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Orexin: Pathways to obesity resistance?

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Abstract

Obesity has increased in prevalence worldwide, attributed in part to the influences of an obesity-promoting environment and genetic factors. While obesity and overweight increasingly seem to be the norm, there remain individuals who resist obesity. We present here an overview of data supporting the idea that hypothalamic neuropeptide orexin A (OXA; hypocretin 1) may be a key component of brain mechanisms underlying obesity resistance. Prior work with models of obesity and obesity resistance in rodents has shown that increased orexin and/or orexin sensitivity is correlated with elevated spontaneous physical activity (SPA), and that orexin-induced SPA contributes to obesity resistance via increased non-exercise activity thermogenesis (NEAT). However, central hypothalamic orexin signaling mechanisms that regulate SPA remain undefined. Our ongoing studies and work of others support the hypothesis that one such mechanism may be upregulation of a hypoxia-inducible factor 1 alpha (HIF-1 α)-dependent pathway, suggesting that orexin may promote obesity resistance both by increasing SPA and by influencing the metabolic state of orexin-responsive hypothalamic neurons. We discuss potential mechanisms based on both animal and *in vitro* pharmacological studies, in the context of elucidating potential molecular targets for obesity prevention and therapy.

Keywords

Orexin (hypocretin); Hypothalamus; Obesity; Spontaneous physical activity

Spontaneous Physical Activity and Orexin

Despite genetic influences and obesigenic environments, propensity for obesity varies among humans and animals, with some individuals resisting obesity [1–6]. Obesity resistance is positively correlated with elevated spontaneous physical activity (SPA), defined as all movement not associated with formal exercise; SPA contributes to obesity resistance

through an increase in non-exercise activity thermogenesis (NEAT) [Reviewed in 7]. The thermogenic effect of subtle but chronic increased movement has an important influence on energy balance, and NEAT contributes significantly to obesity resistance [8,5,9,10]. While noteworthy studies in both humans and rodents have demonstrated that differences in activities of daily living and propensity for general movement can predict obesity resistance [11,12,5,13], the underlying central neural mechanisms driving SPA remain relatively undefined.

Many brain regions, neurotransmitters, and neuropeptides are thought to contribute to SPA and obesity resistance [14]. The hypothalamic neuropeptide orexin (hypocretin), initially of interest in energy balance due to its effect on feeding and arousal, has proven to play an integrative role in energy balance and expenditure [15,16]. Throughout the past decade, work in our laboratory and elsewhere has collectively demonstrated the importance of orexin in obesity resistance [17–19,9]. However, to date the cell signaling mechanisms through which orexin effects long- and short-term change in SPA is unknown. Evidence from studies of ischemia and oxidative stress have suggested that orexin signaling can influence transcription factors involved in resistance to these stressors, and that these pathways might be critically involved in orexin effects on metabolism. The focus of this review will be to evaluate hypothalamic orexin signaling cascades that potentially underlie mechanisms through which orexin confers obesity resistance.

Orexin and Obesity Resistance

The link between susceptibility to obesity and interindividual differences in total body movement between obese and lean subjects was demonstrated in a landmark paper by J.A. Levine *et al.* [13]. Lean individuals were found to expend 270 to 475 more calories (kcal) per day than did obese people. Weight gain or weight loss by either group did not alter basal propensity for movement, suggesting SPA is somehow biologically determined. Support for the notion that intrinsic differences in SPA contribute to obesity resistance has come from work utilizing a polygenic rodent model of obesity, first described over a decade ago by Levin *et al.* [20]. Many rat strains show variability in weight gain when fed a high-energy diet [21]; some rats develop diet-induced obesity (DIO) while diet-resistant (DR) rats remain lean. Inherent variability in body weight among male Sprague-Dawley rats led Levin and colleagues to selectively breed rats with high or low rates of weight gain, ultimately resulting in two lines of animals diverging on body weight gain despite comparable caloric intake [20,22]. While these selectively-bred DIO and DR rats do not show differential SPA or weight gain when fed chow, after exposure to high-fat diet, DIO rats significantly decrease SPA and NEAT and become obese [19]. Levin's original selectively-bred rat lines have been developed into commercially available models, including Levin DIO/DR rats (which retain a high-fat diet requirement for differential development of obesity) and obesity prone (OP) or obesity resistant (OR) rats, which develop differential obesity even on chow diets. Early work with DIO/DR rats (including work leading to the development of selectively bred OP/OR animals) suggests physiological differences in sympathetic activation and monoaminergic function exist between individual rats even prior to exposure to a high-energy diet, and that these differences in CNS function may be related to their propensity for obesity [23–26].

