Neuropharmacology 62 (2012) 978–987

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Pharmacological characterization of MK-6096 – A dual orexin receptor antagonist for insomnia

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ARTICLE INFO

Article history:
Received 1 August 2011
Received in revised form
26 August 2011
Accepted 4 October 2011

Keywords:
Orexin
Hypocretin
Insomnia
Receptor occupancy
Pharmacology
MK-6096

ABSTRACT

Orexin (hypocretin) neuropeptides promote wakefulness by signaling through two G-protein coupled receptors, Orexin 1 Receptor (OX_1R) and Orexin 2 Receptor (OX_2R). MK-6096 is an orally bioavailable potent and selective reversible antagonist of OX_1R and OX_2R currently in clinical development for insomnia. In radioligand binding and functional cell based assays MK-6096 demonstrated potent binding and antagonism of both human OX_1R and OX_2R (<3 nM in binding, 11 nM in FLIPR), with no significant off-target activities against a panel of >170 receptors and enzymes. MK-6096 occupies 90% of human OX_2Rs expressed in transgenic rats at a plasma concentration of 142 nM, and dose-dependently reduced locomotor activity and significantly increased sleep in rats (3–30 mg/kg) and dogs (0.25 and 0.5 mg/kg). DORA-22, an analog of MK-6096, exhibits similar sleep promoting properties that are absent $OX_{1/2}R$ double knockouts, demonstrating the mechanism of action and specificity of these effects. These findings with a novel, structurally distinct class of OxR antagonists provide further validation of the orexin pathway as an effective target to promote normal sleep. Comparative analysis of the biochemical and pharmacokinetic properties of these compounds relative to other OXR antagonists provides a basis for understanding the attributes critical for *in vivo* efficacy. This mechanism is distinct from current standard of care such that MK-6096 represents a novel and selective therapeutic for the treatment of insomnia.

This article is part of a Special Issue entitled 'Post-Traumatic Stress Disorder'.

1. Introduction

With an estimated incidence of 10–15% in the general population and 30–60% in elderly, insomnia is one of the most common neuropsychiatric disorders, and is closely linked with a host of other diseases including depression, obesity, cardiovascular disease, cancer and chronic pain (Benca, 2005; Foley et al., 1995; Kamel and Gammack, 2006; Manabe et al., 2000; Roth, 2001). The economic impact of insomnia has been reported to range between $77 to $107 billion dollars annually, resulting in losses from accidents, health care costs, productivity and absenteeism (Drake et al., 2003; Leger, 2000; Stoller, 1994; Walsh and Engelhardt, 1999). Current pharmacological therapies rely primarily on the use of sedative-hypnotics that modulate GABA receptor function and may produce undesirable effects including dependence, tolerance, next-day hangover, amnesia, rebound insomnia and increased risk of falls. Insomnia remains an unmet medical need, requiring novel approaches for treatment with new mechanisms of action.

The orexin neuropeptides (also known as hypocretins) were first discovered through genetic studies investigating hypothalamic signaling, and subsequently found to regulate arousal and sleep/wake control (Bonnavion and de Lecea, 2010; de Lecea et al., 1998; Gautvik et al., 1996; Lin et al., 1999; Sakurai et al., 2010; Scammell and Winrow, 2011). Prepro-orexin peptide is cleaved to form two orexin peptides (OX-A and OX-B) which activate a pair of G-protein coupled receptors, Orexin 1 Receptor (OX_1R) and Orexin 2 Receptor (OX_2R). OX-A binds with similar affinity to both receptors, whereas OX-B demonstrates preferential activation of OX_2R with approximately 10-fold higher potency relative to OX_1R (Sakurai et al., 1998). Orexin levels exhibit a diurnal pattern,

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doi:10.1016/j.neuropharm.2011.10.003

with orexinergic neurons firing actively during wakefulness and becoming virtually silent during the normal sleep period (Estabrooke et al., 2001; Fujiki et al., 2001; Grady et al., 2006; Lee et al., 2005). When administered exogenously, orexin peptides increase locomotor activity and wakefulness in mice, rats, dogs and monkeys (Deadwyler et al., 2007; Fujiki et al., 2003; Sakurai et al., 1998; Winrow et al., 2010). Maintenance of wake is a key function of the orexin system, as has been demonstrated both genetically and pharmacologically. Dogs lacking functional OX_2R show disrupted sleep/wake behaviors and are prone to narcolepsy (Fujiki et al., 2002; Lin et al., 1999). In mice, chronic loss of orexin signaling results in sleep/wake dysregulation that is most severe with loss of orexinergic neurons or deletion of prepro-orexin gene, and less pronounced in animals lacking functional orexin receptors (Chemelli et al., 1999; Hara et al., 2001; Mochizuki et al., 2004). Small molecule orexin receptor antagonists suvorexant and almorexant have been shown to effectively promote sleep in animals, as well as demonstrating clinical efficacy (Brisbare-Roch et al., 2007; Herring et al., 2010; Winrow et al., 2011). We recently reported the preclinical characterization of suvorexant, a diazepane-based antagonist currently in late clinical development for the treatment of insomnia. Suvorexant is a dual orexin receptor antagonist (DORA) with similar potency toward both OX_1R and OX_2R, and effectively induces sleep across species, including man (Cox et al., 2010; Herring et al., 2010; Winrow et al., 2011). This molecule significantly and dose-proportionally promotes sleep in insomnia patients, thereby establishing clinical proof of concept for DORAs in insomnia (Herring et al., 2010).

