The Effects of Hypoxia, Prolyl Hydroxylase Inhibition And TNF-alpha On Synaptic Transmission in The Rat Hippocampus

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Two key features of cerebral ischemia are hypoxia and inflammation. Hypoxia results in both acute and chronic cellular adaptation. Adenosine is a key acute regulator of neuronal function in response to hypoxia. Chronic adaptation is regulated by hypoxia inducible factor (HIF). Degradation of HIF under normoxic conditions is regulated by proline and asparaginyl hydroxylases. Inhibition of these enzymes has become a novel target to modulate the hypoxic response for therapeutic benefit. Inhibition of prolyl-4-hydroxylase domain (PHD) has been shown to delay neuronal cell death, and protect against glutamate-induced ischemic injury in the hippocampus. Tumour necrosis factor alpha (TNF-α) concentration significantly increases during hypoxia and has been shown to impair the recovery of synaptic signalling following hypoxia. In this study we have examined the acute effects of hypoxia, the PHD inhibitor dimethyloxalyl glycine (DMOG) and TNF-α on synaptic signaling and plasticity in the rat hippocampus.

350µm transverse hippocampal slices were prepared from P21 male Wistar rats (anaesthetized by 4% isoflurane inhalation and decapitated) as has previously been described. Field excitatory postsynaptic potentials (fEPSPs) were elicited by stimulation of the Schaffer collateral pathway in the CA1 region of the hippocampus using a monopolar glass electrode. Hypoxia was induced by substituting 95% O₂ / 5% CO₂ with 95% N₂ / 5% CO₂. PO₂ was measured using O₂ monitor (OXILITE™). Long term potentiation (LTP) was induced by high frequency stimulation with 3 trains separated by 20 s (each train of 1 s at 100Hz).

Oxygen tension was decreased to hypoxic levels within 5 minutes (8.2 ± 4.5mmHg, n=4). PO₂ was returned to baseline within 3 minutes of reoxygenation. Induction of hypoxia resulted in a significant decrease in fEPSP slope compared to controls after 10 minutes (10.99 ± 3.33%, n=5, P<0.001) which was reversed upon reoxygenation (96.47 ± 13.27%). LTP was not significantly different from controls. Application of 1mM DMOG resulted in a significant decrease in fEPSP slope compared to controls (DMOG, 87.71 ± 6.3%, n=9, P<0.001). DMOG significantly decreased LTP (113.69 ± 15.45, n=5, P<0.005). Application of 5ng/ml TNF-α had no significant effect on synaptic signalling (95.66 ± 8.8%, n=6, P>0.05). Induction of hypoxia resulted in a response not significantly different to hypoxia controls (9.8 ± 78%, n=6, P>0.05). Reoxygenation resulted in a return to baseline fEPSP slope which was not significantly different to controls (85.75 ± 23.4%, n=6, P>0.05).

In these experiments we examined the effects of hypoxia and the hypoxic mimetic, DMOG, on synaptic plasticity on synaptic signalling and plasticity. We show that hypoxia induces a significant decrease in fEPSP slope which has been shown to the modulated by adenosine acting on presynaptic A1 receptors. DMOG resulted in a small but significant decrease in synaptic signalling which is not mediated by adenosine and possibly a result of the glycine molecule of the compound acting on surface receptors rather than PHD inhibition. TNF-α had no effect on synaptic signalling nor on the recovery from hypoxia as previously reported. This may be due to the length of the exposure to TNF-α.