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Neuroprotective effects of interleukin-10 and tumor necrosis factor-α against hypoxia-induced hyperexcitability in hippocampal slice neurons

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Abstract

In our previous experiments we have demonstrated that repeated exposures of rat hippocampal slices to brief episodes of hypoxia induce a sustained decrease in the threshold of stimulus-evoked epileptiform discharges in CA1 pyramidal neurons. The aim of this study was to investigate the comparative effects of interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) on the hyperexcitability of CA1 pyramidal neurons induced by brief episodes of hypoxia in the rat hippocampal slices. The method of field potentials measurement in CA1 region of hippocampal slices have been described in our previous work [O. Godukhin, A. Savin, S. Kalemenev, S. Levin, Neuronal hyperexcitability induced by repeated brief episodes of hypoxia in rat hippocampal slices: involvement of ionotropic glutamate receptors and L-type Ca^{2+} channels, Neuropharmacology 42 (2002) 459–466]. The principal results of our work are summarized as follow. Pro-inflammatory cytokine TNF-α (0.8, 4 and 20 ng/ml) and anti-inflammatory cytokine IL-10 (1 and 10 ng/ml) significantly reduced the hyperexcitability in CA1 pyramidal neurons induced by brief episodes of hypoxia in the rat hippocampal slices. The neuroprotective effects of IL-10 and TNF-α against the hypoxia-induced hyperexcitability were mediated by anti-hypoxic actions of these cytokines through, possibly, mechanism of preconditioning.

Keywords: Hippocampal slices; Hypoxia; Hyperexcitability; Tumor necrosis factor-alpha; Interleukin-10; Population spike; Epileptiform activity

It is known that hippocampal formation, especially CA1 region, is highly sensitive to hypoxia/ischaemia, and these conditions can induce long-lasting functional modifications leading to a decrease in the threshold of seizure activity generation [9,20]. In our previous experiments we have demonstrated that repeated exposures of rat hippocampal slices to brief episodes of hypoxia induce a sustained decrease in the threshold of stimulus-evoked epileptiform discharges in CA1 pyramidal neurons [6]. This hypoxia-induced hyperexcitability in CA1 pyramidal neurons critically depends on functional activities of L-type voltage-gated Ca^{2+}-channels and ionotropic glutamate receptors but not $GABA_A$ and $GABA_B$ receptors [6,11].

There is some evidence for the involvement of pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) and anti-inflammatory cytokine interleukin-10 (IL-10) in experimental models of seizures and in human epilepsy [1,7,8,18,19,21]. However, the functional roles of these cytokines in seizure activity either unknown (for IL-10) or controversial (for TNF-α). Expression of IL-10 is elevated during the course of most major diseases in the brain and promotes survival of neurons and all glial cells by blocking the effects of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 [19]. Furthermore, IL-10 blocks the inhibitory effect of IL-1β on long-term potentiation in the hippocampus [10]. Although the functions of IL-10 in the brain are most often associated with increased animal survival, in certain cases the potent anti-inflammatory property of IL-10 leads to the progression of disease and death [19]. Currently, the functional role of IL-10 in seizures is unknown.

The role of pro-inflammatory cytokine TNF-α in the seizure activity development is controversial. Some authors have demonstrated that TNF-α prolonged epileptiform discharges in amygdala kindled rats [16]. While the other investigators have shown that TNF-α inhibited kainic acid-induced seizures in mice, and this neuroprotective action against seizures is mediated by neuronal p75 receptors [2]. It is proposed that this

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cytokine can cause both detrimental and neuroprotective effects on neuronal activity, depending on its local concentration, the type of target brain cells, the receptor subtypes and the experimental models of epileptogenesis [1,2,22].

In our study, we investigated the comparative effects of IL-10 and TNF-α on the hyperexcitability of CA1 pyramidal neurons induced by brief episodes of hypoxia in the rat hippocampal slices.

All experiments were carried out with male Wistar rats (60–70 days old; $n = 60$). The use of animals was in accordance with the UK Animals (Scientific Procedures) Act 1986. Transverse hippocampal slices (250–300 μm thick) were prepared with a tissue chopper and placed into a recording chamber (submersion type). Slices were superfused at 2.5 ml/min with the artificial cerebrospinal fluid (ACSF) maintained at 32 °C. The ACSF composition was (mM): 124 NaCl, 3 KCl, 1.25 KH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose; pH 7.4 was adjusted with 95% O_2/5% CO_2. Slices were allowed to recover for 5 h before data collection. This incubation was necessary because the literature findings have demonstrated that the level of some cytokines was relatively high in hippocampal slices incubated for shorter periods [15]. The method of field potentials measurement in CA1 region of hippocampal slices have been described in our previous works [6,11]. Briefly, population spikes (PSs) of CA1 pyramidal neurons in stratum pyramidale were recorded with glass microelectrode (2–5 MΩ) in response to electrical stimulation (0.1 ms, 50–350 μA) of Schaffer collateral/commissural fibers (SCH). PS amplitude (mV) were measured for series of 7 separate single current pulses with increasing intensity (from minimum to maximum values for PS generation) applied at 10 s interval. A delay of 20 min separated each group of 7 stimuli from the next. Three hypoxic episodes (3 min duration each with the 10 min interval) were produced by switching from ACSF equilibrated with 95% O_2/5% CO_2 to ACSF equilibrated with 95% N_2/5% CO_2. The effectiveness of hypoxic episode (T_h) was evaluated by employing the following formula (Fig. 2A):

T_h = the time point of 50% recovery of PS amplitude (T_h^r)

− the time point of 50% depression of PS amplitude (T_h^d).