Here we describe the characterization of MK-6096, a potent, reversible, orally bioavailable and structurally distinct piperidine-derived DORA that is currently being evaluated in clinical studies for insomnia. MK-6096 as well as a close analog, DORA-22, are highly selective for OX_1R and OX_2R, with desirable pharmacological properties enabling significant reductions in wakefulness and corresponding increases in NREM and REM sleep across species. The absence of these sleep promoting effects in mice lacking OX_1 and OX_2 receptors clearly demonstrates that these effects are mediated through orexin signaling and further illustrates the specificity of these compounds. The favorable potency, selectivity and efficacy observed with MK-6096 support the continued clinical development of this molecule for the treatment of insomnia, and, in comparisons with other DORAs, more clearly define the biochemical (e.g. *in vitro* binding, potency, on/off-rate) and pharmacokinetic ($T_{1/2}$, plasma/CSF exposure) properties critical to achieving efficacy. Together, these findings with compounds that are structurally distinct from others in clinical development validate the orexin pathway as an effective mechanism to specifically promote somnolence having the properties of normal sleep.

2. Materials and methods

All animal studies were performed in accordance with the USDA Guide for the Care and Use of Laboratory Animals and were approved by the Merck Institutional Animal Care and Use Committee. All efforts were made to minimize animal use and suffering.

2.1. Orexin receptor binding and inhibition of activity

Binding of MK-6096, the structural analog DORA-22 and almorexant was assessed with membranes from Chinese hamster ovary (CHO) cells expressing human OX_1R or human OX_2R using a protocol that has been described previously (Coleman et al., 2010a; Cox et al., 2010; Kunapuli et al., 2003; Mosser et al., 2003; Winrow et al., 2011). MK-6096 and DORA-22 binding to CHO cells expressing OX_1R or OX_2R from rat, rhesus, and dog were evaluated by the same protocol. In brief, displacement of tritiated OX_1R or OX_2R ligands from membranes was carried out using MK-6096 or DORA-22 in 20 mM HEPES (pH 7.4), 120 mM NaCl, 5 mM KCl, 5 mg membrane protein for 120 min at room temperature. Determination of non-specific binding was performed using unlabeled OX_1R/OX_2R antagonist at 1 μM, and K_i values determined using the RAB Calc software package.

Functional blockade of human OX_1R and human OX_2R activation by MK-6096, DORA-22 and almorexant was evaluated using CHO cells expressing the receptors as previously described (Coleman et al., 2010a; Cox et al., 2010; Winrow et al., 2011). Briefly, calcium-dependent fluorescence of cells loaded with Fluo-4-AM ester was measured following addition of modified human orexin peptide ligand, OX-A (Ala6,12). Inhibition of the response was evaluated following addition of increasing concentrations of MK-6096, DORA-22 or almorexant delivered 5 min prior to OX-A (Ala6,12) stimulation.

2.2. Receptor occupancy

Transgenic rats over-expressing the human OX_2R protein in rat brain via the rat neuron-specific enolase promoter were used to evaluate MK-6096, DORA-22 and almorexant in an *ex vivo* occupancy assay following an established protocol (Cox et al., 2010; Winrow et al., 2011) using a tritiated compound 9 depicted in Cox et al. (2010) (page 5327) having a reversible K_d for OX_2R = 0.2 nM. Test compounds were administered by i.v. infusion over 30 min, followed by brain collection, lysate preparation and measurement of radioligand displacement (Cox et al., 2010; Winrow et al., 2011). Pharmacokinetic assessments were performed to determine plasma levels of the test articles. The concentrations required to obtain 90% receptor occupancy were derived by nonlinear curve fitting using Prism software ($\%\ \text{Occupancy} = (1 - (\text{slope}_{\text{drug}}/\text{slope}_{\text{vehicle}})) \times 100$).

2.3. Mouse sleep architecture, temperature and activity

Electrocorticogram (ECoG) and electromyogram (EMG) were continuously monitored in single housed adult (~30 g) male wildtype C57BL/6J and OX_1R/OX_2R double mutant mice housed in a 12:12 light:dark cycle (lights on: 14:00, off: 02:00) surgically implanted with TL11M2-F20-EET radio telemetry transmitters (Data Sciences International, Arden Hills, MN). Mice with targeted mutation of both OX_1R (*Hcrtr1*) and OX_2R (*Hcrtr2*) genes were obtained by breeding individual single knockouts (lines T2093 and T1761 from Deltagen Inc.) (San Mateo, CA) to homozygosity for both genes. Individual mutant lines were backcrossed >9 generations onto C57BL/6Ntac prior to generation of double knockouts, and age matched C57BL/6Ntac wildtype animals were used as controls.

Effects of 100 mg/kg DORA-22 relative to vehicle (20% Vitamin E TPGS in water, p.o.) administered in the active phase (ZT 20:00; 10:00 DST) were evaluated simultaneously in wildtype and OX_1R/OX_2R mutants in adjacent cages under identical conditions. The effect of compound treatment was assessed using a counterbalanced crossover design in which all animals were alternatively treated with drug and vehicle daily for 5 consecutive days: a 5 day arm of drug or vehicle followed by 2 days of washout (no treatment) followed by 5 days of conditional crossover. Automated scoring and sleep stage analyses were performed as previously described (Kraus et al., 2010; Renger et al., 2004; Winrow et al., 2011). Averages of each 30 min interval for each condition over 5 days for vehicle/compound treatment were statistically compared as detailed previously (Winrow et al., 2011) and described briefly below. Subcutaneous temperature and locomotor activity measures obtained from implanted TL11M2-F20-EET transmitters was recorded simultaneously with ECoG/EMG. Mean temperature over each 30 min interval from wildtype or mutant animals from 5 days of consecutive vehicle or compound treatment was averaged and plotted on a single 24 h time course. Locomotor activity measured as implant movements, was similarly evaluated except that the activity sums over each 30 min interval were averaged. Compound induced temperature and activity changes for the 4 h following treatment were evaluated by 2-way ANOVA with significant changes during 30 min intervals made by Bonferroni post-hoc analysis.