One of the major findings explaining the difference in weight gain in this model is that OR animals exhibit greater SPA than do more obesity-prone controls [27]. Several lines of evidence suggest orexin contributes to the SPA phenotype. Injection of orexin A (OXA) into the rostral lateral hypothalamus (rLH), paraventricular hypothalamic nucleus, nucleus accumbens, or third ventricle is known to stimulate SPA in both monogenetic and polygenetic rodent models [28–32], and both DR and OR rats show higher SPA response to orexin than do obese controls [19,9]. Indirect calorimetry shows that OXA-induced SPA results in increased oxygen consumption, CO₂ production, and thermogenesis [29]. Additionally, increased OXA responsiveness in OR rats is due in part to higher levels of orexin receptor expression, particularly in the rLH [17,9]. While OXA effects on SPA can be elicited in several specific brain sites, much of our work has thus focused on the rLH.

An interesting aspect of the OP/OR model is that Levin *et al.* did not set out to specifically breed animals that differed in physical activity; this phenotype was an unexpected byproduct of selection for genes promoting weight gain or resistance to obesity. However, the differential orexin responsivity observed in these animals has been found in other rodent models specifically selected for physical activity. Our laboratory recently described a model of high activity (HA) and low activity (LA) rats, using the naturally occurring variability in SD rat phenotypes [33], much as was done with the early DIO/DR studies. Rats were screened for baseline SPA propensity, and animals that exhibited more than 120 min or less than 90 min total SPA over 24 h designated HA and LA, respectively. In these rats, HA animals showed higher baseline energy expenditure, and baseline SPA in all animals was significantly correlated with lean body mass and total body weight [33]. Subsequent tests showed that HA rats were more responsive to rLH orexin than were LA rats, and mRNA analysis showed HA rats had higher lateral hypothalamic prepro-orexin expression [33]. When challenged with high fat diet, HA animals showed greater resistance to fat mass gain (relative to lean mass gain) than did LA animals [33]. Where the HA/LA animals exploited naturally occurring variability, selective breeding similar to that used in development of the OP/OR model has been used to generate rats selectively bred for aerobic capacity and wheel running propensity [34]. Recent investigations of these animals have shown that, as with HA/LA and OP/OR rats, orexin may be critically involved in the phenotype [35]. Where rats with low aerobic capacity (LCR) show propensity for cardiovascular disease and development of metabolic syndrome, those bred for high aerobic capacity (HCR) are obesity resistant, remaining leaner than their LCR counterparts [35], especially on high fat diet. HCR animals are intrinsically more active than LCR animals, and show greater NEAT response to orexin injection [35]. The association of greater orexin expression or sensitivity with resistance to obesity is a common thread in the phenotypes of these animal models. That these models are polygenic is also important. Much work has been performed using monogenic rodent obesity models, but monogenic forms of human obesity are rare [36,37]. As such, the observation that orexin is important in the multiple obesity-resistant polygenic phenotypes suggests this mechanism is more relevant to human obesity phenotypes.

Together, the evidence suggests that underlying genetic differences contribute to obesity resistance, that orexin is an important component of this variability, and that the polygenic rodent models described above are suitable for testing potential orexin-mediated brain

mechanisms of obesity resistance. Work with these animal models has clearly defined a direct link between orexin signaling and neuromodulation of SPA; however, the cellular signaling pathways through which orexin mediates SPA and obesity resistance still remain largely unknown [9,38]. We present here evidence from multiple *in vitro* investigations, including data from our ongoing work and that from other non-orexin focused rodent models, to delineate potential mechanisms through which orexin might affect obesity resistance.

Orexins and Signal Transduction

The orexins consist of two peptides, OXA and orexin B (OXB; hypocretin 2); both are produced by post-translation modification from a common precursor, prepro-orexin [39–41]. Expression of prepro-orexin within the CNS appears to be limited to a subset of cells in the lateral and perifornical hypothalamus [40,42], while OXA- and OXB-immunoreactive fibers are abundant in both hypothalamic and extra-hypothalamic regions [42–44]. Orexin peptides are endogenous ligands for two G protein-coupled receptors, orexin receptors 1 and 2 (OX1R and OX2R; respectively); orexin receptors are widely and differentially distributed throughout the brain [39,45,46]. Pharmacological data supports that OX1R has a higher selectivity for OXA, while OX2R binds with similar affinity to both orexins [39]. Although it appears that OXB can induce SPA to some degree [32], and we do not dismiss a potential role for OXB in obesity resistance, at this time the contribution of OXB in promotion of energy expenditure is unclear. This review therefore focuses on OXA; as such only potential OXA signal transduction mechanisms will be directly addressed here.

