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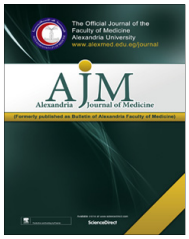

Targeting the orexinergic system: Mainly but not only for sleep-wakefulness therapies

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Abstract Orexin receptors belong to the big family of G protein coupled receptors (GPCRs) that constitute the main targets in the modern pharmacological approaches. Although the orexinergic system is involved in a variety of processes, treating sleep-wakefulness disorders such as narcolepsy and insomnia, remains the main therapeutic implication of targeting orexinergic receptors. After novel advances, such as the description of the binding pockets, and ligand developments, more researchers are focusing on orexin receptors as promising targets. Furthermore, targeting these receptors may provide therapeutic solutions for some health problems, other than sleep-wakefulness disorders including some psychiatric disorders and neurodegenerative diseases. Within this paper, we put a spotlight on the orexins' physiology, pathophysiology and pharmacology of mainly sleep-wakefulness. We have also reviewed examples about other orexinergic system-related disorders. We further illustrated recent development in orexin receptors' agonists and antagonists. In addition, we discussed selected progresses in orexinergic receptors' ligands.

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Contents

1. Introduction

Among the emerging targets in neuropharmacology, orexin receptors are objects of more focus. A food intake study had led, in 1998, to the discovery of neurotransmitter orexins (OX), also called hypocretins (hcrt), in the rat lateral hypothalamic area (LHA).[1,2] The hypothalamic neuropeptides orexin-A and orexin-B interact with two orphan GPCRs.[3] Indeed, orexinergic receptors (OXR) type 1 and type 2 (OX1R and OX2R) are G-protein-coupled receptors (GPCRs).[1,2,4] Orexin-B has been pointed as showing 10–100-fold higher potency at the OX2 receptor than at the OX1 receptor[2,5], whereas OX-A binds with similar affinity to both receptors.[4] It has also been reported that during proestrous afternoon there is an increased expression of OX1R and OX2R,[6] and, in sleep, the secretion of orexin is seasonally dependent,[7] which shows a time-dependant activity of the orexinergic system. Studies on the intracellular pathways and signal transductions have shown that Ox/hcrt-R1 is coupled to Gq-type G protein, whereas ox/hcrt-R2 is coupled to both Gi/o and Gq proteins.[8] GPCRs are receptors that are characterized by the factors that can influence their activity[9] and the signal transduction signal they implicate.[10] Regarding the corresponding intracellular downstream, orexin G protein-coupled receptors couple to different proteins including phospholipases and kinases.[11,12] The cellular and molecular bases of the orexinergic system's functions lie on both the orexinergic receptors and the corresponding pathway that involves diverse intracellular mechanisms such as the inhibition of the cyclic AMP(cAMP) synthesis.[13] Orexins and their receptors have been described both inside and outside the brain, for instance, the presence of both receptors in the ovary was demonstrated.[6]

Within this mini-review, we describe, via illustrative examples, some of the possible therapeutical applications and pharmacological properties that have been linked to the orexinergic system. Although treatment of sleep-wakefulness disorders, like insomnia,[14] remains the main pharmacological application, other therapeutical implications seem interesting.

2. Physiological and pathophysiological overview of the orexinergic system

Orexin system (ox/hcrt system) has been described in several physiological and pathophysiological processes, mainly in the regulation of sleep and wakefulness.[15–20] Indeed, the ventrolateral preoptic nucleus (VLPO) is suggested to send inhibitory signals to both orexin neurons and the "arousal center", whereas orexin neurons depolarize the "arousal center" during the wake stage.[21,22] Other functions and properties, among which selected examples are summarized in Table 1, have also been linked to the orexinergic system. Furthermore, since peripheral organs have been shown to involve orexins, we suggest that orexins play other roles in more physiological functions[23] which suppose novel possibilities for the related pharmacotherapies.

Table 1 Mechanisms and effects in which the orexinergic system has been shown, or hypothesized, to play roles.

Mechanisms/effects	References
Regulation of appetite and stress response	24–26
Respiratory effects	27–29
Addiction	11,25,26,30–38
Behavioral changes associated with cocaine administration	39
Analgesia	11,40
Food intake regulation	23,41
Central regulation of feeding (of blind cavefish)	42
Regulation of glucose metabolism	43,44
Energy balance regulation	45,46
Insulin secretion regulation	44
Variability in diet-induced obesity sensitivity	47
Proliferation and viability of 3T3-L1 preadipocytes	48
Body temperature regulation	46,49
Neuroendocrine function	6,49,50
Pituitary hormone secretion	51
Cardiovascular activities	49,52–54
Post-ischemic attenuation of inflammatory responses (neuroprotection)	55
Prevention of cerebral ischemic neuronal damage (neuroprotection)	43
Reproduction	56–62
Motor activity	50,54
Migraine	63
Arousal states	45,64,65
Narcolepsy and cataplexy	45,64–68
Major depression	69
Epileptic activity	40