2.4. Rat sleep architecture studies

Rat sleep studies were conducted in a similar format to those previously published (Renger et al., 2004; Whitman et al., 2009; Winrow et al., 2011). In summary, electrocorticogram/electroencephalogram (ECoG/EEG), electromyogram (EMG) and locomotion were continuously monitored in male Sprague Dawley rats (n = 8/study; age: 3–6 months; weight: 450–600 g), that had been surgically implanted with radio telemetric physiologic monitors (TL10M3-F50-EEE; Data Sciences International, Arden Hills, Minnesota, U.S.A.). The animals were singly housed with water and food *ad libitum* and a 12 h light: 12 h dark cycle with lights on at 04:00 and off at 16:00.

Sleep studies were conducted to evaluate MK-6096 (3 and 10 mg/kg, p.o.), DORA-22 (10 mg/kg, p.o.) and almorexant (3 and 30 mg/kg, p.o.), employing a counterbalanced crossover design in which all animals were alternatively treated with drug and vehicle daily for either 3 or 7 consecutive days (for DORA-22 and MK-6096/almorexant, respectively): 2 baseline days (no dosing), a 2 day vehicle-only run-in, a 3 or 7-day arm of drug or vehicle followed by 3 or 7 days of conditional crossover. Effects of compound treatments relative to vehicle (20% Vitamin E TPGS, p.o.) were evaluated following administration in the active phase (ZT 17:00–17:30; (09:00–09:30 DST)). Results for all 8 animals were averaged by condition over 7 days of compound administration as described previously (Winrow et al., 2011) and detailed briefly below.

2.5. Canine sleep architecture studies

Sleep studies with canines were conducted as previously described (Winrow et al., 2011). Briefly, ECoG/EEG, EMG and electrooculogram (EOG) were recorded continuously from healthy male Beagle dogs ($n = 6$/study; age: 6–12 years; weight: 9–17 kg) surgically implanted with radio telemetric physiologic monitors (DC-70-EEE; Data Sciences International, Arden Hills, Minnesota, U.S.A.). Acclimated animals were housed individually in standard canine runs with water *ad libitum* and food once/day in a 12 h light: 12 h dark cycle with lights on at 05:00 and off at 17:00.

MK-6096 (0.25 and 0.5 mg/kg, p.o.), DORA-22 (3 and 30 mg/kg) and vehicle (20% vitamin E TPGS) were administered by oral gavage once daily at ZT 3:00 (08:00) in a counterbalanced crossover design in which all animals were alternatively treated with drug and vehicle: 3 days of vehicle-only run-in, 5 days of drug or vehicle, 2 day vehicle washout, then 5 days of conditional crossover. Results were grouped into 30 min periods across 24 h. The results for all six animals were averaged by treatment in 30 min bins over 5 administration nights and analyzed as detailed previously (Winrow et al., 2011) and detailed briefly below.

2.6. Sleep stage data collection and analysis

Automated data collection and analysis was carried out in a manner similar to that described previously for rodents and dogs (Cox et al., 2010; Renger et al., 2004; Whitman et al., 2009; Winrow et al., 2010). ECoG/EEG and EMG signals (mice, rats and dogs) and EOG signals (dogs) were collected and used to characterize 4 sleep/wake states (active wake, slow wave sleep I) (SWS I - light non-REM sleep), slow wave sleep II (SWS II - deep delta non-REM sleep), and REM sleep. Sleep architecture results for each 30-min bin were evaluated for changes between MK-6096, DORA-22, almorexant and vehicle-treated animals. Percent latency to sleep stages and percent duration in sleep stages were analyzed from raw sleep stage time and bout data for the cumulative first 3 h post-dosing in dogs and the cumulative first 4 h post-dosing in rodents, with each animal as their own control in the crossover design. Percentage change values were statistically evaluated using a population *t*-test, 2-tailed, using Graphpad Prism (GraphPad Software, LaJolla, CA, U.S.A.). Latency to SWS I or SWS II was defined as latency to the first consecutive 60-s episode; latency to REM was the first consecutive 30-s episode as previously described (Winrow et al., 2011).