Two central questions have persisted in studying the role of the orexin system in obesity. First, what pathways are activated by OXA action at the orexin receptors, and second, what are the functional outcomes of this activation? Data from *in vitro* work has begun to address these issues. In neuronal cultures and recombinant cell lines, the most immediate response upon OXA binding to either receptor is an increase of intercellular Ca^{2+} , via phospholipase C (PLC) and protein kinase C (PKC) activation [47]. As with any G-protein coupled receptor, the pathways activated by OX1R and OX2R depend on the specific G-subunits coupled to each. Early investigations using transfection models demonstrated that an OXA/OX1R response was non-pertussis toxin (PTX) sensitive, indicating $G_{i/o}$ activation, while an OXA/OX2R response appeared to involve both PTX-sensitive and non-PTX sensitive G-proteins inhibiting adenylyl cyclase [48–50]. Further work shows that OX1/2R can couple to excitatory $G_{q/11}$ subunits, whereas OX2R can couple to inhibitory G_i/G_o subunits thought to be responsible for K^+ efflux and membrane hyperpolarization in neurons [51]. In rat adrenocortical cells, OXA has been shown to activate adenylyl cyclase/protein kinase A (PKA), indicating a G_s response [51]. Studies with hypothalamic tissue from food deprived Wistar rats demonstrate that energy status can modify orexin signaling responses by stimulating distinct G- α subunit responses [52].

Recently, OXA has been demonstrated to activate mitogen-activated protein kinase (MAPK) pathways [53]. Signal profiling of recombinant human OX1R and OX2R receptors has demonstrated that orexin can activate extracellular receptor kinase 1/2 (ERK1/2) and p38, protein members of the MAPK cascade [54]. An OX1R overexpression model in Chinese

hamster ovary cells has shown that orexin activation of ERK1/2 can take place via a PLC/PKC, Ras, Src and PI3K pathway [55]. Studies of human embryonic kidney (HEK-293) cells transfected with human OX2R demonstrate that OXA activation of ERK1/2 is predominantly a G_q/11, G_s and G_i process, while p38 initiation is independent of G_q/11 and G_i activation [54]. Collectively, these findings suggest that OX1R and OX2R G- α subunit activation by OXA may regulate regionally distinct and tissue specific orexin responses.

Orexin-mediated signaling and energy balance

OXA Activates MAPKs

Given the multiple physiological processes and second messenger pathways potentially activated by orexin, it is not surprising that OXA has pleiotropic effects [31,56,57]. However, little is known about the short- and long-term effects of OXA signaling on intracellular neuronal metabolic status or the physiological relevance of this signaling to SPA. Collectively, emerging evidence indicates that activation of OX1R/OX2R by OXA alters proteins involved in intracellular metabolic function [39]. The *in vitro* models described above suggest that OXA activation of MAPKs might represent one link between orexin and cellular mechanisms mediating long-term energy balance. MAPKs, a kinase family of signaling kinases integrating signaling transduction cascades, are traditionally known for their participation in cellular proliferation, but are continually being identified in novel CNS roles. The *in vitro* data discussed above shows that OXA can activate MAPKs, and several studies have shown that increased MAPK activity is correlated with increased obesity resistance. The MAPK inhibitor MAPK-phosphatase-1 (MKP-1) is an immediate-early gene that dephosphorylates MAPKs and inhibits their activity in the nucleus. Mice globally lacking MKP-1 thus have increased MAPK activity. These mice express higher levels of peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α) in skeletal muscle, show increases in skeletal muscle oxidative metabolism, have enhanced energy expenditure and physical movement, and are resistant to diet-induced obesity [58,59]. Pharmacological experiments exploring effects of OXA activated MAPKs, and the distinct contributions of the two receptor subtypes in these processes, are now ongoing in our lab.