3. Pharmacology and potential therapeutics

Orexinergic system-related pharmacological and therapeutic researches have recently started to overcome the struggle of the lack of developed synthetic antagonists.[70] However, the majority of those synthetic antagonists are not commercially available.[71] Agonist and antagonist binding pockets of orexin receptors are gradually being unveiled, therefore, we expect more advances in ligand researches.[72] Sleep-wakefulness

remains the main topic among orexin system-related researches. In fact, selective activation of ox/hcrt-R2 promotes wakefulness.[73] In addition, whereas orexin neuron inactivation was linked with sleep, their activation promotes the wakefulness.[74,75] Therefore, pharmaceutical companies are aiming to develop agents that would target the Hcrt system *in vivo*[70] as new therapies, not only for sleep disorders, but also for other diseases and disorders.[76] Such advances highlight the progress of the pharmaceutical industry in the pharmacological targeting of the orexinergic system. For example, orexin receptors' blocking could constitute a treatment for insomnia.[77] Following this idea, Johnson & Johnson has pointed that blockade of OX1R can reduce OX2R antagonist-induced sleep promotion in rats.[78] More importantly, due to insomnia statistics and the pathologies that have been linked to this disorder, we realize that developing treatment for insomnia will constitute an important advancement in neuropsychiatry. Insomnia is a common neuropsychiatric disorder with a chronic form that affects 20% of the adult population.[79] Importantly, insomnia has been linked to depression, obesity, cardiovascular disease, cancer and chronic pain.[80–84] Moreover, insomnia may lead to depression[85] and sleep disturbances are frequently observed in multiple sclerosis (MS) patients.[86] Studies of diverse new agents and receptors have reported numerous compounds and targets for developing novel insomnia treatments, among them we mention central nervous system (CNS) histamine receptors, GABA(A), melatonin and serotonin modulators.[87] However, sedative-hypnotics that target GABA receptors represent the main current therapy for insomnia.[18] Starting from the hypothesis that the new compounds that are potentially effective in various types of insomnia, which is associated with some disease, can, via the alleviation of insomnia, directly result in the improvement of those diseases, we suppose that treating insomnia using novel approaches might have three main consequences: {1} Reduce the incidence of the above mentioned disorders,[1]{2}improve the prognosis of insomnia-related pathologies and {3} provide an alternative to the agents that target GABA receptors and thus, avoid the side effects of such class of drugs (see: Fig. 1).

In mammals, the hypothalamus is the unique brain region orexin neurons are restricted to[49,50,88–90], but brain anatomy shows an important distribution of orexin neurons[49,89,91], and many brain regions, in addition to other extra cerebral structures, have orexin neuron fibers including the median eminence, arcuate nucleus, pituitary, olfactory bulb, cerebral cortex, thalamus subfornical organ, area postrema, hippocampus, amygdala, indusium griseum, brainstem and spinal cord[49,89,91–94], thus pharmacological benefits of targeting orexinergic neurons might be extended into those structures in the future.

Orexinergic neurons, that form a unique central orexinergic system,[50] project to regions associated with different cerebral functions such as reward, learning and memory, emotion and attention,[49] supposing a possible modification of these functions by orexin receptors' ligands, either as an eventual therapeutical approach or as a side effect of orexin receptors' ligands. Importantly, interactions of the orexinergic system with other neurotransmitters have also been reported. For instance, in anesthetized rats, the activation of orexin neurons can be obtained via a local disinhibition of neurons of perifornical region[95] of the posterior hypothalamus neurons by GABA (A) receptor antagonists.[53] Interestedly, the implication of orexin A and orexin B in the regulation of monoaminergic and cholinergic neuron function in wakefulness maintenance has also been reported.[3] Furthermore, orexin application to the rat brain produces serotonergic and cholinergic neuron depolarization,[96,97] and orexin has been shown to modify the synaptic activity of dopamine neurons.[30] Moreover, a recent publication has shown that the increase of glutamatergic synaptic transmission in the ventral tegmental area (VTA) has been linked to OX2R activity.[30] Finally, whereas OxA/hcrt-1N-methyl-D aspartate receptor (NMDAR)-mediated synaptic transmission of dopamine neurons in the VTA has also been reported,[95,98–102] intra-VTA administration of oxA/hcrt-1 results in an increased local dopamine and glutamate release.[103,104] These interactions between the orexinergic system and other neurotransmitters could be exploited either to a better understanding of the orexinergic system underlying pathways or to develop more selective drugs. At the same time such interactions can lead to complex side effects of the orexinergic receptors' ligands.

Based on the orexin related-pharmacological properties, pharmaceutical companies are trying to develop drugs that target orexin receptors, mainly to treat sleep disorders.[3] It was reported that small molecule orexin receptor antagonists can help patients suffering from insomnia by promoting sleep.[105] Herein, we state some orexin receptors' ligands that have been either pointed in clinical trials or that may be effective for

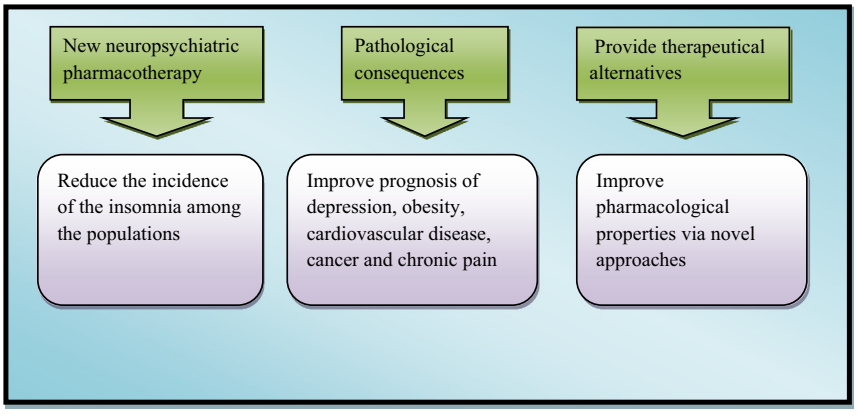

Figure 1 Some possible consequences of targeting orexin receptors in insomnia.

