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Research report

Acute hypoxic exposure and prolyl-hydroxylase inhibition improves synaptic transmission recovery time from a subsequent hypoxic insult in rat hippocampus

Sinead Lanigan[a], Alan E. Corcoran[a], Audrey Wall[a], Gatambwa Mukandala[b], John J. O'Connor[a,*]

[a] UCD School of Biomolecular & Biomedical Science, UCD Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland
[b] College of Natural and Applied Sciences, University of Dar-Es-Salaam (UDSM), P.O Box 35064, Dar-Es-Salaam, Tanzania

HIGHLIGHTS

- 30 min hypoxia gives rise to a reversible decrease in EPSP slope in CA1 hippocampus.
- This hypoxia was sufficient to induce stabilization of HIF-1α in hippocampal slices.
- A second hypoxic insult results in a greater rate of recovery of the EPSP.
- DMOG applied before hypoxia results in a greater rate of recovery of the EPSP.
- Preconditioning improves the rate of recovery of synaptic transmission.

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ABSTRACT

In the CNS short episodes of acute hypoxia can result in a decrease in synaptic transmission which may be fully reversible upon re-oxygenation. Stabilization of hypoxia-inducible factor (HIF) by inhibition of prolyl hydroxylase domain (PHD) enzymes has been shown to regulate the cellular response to hypoxia and confer neuroprotection both *in vivo* and *in vitro*. Hypoxic preconditioning has become a novel therapeutic target to induce neuroprotection during hypoxic insults. However, there is little understanding of the effects of repeated hypoxic insults or pharmacological PHD inhibition on synaptic signaling. In this study we have assessed the effects of hypoxic exposure and PHD inhibition on synaptic transmission in the rat CA1 hippocampus. Field excitatory postsynaptic potentials (fEPSPs) were elicited by stimulation of the Schaffer collateral pathway. 30 min hypoxia (gas mixture 95% N_2/5% CO_2) resulted in a significant and fully reversible decrease in fEPSP slope associated with decreases in partial pressures of tissue oxygen. 15–30 min of hypoxia was sufficient to induce stabilization of HIF in hippocampal slices. Exposure to a second hypoxic insult after 60 min resulted in a similar depression of fEPSP slope but with a significantly greater rate of recovery of the fEPSP. Prior single treatment of slices with the PHD inhibitor, dimethyloxalylglycine (DMOG) also resulted in a significantly greater rate of recovery of fEPSP post hypoxia. These results suggest that hypoxia and 'pseudohypoxia' preconditioning may improve the rate of recovery of hippocampal neurons to a subsequent acute hypoxia.

1. Introduction

In the central nervous system approximately 40% of cerebral oxygen is utilised for synaptic transmission (Astrup et al., 1981). Given the high demand for O_2, the relationship between hypoxia and synaptic signaling is very important whereby neurons can alter synaptic transmission in response to hypoxic conditions within minutes. Depending on many factors including the duration of hypoxia, neurons can fully recover upon reoxygenation (Fowler et al., 2003; Lipton and Whittingham, 1978). The depression of synaptic transmission during hypoxia is primarily mediated by adenosine, the concentration of which is greatly increased during cerebral ischemia (Laghi Pasini et al., 2000). The release of adenosine from cells in response to reduced regional blood flow, which is not significant to induce glutamate excitotoxicity,

Abbreviations: EPO, erythropoietin; fEPSP, field excitatory post synaptic potential; DMOG, dimethyloxalylglycine; HIF, hypoxia inducible factor; PHD, prolyl hydroxylase domain; VEGF, vascular endothelial growth factor

* Corresponding author.
E-mail address: John.oconnor@ucd.ie (J.J. O'Connor).

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suggests adenosine may play some role in alleviating the potential for excitotoxicity (Matsumoto et al., 1992; Duarte et al., 2016).

