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Dynamic aspects of cerebral hypoxic preconditioning measured in an in vitro model

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HIGHLIGHTS

- We studied kinetic aspects of oxygen/glucose deprivation (OGD) in hippocampal slices.
- OGD led to a reduction in the population spike amplitude.
- Preconditioning requires a time window of ca. 60 min.

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ABSTRACT

Preconditioning increases the neurons' resistance to subsequent hypoxia. An in vitro study was conducted to explore kinetic aspects of hypoxic preconditioning. Hippocampal slices were exposed to one single or repeated episodes of oxygen and glucose deprivation (OGD). The interval between OGD episodes varied between 30 min and 180 min. OGD led to a significant reduction in the population spike amplitude. Subsequent episodes of OGD did not result in a further reduction in the population spike amplitude if the interval between the episodes was ca. 60 min, which demonstrated that there were preconditioning effects. In the experiment using an interval of 30 min, population spike amplitude decreased after each OGD episode. The set-up described is useful for detecting damaging effects of OGD as well as preconditioning effects. A time window of ca. 60 min is required to induce protective mechanisms.

1. Introduction

Cerebral hypoxia refers to a condition in which there is a decrease of oxygen supply to the brain even though there is adequate blood flow. There are numerous causes of cerebral hypoxia. These include high altitudes, suffocation, some types of congenital heart disease, cardiac arrest, various types of pulmonary diseases, head trauma, carbon monoxide poisoning, and complications of general anaesthesia. Depending on the duration of deprived oxygen supply, brain damage may occur because neurons are especially sensitive to hypoxic injury.

Oxygen and glucose deprivation (OGD) deplete intracellular ATP stores and lead to the development of neuronal damage and a depression of synaptic transmission [19]. The brain's resistance to hypoxic injury can be transiently augmented by prior exposure to a sublethal preconditioning stimulus. Preconditioning triggers a cascade of intrinsic protective mechanisms which increase the neurons' resistance to subsequent hypoxia. This cascade includes changes in apoptosis, bcl-2 oncoprotein, tumor necrosis factor α, mitogen-activated protein kinase, hypoxia inducible factor α, survivin, stanniocalcin-1, inhibition of iNOS proteins, heat shock proteins, mitochondrial functioning, and others [4,5,7–9,11,16,24,29,32,34,39,41,43,45,46]. According to Nandagopal et al., there are two different types of protection afforded by preconditioning stimuli, i.e. acute and delayed preconditioning. The protective effects of acute preconditioning are protein synthesis independent and are short lived. The effects of delayed preconditioning require new protein synthesis and are sustained for days and weeks [23]. The mechanisms underlying protection by preconditioning are incompletely understood. This is due to comprehensive and dynamic interactions between factors of the cascade. In terms of dynamic aspects, two separate forms of cerebral preconditioning can be identified. Rapid preconditioning occurs when the preconditioning stimuli precede the insult by a brief period (minutes or several hours), whereas delayed preconditioning requires hours or days [2].

Different in vivo and in vitro models were developed to study the mechanisms underlying hypoxic preconditioning. Previous studies have shown that brief reversible hypoxia/hypoglycemia causes immediate loss of evoked potentials in the CA1 region of

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hippocampal slices, and that the extent of restoration of the evoked field potentials following reoxygenation could serve as a parameter for restored cell function [1,27,28]. Recently, it was shown that hypoxia/hypoglycemia can be protected against further impairment in cellular function by introducing a specific interval between the deprivation episodes [30]. The question arose whether variations in the duration of the interval could modify the resulting protection. We have chosen this model to study dynamic aspects of preconditioning effects.

2. Materials and methods

The experiments reported here were conducted in accordance with the regulations of The National Act on the Use of Experimental Animals (Germany) and EC guidelines.

2.1. Animals

Animals were male Wistar rats [Shoe:Wist(Shoe), DIMED Schönwalde, Germany]. The rats were kept under controlled laboratory conditions with a light/dark cycle 12:12 (lights on at 6:00 am), temperature 20 ± 2 °C, and air humidity 55–60%. The animals had free access to commercial rat pellets (TEKLAD Global Diet, Harlan-Teklad, Blackthorn, UK) and tap water. The animals were housed in groups of five in Macrolon IV cages. At the beginning of the experiments, the rats were aged 8 weeks.

The experiments were carried out between 9:00 am and 3:00 pm.

2.2. Experimental set-ups

2.2.1. Recording of evoked field potentials on hippocampal slices prior to and after one hypoxic episode

The animals were killed by a blow to the neck. Transverse hippocampus slices 400 μm thick (one slice per rat for recording) were prepared using a tissue chopper, placed in a submerged-type perfusion chamber at 32.5 °C, and superfused with a medium containing (mM): NaCl 124, KCl 4.9, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.5, $NaHCO_3$ 25.6, D-glucose 10.6, bubbled with 95% O_2 and 5% CO_2 (carbogen). The flow rate of the medium was 1.4 ml/min.

