Role of P1 and P2 purinergic receptors during severe in vitro ischemia in the rat CA1 hippocampus and dentate gyrus

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The hippocampus is comprised of two distinct subfields that show different responses to hypoxic/ischemic brain injury: the CA1 region is particularly susceptible, whereas the dentate gyrus (DG) is quite resistant. A major resistance of the DG to ischemia in adulthood is probably due to the regenerative capacity of the neural stem cells of the subgranular zone (SGZ) that proliferate and maturate into neurons, astrocytes and oligodendrocytes in response to multiple factors, including ischemic injury. During ischemia, ATP intracellular concentrations decline to refurnish energy to cells. However, in this condition ATP outflow from cells increases, as demonstrated in in vivo or in vitro ischemic models. Extracellularly ATP acts on P2 receptors that are subdivided into ligand-gated ion channels, P2X, and metabotropic P2Y receptors. Several data highlight the involvement of P2X7 and P2Y1 subtypes in the control of ischemic brain damage. The synaptic and proliferative response of the DG to severe oxygen and glucose deprivation (OGD) in acute rat hippocampal slices and the contribution of P1 and P2 purinergic receptors to these phenomena was investigated (Maraula et al., 2013; 2014). Nine min OGD always induced the appearance of anoxic depolarization (AD), a clear sign of tissue damage, and the irreversible block of synaptic activity up to 24 hours from the end of the insult, as assessed by extracellular recordings of field excitatory post-synaptic potentials (fEPSPs) in the dentate molecular layer of the hippocampal slices. The selective antagonist of adenosine A_{2A} receptors ZM241385 (4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol), applied before and during OGD, prevented or delayed the appearance of AD and protected from the irreversible block of neurotransmission induced by the 9-min OGD. Similar effects were obtained in the presence of the selective antagonists of $P2Y_1$ receptor MRS2179 (2'-Deoxy-N^6-methyladenosine 3',5'-bisphosphate) and of $P2X_7$ receptor BBG (Brilliant Blue G). In hippocampal slices prepared from bromodeoxyrudine (BrdU)-treated rats and incubated with the immature neuronal marker doublecortin (DCX), the number of $BrdU^+$ cells of the SGZ was significantly decreased 6 hours after OGD, but returned to control values 24 hours thereafter, when a significant increase of DCX immunofluorescence was also observed. The decrease of the number of $BrdU^+$ cells 6 hours after OGD was antagonized by ZM241385 and BBG, but not by MRS2179 that, 24 hours after OGD, reduced the number of $BrdU^+$ cells.
Data indicate that A_{2A}, $P2Y_1$ and $P2X_7$ receptors contribute to the early damage induced by OGD in the DG likely contributing to glutamate-induced excitotoxic effects that causes irreversible synaptic failure after severe OGD. A_{2A} and $P2X_7$ receptors are also involved in the decreased proliferation of immature neuronal cells at a precocious time after OGD.

Maraula et al. (2013). *Neuropharmacology* 67:511-20.
Maraula et al. (2014) *PLoS One* 9(12):e115273.