Antagonism of $P2X_7$ and $P2Y_1$ purinergic receptors prevents synaptic failure induced by oxygen and glucose deprivation in rat dentate gyrus

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Adenosine 5'-triphosphate (ATP) is attracting increasing attention as a messenger in the central nervous system during different physiological and pathological events, exerting its effects via activation of P2 purinergic receptors, subdivided into the ligand-gated ion channels $P2X_{(1-7)}$ and metabotropic $P2Y_{(1,2,4,6,11,12,13,14)}$. In the central nervous system, including dentate gyrus (DG), P2X and P2Y receptors are broadly expressed on both neurons and glial cells.
In this study we investigated the role of P2 purinergic receptors during oxygen and glucose deprivation (OGD) in the DG. We conducted extracellular recordings of DG field excitatory post-synaptic potentials (fEPSPs) from slices acutely prepared from male Wistar rats (150-200g body weight).
In agreement with our previous results (*Maraula et al., 2013*), in the DG, 9-min OGD elicited an irreversible loss of fEPSP and was invariably followed by the appearance of anoxic depolarization (AD), an unambiguous sign of neuronal damage. The application, before and during OGD, of two selective P2 antagonists, N^6-methyl-2'-deoxyadenosine-3',5'-bisphosphate (MRS2179, selective for $P2Y_1$ receptor, 10 μM, *n*=21) and Brilliant Blue G (BBG, selective for $P2X_7$ receptor, 10 μM, *n*= 19) did not modify fEPSP outcome before and during OGD, but prevented AD appearance and allowed a significant recovery of neurotransmission after 9-min OGD, when slices were reperfused in oxygenated, glucose containing, solution (85.0 ± 4.3% of pre-OGD values in BBG, and 98.7 ± 8.5% of pre-OGD values in MRS2179, in comparison to 3.9 ± 4.4%, n=34, found in untreated OGD slices).
The effects of 9-min OGD on proliferation and maturation of cells localized in the subgranular zone of DG of slices prepared from 5-bromo-2'-deoxyuridine (BrdU) treated rats were investigated at different times (3, 6 and 24 hours) from the end of 9-min OGD. In order to study cell maturation, slices were incubated with an immature neuronal marker, doublecortin (DCX).
The number of $BrdU^+$ cells in the SGZ was significantly decreased 6 hours after OGD (from 23.6±2.7 before, *n*=14, to 15.5±1.6 after OGD, *n*=12). This effect was antagonized by MRS2179 (10 μM, *n*=4) and BBG (10 μM, *n*=4). Twenty-four hours after 9-min OGD, the number of $BrdU^+$ cells returned to control values and an increased arborisation of tertiary dendrites of DCX^+ cells was observed, indicating cell maturation. MRS2179 and BBG did not modify the effect of OGD on cell maturation.
Data indicate that the selective antagonism of $P2X_7$ and $P2Y_1$ receptors has a protective effect on neurotransmission, preventing the depression of synaptic activity and AD appearance induced by a severe OGD in the rat DG.

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