Possible Role of PGC-1 α

Previous and ongoing work suggests orexin MAPK pathways involve PGC-1 α , a tissue-specific and inducible transcriptional coactivator for several nuclear receptors. However, whether PGC-1 α is a critical component of orexin effects on neuronal metabolism remains to be explored. PGC-1 α is expressed in heart, kidney, brown adipose tissue, skeletal muscle, and throughout the brain [60]. PGC-1 α is a key transcriptional regulator of mitochondria and oxidative metabolic pathways [61]. PGC-1 α functions as a direct link between external physiological stimuli and regulation of mitochondrial biogenesis, can simultaneously upregulate genes that protect against oxidative stress, and is a key facilitator for production of ATP [62–65]. This cofactor is also known to play key roles in energy metabolism, hepatic gluconeogenesis, and cholesterol homeostasis. Alterations in PGC-1 α are associated with pathologies such as obesity, diabetes, and chronic neurodegenerative diseases [62–65]. While the specific function of PGC-1 α in brain and neuronal metabolism is still under investigation, there is increasing interest in its role in neuronal survival and systemic energy

balance [66,60,62,67,68,61,64,69,65]. Data suggest that global inactivation of PGC-1 α leads to neurodegeneration, partially due to the loss of protection against oxidative stress damage and impaired nutritional regulation of hypothalamic expression of genes that regulate systemic energy balance [68,64,69].

OXA Activates HIF1 α

While a specific role for PGC-1 α in the hypothalamus and how it may contribute to the central control of obesity is unclear at this time, data suggests that PGC-1 α might be an important part of a recently described link between OXA and the transcription factor hypoxia-inducible factor 1 α (HIF-1 α). OXA has recently been shown to increase the expression of HIF-1 α in hypothalamic tissue [70]. While increases in HIF-1 α are usually associated with hypoxia, OXA induction of HIF-1 α occurs in normal, non-hypoxic hypothalamic tissue [70]. OXA has been shown to be neuroprotective in cerebral cortex and in hypothalamic cell culture following oxidative stressors [71,38], potentially through activation of HIF-1 α . Recent studies have shown that in conjunction with MAPK, PGC-1 α is important in regulating HIF-1 α expression [72,62,73,61]. As described above, OXA effects on MAPK pathways might result in increased PGC-1 α . PGC-1 α is known to participate in the regulation of HIF-1 α in peripheral tissue [61] and presumably could do so in the hypothalamus as well.

Several lines of evidence suggest OXA effects on HIF-1 α could represent another link between orexin and cellular metabolic signaling pathways relevant to obesity. We and others have linked OXA to energy metabolism, showing that OXA induces HIF-1 α expression in hypothalamic tissue *in vitro* (Figure 1), and that this results in increased ATP production via oxidative phosphorylation [74,70,38]. In separate rodent studies, mice with the neuron-specific loss of the HIF-1 α inhibitor asparaginyl hydroxylase factor (FIH) had reduced body weight and were protected against high-fat-diet-induced weight gain [75]. Additionally, mice lacking functional HIF-1 α and HIF-2 α proteins in arcuate nucleus pro-opiomelanocortin (POMC) neurons (POMC/HIF β mice) have impaired energy expenditure, hyperphagia, and increased fat mass [76]. In the same study, viral overexpression of HIF-1 α in the mediobasal hypothalamus resulted in obesity resistance during HFD feeding. The specific role of OXA in these models has not been fully evaluated, but current literature suggests that OXA effects on HIF signaling cascades could alter central mechanisms of energy expenditure in response to various metabolic stressors such as high fat diets.

Conclusion

The brain is exceptionally sensitive to oxidative stress caused by changes in both peripheral and intra-neuronal metabolism. The relationship between intracellular energy sensing within specific responsive neurons or brain sites is still poorly understood, but is an emerging field of study in which orexin might play a pivotal role. The hypothalamus is a complex neuroendocrine tissue, and while mechanistic studies within this region of the brain are exceptionally challenging, *in vitro* pharmacology models utilizing hypothalamic cell lines provide very powerful molecular tools when integrated with relevant animal models. Determining the mediators of orexin effects on cellular signaling pathways influencing

energy expenditure could translate into therapies for physiological disorders in which orexin plays a role, such as those designed to increase obesity resistance by increasing responsiveness to orexin-induced SPA.

