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Research Report

Mild hypoxia affects synaptic connectivity in cultured neuronal networks

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ABSTRACT

Eighty percent of patients with chronic mild cerebral ischemia/hypoxia resulting from chronic heart failure or pulmonary disease have cognitive impairment. Overt structural neuronal damage is lacking and the precise cause of neuronal damage is unclear. As almost half of the cerebral energy consumption is used for synaptic transmission, and synaptic failure is the first abrupt consequence of acute complete anoxia, synaptic dysfunction is a candidate mechanism for the cognitive deterioration in chronic mild ischemia/hypoxia. Because measurement of synaptic functioning in patients is problematic, we use cultured networks of cortical neurons from new born rats, grown over a multi-electrode array, as a model system. These were exposed to partial hypoxia (partial oxygen pressure of 150 Torr lowered to 40–50 Torr) during 3 ($n=14$) or 6 ($n=8$) hours. Synaptic functioning was assessed before, during, and after hypoxia by assessment of spontaneous network activity, functional connectivity, and synaptically driven network responses to electrical stimulation. Action potential heights and shapes and non-synaptic stimulus responses were used as measures of individual neuronal integrity. During hypoxia of 3 and 6 h, there was a statistically significant decrease of spontaneous network activity, functional connectivity, and synaptically driven network responses, whereas direct responses and action potentials remained unchanged. These changes were largely reversible. Our results indicate that in cultured neuronal networks, partial hypoxia during 3 or 6 h causes isolated disturbances of synaptic connectivity.

Abbreviations: MEA, multi electrode array; AWFR, array wide firing rate; PSTH, post-stimulus time histogram

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1. Introduction

Chronic mild cerebral ischemia/hypoxia results from prevalent diseases, such as chronic heart failure or obstructive pulmonary disease (Thakur et al., 2010; Vogels et al., 2007c). In chronic mild cerebral ischemia, cerebral perfusion is probably reduced to 35–50 ml/100 g/minute and in chronic hypoxemia oxygen saturation is just below 90% (Bandera et al., 2006; NVALT, 2010). Approximately 80% of patients affected by these conditions have cognitive impairment (Thakur et al., 2010; Vogels et al., 2007c). Unlike acute severe cerebral ischemia, in which neurological impairment is associated with structural damage of neurons as visualized with MRI (Schellinger et al., 2010), in chronic mild cerebral ischemia such signs of damage are lacking (Vogels et al., 2007a), and the exact cause of neuronal dysfunction leading to cognitive impairment is unclear.

Research on the nature of neuronal damage in patients with chronic mild cerebral ischemia so far has focused on the detection of structural (micro) lesions (Vogels et al., 2007a, 2007d). However, in partial as opposed to complete anoxia, functional rather than structural damage probably plays an important role. Already in the 1950s "perfusion thresholds for the brain" were introduced (Symon et al., 1977). It was shown that neuronal activity became impaired with partially reduced perfusion (14–35 ml/100 g/min), along with electroencephalographic (EEG) and evoked potential disturbances, whereas loss of ion gradients across the plasma membrane and cell swelling occur at lower perfusion levels around 4.8–8.4 ml/100 g/min (Bandera et al., 2006; Symon et al., 1977).

The human brain uses approximately 20% of the body's resting oxygen consumption, of which 75% is for signaling processes, including generation of action potentials and synaptic neurotransmission (Attwell and Laughlin, 2001). In rodents, approximately half of the energy used for signaling is for synaptic neurotransmission, which makes synaptic function viable for effects of energy depletion (Attwell and Laughlin, 2001). In humans, the density of neurons is three-to tenfold lower than in rodents with an unchanged density of synapses, implying a three- to tenfold higher number of synapses per neuron (Abeles, 1991). The expected share of synaptic consumption relative to other energy dependent processes is therefore probably also higher.

In patients with chronic mild cerebral ischemia or hypoxemia, the pattern of cognitive decline supports disturbances of synaptic connectivity as a cause of neuronal dysfunction. In these patients, the profile of cognitive disturbances is dominated by disturbed learning (Vogels et al., 2007b), which indeed strongly depends on synaptic integrity (Kandel, 2004). However, the association between cognitive impairment and synaptic connectivity disturbances in these conditions has not been studied yet. This is in part explained by the technical limitations to study synaptic function in patients in a direct way.

Depression of excitatory synaptic transmission during and after variable episodes of acute severe anoxia has been demonstrated in vitro (Fujiwara et al., 1987; Lobner and Lipton, 1993) and in vivo (Bolay et al., 2002; Sun et al., 2002). Isolated synaptic disturbances were even irreversible after transient anoxia, if the depth or duration of hypoxia was sufficiently severe (Bolay et al., 2002; Gao et al., 1999). However, the effect of slowly induced and lasting partial hypoxia has not been studied (Hofmeijer and van Putten, 2012).

Here we use networks of cultured cortical neurons as a model system to study the effect of partial, but not complete hypoxia on neurons' electrophysiological properties. In cultured neuronal networks, interneuronal connections exclusively consist of chemical synapses (Müller et al., 1997). An important advantage of the model to study effects of slowly induced lasting hypoxia is that, unlike acute brain slices, cultures can stay alive for several weeks up to months. We hypothesize that partial hypoxia of various duration causes isolated synaptic connectivity failure, while membrane potential and ability to generate action potentials remain unaffected.

2. Results

2.1. Cultured neuronal networks

Twenty-two cortical neuronal networks were obtained from 22 newborn Wistar rats (male and female) and used for 22 experiments. At the time of the experiments, the cultures' mean age ± standard deviation (SD) was 32±6 days. Osmolarity of the medium of active cultures ranged from 0.319 to 0.511 osmol/kg.

2.2. Induced hypoxia

Replacement of 95% ambient air and 5% CO_2 by 95% N_2 and 5% CO_2 quickly reduced the oxygen concentration under the hood. The partial oxygen pressure of the medium changed slowly by diffusion, from 150 Torr to ~50 Torr after 3 h and a stable value of ~40 Torr after 6 h of hypoxia. Osmolarity of the medium was 0.319 osmol/kg before, and 0.322 osmol/kg after 3 h of hypoxia.

2.3. Before hypoxia

Before hypoxia, all cultures were spontaneously active, with activity at 36 ±16 electrodes. On average we recorded 61,271 ± 55,296 action potentials in the hour immediately before the hypoxic period. All activity patterns contained network bursts (Fig. 1).

2.4. During hypoxia

During both 3 and 6 h of hypoxia, there was a decrease of the three measures of synaptic functioning in all cultures with a statistically significant mean decrease. During 3 h, AWFR decreased to 19 ± 19% at approximately 100 min after the induction of hypoxia ($P<0.0001$, Fig. 2), and functional connectivity expressed as conditional firing probability decreased to 38±13% ($P<0.0001$, Fig. 3). The absolute number of active connections within a culture tended to decrease from 292±376 to 138±163 ($P=0.25$, Fig. 3), and the late response after stimulation decreased to 29±50% ($P=0.005$, Fig. 4) at the