Mechanism and Consequences of Extracellular Adenosine Accumulation in the Hypoxic Hippocampal Slice

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ABSTRACT

This thesis examines the alterations in electrophysiological function during hypoxia in the rat hippocampal slice, in particular those alterations induced by extracellular accumulation of adenosine. Evaluation of electrophysiological responses in the *in vitro* rat hippocampal slice is a standard model for neurophysiological investigations, and has been used extensively in the study of hypoxia. Rat hippocampal CA_1 pyramidal neurons are considered to be selectively vulnerable to hypoxic damage; however, adenosine A_1 receptor-mediated depression of excitatory synaptic transmission during hypoxia may protect neurons. Enhanced excitability of neurons is reported following hypoxia, and is proposed to contribute to neuronal damage.

In the present studies, the post-hypoxic recovery of synaptically evoked field potentials was used to measure the survival of CA_1 pyramidal neurons, and to assess any functional alterations precipitated by hypoxia. Excitatory synaptic transmission in the hippocampal CA_1 area was depressed during 30 minutes of hypoxia due to activation of adenosine A_1 receptors, this depression being sensitive to A_1 receptor antagonism. Synaptic transmission always recovered upon reoxygenation, indicating that CA_1 neurons survived prolonged inhibition of respiration, and that adenosine A_1 receptor activation during hypoxia was not necessary for this neuronal recovery. Following prolonged hypoxia, recovered postsynaptic potentials manifested a permanent hyperexcitability, obvious as multiple population spikes. A method for quantifying this hyperexcitability was developed, which established that it results from a postsynaptic alteration in CA_1 neuronal excitability. Similar hyperexcitability also followed normoxic adenosine exposure, but was not produced by agonists for the A_1 or A_2 adenosine receptor subtypes. Apparently, adenosine activation of an unidentified receptor subtype produces postsynaptic hyperexcitability, and this may be the mechanism of hyperexcitability following hypoxic adenosine accumulation.

This work also examined the mechanism of adenosine accumulation during hypoxia, by assessing A_1 receptor-mediated depression of synaptically evoked field potentials. Hypoxia, respiratory inhibitors and mitochondrial uncoupling agents all caused an adenosine induced depression of synaptic transmission, whereas inhibitors of oxidative phosphorylation did not. Furthermore, alleviation of ATP depletion failed to increase the latency of synaptic depression during mitochondrial uncoupling. It seems likely, therefore, that the massive adenosine accumulation during energy deprivation is not a direct consequence of ATP depletion, but is most likely a result of stimulation of cytosolic 5'-nucleotidase and inhibition of adenosine kinase, possibly by changes in free AMP, pH or cytosolic calcium.