While some of the mechanisms above have not yet been confirmed in brain tissue, the likelihood they are present in neurons is high. As outlined above and summarized in Figure 2, current data from multiple independent *in vitro* and rodent models support the hypothesis that OXA-mediated increases in energy expenditure, and thus the obesity resistance properties of orexin, could depend in part on signaling cascades involving MAPKs, PGC-1 α , and HIF-1 α . Collectively, these independent lines of evidence support the idea that OXA actions on responsive neurons trigger pleiotropic effects on gene expression and second messenger pathways important in regulating intracellular neuronal metabolism, which is ultimately manifested in increased SPA and obesity resistance. Further investigation of orexin involvement in the signaling pathways outlined here will provide insight into mechanisms influencing metabolic status of OXA-responsive neurons, and elucidate how this ultimately influences energy expenditure and propensity for obesity.

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ABBREVIATIONS

DIO	Diet-induced obese
DR	Diet-resistant
ERK1/2	Extracellular receptor kinase 1 and 2
FIH	Factor inhibiting HIF
HA	High-activity
HCR	High caloric restriction
HEK	Human embryonic kidney
HIF-1α	Hypoxia-inducible factor 1 alpha
LA	Low-activity
LCR	Low caloric restriction
MAPK	Mitogen-activated protein kinase
MKP-1	MAPK-phosphatase-1
NEAT	Non-exercise activity thermogenesis
OP	Obesity-prone
OR	Obesity-resistant
OX1R	Orexin/hypocretin 1 receptor

OX2R	Orexin/hypocretin 2 receptor
OXA	Orexin A (Hypocretin 1)
OXB	Orexin B (Hypocretin 2)
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
POMC	Pro-opiomelanocortin
PTX	Pertussis toxin
rLH	Rostral lateral hypothalamic area
SPA	Spontaneous physical activity

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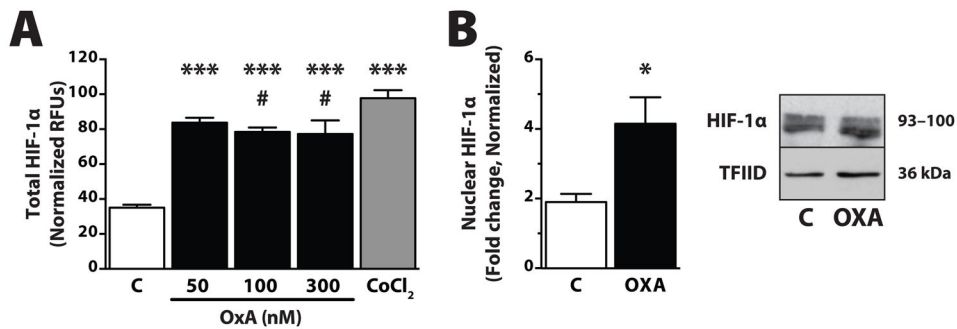
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**Fig. 1.**

OXA increases both total and nuclear HIF-1 α protein within 2 h in an immortalized hypothalamic cell line. **(A)** Protein levels were determined by an in-cell ELISA assay (R&D Systems, Minneapolis, MN USA) following OXA treatment (Phoenix Pharmaceuticals, Burlingame, CA USA; 50, 100, or 300 nM) or the positive control CoCl₂ (Sigma, St. Louis, MO USA; 150 μ M). Briefly, intact cells were fixed with 4% formalin following OXA treatment, incubated with primary antibody (anti-HIF-1 α or anti-cytochrome-C) followed by secondary antibody (each containing two different distinctly labeled conjugates). Cells were assayed by reading the relative fluorescent units (RFU) for each secondary antibody. Values normalized to cytochrome-C housekeeping protein; n = 3–6/group; *** p < 0.0001 vs. control; # p = 0.22 vs. CoCl₂. **(B)** Western blot analysis for nuclear HIF-1 α , following OXA (300 nM for 2 h) treatment. Nuclear fractions were prepared (Thermo-Pierce, Rockford, IL USA) and 30 μ g total protein was used to determine changes in nuclear (activated) HIF-1 α (Novus, Littleton, CO USA; 1:1000) compared to control (C). Nuclear TATA binding protein TFIID (Santa Cruz Biotechnology, Dallas, TX USA) used as loading control. n = 4/group; * p < 0.05 vs. control