The slices were allowed to equilibrate for at least 2 h before any recording was carried out. Field potentials were recorded extracellularly in the CA1 region in response to constant current stimulation of the Schaffer collaterals with biphasic rectangular pulses of 0.1 ms duration per half wave, it means stimulation was in total 0.2 ms. For this purpose, an electrolytically sharpened and lacquer-coated stainless-steel electrode was positioned in the Schaffer collateral–commissural pathway for stimulation, and a glass microelectrode filled with physiological saline was placed in the pyramidal layer. Four evoked potentials were recorded and amplified with a D/C-coupled amplifier (Axoclamp 2A, Axon Instruments), averaged by a personal computer and stored on a hard disc and analysed with custom-made software. The amplitude of the population spike (pop-spike) was determined as the difference between spike onset and potential peak.

Prior to the experiment, baseline input/output (I/O) curves were determined with four stimulation intensities (beginning with the appearance of potential, then this stimulation intensity multiplied by 1.5, 2 and 2.5). The 1.5 value was determined as the baseline stimulation for this experiment (approximately 40% of the maximum response). After the first I/O curve, the mean of three values was set as 100%. During a period of 1 h before hypoxia, six values were determined. At the end of the experiment, a further I/O curve was recorded.

Oxygen and glucose deprivation (OGD) was induced by changing the perfusion with O_2/glucose-free medium bubbled with 95%N_2/5%CO_2. During OGD, potentials were recorded at an interval of one minute. After the population spike amplitude reached zero, the slices were kept for 7 min in the OGD state. Thereafter they were reperfused with normal medium and carbogen-bubbling.

2.3. Statistics

Input/output curves were evaluated with ANOVA repeated measures using SPSS13+ software. Within-subject variable was time and between-subject factor was OGD. The three evoked potentials obtained prior to stimulation and the final three out of six values obtained after each stimulation were assessed using the same procedure and the within-subject variable was stimulus intensity and the between-subject factor group (i.e. baseline vs. final measurement). The significance threshold was set at $p < 0.05$.

Data were presented as mean ± SEM.

3. Results

3.1. Experiment 1: Recording of evoked field potentials following a single OGD episode

As shown in Fig. 1, there is a significant difference in the course of the population spike (pop-spike) after OGD ($F_{1,48} = 30.54$, $p = 0.001$). The amplitude after OGD is significantly lower ($F_{1,8} = 372.52$, $p < 0.001$).

An analysis of I/O curves showed a significant stimulus strength × test condition interaction (i.e. prior to vs. post hypoxia, $F_{1,16} = 48.12$, $p < 0.001$). This clearly indicates that (i) one period of 7 min hypoxia induced long-term damage to the hippocampal slice preparation; (ii) within a period of 3 h, the pop-spike amplitude did not return to values similar to those prior to OGD; (iii) the set-up is useful for studying OGD in the slice preparation.

3.2. Experiment 2: Recording of evoked field potentials following subsequent OGD episodes at an interval of 3 h

It is known from the literature [26] that pre-exposure to 8% oxygen for 3 h protects the brain against combined hypoxia/ischaemia 24 h later. To find out whether a 7 min period of hypoxia can act as a preconditioning stimulus which induces protective mechanisms, we studied dynamic changes of evoked field potentials in the slice preparation. For that purpose, hippocampal slices were exposed to two 7 min periods of OGD 3 h apart. Evoked potentials were recorded 5 min and afterwards every 15 min over a period of 3 h after OGD.

As shown in Fig. 2, there is a significant difference in the course of the pop-spike after OGD ($F_{2,60} = 19.4$, $p = 0.001$). The amplitude after OGD is significantly lower ($F_{1,10} = 36.89$, $p < 0.001$).

Following the first OGD period, the pop-spike amplitude was significantly lower compared with pre-exposure conditions ($F_{1,10} = 21.36$, $p = 0.001$). Similarly, pop-spike amplitude after the second OGD period was lower compared with pre-exposure conditions ($F_{1,10} = 16.17$, $p = 0.002$). There was no significant difference between the pop-spikes amplitude after OGD ($F_{1,10} = 1.28$, $p = 2.84$).

The course of the I/O curves differed significantly ($F_{2,20} = 19.4$, $p < 0.001$). Compared with pre-exposure conditions, the amplitude was significantly lower following the first ($F_{1,10} = 21.36$, $p < 0.001$) and the second ($F_{1,10} = 16.2$, $p = 0.002$) OGD episode. Data obtained after the first and the second OGD episode did not differ from one another. This indicates that the first episode induced damage to the slice, but there was no further decrease in the pop-spike amplitude following repeated OGD, which might be a result of protective mechanisms induced by the first OGD episode.