insomnia treatment. SB-334867 constituted the most often used orexinergic antagonist in the literature.[106] Whereas SB-649868 constitutes an emerging antagonist in development by GlaxoSmithKline,[107] a new selective and highly potent spiropiperidine-based OX2R antagonist was discovered in 2011.[77] On the other hand, several papers have pointed to the two orexin receptor antagonists: suvorexant and almorexant (or ACT-078573), these small molecules have, in addition to their ability to promote sleep in animals, clinical efficacy.[19,108] Furthermore, for the treatment of primary insomnia, the phase III of clinical trials of suvorexant has been completed.[3] Previously suvorexant, considered as the most advanced dual orexin receptor antagonist (DORA),[76] was reported to induce sleep in humans[109] with analogous potency toward both OX1R and OX2R.[108,110] Among suvorexant, MK-6096 (a piperidino-derived) and DORA-22 that are dual orexin receptor antagonists (DORAs),[18] MK-6096 and DORA-22 show a high selectivity for OX1R and OX2R therefore, have the pharmacological properties to decrease wakefulness and thus, represent potential agents for sleep/wake dysregulation treatment.[18]

Previously, eszopiclone (ESZ), a cyclopyrrolone targeting GABA-A receptor[111,112], was pointed to suppress orexinergic neurons' activity with a possible use in insomnia treatment.[113-117] This datum gets its importance from the fact that many hypnotic agents target GABA-A receptor,[118,119] which strengthens the theory that links orexin system to insomnia. Another class of orexin 2 antagonists, that includes 2-methyl-3-furanyl-4H-1,2,4-triazol-3-ylthioamides, has been described as well.[120] Pharmacokinetics described different routes of administration of orexin receptors' antagonists. For instance, almorexant can be administered orally, while SB-334867 can be injected systemically[121], thus gives a clinical use flexibility of such drugs.

Since orexin receptors are subject to active drug development, more advances are emerging and many orexin receptors antagonists are mentioned in the literature.[105,120] Whereas, in 2007, GlaxoSmithKline brought out data about an orexin receptor antagonist for the clinical treatment of insomnia,[70] the two dual OX1R/OX2R receptor antagonists: almorexant and MK-4305 were entered in Phase-III, but almorexant was discontinued.[77] Herein, we mention that pharmacokinetic interaction of almorexant with both simvastatin and atorvastatin has been recently reported.[122] It has been reported that orexin receptors' non-subtype-selective antagonists may provide agents with hypnotic properties as well.[70] Importantly, it has been shown that intraperitoneal injection of OX1R antagonist can inhibit OXA-triggered food intake, feeding behavior and weight gain.[123,124] In addition, the study of the role of oxA/hcrt-1 in addiction-associated behaviors might help to develop agents for the treatment of pathological stress-related behaviors.[30] Researches have also given indications about the impact of the molecular structure and receptor type on the drug activity. Indeed, D-Leu15-orexin-B (A11, DL15-orexin-B) may be more selective for OX2 receptors compared to OX1 receptors.[125] In addition, factors including expression level, coupling efficiency and signal pathways have been linked to agonist potency[126] thus, taking into account such data will be a good contribution to drug design especially of chemical-derived drugs.

Genetic therapy has also found its place among the orexin-related therapies. Indeed, a recent study pointed that it is possible to both control cataplexy attacks and modestly improve wake maintenance by orexin gene transfer into the dorsolateral pontine neurons.[127] Finally, animal experiments contribute strongly in the evaluation of potential orexin receptor ligands. For instance, orexin-2 receptor antagonist TCS-OX2-29 was reported to induce a decreased wakefulness in rats.[20] In addition, the organization of the hypothalamus orexinergic system has been studied in both giraffe (*Giraffa camelopardalis*) and harbor porpoise (*Phocoena phocoena*), which belong to the mammalian order[128], thus allow a better understanding of the human orexinergic system and provide important orientations for future trials.

4. Perspectives

In summary, we highlight the main pharmacological properties of orexin receptors' ligands: Antagonists have hypnotic properties and offer a new approach to insomnia treatment,[129] while agonists may have benefits for hypersomnia and narcolepsy.[130] Because of some struggles, such as the non-commercial availability of most of the developed antagonists,[71] targeting orexin receptors still constitutes a new area of research. Importantly, several papers have highlighted the possible therapeutic usages of orexin receptors' ligands in a variety of other cerebral and neurological disorders including the use of orexin receptor antagonist as cognitive enhancer in post-traumatic stress disorder (PTSD),[77] eating disorder,[131,132] Alzheimer's disease (via reducing amyloid-β),[133] panic anxiety[134] and addiction.[135,136] In addition, as orexin (hypocretin) has been linked to both nicotine withdrawal (130) and addiction,[137,138] it was supposed that further investigations on this property may lead to develop agents that can help smoking cessation.[139] Moreover, in tetrahydrocannabinol (THC) as well as nicotine dependence, the orexinergic system constitutes a potential target,[137] which may give more importance to this emerging aspect of cerebral pharmacology. Moreover, whereas in some clinical anxiety disorders, characterized by intense emotional arousal, orexin receptor antagonists might turn out to be a therapy,[140] the orexin receptor antagonist (MK-6096) has been reported as a potential treatment of chronic migraine.[141] Importantly, pharmacological blockade of the orexinergic system leads to an antidepressant-like effect.[69] The studied chemical properties of the potential OXR ligands may provide a starting point to future drug development through compound screening say for example. In addition, molecules, such as some laboratory reagents and solvents, that have been reported to have biological or pharmacological effects on GPCRs,[142,143] could constitute candidates in drug design or experimental developments in orexinergic system-related researches. All these pharmacological and clinical data point out clearly how this new area is underestimated, thus further investigations are strongly recommended especially within the context that could clearly clarify the toxicology and pharmacology of the drugs[144,145] that may be provided by natural products[146,147]