The recovery of synaptic transmission after a period of hypoxia and repeated hypoxia has been previously investigated by a number of laboratories. We have previously demonstrated that rats treated with intermittent hypoxia for 7 days showed improved recovery times for synaptic transmission in both the CA1 and dentate gyrus regions of the hippocampus (Wall et al., 2014). Sebastião et al., (2001) demonstrated an inhibitory role for adenosine in the recovery from 90 min hypoxia, an effect that was reversed by tetrodotoxin. Frenguelli et al., (2003) also demonstrated a role for adenosine A_1 receptors (desensitization) in the recovery from hypoxia, although Vlkolinský and Stolc (1999) showed some of these effects to be irreversible.

Other previous studies have demonstrated that application of hypoxic or sub-lethal ischemic conditions prior to stroke significantly reduces infarct severity in neonates and adult rats (Vannucci et al., 1998; Gidday et al., 1999; Miller et al., 2001; Bernaudin, et al., 2002a). It has been proposed that tolerance to ischemic insults by hypoxia preconditioning is due to activation of hypoxia inducible factors (HIF) and HIF target genes including VEGF, EPO, GLUT-1 and adrenomedullin (Bergeron et al., 1999; Bernaudin, et al., 2002b). Under normoxic conditions the activation of HIF-1∝ is blocked by prolyl hydroxylase domain proteins (PHDs). Whether these factors would have a role to play in acute hypoxia (30–60 min) remains to be seen.

Since their discovery, PHDs have become a novel therapeutic target for hypoxic injuries. Pharmacological inhibition of PHDs via 2-OG competitive antagonism or iron chelation has become an attractive strategy to precondition neurons for a subsequent hypoxic stress. *In vitro*, various types of PHD inhibitors have been shown to stabilize HIF-1α, either by 2-OG antagonism (N-oxalylglycine, DMOG, 3,4-dihydroxybenzoate, DHB), iron chelation (deferoxamine, DFO) or heavy metal substitution of iron (CoCl) (Epstein et al., 2001; Huang et al., 2003; Siddiq et al., 2005). We have recently demonstrated fast acting (within minutes) mechanisms of action of PHD inhibition on synaptic signaling and plasticity (Corcoran et al., 2013; Corcoran and O'Connor, 2013; Lanigan and O'Connor, 2018). In the following studies we have set out to investigate the effects of an initial hypoxic (95% N_2/5% CO_2) exposure or pre-treatment with the PHD inhibitor, DMOG, on the rate of recovery of synaptic transmission from a subsequent hypoxic exposure (see methods).

2. Results

2.1. Acute hypoxia decreases synaptic signaling and increases HIF stabilization

We recorded fEPSPs from the stratum radiatum pyramidal neurons of the CA1 region of the rat hippocampus every 30 s. During hypoxia (95% N_2/5% CO_2) superfusion, the fEPSP slope was significantly decreased to 8.0 ± 3.5% control (n = 7, P < 0.001) within 15 min. 20 min after re-oxygenation the fEPSP slope recovered to 92.4 ± 9.5% control (Fig. 1A). Using fluorescence quenching oxymetry, we monitored the oxygen tension at the surface and within the slice at a depth similar to our recording electrode (~100 μm). Partial pressures of oxygen (PO_2) were measured during control O_2, hypoxia and subsequent reoxygenation. 100 μm below the surface we detected a rapid reduction of PO_2 which reached 7.8 ± 5.9 mmHg 5 min after hypoxia superfusion (Fig. 1Bi). At the surface of the slice PO_2 fell to 45.4 ± 6.7 mmHg (Fig. 1Bii). We then measured the levels of HIF-1α to determine if our hypoxia paradigm resulted in phenotypic changes associated with hypoxia superfusion. Immunoblotting of hippocampal slices exposed to 30 min hypoxia showed a 6.1 ± 0.8 fold increase in HIF-1α expression compared to control slices (n = 4, P < 0.01). 30 min reoxygenation of slices resulted in a significant degradation of HIF-1α compared to hypoxia, although still significantly higher than control tissue. Application of DMOG (1 mM) for 30 min also significantly increased HIF-1α expression (Fig. 1C).