3. Results

3.1. MK-6096 selectively binds and antagonizes orexin receptors in vitro

MK-6096 (((2R,5R)-5-((5-fluoropyridin-2-yloxy)methyl)-2-methylpiperidin-1-yl) (5-methyl-2-(pyrimidin-2-yl)phenyl)methanone) is a highly selective and potent antagonist of both OX_1R and OX_2R, developed from a piperidine-series of compounds (Coleman et al., unpublished) MK-6096 bound with similar affinity in radioligand binding assays to human OX_1R (Ki = 2.9 nM, $n = 12$) and OX_2R (Ki = 0.31 nM, $n = 13$) (Table 1). The affinity of MK-6096 for OX_1R and OX_2R from other species was evaluated and found to show similar potency in rat (OX_1R Ki = 2.48 nM, OX_2R Ki = 0.22 nM), dog (OX_1R Ki = 2.7 nM, OX_2R Ki = 0.36 nM), mouse (OX_1R Ki = 2.9 nM, OX_2R Ki = 0.56 nM), rabbit (OX_1R Ki = 2.61 nM, OX_2R Ki = 0.28 nM) and rhesus (OX_1R Ki = 12.5 nM, OX_2R Ki = 0.44 nM). Similar affinities in the nanomolar range were observed across species with the piperidine analog DORA-22 (Table 1). Consistent with binding data, MK-6096 blocked orexin A–stimulated calcium response in CHO cells expressing human and rat orexin receptors demonstrating equivalent potency for both receptor isoforms (Kb: hOX_1R = 11 nM, hOX_2R = 11 nM; rOX_1R = 10 nM, rOX_2R = 22 nM) as well as orexin receptors from dog (OX_1R Kb = 10 nM, OX_2R Kb = 10 nM), mouse (OX_1R Kb = 9 nM, OX_2R Kb = 10 nM), rabbit (OX_1R Kb = 13 nM, OX_2R Kb = 16 nM) and rhesus (OX_1R Kb = 21 nM, OX_2R Kb = 14 nM) (Table 1). DORA-22 exhibited similar potency for human and rat OX_1R (Kb: hOX_1R = 32 nM, rOX_1R = 77 nM) and OX_2R (Kb: hOX_2R = 10 nM, rOX_2R = 19 nM), as well as in four other species (Table 1). Evaluation of almorexant *in vitro* binding (hOX_1R = 2.7 nM, hOX_2R = 0.19 nM) and functional potency (hOX_1R = 128 nM, hOX_2R = 119 nM; rOX_1R = 154 nM, rOX_2R = 190 nM) was also conducted. MK-6096 and DORA-22 are highly selective for OX_1R/OX_2R antagonism with no significant interactions against known receptors and enzymes as demonstrated by *in vitro* assay panels including (170 targets screened for MK-6096 and 116 targets screened for DORA-22; MDS Pharma) including those associated with cardiovascular, renal, oncogenic, autonomic and CNS function nor was any activity on cardiac hERG K^+ channels up to 15 μM were detected (Cox et al., 2010). Due to inherently low endogenous levels of orexin receptors in brain, a transgenic line of rats overexpressing human OX_2R throughout the brain was established to enable *ex vivo* receptor occupancy studies. Although these animals express extraphysiological levels OX_2R, they not only allow for detectable ligand displacement signal, but also evaluate binding of compounds to the human Ox2R protein (Cox et al., 2010; Winrow et al., 2011). In practice, a plasma concentration required to achieve 90% OX_2R occupancy (Occ_{90}) is typically associated with the appearance of somnolence promoting effects of OXR antagonists. MK-6096 showed radioligand displacement to occupy OX_2R in transgenic rat brain in a dose-dependent manner (Fig. 1A). Following peripheral dosing, MK-6096 achieved 90% occupancy at an exposure of 142 nM in plasma, showing 3–14 fold higher potency than DORA-22 (Occ_{90} = 1139 nM) and almorexant (Occ_{90} = 1922 nM) (Fig. 1A–C). MK-6096 binding is fully reversible as demonstrated by *in vitro* kinetic displacement assays using radio-labeled MK-6096 with a $T_{1/2}$ ON = 63 min and $T_{1/2}$ OFF = 118 min. This was compared to the structurally distinct DORA, almorexant, which exhibited significantly longer on-rates ($T_{1/2}$ ON = 162 min) and off-rates ($T_{1/2}$ OFF = 262 min) (Fig. 2).

Table 1
In vitro binding and potency of MK-6096 and DORA-22 to OX_1R and OX_2R across species.

Compound	Assay	OX_1R						OX_2R					
		Mouse	Rat	Rabbit	Dog	Rhesus	Human	Mouse	Rat	Rabbit	Dog	Rhesus	Human
MK-6096	Binding[a] (K_i, nM)	2.9 ($n = 3$)	2.48 ($n = 3$)	2.61 ($n = 3$)	2.7 ($n = 3$)	12.5 ($n = 3$)	2.5 ($n = 3$)	0.56 ($n = 4$)	0.22 ($n = 3$)	0.28 ($n = 3$)	0.36 ($n = 3$)	0.44 ($n = 3$)	0.31 ($n = 5$)
DORA-22	Binding[a] (K_i, nM)	21 ($n = 3$)	13.21 ($n = 3$)	9.07 ($n = 3$)	22 ($n = 3$)	69.7 ($n = 3$)	9.7 ($n = 3$)	1.14 ($n = 4$)	0.44 ($n = 3$)	0.75 ($n = 3$)	0.36 ($n = 3$)	1.23 ($n = 3$)	0.61 ($n = 5$)
Almorexant	Binding[a] (K_i, nM)						2.7 ($n = 8$)						0.19 ($n = 15$)
MK-6096	FLIPR[b] (K_b, nM)	9 ($n = 5$)	10 ($n = 4$)	13 ($n = 3$)	10 ($n = 7$)	21 ($n = 4$)	11 ($n = 4$)	10 ($n = 4$)	22 ($n = 5$)	16 ($n = 5$)	10 ($n = 6$)	14 ($n = 5$)	11 ($n = 6$)
DORA-22	FLIPR[b] (K_b, nM)	11 ($n = 4$)	77 ($n = 3$)	19 ($n = 4$)	6 ($n = 7$)	84 ($n = 4$)	32 ($n = 4$)	10 ($n = 5$)	19 ($n = 4$)	21 ($n = 5$)	12 ($n = 6$)	19 ($n = 5$)	10 ($n = 6$)
Almorexant	FLIPR[b] (K_b, nM)		154.1 ($n = 22$)				128.4 ($n = 22$)		190 ($n = 21$)				118.9 ($n = 22$)

[a] Specific and non-specific binding to OX_1 and OX_2 receptors was evaluated by displacement of tritiated small molecule ligands and unlabeled compounds, respectively, as previously described (Coleman et al., 2010a,b; Cox et al., 2010; Winrow et al., 2011).

[b] Inhibition of OX-A[ala6,12]-mediated induction of receptor activity measured fluorescent calcium signal in recombinant cells expressing the indicated OX receptor (Coleman et al., 2010a,b; Cox et al., 2010; Winrow et al., 2011).

