Orexin-A Protects Against Oxygen–Glucose Deprivation-Induced Neuronal Injury By Up-Regulating Endocannabinoid Synthesis In Primary Cortical Neurons

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Orexin-A, a neuropeptide that is widely distributed in the central nervous system and peripheral tissues, plays an important role in many physiological functions including the control of food intake, sleep–wake behavior and energy balance (Sakurai. 2014). This neuropeptide also increases neuronal viability and protects cortical neurons against oxidative stress (Soko?owska et al., 2014). It is well established that, in the hypothalamus, a functional interaction exists between endocannabinoids (ECs) and orexin-A (OX-A) signaling, both of which play modulate food intake. In a recent study, we found a positive control by OX-A on the levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) in the hypothalamus of obese mice (Cristino et al., 2015 submitted). We also demonstrated that in hypothalamic neurons an increased endocannabinoid tone, as well as exogenous treatment with a CB_1 agonist prevent leptin-induced radical oxygen species (ROS) accumulation (Palomba et al., 2015), one of the major causes of neuronal damage. The aim of the present study was to investigate the role of the endocannabinoid/orexin-A interaction in ROS-mediated neuronal injury induced by oxygen-glucose deprivation (OGD) in primary cortical neurons. We found that in these neurons ROS production as well as cell death induced by OGD were prevented by both OX-A and ACEA (a selective CB_1 receptor agonist), in a manner sensitive to either OX-A receptor-1 or CB_1 receptor antagonists, respectively. Interestingly, AM251 (a CB_1 receptor antagonist/inverse agonist), also prevented the protective effect of OX-A, indicating a role of CB_1 activation in OX-A-mediated cell survival. Moreover, OGD failed to induce ROS production and toxicity in primary cortical neurons isolated from monoacylglycerol lipase (MAGL, the 2-AG hydrolyzing enzyme) knock-out mice, where we found high levels of 2-AG. This effect was most likely due to increased 2-AG levels since AM251 treatment restored the OGD-induced ROS formation and cell death. OX-A-induced EC-mediated activation of the ERK (extracellular-regulated kinase) and PI3K/Akt (phosphoinositide-3-kinase/Akt) survival/neuroprotection pathway might be involved in the counteraction of OGD-induced injury since both OX-A and ACEA activated ERK1/2 and Akt. This activation was prevented by either SB334867(an OX-A receptor-1 antagonist) or AM251. Furthermore, OX-A and ACEA protective effects were prevented by PD98059 (a MAPK inhibitor) and LY294002 (a PI3K inhibitor).
Taken together, these results strongly suggest that, at least in vitro, OX-A leads to protection against ROS-induced neuronal injury through the stimulation of 2-AG production.

Sakurai T. (2014) *Nat Rev Neurosci.* 15, 719-731.
Sokolowska et al, (2014) *J Mol Neurosci.* 52, 48-55.
Cristino et al, (2015) *Neuropsychopharmacol. in press.*
Palomba et al, (2015) *J Biol Chem.* 290, 13669-13677.