Role of Orexin A Signaling in Dietary Saturated Fatty Acid–Activated Microglial Cells

Cayla M. Duffy1,2, Charles J. Billington1,3,4, Catherine M. Kotz1,2,4, Joshua P. Nixon1,2, and Tammy A. Butterick1,2

1Veterans Administration Medical Center, Minneapolis, MN, USA 55417
2Department of Food Science and Nutrition, University of Minnesota, St Paul, MN USA 55010
3Department of Medicine, University of Minnesota, Minneapolis, MN
4Minnesota Obesity Center, St Paul, MN

Excess saturated fatty acids (SFAs) such as palmitic acid (PA) contribute to hypothalamic inflammation and subsequent metabolic dysregulation. 1, 2 In rodent models, high fat diets activate microglial nuclear factor-kB (NFkB) nuclear translocation, initiating release of the pro-inflammatory cytokines tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6). 3, 4 Further, SFA–activated microglia induce cell death in nearby neurons. 5 Conversely, the neuroprotective peptide orexin A (OXA, hypocretin) prevents neurodegeneration and inflammation through a microglial mediated pathway, reducing apoptosis through activation of orexin receptors 1 and 2 (OX1R, OX2R). 6, 7 We present here results of a study investigating modulation of microglial activation by OXA after exposure to SFAs.

Overall Hypothesis

Orexin A attenuates palmitic acid-induced microglial activation and reduces release of pro-inflammatory cytokines.

Aim 1: Characterize PA induced inflammatory changes in microglial cells.

Aim 2: Determine if OXA reduces pro-inflammatory cytokine release in microglial cell model.

Cryopreserved microglial cells were treated with 0.1 mM palmitic acid (PA) and 1 mM OXA for 24 hours. This treatment was repeated with or without 10 ng/mL lipopolysaccharide (LPS) stimulation to determine whether OXA inhibition of pro-inflammatory cytokine production from PA-stimulated microglia is increased in the presence of LPS.

Methods

Lymphocytes were isolated from peripheral blood mononuclear cells (PBMCs) using Ficoll-hypaque density techniques. Cells were cultured for 7 days in RPMI-1640 media supplemented with 10% FBS and 1% PSN (DMEM; Invitrogen) and maintained at 37°C with 5% CO2. Cells (7.8 × 10^6) were cultured in 24 wells in 2 mL of media for 24 hours prior to 1× OXA, 0.001× OXA pretreatment and 1× OXA (0.1 mM) or hypomelastatin (LPS, 0.1 μg). Western blotting: Protein samples (15 μg) separated on polyacrylamide-SDS gels, transferred to nitrocellulose membrane, and blocked using Snap ID system (Millipore). Antibodies: Iba-1 (ab5076; Abcam) and GAPDH (NB100-56875; Novus) primary; HRP conjugated anti-goat IgG (ab97100; Abcam) or anti-rabbit IgG (ab97051; Abcam) secondary. All filters for HRP-antibody filters were developed and band density was determined (Eagle Eye II, Stratagene); data normalized to GAPDH (Fig 1).

Orexin A attenuates palmitic acid-induced cytokine release in microglia.

Cell Viability Assay for Hypothalamic Cells:

Cell Viability Assay for Hypothalamic Cells: Cell survival was determined using the resazurin-based assay producing a fluorescent signal proportional to number of living cells. 8 Activity was determined using SpectraMax M5, data reported as relative fluorescence units (RFU) % changes control.

Statistical Methods: Statistical differences were determined by repeated, two-tailed t-test using GraphPad Prism 5. *p<0.01 vs. control, **p<0.01 vs. OXA only, ***p<0.01 vs. OXA only, +++p<0.01 vs. OXA only, +++p<0.001 vs. OXA, p<0.001 vs. 0.01, p<0.001 vs. PA only. 

Results

1. OXA pretreatment reduces TNFα secretion compared to PA only treatment.
2. Orexin A increases OX1R expression in microglia. Orexin receptor 1 (A) expression but not OX2R (B) is increased following PA and LPS stimulation.
3. Orexin A attenuates hypothalamic neuronal cell death in microglia–conditioned media. Adult mouse hypothalamic cells (A12) have increased viability when conditioned media (CM) from OXA stimulated microglia is added. Cell viability is reduced following the addition of PA and LPS treatment (0.1 mM) significantly increases Iba-1 protein in BV-2 microglial cells.
4. Orexin A decreases lipid peroxidation and apoptosis in a novel hypothalamic cell model. Neuron–microglia crosstalk is essential to maintain brain homeostasis. To our knowledge, this is the first report demonstrating PA increases OX1R expression in microglia. Increased microglial OX1R expression in response to pro-inflammatory stimuli could represent a compensatory response to reduce release of inflammatory cytokines. As OXA is neuroprotective, 4 acting upon microglia may be one mechanism through which OXA protects against apoptosis and lipid peroxidation. Future studies will further explore this mechanism and focus on defining the role of OXA as an immunomodulator.

Summary

- PA increases microglial activation through increased IL-6 (Fig 2).
- Orexin receptor 1, but not OX2R is increased with PA and LPS stimulation (Fig 3A-B), suggesting OX1R primarily participates in inflammatory responses.
- Pro-inflammatory cytokines IL-6 (Fig 4A) and TNFα (Fig 5) are increased by PA and LPS.
- OXA pretreatment decreases IL-6 and reduces TNFα expression (Figs. 4A, 5).
- Anti-inflammatory IL-4 expression is reduced by PA and LPS (Fig 4B).
- OXA attenuates PA–activated microglia–induced cell death in neurons exposed to conditioned media (Fig 6).

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References