In contrast, orexinergic neuron distribution and the interactions of the orexinergic system with other neurotransmitters might result in influences of orexin receptors' ligands on different physiological and pathophysiological mechanisms, mainly those in which orexin system is implicated. Especially if we consider the cerebral neuro-system as a complex network within which different neurotransmitters are in continuous

interactions.[148,149] Therefore, a particular pharmacovigilance for agents targeting the orexinergic system remains highly important.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

1. de Lecea L, Kilduff TS, Peyron C, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 1998;**95**:322–7.
2. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998;**92**:573–85.
3. Mieda M, Sakurai T. Orexin (Hypocretin) receptor agonists and antagonists for treatment of sleep disorders: rationale for development and current status. *CNS Drugs* 2013.
4. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998;**92**, 1 page following 696.
5. Holmqvist T, Akerman KE, Kukkonen JP. Orexin signaling in recombinant neuron-like cells. *FEBS Lett* 2002;**526**:11–4.
6. Cataldi NI, Lux-Lantos VA, Libertun C. Effects of orexins A and B on expression of orexin receptors and progesterone release in luteal and granulosa ovarian cells. *Regul Pept* 2012;**178**:56–63.
7. Kirsz K, Szczesna M, Molik E, Misztal T, Wojtowicz AK, Zieba DA. Seasonal changes in the interactions among leptin, ghrelin, and orexin in sheep. *J Anim Sci* 2012;**90**:2524–31.
8. Zhu Y, Miwa Y, Yamanaka A, et al. Orexin receptor type-1 couples exclusively to pertussis toxin-insensitive G-proteins, while orexin receptor type-2 couples to both pertussis toxin-sensitive and -insensitive G-proteins. *J Pharmacol Sci* 2003;**92**:259–66.
9. Ghanemi A, He L, Yan M. New factors influencing G protein coupled receptors' system functions. *Alexandria J Med* 2013;**49**:1–5.
10. Ghanemi A. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. *Saudi Pharm J* 2013.
11. Kukkonen JP. Physiology of the orexinergic/hypocretinergic system: a revisit in 2012. *Am J Physiol Cell Physiol* 2013;**304**:C2–C32.
12. Jantti MH, Putula J, Somerharju P, Frohman MA, Kukkonen JP. OX1 orexin/hypocretin receptor activation of phospholipase D. *Br J Pharmacol* 2012;**165**:1109–23.
13. Urbanska A, Sokolowska P, Woldan-Tambor A, et al. Orexins/hypocretins acting at Gi protein-coupled OX 2 receptors inhibit cyclic AMP synthesis in the primary neuronal cultures. *J Mol Neurosci MN* 2012;**46**:10–7.
14. Steiner MA, Lecourt H, Jenck F. The dual orexin receptor antagonist almorexant, alone and in combination with morphine, cocaine and amphetamine, on conditioned place preference and locomotor sensitization in the rat. *Int JNeuropsychopharmacol* 2013;**16**:417–32.
15. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999;**98**:437–51.
16. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 1999;**98**:365–76.
17. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 2000;**355**:39–40.
18. Winrow CJ, Gotter AL, Cox CD, et al. Pharmacological characterization of MK-6096 - a dual orexin receptor antagonist for insomnia. *Neuropharmacology* 2012;**62**:978–87.
19. Brisbare-Roch C, Dingemanse J, Koberstein R, et al. Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat Med* 2007;**13**:150–5.
20. Kummangal BA, Kumar D, Mallick HN. Intracerebroventricular injection of orexin-2 receptor antagonist promotes REM sleep. *Behav Brain Res* 2013;**237**:59–62.
21. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci* 2001;**24**:726–31.
22. Yoshida K, McCormack S, Espana RA, Crocker A, Scammell TE. Afferents to the orexin neurons of the rat brain. *J Comp Neurol* 2006;**494**:845–61.
23. Wong KK, Ng SY, Lee LT, Ng HK, Chow BK. Orexins and their receptors from fish to mammals: a comparative approach. *Gen Comp Endocrinol* 2011;**171**:124–30.
24. Kukkonen JP, Holmqvist T, Ammoun S, Akerman KE. Functions of the orexinergic/hypocretinergic system. *Am J Physiol Cell Physiol* 2002;**283**:C1567–1591.
25. Boutrel B, Kenny PJ, Specio SE, et al. Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc Natl Acad Sci U S A* 2005;**102**:19168–73.
26. Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 2005;**437**:556–9.
27. Wang W, Pan Y, Li Q, Orex Wang L. A potential role in the process of obstructive sleep apnea. *Peptides* 2013.
28. Iigaya K, Horiuchi J, McDowall LM, et al. Blockade of orexin receptors with Almorexant reduces cardiorespiratory responses evoked from the hypothalamus but not baro- or chemoreceptor reflex responses. *Am J Physiol Regul Integr Comp Physiol* 2012;**303**:R1011–1022.
29. Wang W, Li Q, Pan Y, Zhu D, Wang L. Influence of hypercapnia on the synthesis of neuropeptides and their receptors in murine brain. *Respirology* 2013;**18**:102–7.
30. Borgland SL, Ungless MA, Bonci A. Convergent actions of orexin/hypocretin and CRF on dopamine neurons: emerging players in addiction. *Brain Res* 2010;**1314**:139–44.
31. Lawrence AJ, Cowen MS, Yang HJ, Chen F, Oldfield B. The orexin system regulates alcohol-seeking in rats. *Br J Pharmacol* 2006;**148**:752–9.
32. Narita M, Nagumo Y, Hashimoto S, et al. Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J Neurosci* 2006;**26**:398–405.
33. Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ. Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci U S A* 2008;**105**:19480–5.
34. Richards JK, Simms JA, Steensland P, et al. Inhibition of orexin-1/hypocretin-1 receptors inhibits yohimbine-induced reinstatement of ethanol and sucrose seeking in Long-Evans rats. *Psychopharmacology* 2008;**199**:109–17.