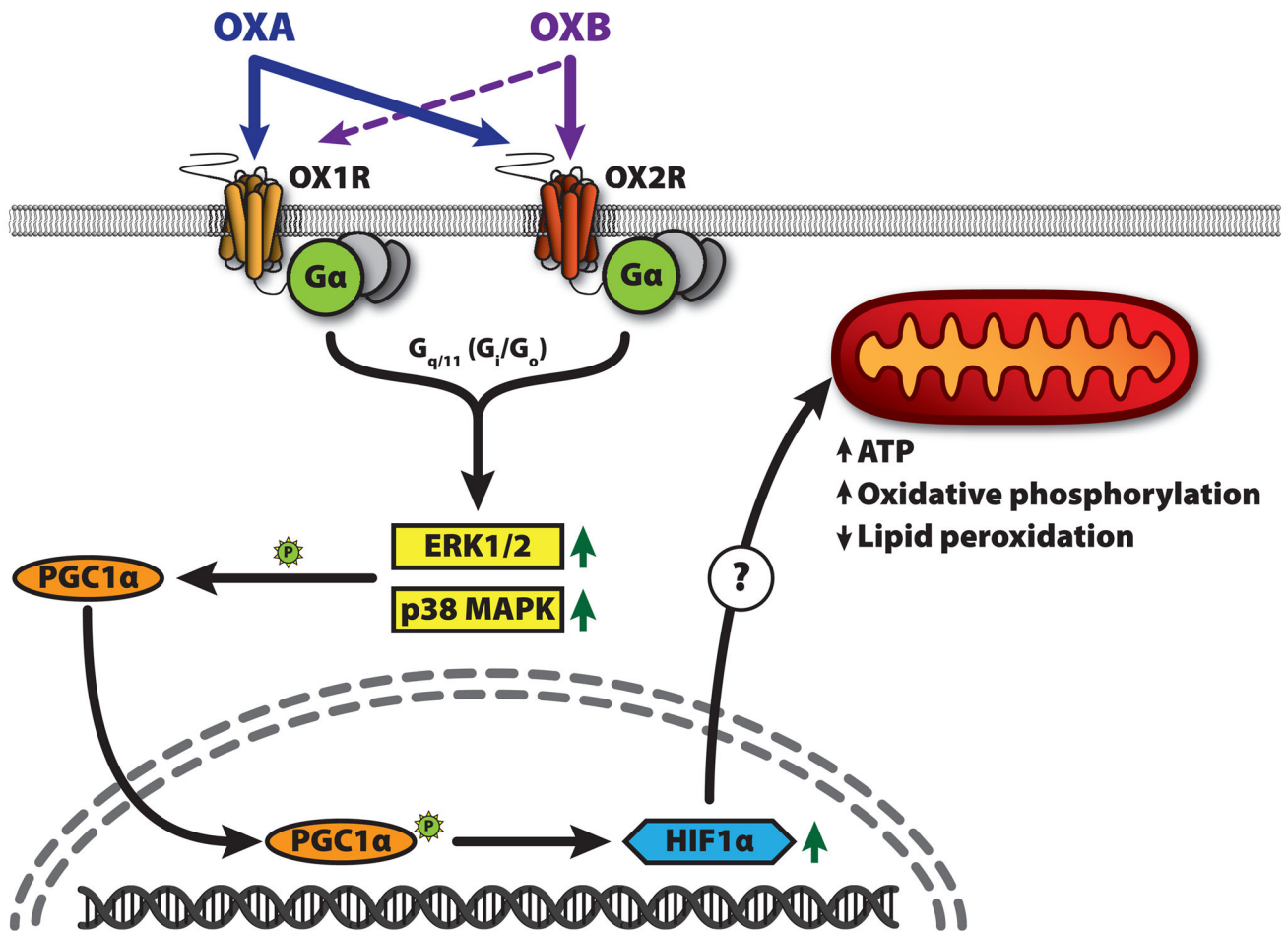


Fig. 2.

Schematic representation of the hypothesized orexin signaling pathways. Orexins A and B (OXA, OXB) act on orexin receptors 1 and 2 (OX1R, OX2R). Orexin is known to increase activation (phosphorylation) of the MAP kinases ERK1/2 and p38 MAPK. This activation appears to be primarily mediated by G_{q/11} signaling, but G_s/G_o may be more important for OX2R. Both OXA and p38 MAPK can increase HIF-1α activation and expression; the mechanism may rely on the transcriptional coactivator PGC-1α. PGC-1α functions as a direct link between external physiological stimuli and regulation of mitochondrial biogenesis, and is a key facilitator for ATP production. PGC-1α is activated by p38 MAPK, and is a known regulator of HIF-1α expression in peripheral tissue. Increased HIF-1α results in gene expression changes leading to increased oxidative phosphorylation and decreased lipid peroxidation.