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Influence of cooling rate on activity of ionotropic glutamate receptors in brain slices at hypothermia

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ABSTRACT

Hypothermia is a known approach in the treatment of neurological pathologies. Mild hypothermia enhances the therapeutic window for application of medicines, while deep hypothermia is often accompanied by complications, including problems in the recovery of brain functions. The purpose of present study was to investigate the functioning of glutamate ionotropic receptors in brain slices cooled with different rates during mild, moderate and deep hypothermia. Using a system of gradual cooling combined with electrophysiological recordings in slices, we have shown that synaptic activity mediated by the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptors in rat olfactory cortex was strongly dependent on the rate of lowering the temperature. High cooling rate caused a progressive decrease in glutamate receptor activity in brain slices during gradual cooling from mild to deep hypothermia. On the contrary, low cooling rate slightly changed the synaptic responses in deep hypothermia. The short-term potentiation may be induced in slices by electric tetanization at 16 °C in this case. Hence, low cooling rate promoted preservation of neuronal activity and plasticity in the brain tissue.

1. Introduction

Brain functions are highly sensitive to relatively small changes in temperature. As it is shown in models in vitro, the changes the temperature by several degrees alter the excitability of nerve cells and duration of their responses (Fujii, 1977; Shen and Schwartzkroin, 1988; Lomber et al., 1999; Volgushev et al., 2000; Aihara et al., 2001; Pasikova et al., 2010; Hedrick and Waters, 2012). While lowering the temperature the evoked activity of neurons (field potentials, FPs) is different in hippocampus of non-hibernated and hibernated animals (Schiff and Somjen, 1985; Thompson et al., 1985; Pakhotin et al., 1993; Moser and Andersen, 1994; Anderson and Moser, 1995; Pakhotin et al., 1996; Gabriel et al., 1998). The amplitudes of excitatory postsynaptic potentials (EPSPs) of FPs are usually increased in brain tissue of non-hibernated animals in temperature range 30–35 °C (mild hypothermia) (Schiff and Somjen, 1985; Thompson et al., 1985; Tanimoto and Okada, 1987; Moser et al., 1993; Itoh, 1999; Fujii et al., 2002). Activity-dependent changes in excitatory transmission mediated through ionotropic glutamatergic, the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptors (AMPARs and NMDARs) are involved in synaptic plasticity. However it is not determined how lowering the temperature modifies activity of these receptors.

Mild hypothermia is neuroprotective, as established in animal models of a stroke and is used clinically to prevent brain damage (Gluckman et al., 2005; Shankaran et al., 2005; MacLellan et al., 2009; Dietrich and Bramlett, 2010; Yenari and Han, 2012; Wu and Grotta, 2013). Neuronal dysfunction induced by deregulated glutamatergic neurotransmission, with changes in NMDARs following brain ischemia is attenuated by moderate post-ischemic hypothermia (Nakamura et al., 2001) and intra-ischemic hypothermia (Friedman et al., 2001). Post-asphyxic and intra-ischemic mild hypothermia prevent damage induced changes in tyrosine phosphorylation of NR1 and NR2A subunits of NMDARs (Mueller-Burke et al., 2008; Hu et al., 2011), whereas prolonged post-ischemic cooling regulates the AMPAR GluR2 subunit expression (Colbourne et al., 2003). Cellular protection that is expected during therapeutic hypothermia depends on many factors, including the method, duration and the rate of cooling, because side effects of hypothermia also appear to occur more frequently with each degree reduction in temperature (van der Worp et al., 2010). Hypothermia lowers metabolic and energy balance sufficient to the maintenance of normal transmembrane ion and neurotransmitter gradients (Choi et al., 2012). It is therefore important to understand

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how the AMPAR and NMDAR-dependent ionotropic channel receptors function at hypometabolic state of neurons and their role in cerebral function during every phase of hypothermia.

The effect of deep hypothermia on neuronal synaptic function of non-hibernated animals are even more ambiguous. Deep hypothermia (21 °C) induced a much more protective effect than moderate hypothermia (28 °C) for FPs preservation against deprivation of oxygen and glucose and anoxia in guinea pig hippocampal slices (Okada et al., 1988; Kurihara et al., 1996). Experimental seizure activity in mice brain slices is inhibited by deep cooling to 24 °C, and complete cessation of epileptiform discharges is obtained at temperatures of 20–22 °C. In this case, the efficacy of seizure suppression depended on high cooling rate (Motamedi et al., 2012). In a swine model of lethal hemorrhage a rapid induction of deep (15 °C) and profound (10 °C) hypothermia promotes the survival of animals that displayed normal learning capacity (Alam et al., 2006). On the other hand, deep hypothermia is associated with neurological complications, produced by neuronal loss and degeneration in the brain of dogs with or without circulatory arrest (Watanabe et al., 1982; DeLeon et al., 1998). It is proposed that mechanism of neuronal injury during profound hypothermia/rewarming in hippocampal slices is a result of increase in intracellular Ca^{2+} mediated by NMDAR excitotoxicity that activates necrotic processes (Bickler et al., 2012; Warren et al., 2012). Induction of deep hypothermia had a significant effect on the gene expression profiles of key genes, with an overall up-regulation of pro-survival pathways and a down-regulation of metabolic pathways (Qin et al., 2008; Alam et al., 2010). Such adaptive modifications apparently occur before a certain threshold and then great metabolic and functional changes may lead to loss of cerebral autoregulation (Ehrlich et al., 2002). The molecular mechanism of hypothermic neuroprotection remains unclear and the ascertainment of target hypothermic temperature, time to achieve target temperature, duration and rate of cooling, is on the agenda (Moore et al., 2011). The aim of present study was to determine whether the ionotropic glutamate AMPARs and NMDARs are targets of hypothermic impact, whether there is a temperature point at which these receptors do not remain active, and whether it depends on the cooling rate.

