Regulation of aerobic glycolysis by agonists selective for the canonical L-lactate receptor HCAR1

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Astrocytes provide energy to support neurons, which involves astroglial aerobic glycolysis, where the metabolite pyruvate is converted to L-lactate by lactate dehydrogenase (Dienel GA (2012) ASN Neuro 4(5); Vardjan N et al., (2018) Front Mol Neurosci 11:148). We are interested to understand the regulation of astroglial aerobic glycolysis by L-lactate, which is not only fuel but also likely represents an extracellular signal. L-lactate signaling may occur through several mechanisms, including the activation of the L-lactate-sensitive receptors, such as the G_i-protein coupled receptors HCAR1 or the yet unidentified plasma membrane receptor(s). It is known that extracellular L-lactate in astrocytes activates adenylyl cyclase, elevates cytosolic cAMP and accelerates aerobic glycolysis. Interestingly, by using selective agonists for HCAR1, even in the absence of this receptor, in astrocytes from HCAR1-knockout (KO) mice, elevation in cytosolic cAMP was still recorded. This indicates that in addition to the HCAR1, these agonists activate also a yet unidentified L-lactate receptor-like mechanism, which is also present in undifferentiated 3T3-L1 cells (Vardjan N et al., (2018) Front Mol Neurosci 11:148). To identify the new L-lactate receptor we determined potential candidates by using bioinformatic analyses and molecular dynamics. Furthermore, by using the CRISPR/Cas9, 3T3-KO cell lines were made. We used a FRET (fluorescence resonance energy transfer) nanosensor to measure the elevation of the intercellular L-lactate, upon stimulation of the knocked-out cells with different concentrations of selective agonists. On the basis of our preliminary results, we noticed differences in dose-dependent responses and responsiveness between wildtype and KO cells. This research will help us to better understand the dynamics of the mechanisms of GPCR-mediated signaling pathways in astrocytes and in 3T3 cells.