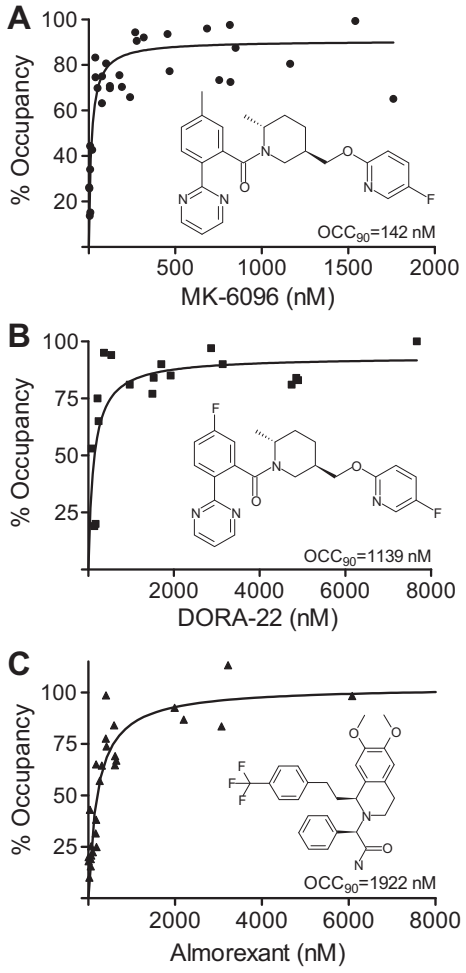

Fig. 1. Dose-dependence of OX_2R occupancy by MK-6096, DORA-22, and almorexant, in transgenic rat brain expressing human OX_2R. The percentage of human OX_2R assessed *ex vivo* by small molecule radioligand displacement is plotted relative to plasma levels of MK-6096 (A), DORA-22 (B), or almorexant (C) following i.v. treatment. *Insets*, chemical structures for the indicated compounds.

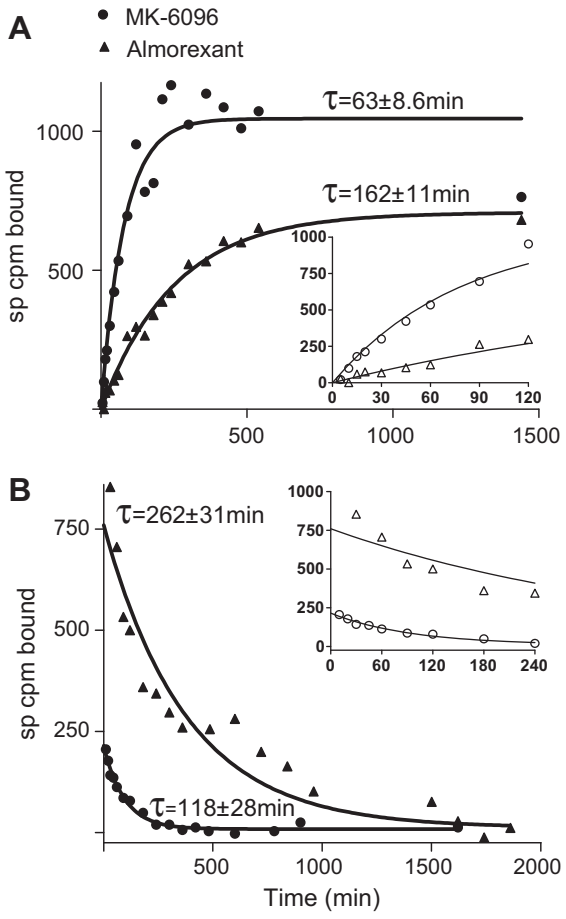

Fig. 2. MK-6096 exhibits accelerated binding kinetics relative to almorexant. CHO cells expressing human Ox2R were incubated with labeled MK-6096 (circles) or almorexant (triangles). A. On rate kinetics ($T_{1/2}$On = 63 ± 8.6 and 162 ± 10.5 min; K_{ON} = 1.57 × 10^{-19} and 4.868 × 10^{-20} mol/min) for MK-6096 and almorexant, respectively. *Inset*, first 2 h of binding. B. Off rate kinetics ($T_{1/2}$Off = 118 ± 27.8 and 262 ± 30.6 min; K_{OFF} = 1.35 × 10^{-19} and 3.48 × 10^{-20} mol/min) for MK-6096 and almorexant, respectively. *Inset*, first 4 h of the experiment. In both experiments, MK-6096 was evaluated at concentrations approximating its K_d, while almorexant was assessed at ~3× K_d, driving more rapid association/dissociation kinetics.

3.2. Orexin receptor antagonism selectively promotes sleep in mice

Wildtype mice treated with 100 mg/kg, (p.o.) of DORA-22 4 h prior to the inactive phase responded with reductions in both locomotor activity and body temperature within the normal physiological range (Fig. 3A). These changes were significant for 4 h following treatment ($F_{1,464}$ = 17.25, p = 0.0001; $F_{1,464}$ = 11.02, p = 0.0016, respectively; 2-way ANOVA) and were reduced to levels similar to that seen during the normal inactive phase. Consistent with these changes were significant 4 h reductions in active wake (−26.4%) and corresponding increases in both non-REM (58.9% increase in SWS) and REM sleep (122.2%) (Fig. 3B and C). Latency to REM sleep was also reduced (−46%). In contrast, animals lacking functional Ox_1R and Ox_2R, showed no significant activity or temperature responses to DORA-22 (Fig. 3A, right panels ($F_{1,304}$ = 3.01, p = 0.0907; $F_{1,304}$ = 0.13, p = 0.7162, respectively; 2-way ANOVA)), nor did they show consistent active wake decreases or sleep increases similar to that of wildtype animals (Fig. 3B and C, right panels). In these and other trials with DORA-22, no tachyphylaxis was observed over the course of these 5 day experiments (not shown). These results demonstrate the capacity of a DORA to promote sleep in mice selectively through antagonism of OX_1R and OX_2R.

3.3. DORAs increase sleep in rats

MK-6096 and DORA-22 altered locomotor activity and sleep/wake architecture in rats compared to vehicle as assessed using multi-day crossover studies following daily oral administration during the normal rat active phase. Significant reductions in locomotor activity were observed over 4 h post-dose with both DORA-22 (30 mg/kg, $F_{1,483}$ = 7.18, p = 0.0092, 2-way ANOVA) and MK-6096 (10 mg/kg, $F_{1,490}$ = 36.01, p < 0.0001, 2-way ANOVA) compared to vehicle-treated rats (Fig. 4A).