In the present experiments, we have investigated the responses of AMPAR and NMDAR-dependent mechanisms in piriform cortex slices under the gradual cooling with high (2.0 °C /min) and low (0.125 °C/min) rates from 37 °C to 16 °C. The effects of mild, moderate and deep hypothermia on synaptic receptor activity of piriform cortex were examined by using extracellular electrophysiological recording techniques. To ascertain the influence of cooling rate on modification of neuronal plasticity during deep hypothermia we tested the development of long-term potentiation (LTP) in slices.

2. Materials and methods

2.1. Tissue preparation

All animals used in this study were treated with observance of recommendations on ethics of work with the animals offered European Communities Council Direction (86/609 EEC). The experiments with rats were approved in strict accordance with the Russian Federation Council's Guide for the Care and Use of Laboratory Animals (1994) and with the guidelines of the IP Pavlov Institute Physiology Russian Academy of Sciences of the ethical code (1996).

Male Wistar rats (200–250 g, 4–5 months) were housed four per cage on a 12 h dark/light cycle in a temperature-controlled environment with free access to food and water. All efforts were made to minimize animal suffering and reduce the number of animals used.

Tangential slices of olfactory cortex about 400–500 μm in thickness prepared within 1 min were maintained in artificial cerebrospinal fluid (ACSF), consisted of (mM): NaCl—124; KCl—5; $CaCl_2$—2.6; KH_2PO_4—1.24; $MgSO_4$—1.2; $NaHCO_3$—3; glucose—10; and Tris–HCl—23 equilibrated with O_2. The use of Tris–HCL allowed to conduct the experiments in an atmosphere of O_2, pH in these conditions was within 7.20–7.25. The brain slice was preincubated for 2 h to recover in oxygenated ACSF at 37 °C in water bath and then transferred to a recording chamber. The brain slice was continuously perfused with oxygenated ACSF at the rate of 2 ml/min tthroughout the temperature range of 37–16 °C. The recording chamber was purged with oxygen too.

2.2. Controlling temperature

All construction details and the appropriate electrical circuits are provided. Using this home-made device, the steady-state chamber temperature could be precisely monitored with a thermometer (TPEM-1, Russia) a resolution of ±0.2 °C in a range of 0–40 °C. The chamber temperature was continuously measured directly near the tissue slice. The temperature was decreased from 37 °C to 16 °C at a controlling rate of 0.125 °C/min (low rate) and 2.0 °C/min (high rate). Rewarming was made with the same rates. In some series of experiments rate of cooling was 4.0 and 6.0 °C/min.

2.3. Electrophysiological recordings

Basal AMPAR and NMDAR-dependent ionotropic glutamatergic synaptic transmission were registered after orthodromic stimulation of the lateral olfactory tract (LOT) conducted by rectangular pulses with a duration of 0.1–0.3 ms and an intensity of 1–3 V and with frequency equal to 0.003 Hz (ESU-1, Russia) through platinum bipolar electrodes. FPs from slices were recorded using glass microelectrodes filled with 1 M NaCl (resistance 1–5 mΩ) conducted with amplifier (NTO, Russia). The indifferent silver electrode was located at the bottom of the recording chamber.

The development of LTP in slices was tested at 16 °C after the LOT fibers fourfold tetanization: frequency 100 Hz for 5 s with the interval 5 s (the θ burst stimulation-TBS). After 3 and 5 min of tetanization the modifications of the AMPA and NMDA EPSPs were tested at the single LOT stimulation.

2.4. Data analysis and pharmacological isolation of AMPAR and NMDAR-dependent mechanisms in control conditions and during lowering the temperature

We used the technique of controlled experiment by computer to standardize conditions of individual experiments. The intensity of stimulation and the intervals and quantity of stimuli delivered to LOT were specified. After each stimulus, the FPs were processed in the on-line mode. The FPs were amplified with the analog-digital converter MD-32 (Russia) (sample rate 25 kHz), and transmitted to computer for registration and subsequent analysis, using special homemade software. Amplitudes of FPs components we estimated from the isoline to the peak level. The amplitudes of AMPA EPSP we assessed within a 2.0 ms window centered at the peak of the response. Peak NMDA EPSP was measured as the average potential observed in a 8.0 ms window (Fig.1A).

To make sure the authenticity of AMPA and NMDA EPSP components of FPs and to identified the possible latency shifts of these postsynaptic excitatory components of the FPs in the rat olfactory cortex slices we tested their amplitudes at 37 °C (Fig.1) before cooling the temperature and in the time point of the maximal EPSPs changes using competitive blockers of the ionotropic AMPA and NMDARs: CNQX (6-cyano-7-nitroquinoxaline-2,