35. Aston-Jones G, Smith RJ, Moorman DE, Richardson KA. Role of lateral hypothalamic orexin neurons in reward processing and addiction. *Neuropharmacology* 2009;**56**(Suppl. 1):112–21.
36. Jupp B, Krivdic B, Krstew E, Lawrence AJ. The orexin receptor antagonist SB-334867 dissociates the motivational properties of alcohol and sucrose in rats. *Brain Res* 2011;**1391**:54–9.
37. Cannella N, Kallupi M, Ruggeri B, Ciccocioppo R, Ubaldi M. The role of the neuropeptide S system in addiction: focus on its interaction with the CRF and hypocretin/orexin neurotransmission. *Prog Neurobiol* 2013;**100**:48–59.
38. Yeoh JW, James MH, Jobling P, Bains JS, Graham BA, Dayas CV. Cocaine potentiates excitatory drive in the perifornical/lateral hypothalamus. *J Physiol* 2012;**590**:3677–89.
39. Rao Y, Mineur YS, Gan G, et al. Repeated in vivo exposure of cocaine induces long-lasting synaptic plasticity in hypocretin/orexin neurons in the lateral hypothalamus in mice. *J Physiol* 2013.
40. Erken HA, Erken G, Genc O, et al. Orexins cause epileptic activity. *Peptides* 2012;**37**:161–4.
41. Barson JR, Karatayev O, Gaysinskaya V, Chang GQ, Leibowitz SF. Effect of dietary fatty acid composition on food intake, triglycerides, and hypothalamic peptides. *Regul Pept* 2012;**173**:13–20.
42. Wall A, Volkoff H. Effects of fasting and feeding on the brain mRNA expressions of orexin, tyrosine hydroxylase (TH), PYY and CCK in the Mexican blind cavefish (Astyanax fasciatus mexicanus). *Gen Comp Endocrinol* 2013;**183C**:44–52.
43. Harada S, Yamazaki Y, Tokuyama S. Orexin-A suppresses postischemic glucose intolerance and neuronal damage through hypothalamic brain-derived neurotrophic factor. *J Pharmacol Exper Ther* 2013;**344**:276–85.
44. Yilmaz E, Celik O, Celik N, et al. Maternal and fetal serum orexin-A levels in gestational diabetes mellitus. *J Obst Gynaecol Res* 2013;**39**:139–45.
45. Lopez M, Tena-Sempere M, Dieguez C. Cross-talk between orexins (hypocretins) and the neuroendocrine axes (hypothalamic-pituitary axes). *Front Neuroendocrinol* 2010;**31**:113–27.
46. Madden CJ, Tupone D, Morrison SF. Orexin modulates brown adipose tissue thermogenesis. *Biomol Concepts* 2012;**3**:381–6.
47. Perez-Leighton CE, Boland K, Teske JA, Billington C, Kotz CM. Behavioral responses to orexin, orexin receptor gene expression, and spontaneous physical activity contribute to individual sensitivity to obesity. *Am J Physiol Endocrinol Metab* 2012;**303**:E865–74.
48. Skrzypski M, Kaczmarek P, Le TT, et al. Effects of orexin A on proliferation, survival, apoptosis and differentiation of 3T3-L1 preadipocytes into mature adipocytes. *FEBS Lett* 2012;**586**:4157–64.
49. Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998;**18**:9996–10015.
50. Zhang XY, Yu L, Zhuang QX, Zhu JN, Wang JJ. Central functions of the orexinergic system. *Neurosci Bull* 2013.
51. Jawiarczyk-Przybylowska A, Bolanowski M. The role of orexin A in metabolic disturbances in patients with acromegaly. *Endokrynol Pol* 2012;**63**:463–9.
52. Xiao F, Jiang M, Du D, et al. Orexin A regulates cardiovascular responses in stress-induced hypertensive rats. *Neuropharmacology* 2013;**67**:16–24.
53. Stettner GM, Kubin L. Antagonism of orexin receptors in the posterior hypothalamus reduces hypoglossal and cardiorespiratory excitation from the perifornical hypothalamus. *J Appl Physiol* 2013;**114**:119–30.
54. Wu MF, Nienhuis R, Maidment N, Lam HA, Siegel JM. Cerebrospinal fluid hypocretin (orexin) levels are elevated by play but are not raised by exercise and its associated heart rate, blood pressure, respiration or body temperature changes. *Arch Ital Biol* 2011;**149**:492–8.
