Leptin-controlled orexin/endocannabinoid interactions in the mouse periaqueductal grey: role in the regulation of the descending antinociceptive pathway

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Activation of excitatory output neurons in the ventrolateral periaqueductal gray (vlPAG) projecting to the rostral ventromedial medulla (RVM) causes antinociceptive responses via OFF cells stimulation and ON cell inhibition. We demonstrated that this descending nociceptive pathway is under the control of cannabinoid receptor type-1 (CB1). Moreover, 2-AG deeply affects nociception via CB1 stimulation, and its concentration is higher in the PAG and RVM of mice in neuropathic pain conditions. Orexins are hypothalamic peptides known to modulate arousal, feeding, reward and antinociception via orexin receptors (OX-R). In obese *ob/ob* leptin knock-out mice, orexin-A (OX-A) expression increases in the fibers projecting to vlPAG and 2-AG levels increase in the vlPAG. Recently, Ho and collaborators demonstrated that OX-A, by activating OX-AR (OX-A receptor) in the vlPAG of rats, stimulates the synthesis of 2-AG and retrograde inhibition of the tonically active GABAergic circuit (disinhibition) thus inducing activation of descending nociceptive pathway. On this basis we hypothesized the existence of a leptin-controlled orexin/endocannabinoid interaction in the modulation of the pain network leading to nociception. In this study we have validated this hypothesis using a combination of electrophysiological (*in vivo* extracellular recordings), immunohistochemical (OX-A, OX-AR and CB1 single and multiple localization), ultrastructural (CB1/OX-A immunogold labeling on symmetric or asymmetric synapses) and behavioral (nociception in the "plantar test' and in spontaneous and tail-flick test approaches in wt and *ob/ob* mice.
We observed that OFF (anti-nociceptive) and ON (pro-nociceptive) cells are more and less active, respectively, in*ob/ob*compared to wt. We found a significant increase of number and intensity of OX-A fibers in the PAG of*ob/ob*mice and this was accompanied by a two-fold increase of pre-prorexin mRNA expression in the LH compared to wt mice. OX-AR/DAGLalpha expression colocalized in a limited subset of PAG neurons through a electron microscopy approach. Moreover, CB1 receptors were expressed at symmetric synapses to OX-AR-expressing neurons thus suggesting an heterosynaptic pathway. The pharmacological blockade of the OXR1 into the PAG produced pro-nociceptive effect in wt mice detected by both paw withrawal and ON OFF cell activity. Interestingly, in the ob/ob mice the dose of the OX-AR antagonist able to generate the pronociceptive effect were two fold that used in wt mice suggesting a change of this system in the absence of leptin. On the other hand, AM251, a selective CB1 antagonist also induced pro-nociceptive effect in wt mice and needed of lower dose in the ob/ob mice suggesting a tight cross-talk between leptin-orexin and cannabinoid systems. The endocannabinoid level measurements further confirmed the data.
CONCLUSIONS: Here we provide evidence supporting that the heterosynaptic endocannabinoid spread in the vlPAG after OX-AR activation is modulated by leptin. The leptin-related increase of OX-A signalling in PAG is accompained by incresed activation of OX-AR which are GqPCRs and could initiate the GqPCR-PLC-DAGL-2AG retrograde inhibition onto tonic GABAergic transmission in the vlPAG , leading to the potentiation of antinociception. Finally, we show that, beside the feeding and arousal, the orexin system could be highly involved in the pain modulation and is activity is possibly regulated by the leptin-cannabinoid system interaction.

References

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