DORA-22 administered at 10 mg/kg, p.o. (C_{max}, = 0.67 μM, AUC = 2.52 μM h, $T_{1/2}$ = 0.5 h) significantly decreased active wake (−27.6%) immediately upon administration, lasting approximately 2.5 h post-dose with corresponding significant increases in non-REM SWS (20.6%) and REM sleep (48.5%) (Fig. 4B and C). Latencies to non-REM and REM sleep were reduced by −29.7% and −53.7% respectively. Oral administration of 10 mg/kg DORA-22 yielded plasma exposures similar to levels necessary for 90% receptor occupancy and are consistent with the somnolence promoting effects observed. MK-6096 dosed during the active phase, at 3 mg/kg, p.o. (C_{max}, = 0.29 μM, AUC = 0.47 μM h) or 10 mg/kg p.o. (C_{max}, = 1.48 μM, AUC = 1.8/3.33 μM h) in 7-day crossover rat EEG studies caused significant sleep effects (Fig. 4B and C). The plasma

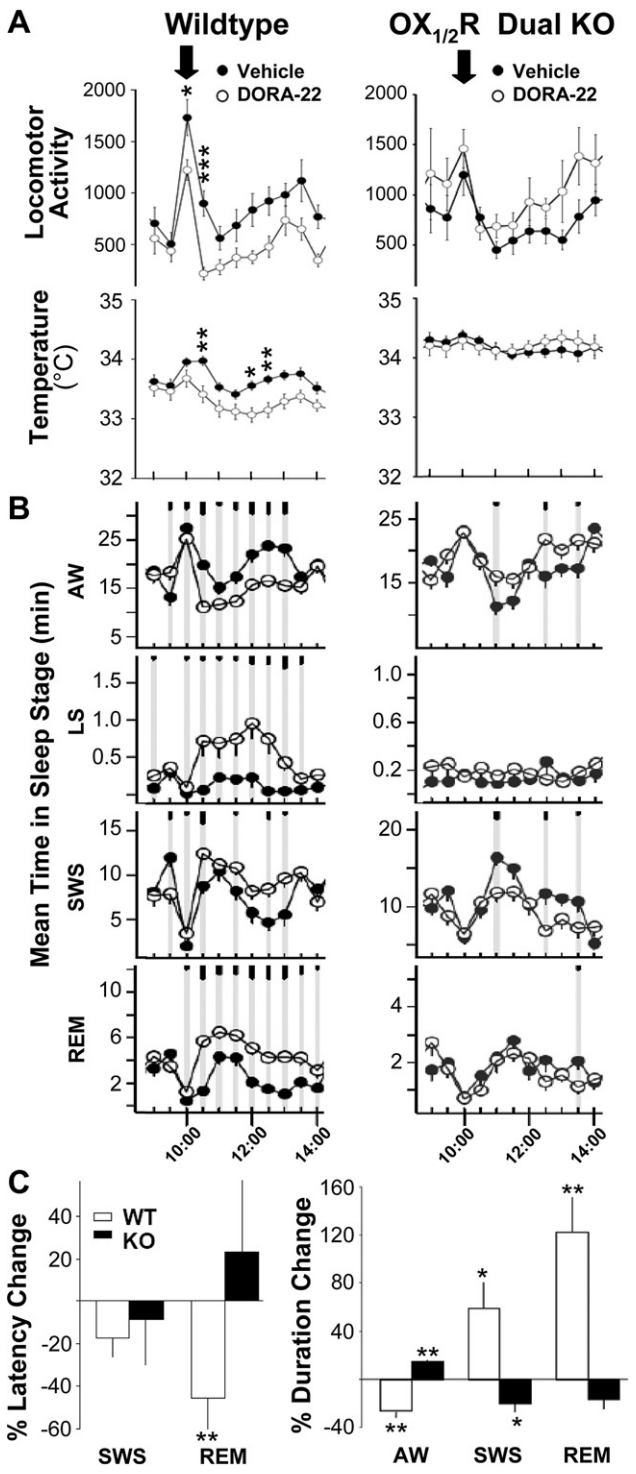

Fig. 3. Activity and wake suppressing effects of MK-6096 analog, DORA-22, in wildtype mice ($n = 6$) are absent in $OX_{1/2}R$ double knockouts ($n = 5$). A. Locomotor activity and body temperature of wildtype (*left panels*) and $OX_{1/2}R$ double mutant animals (*right panels*) following treatment with 100 mg/kg (p.o.) DORA-22. DORA-22 significantly reduced activity and temperature during the 4 h following treatment in wildtype mice ($F_{1,464} = 17.25$, $p = 0.0001$, $F_{1,464} = 11.02$, $p = 0.0016$, respectively; 2-way ANOVA), but had no significant effect on activity or temperature in $Ox_{1/2}R$ dKOs during this time-frame ($F_{1,304} = 3.01$, $p = 0.0907$, $F_{1,304} = 0.13$, $p = 0.7162$, respectively; 2-way ANOVA). *, **, ***, $p < 0.05$, 0.01, 0.001 (Bonferroni Post test). B. ECoG/EMG recordings from radio telemetry-implanted wildtype and $Ox_{1/2}R$ double knockout mice (*left*, *right panels*, *respectively*) were used to evaluate the effect of 100 mg/kg (p.o.) DORA-22 (open symbols) relative to vehicle (20% Vitamin E TPGS, closed symbols) on time spent in the indicated sleep stages. Values represent the mean responses over 30 min intervals over 5 days of consecutive treatment during the active phase (10:00 AM; ZT 20:00, black arrows). AW, Active Wake; LS, Light Sleep; SWS, slow wave sleep; REM, REM sleep. Time points at which significant differences exist between vehicle and DORA-22 responses are indicated by gray vertical lines and tic marks (short, medium, long marks, $p < 0.05$, 0.01, 0.001, respectively, Linear Mixed Effects Model for Repeated Measures). C. Latency to persistent sleep and sleep stage duration for wildtype (open bars) and $OX_{1/2}R$ double knockout animals (filled bars) induced by 100 mg/kg DORA-22 expressed in percent change relative to vehicle (*, **, ***, $p < 0.05$, 0.01, 0.001, population *t*-test).