2.2. Hypoxic preconditioning improves the recovery rate from a subsequent hypoxic insult

In another set of experiments fEPSPs were evoked every 30 s in the CA1 region of the hippocampus for 90 min before superfusion of media equilibrated with 95% N_2/5% CO_2 (hypoxia). fEPSP slope decreased significantly to 9.1 ± 0.9% control (n = 6, P < 0.001) 15 min after hypoxia. fEPSP slope returned to baseline (99.5 ± 4.4%) 15 min after reoxygenation (Fig. 2A). In a parallel set of experiments an initial 30 min superfusion with 95% N_2/5% CO_2 resulted in a significant decrease in fEPSP slope to 9.6 ± 1.7% control (not significantly different from the first set of experiments). Again fEPSP slope returned to baseline upon reoxygenation (95.5 ± 3.3%). 30 min after reoxygenation, a second 30 min hypoxia was initiated. The second insult resulted in a similar decrease in fEPSP slope (8.1 ± 2.8% after 15 min). The fEPSP slope returned to 100.1 ± 6.3% control with a higher recovery rate upon reoxygenation. To determine changes in recovery rate we analysed the time taken to return to pre hypoxia baseline synaptic transmission (t_{max}) and the time taken to reach 50% of the maximum recovery (t_{50}). The t_{50} was significantly reduced to 4.7 ± 0.6 min (versus 10.4 ± 1.5 min in single hypoxia perfused slices) following reoxygenation of preconditioned slices (Fig. 2B and C). Additionally, t_{max} was significantly reduced to 6.8 ± 0.7 min (versus 13.9 ± 1.8 min in single hypoxia perfused slices; n = 6, P < 0.01) after reoxygenation (Fig. 2B and C).

2.3. DMOG preconditioning improves the recovery rate from acute hypoxia

Application of DMOG resulted in a 10% depression of fEPSP similar to that observed by Batti et al. (2010). Therefore in these experiments the baseline was normalised to 100% before the hypoxia exposure. In the presence of DMOG, 30 min hypoxia resulted in a significant decrease in fEPSP slope to 16.2 ± 2.4% which was not significantly different to hypoxia alone treated slices (n = 6, P > 0.05). Reoxygenation resulted in an increase of fEPSP slope to 90.6 ± 3.8% control which was not significantly different to hypoxia alone treated slices 30 min after reoxygenation (Fig. 3A). To determine changes in recovery rate we analysed the time taken to return to pre-hypoxia baseline (t_{max}) and the time taken to reach 50% of the maximum recovery (t_{50}). In DMOG treated slices t_{50} was significantly decreased to 6.5 ± 0.4 min (n = 6, P < 0.01) following reoxygenation. t_{max} was also significantly decreased to 8.7 ± 0.5 min (n = 6, P < 0.0, compared to single hypoxia perfused slices) after reoxygenation (Fig. 3B and C).

3. Discussion

In these experiments we have observed a significant increase in the recovery rate of synaptic transmission in the hippocampal CA1 region following a prior acute superfusion of 95% N_2/5% CO_2 (hypoxia) or DMOG application. These responses were all associated with an up-regulation of HIF-1∝ and a reduction in PO_2 levels both on the surface and 100 μm below the surface of the slices.

In vitro electrophysiological recordings require the perfusate to be constantly bubbled with 95%O_2/5%CO_2 gas. Whilst the high O_2 content of this gas mixture can produce a hyperoxic environment, this methodology is routinely used for electrophysiological recording in acute hippocampal slices and has been shown to be best practice to preserve function within the slice (Aitken et al., 1995). PO_2 in the intact CNS ranges from below 10–35 mmHg in normobaric air but there is significant variance and in particular for the hippocampus (Garcia et al., 1985). Therefore brain tissue exceeding 40 mmHg in the CNS might be considered hyperoxic (D'Agostino et al., 2007). However the partial pressure of O_2 decreases from the surface of the slice to the middle layers (see Fig. 1). Experiments carried out in our laboratories show

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