b).

DISCUSSION

LTP of ST in the hippocampus can be obtained in two LTP associative pathways: Schäffer collateral fiber pathway and perforant fiber pathway (Rison & Stanton, 1995) by many different protocols, of which LTP induction by HFS of 100 Hz frequency and 1 s duration is often used and recommended (Bortolotto *et al*., 2001). The probability of inducing LTP in CA3–CA1

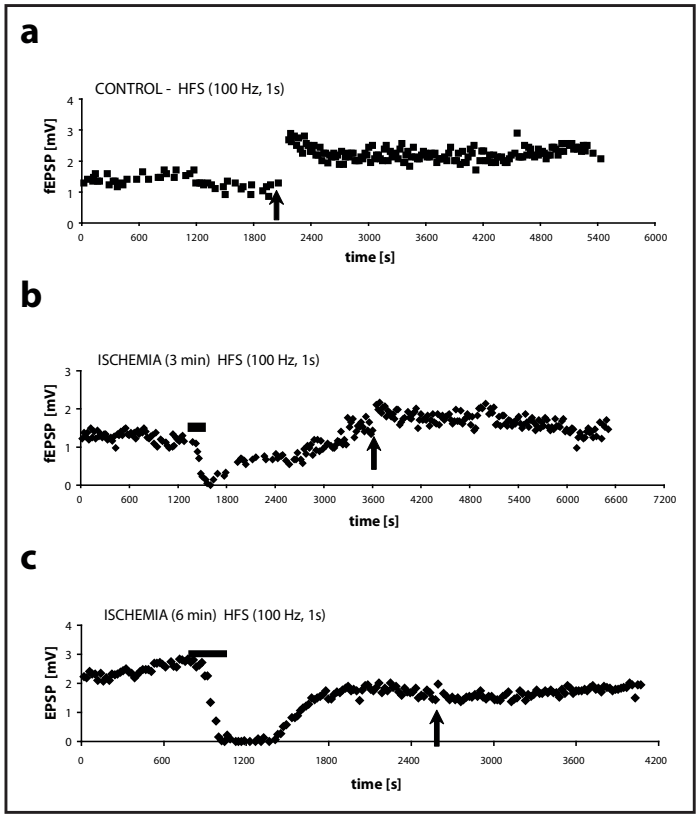

Figure 1. Representative recording from individual rat hippocampal slices.
a) LTP induced by HFS (100 Hz, 1s) in control slice,
b) LTP induced by HFS (100 Hz, 1s) 35 min after exposure of slice to 3-min IS (hypoxia with hypoglycemia), and
c) failure in induction of LTP by HFS (100 Hz, 1s) 30 min after 6-min IS. Duration of IS is expressed by full line and site of HFS is expressed by arrow.

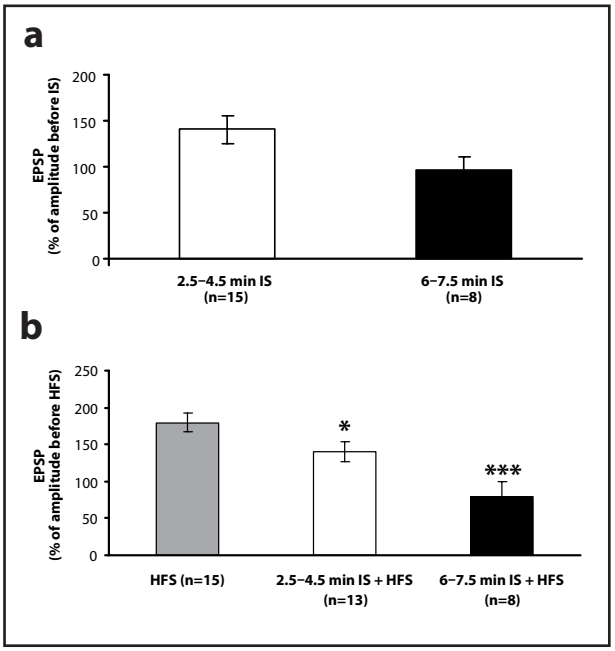

Figure 2. Ischemia-induced LTP without application HFS and HFS-induced LTP with previous ischemia.
a) The mean values ± SEM of fEPSP amplitude calculated from recordings 30–40 min after 2.5–4.5 or 6–7.5-min IS (hypoxia with hypoglycemia), and
b) the mean values ± SEM calculated from recordings 40–60 min after HFS, where IS was applied 40 min earlier. The n values represent the number of slices used in each experimental group. The differences in fEPSP amplitude measured 40–60 min after HFS in controls compared to fEPSP amplitude in slices exposed to 2.5–4.5-min IS (*$p<0.05$) or 6–7.5-min IS (***$p<0.001$) were examined using the Student´s t-test.

pathway depends also on the layer of the CA1 area from which the response is recorded (Arai *et al*., 2004, Kopanitsa *et al*., 2006). Thus one of the principal feature of LTP – its magnitude – differs from author to author, probably due to many other factors including conditioning of slices, such as their cutting, incubating, type and parameters of measuring chamber (interface or submerged), as well as the age of animals, etc. Crepel *et al*., (1993) were the first to characterize a pathological form of synaptic plasticity induced by 1–3 min of anoxia and aglycemia *in vitro*. Anoxia-induced LTP on glutamatergic synaptic transmission in the CA1 region of rat hippocampus was further found by using intracellular recordings *in vitro* by Hsu and Huang (1997) and concurrently by performing extracellular recordings using a two-pathway design by Lyubkin *et al*., (1997). Last mentioned authors studied also only short 2-min hypoxia where dextrose concentration was not reduced during hypoxia. Hsu and Huang (1997) tested 15-min episode of anoxia on anoxia-induced-LTP, where content of glucose was also not reduced. We found hypoxia/hypoglycemia-induced LTP after 2.5–4.5-min IS but not after 6–7.5-min IS. Thus presence of energy source seems to be crucial in ischemia-induced LTP. Accordingly cognitive decline was found due to hypoglycemia (Akyol *et al*., 2003, Umegaki *et al*., 2006). In our previous research where resistance of hippocampal slices to 6-min IS followed by 20-min reoxygenation *in vitro* was improved by tested antioxidants (Gáspárová *et al*., 2006; Štolc *et al*., 2006), in control slices without drugs tested we observed almost no recovery of ST at the end of 20-min reoxygenation in rat hippocampal slices under the same experimental conditions with IS , except the stimulation which was supramaximal for the first population spike at 0.2 Hz frequency. We thus further suggest that the sensitivity of ST (only EPSP) to oxidative stress differs from ST with stimulated excitability (EPSP with population spike of its maximal amplitude). In the present paper, we further showed that 6–7.5-min IS interfered with the induction of LTP of ST in rat hippocampal slices. From literature it is known that neonatal anoxic insult in rats resulted in hyperactivity (Ujházy *et al*., 2006) and there may be some expectation of attention and memory impairment later on as well. There is suggestion that extended brain IS can have deleterious effect on synaptic plasticity.

CONCLUSION

The results revealed that extended IS influenced induction of LTP of ST in hippocampus and thus it could have deleterious effect on learning and memory. These findings may have clinical implications in stroke, brain IS, sleep apnoe, etc. and call for further studies of effects of neuroprotectants and antioxidants on the induction of LTP in hippocampal slices exposed to oxidative stress.

ACKNOWLEDGEMENTS

This work was supported by scientific grants APVV 51-017905, VEGA 2/0083/08 and VEGA 2/0093/08.

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