levels at these doses exceed the levels required for 90% occupancy and the half-life of MK-6096 was 0.5 h, with 20% oral bioavailability and high clearance (46.1 mL/min/kg) in rats. MK-6096 treatment led to significant dose-dependent reductions in active wake (−30.8 to −49.2%) and corresponding increases in non-REM (9.9–21%) and REM sleep (28.9–48.4%) (Fig. 4B and C). Significant reductions in latency to non-REM (−40.5 to −51.4%) and REM sleep (−54.5 to −65.2%) were also observed. Administration of almorexant produced significant effects on sleep at 30 mg/kg, but not at 3 mg/kg (Fig. 5) and was consistent with published results (Brisbare-Roch et al., 2007), however the magnitude of effects was less than that observed with DORA-22 or MK-6096 and no effects on SWS or REM latency were observed (Fig. 5B). In no case did we observe detectable attenuation or potentiation in the somnolence promoting in efficacy of either MK-6096, DORA-22 or almorexant between the first and last day of treatment in these studies (not shown). Overall, the somnolence inducing properties of DORA-22 and MK-6096 on rat sleep architecture were similar, but more potent than have been reported in experiments with other orexin receptor antagonists from diverse unrelated series (Bergman et al., 2008; Brisbare-Roch et al., 2007; Coleman et al., 2010a,b; Cox et al., 2009; Cox et al., 2010; Whitman et al., 2009; Winrow et al., 2010, 2011).

3.4. MK-6096 is a potent sleep promoting compound in canines

Orally administered MK-6096 and DORA-22 were evaluated during the normal active phase for effects on dog sleep architecture in counterbalanced crossover studies. In these studies healthy male beagle dogs showed significant dose-dependent increases in sleep immediately after treatment (Fig. 6). An oral dose of 3 mg/kg DORA-22 provided a $C_{max} = 1.14$ μM, AUC = 4.6 μM h and $T_{max} = 0.75$ h, and 30 mg/kg yielded a $C_{max} = 7.3$ μM, AUC = 64.3 μM h, $T_{max} = 1.0$ h, with a half-life of 2.5 h. Administration of DORA-22 (3 mg/kg, p.o.) significantly reduced active wake (−36.7%) and trended toward increased SWS I (331.3%), SWS II (896.1%) and REM sleep (83.7%), with reduced latencies to SWS I (−31.9%), SWS II (−41.0%) and REM sleep (−28.2%).

MK-6096 doses of 0.25 or 0.5 mg/kg, (p.o.) were evaluated in dog sleep studies ($C_{max} = 0.194$ μM, AUC = 0.38 μM h, $T_{max} = 0.75$ h; $C_{max} = 0.47$ μM, AUC = 1.3 μM h, $T_{max} = 0.38$ h, respectively). MK-6096 has a half-life of 1.7 h, moderate clearance of 11.1 mL/min/kg and oral bioavailability of 49%. Following dosing at 0.25 or 0.5 mg/kg dogs assumed sleep postures and had a significant decrease in active wake (−16.9 to −22.7%) and a corresponding increase in SWS I trends (15.3–39.7%), significant increase in SWS II (88.8–371.3%) and trending increases in REM sleep (70.7–133.6%) (Fig. 6). SWS I sleep latency was reduced (−18.5 to −29.2%), as was SWS II latency (−24.2 to −49.3%); REM sleep latency showed a trended decrease (−23.1 to −20.2%). Whereas not every sleep parameter reached statistical significance (due to the large range of response probably accounted for by the smaller sample size, increased heterogeneity of beagles vs rodents and the large plasma exposure variability for DORAs in beagles dosed with Vitamin E vehicle, unpublished data) all sleep stage trends mirrored the results seen in rodents. As seen in other species, no differences in the magnitude of sleep promoting responses of animals treated with either DORA-22 or MK-6096