55. Xiong X, White RE, Xu L, et al. Mitigation of murine focal cerebral ischemia by the hypocretin/orexin system is associated with reduced inflammation. *Stroke J Cereb Circ* 2013.
56. Iqbal J, Pompolo S, Sakurai T, Clarke IJ. Evidence that orexin-containing neurones provide direct input to gonadotropin-releasing hormone neurones in the ovine hypothalamus. *J Neuroendocrinol* 2001;**13**:1033–41.
57. Martynska L, Polkowska J, Wolinska-Witort E, et al. Orexin A and its role in the regulation of the hypothalamo-pituitary axes in the rat. *Reprod Biol* 2006;**6**(Suppl 2):29–35.
58. Pu S, Jain MR, Kalra PS, Kalra SP. Orexins, a novel family of hypothalamic neuropeptides, modulate pituitary luteinizing hormone secretion in an ovarian steroid-dependent manner. *Regul Pept* 1998;**78**:133–6.
59. Sasson R, Dearth RK, White RS, Chappell PE, Mellon PL. Orexin A induces GnRH gene expression and secretion from GT1-7 hypothalamic GnRH neurons. *Neuroendocrinology* 2006;**84**:353–63.
60. Tafuri S, Pavone LM, Lo Muto R, et al. Expression of orexin A and its receptor 1 in the rat epididymis. *Regul Pept* 2009;**155**:1–5.
61. Zhang S, Blache D, Vercoe PE, et al. Expression of orexin receptors in the brain and peripheral tissues of the male sheep. *Regul Pept* 2005;**124**:81–7.
62. Barreiro ML, Pineda R, Navarro VM, et al. Orexin 1 receptor messenger ribonucleic acid expression and stimulation of testosterone secretion by orexin-A in rat testis. *Endocrinology* 2004;**145**:2297–306.
63. Verrotti A, Di Fonzo A, Agostinelli S, Coppola G, Margiotta M, Parisi P. Obese children suffer more often from migraine. *Acta Paediatr* 2012;**101**:e416–421.
64. Siegel JM. Hypocretin (orexin): role in normal behavior and neuropathology. *Annu Rev Psychol* 2004;**55**:125–48.
65. Adamantidis A, de Lecea L. The hypocretins as sensors for metabolism and arousal. *J Physiol* 2009;**587**:33–40.
66. Chen L, McKenna JT, Bolortuya Y, Brown RE, McCarley RW. Knockdown of orexin type 2 receptor in the lateral pontomesencephalic tegmentum of rats increases REM sleep. *Eur J Neurosci* 2013.
67. Santamaria-Cano J. Diagnostic and therapeutic update in narcolepsy. *Rev Neurol* 2012;**54**(Suppl 3):S25–30.
68. Overeem S, Scammell TE, Lammers GJ. Hypocretin/orexin and sleep: implications for the pathophysiology and diagnosis of narcolepsy. *Curr Opin Neurol* 2002;**15**:739–45.
69. Nollet M, Gaillard P, Tanti A, Girault V, Belzung C, Leman S. Neurogenesis-independent antidepressant-like effects on behavior and stress axis response of a dual orexin receptor antagonist in a rodent model of depression. *Neuropsychopharmacology* 2012;**37**:2210–21.
70. Roecker AJ, Coleman PJ. Orexin receptor antagonists: medicinal chemistry and therapeutic potential. *Curr Top Med Chem* 2008;**8**:977–87.
71. Putula J, Turunen PM, Jantti MH, Ekholm ME, Kukkonen JP. Agonist ligand discrimination by the two orexin receptors depends on the expression system. *Neurosci Lett* 2011;**494**:57–60.
72. Putula J, Kukkonen JP. Mapping of the binding sites for the OX1 orexin receptor antagonist, SB-334867, using orexin/hypocretin receptor chimaeras. *Neurosci Lett* 2012;**506**:111–5.
73. Akanmu MA, Honda K. Selective stimulation of orexin receptor type 2 promotes wakefulness in freely behaving rats. *Brain Res* 2005;**1048**:138–45.
74. Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J Neurosci* 2005;**25**:6716–20.
75. Estabrooke IV, McCarthy MT, Ko E, et al. Fos expression in orexin neurons varies with behavioral state. *J Neurosci* 2001;**21**:1656–62.