The appearance of multiple PSs in the PS response to single electrical stimulus was taken as indication of the development of epileptiform activity. Two parameters of such activity were measured: (1) the stimulus intensity (μA) of the appearance of the additional (second) PS was characterized as the threshold of generation of an additional PS in the PS response (TASG), and (2) the number of PSs in the PS response (NPS) measured for the current intensity that induced an additional PS before hypoxic episodes or in the same time points (−20 to 0 min) in the control experiments (without hypoxic episodes applied). IL-10 (1 and 10 ng/ml; from Chemicon, catalog # IL035) and TNF-α (0.8, 4 and 20 ng/ml; from Chemicon, catalog # GF046) were applied for 10 min before and together with hypoxic episodes (the total time—40 min).

All electrophysiological data were digitized at 20 kHz and analyzed using a computer with software developed in house for the measurements of PS amplitude, TASG and NPS. All values in the Results section are given as mean ± S.E.M. The effects of treatments were analyzed by one-way analysis of variance (ANOVA) followed by multiple-comparisons tests (Scheffe's tests). A $P \leq 0.05$ was considered as significant.

Under normal conditions (without hypoxic episodes applied), IL-10 (1 and 10 ng/ml) and TNF-α (0.8, 4 and 20 ng/ml) did not change the amplitude of PS and the values of TASG and NPS (data is not shown). Hypoxic episodes abolished the PSs (during episodes) (Fig. 1A) and induced a post-hypoxic sustained decrease in the TASG (data is not shown) and an increase in the NPS (Fig. 1B). Fig. 1B illustrates the effects of IL-10 (1 ng/ml) and TNF-α (0.8 ng/ml) on the time courses of NPS produced by repeated brief hypoxic episodes. These cytokines significantly depressed an increase in the NPS in a dose-dependent manner. Fig. 1C shows that IL-10 (1 ng/ml) more effectively depressed a hypoxia-induced increase in the NPS than IL-10 (10 ng/ml): 1 ng/ml—on 60% and 10 ng/ml—on 38.5% (140 min after hypoxia). In contrast to IL-10, the depressive effect of TNF-α had a reversed dose–response relationship (Fig. 1C). TNF-α more effectively depressed a hypoxia-induced increase in the NPS in the concentration of 20 ng/ml than in concentrations of 4 and 0.8 ng/ml: 0.8 ng/ml—on 33.3%, 4 ng/ml—on 45% and 20 ng/ml—on 55% (140 min after hypoxia).

However, from the above-mentioned results and the findings received in our previous work [12] it is not clear whether these cytokines depress the mechanisms of hypoxia-induced hyperexcitability (the production of multiple PSs) expressed after hypoxic episodes or depress the effectiveness of hypoxia on functional activity of hippocampal slice tissue during hypoxic episode (antihypoxic action) leading to an abolishment of post-hypoxic hyperexcitability. To examine this issue, we determine the effects of IL-10 and TNF-α on the time courses of PS amplitude depression during hypoxic episodes. Fig. 2A demonstrates the time courses of PS amplitude depression during: single hypoxic episode, single hypoxic episode + IL-10 (1 ng/ml) and single hypoxic episode + TNF-α (0.8 ng/ml). The effectiveness of hypoxic episodes (T_h) were: (1) single hypoxic episode − 293 ± 10 s; (2) single hypoxic episode + IL-10 (1 ng/ml) − 218 ± 11 s and (3) single hypoxic episode + TNF-α (0.8 ng/ml) − 189 ± 27 s. Thus, these results indicated that both cytokines significantly reduce the depressive effect of hypoxia on PS amplitude.

This neuroprotective function of IL-10 and TNF-α against the hypoxia-induced depression of PS amplitude looks like preconditioning. Fig. 2B demonstrates that there are significant delays in the time points of a 50% depression of PS amplitude (T_h^d) during the second and the third hypoxic episodes relatively to the T_h^d during the first episode (preconditioning by the first episode against the second and the third hypoxic episodes): T_h^d (1 episode) = 56 ± 4 s; T_h^d (2 episode) = 89 ± 5 s ($P < 0.05$); T_h^d (3 episode) = 100 ± 7 s ($P < 0.05$). However, an application of IL-10 or TNF-α prolonged the T_h^d during the first hypoxic episode (preconditioning by the cytokines against the first hypoxic episode) (Fig. 2C and D). The values of T_h^d during the first, second and third hypoxic episodes under the cytokine treatment were approximately the same: (1) IL-10: 1