76. Christopher JA, Congreve MS. Treatment and prevention of various therapeutic conditions using OX receptor antagonistic activity (WO2012081692). *Expert Opin Ther Pat* 2013;**23**:273–7.
77. Fujimoto T, Tomata Y, Kunitomo J, Hirozane M, Marui S. Discovery of spiropiperidine-based potent and selective Orexin-2 receptor antagonists. *Bioorg Med Chem Lett* 2011;**21**:6409–13.
78. Dugovic C, Shelton JE, Aluisio LE, et al. Blockade of orexin-1 receptors attenuates orexin-2 receptor antagonism-induced sleep promotion in the rat. *J Pharmacol Exp Ther* 2009;**330**:142–51.
79. Dauvilliers Y, Morin C, Cervena K, et al. Family studies in insomnia. *J Psychosom Res* 2005;**58**:271–8.
80. Benca RM. Diagnosis and treatment of chronic insomnia: a review. *Psychiatr Serv* 2005;**56**:332–43.
81. Foley DJ, Monjan AA, Brown SL, Simonsick EM, Wallace RB, Blazer DG. Sleep complaints among elderly persons: an epidemiologic study of three communities. *Sleep* 1995;**18**:425–32.
82. Kamel NS, Gammack JK. Insomnia in the elderly: cause, approach, and treatment. *Am J Med* 2006;**119**:463–9.
83. Manabe K, Matsui T, Yamaya M, et al. Sleep patterns and mortality among elderly patients in a geriatric hospital. *Gerontology* 2000;**46**:318–22.
84. Roth T. New developments for treating sleep disorders. *J Clin Psychiatry* 2001;**62**(Suppl. 10):3–4.
85. Staner L. Comorbidity of insomnia and depression. *Sleep Med Rev* 2010;**14**:35–46.
86. Caminero A, Bartolome M. Sleep disturbances in multiple sclerosis. *J Neurol Sci* 2011;**309**:86–91.
87. Sullivan S. Update on emerging drugs for insomnia. *Expert Opin Emerg Drugs* 2012;**17**:295–8.
88. Moore RY, Abrahamson EA, Van Den Pol A. The hypocretin neuron system: an arousal system in the human brain. *Arch Ital Biol* 2001;**139**:195–205.
89. Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K. Distribution of orexin neurons in the adult rat brain. *Brain Res* 1999;**827**:243–60.
90. Nixon JP, Smale L. A comparative analysis of the distribution of immunoreactive orexin A and B in the brains of nocturnal and diurnal rodents. *Behav Brain Funct* 2007;**3**:28.
91. Date Y, Ueta Y, Yamashita H, et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci U S A* 1999;**96**:748–53.
92. Cutler DJ, Morris R, Sheridhar V, et al. Differential distribution of orexin-A and orexin-B immunoreactivity in the rat brain and spinal cord. *Peptides* 1999;**20**:1455–70.
93. Date Y, Mondal MS, Matsukura S, et al. Distribution of orexin/hypocretin in the rat median eminence and pituitary. *Brain Res Mol Brain Res* 2000;**76**:1–6.
94. Shibata M, Mondal MS, Date Y, Nakazato M, Suzuki H, Ueta Y. Distribution of orexins-containing fibers and contents of orexins in the rat olfactory bulb. *Neurosci Res* 2008;**61**:99–105.
95. Wanat MJ, Hopf FW, Stuber GD, Phillips PE, Bonci A. Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. *J Physiol* 2008;**586**:2157–70.
96. Brown RE, Sergeeva O, Eriksson KS, Haas HL. Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. *Neuropharmacology* 2001;**40**:457–9.
97. Takahashi K, Koyama Y, Kayama Y, Yamamoto M. Effects of orexin on the laterodorsal tegmental neurones. *Psychiatry Clin Neurosci* 2002;**56**:335–6.
98. Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. *J Neurosci* 2003;**23**:7–11.
99. Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, Bonci A. Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. *Neuron* 2003;**39**:401–7.
100. Borgland SL, Taha SA, Sarti F, Fields HL, Bonci A. Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. *Neuron* 2006;**49**:589–601.
101. Korotkova TM, Brown RE, Sergeeva OA, Ponomarenko AA, Haas HL. Effects of arousal- and feeding-related neuropeptides on dopaminergic and GABAergic neurons in the ventral tegmental area of the rat. *Eur J Neurosci* 2006;**23**:2677–85.
102. Muschamp JW, Dominguez JM, Sato SM, Shen RY, Hull EM. A role for hypocretin (orexin) in male sexual behavior. *J Neurosci* 2007;**27**:2837–45.
103. Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You ZB. Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. *J Neurosci* 2005;**25**:5389–96.
104. Wang B, You ZB, Wise RA. Reinstatement of cocaine seeking by hypocretin (orexin) in the ventral tegmental area: independence from the local corticotropin-releasing factor network. *Biol Psychiatry* 2009;**65**:857–62.
105. Coleman PJ, Renger JJ. Orexin receptor antagonists: a review of promising compounds patented since 2006. *Expert Opin Ther Pat* 2010;**20**:307–24.
106. Smart D, Sabido-David C, Brough SJ, et al. SB-334867-A: the first selective orexin-1 receptor antagonist. *Br J Pharmacol* 2001;**132**:1179–82.
107. Chalmeau J, le Grimellec C, Sternick J, Vieu C. Patterned domains of supported phospholipid bilayer using microcontact printing of Pll-g-PEG molecules. *Colloids Surf B* 2012;**89**:188–95.
108. Winrow CJ, Gotter AL, Cox CD, et al. Promotion of sleep by suvorexant-a novel dual orexin receptor antagonist. *J Neurogenet* 2011;**25**:52–61.
109. Sun H, Kennedy WP, Wilbraham D, et al. Effects of suvorexant, an orexin receptor antagonist, on sleep parameters as measured by polysomnography in healthy men. *Sleep* 2013;**36**:259–67.
110. Cox CD, Breslin MJ, Whitman DB, et al. Discovery of the dual orexin receptor antagonist [(7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2H–1,2,3-triazol-2-yl)phenyl]methanone (MK-4305) for the treatment of insomnia. *J Med Chem* 2010;**53**:5320–32.
111. Brielmaier BD. Eszopiclone (Lunesta): a new nonbenzodiazepine hypnotic agent. *Proc (Bayl Univ Med Cent)* 2006;**19**:54–9.
112. Davies M, Newell JG, Derry JM, Martin IL, Dunn SM. Characterization of the interaction of zopiclone with gamma-aminobutyric acid type A receptors. *Mol Pharmacol* 2000;**58**:756–62.
113. McCall WV, Erman M, Krystal AD, et al. A polysomnography study of eszopiclone in elderly patients with insomnia. *Curr Med Res Opin* 2006;**22**:1633–42.
114. Roth T, Walsh JK, Krystal A, Wessel T, Roehrs TA. An evaluation of the efficacy and safety of eszopiclone over 12 months in patients with chronic primary insomnia. *Sleep Med* 2005;**6**:487–95.
115. Scharf M, Erman M, Rosenberg R, et al. A 2-week efficacy and safety study of eszopiclone in elderly patients with primary insomnia. *Sleep* 2005;**28**:720–7.
116. Soares CN, Joffe H, Rubens R, Caron J, Roth T, Cohen L. Eszopiclone in patients with insomnia during perimenopause and early postmenopause: a randomized controlled trial. *Obstet Gynecol* 2006;**108**:1402–10.
117. Walsh JK, Krystal AD, Amato DA, et al. Nightly treatment of primary insomnia with eszopiclone for six months: effect on sleep, quality of life, and work limitations. *Sleep* 2007;**30**:959–68.
118. Harrison NL. Mechanisms of sleep induction by GABA(A) receptor agonists. *J Clin Psychiatry* 2007;**68**(Suppl 5):6–12.
119. Roth T. A physiologic basis for the evolution of pharmacotherapy for insomnia. *J Clin Psychiatry* 2007;**68**(Suppl 5):13–8.

120. Micheli F, Antolini M, Di Fabio R, Pellacani A, Pozzan A. 2-Methyl-3-furanyl-4H-1,2,4-triazol-3-ylthioamides: a new class of selective orexin 2 antagonists. *Bioorg Med Chem Lett* 2010;**20**:6405–7.
121. Ohno K, Sakurai T. Orexin neuronal circuitry: role in the regulation of sleep and wakefulness. *Front Neuroendocrinol* 2008;**29**:70–87.
122. Hoch M, Hoever P, Theodor R, Dingemanse J. Almorexant effects on CYP3A4 activity studied by its simultaneous and time-separated administration with simvastatin and atorvastatin. *Eur J Clin Pharmacol* 2013.
123. Haynes AC, Jackson B, Chapman H, et al. A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept* 2000;**96**:45–51.
124. Rodgers RJ, Halford JC, Nunes de Souza RL, et al. SB-334867, a selective orexin-1 receptor antagonist, enhances behavioural satiety and blocks the hyperphagic effect of orexin-A in rats.. *Eur J Neurosci* 2001;**13**:1444–52.
125. Asahi S, Egashira S, Matsuda M, et al. Development of an orexin-2 receptor selective agonist, [Ala(11), D-Leu(15)]orexin-B. *Bioorg Med Chem Lett* 2003;**13**:111–3.
126. Fujimoto T, Kunitomo J, Tomata Y, et al. Discovery of potent, selective, orally active benzoxazepine-based Orexin-2 receptor antagonists. *Bioorg Med Chem Lett* 2011;**21**:6414–6.
127. Blanco-Centurion C, Liu M, Konadhode R, Pelluru D, Shiromani PJ. Effects of orexin gene transfer in the dorsolateral pons in orexin knockout mice. *Sleep* 2013;**36**:31–40.
128. Dell LA, Patzke N, Bhagwandin A, et al. Organization and number of orexinergic neurons in the hypothalamus of two species of Cetartiodactyla: a comparison of giraffe (Giraffa camelopardalis) and harbour porpoise (Phocoena phocoena). *J Chem Neuroanat* 2012;**44**:98–109.
129. Herring WJ, Snyder E, Budd K, et al. Orexin receptor antagonism for treatment of insomnia: a randomized clinical trial of suvorexant. *Neurology* 2012;**79**:2265–74.
130. Putula J, Turunen PM, Johansson L, et al. Orexin/hypocretin receptor chimaeras reveal structural features important for orexin peptide distinction. *FEBS Lett* 2011;**585**:1368–74.
131. White CL, Ishii Y, Mendoza T, et al. Effect of a selective OX1R antagonist on food intake and body weight in two strains of rats that differ in susceptibility to dietary-induced obesity. *Peptides* 2005;**26**:2331–8.
132. Haynes AC, Chapman H, Taylor C, et al. Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul Pept* 2002;**104**:153–9.
133. Kang JE, Lim MM, Bateman RJ, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science* 2009;**326**:1005–7.
134. Johnson PL, Truitt W, Fitz SD, et al. A key role for orexin in panic anxiety. *Nat Med* 2010;**16**:111–5.
135. de Lecea L, Jones BE, Boutrel B, et al. Addiction and arousal: alternative roles of hypothalamic peptides. *J Neurosci* 2006;**26**:10372–5.
136. Tabaeizadeh M, Motiei-Langroudi R, Mirbaha H, et al. The differential effects of OX1R and OX2R selective antagonists on morphine conditioned place preference in naive versus morphine-dependent mice. *Behav Brain Res* 2013;**237**:41–8.
137. Rotter A, Bayerlein K, Hansbauer M, et al. Orexin A expression and promoter methylation in patients with cannabis dependence in comparison to nicotine-dependent cigarette smokers and nonsmokers. *Neuropsychobiology* 2012;**66**:126–33.
138. Bruijnzeel AW. Tobacco addiction and the dysregulation of brain stress systems. *Neurosci Biobehav Rev* 2012;**36**:1418–41.
139. Plaza-Zabala A, Flores A, Maldonado R, Berrendero F. Hypocretin/orexin signaling in the hypothalamic paraventricular nucleus is essential for the expression of nicotine withdrawal. *Biol Psychiatry* 2012;**71**:214–23.
140. Rodgers RJ, Wright FL, Snow NF, Taylor LJ. Orexin-1 receptor antagonism fails to reduce anxiety-like behaviour in either plus-maze-naive or plus-maze-experienced mice. *Behav Brain Res* 2013.
141. Lionetto L, Negro A, Palmisani S, et al. Emerging treatment for chronic migraine and refractory chronic migraine. *Expert Opin Emerg Drugs* 2012;**17**:393–406.
142. Ghanemi A. Biological properties and perspective applications of ''Bio-neuter'' chemicals? *Saudi Pharm J* 2014;**22**:1–2.
143. Ghanemi A. Toward overcoming the challenges facing biomedical analyses. *Alexandria J Med* 2015;**51**(3):277–8.
144. Ghanemi A. Is mapping borders between pharmacology and toxicology a necessity? *Saudi Pharm J* 2014.
145. Ghanemi A. Cell cultures in drug development: Applications, challenges and limitations. *Saudi Pharm J* 2014.
146. Ghanemi A, Boubertakh B. Shorter and sturdier bridges between traditional Chinese medicines and modern pharmacology. *Saudi Pharm J* 2014.
147. Boubertakh B, Liu X-G, Cheng X-L, Li P. A spotlight on chemical constituents and pharmacological activities of Nigella glandulifera Freyn et Sint seeds. *J Chem* 2013;**2013**:12.
148. Ghanemi A. Psychiatric neural networks and neuropharmacology: Selected advances and novel implications. *Saudi Pharm J* 2014;**22**:95–100.
149. Ghanemi A. Schizophrenia and Parkinson's disease: selected therapeutic advances beyond the dopaminergic etiologies. *Alexandria J Med* 2013;**49**:287–91.