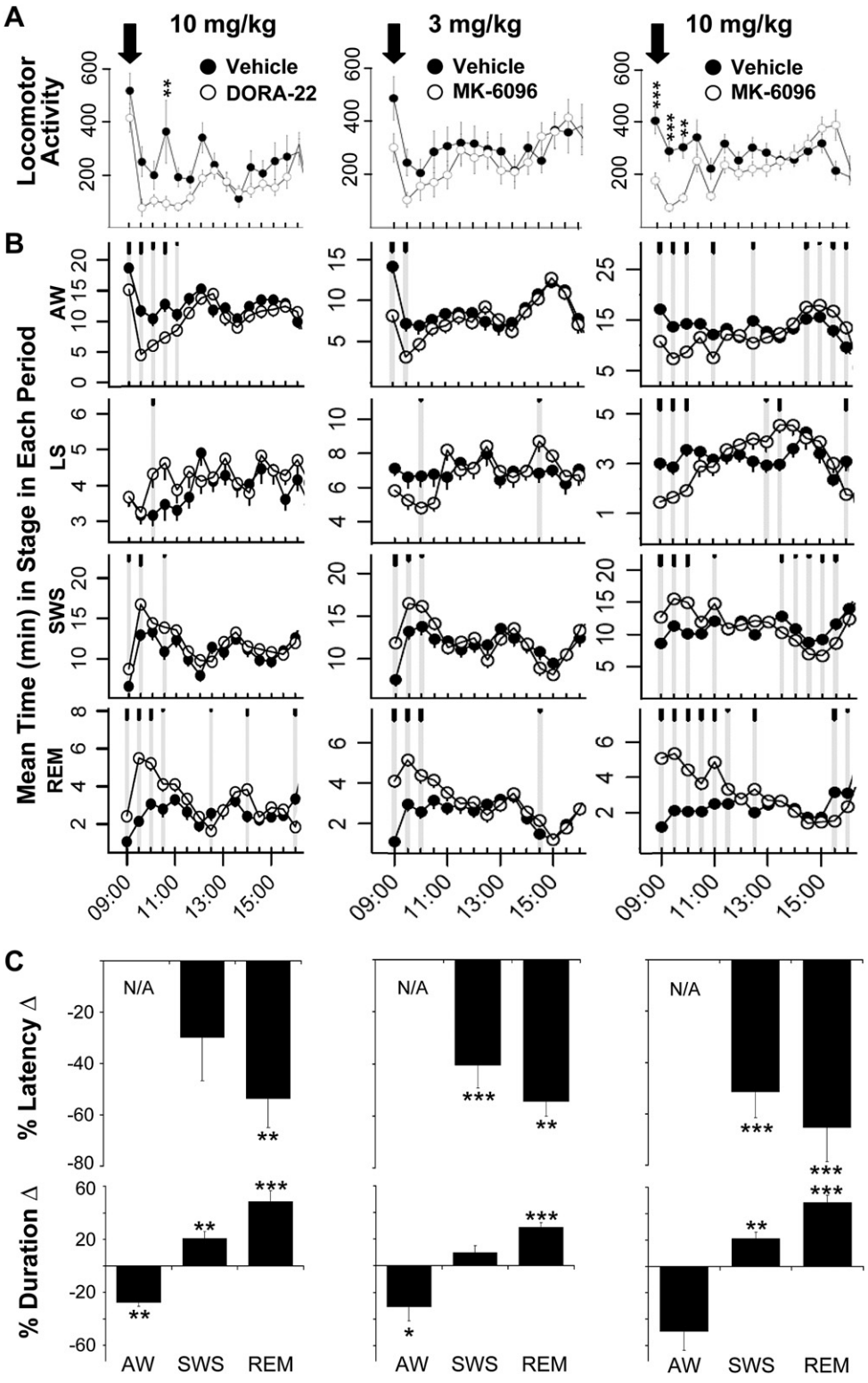

Fig. 4. Activity and wake suppressing effects of DORA-22 and MK-6096 in rats. A. Locomotor suppression following administration of 10 mg/kg DORA-22 was highly significant relative to vehicle (20% Vitamin E TPGS, p.o.) in the 4 h following treatment ($F_{1,483} = 7.18$, $p = 0.0092$, 2-way ANOVA), and 3 and 10 mg/kg MK-6096 exhibited no significant difference at the low dose ($F_{1,665} = 2.41$, $p = 0.124$, 2-way ANOVA), while the 10 mg/kg dose exhibited a highly significant change ($F_{1,490} = 36.01$, $p < 0.0001$, 2-way ANOVA). **, ***, $p < 0.01$, 0.001 (Bonferroni post test). B. Effects of 30 mg/kg DORA-22 and MK-6096 at 3 and 10 mg/kg on mean time in sleep stage over 30 min intervals following treatment (short, medium, long marks: $p < 0.05$, 0.01, 0.001, respectively, Linear Mixed Effects Model for Repeated Measures). C. Quantification of latency to sleep stage and sleep stage duration expressed as mean percent change relative to the vehicle condition in the 4 h following either DORA-22 or MK-6096 treatment (*, **, ***, $p < 0.05$, 0.01, 0.001, population t-test).

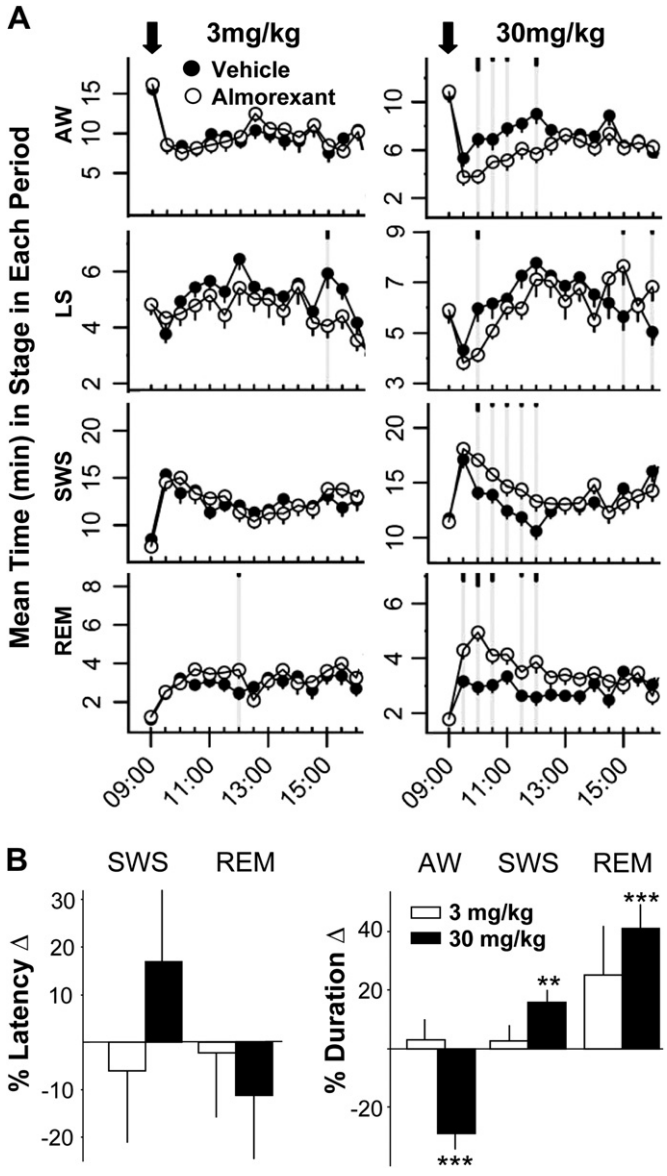

Fig. 5. Effects of almorexant on rat sleep. A. Effects of 3 and 30 mg/kg almorexant on mean time in sleep stage over 30 min intervals following treatment relative to the vehicle (20% Vitamin E TPGS, p.o.) and condition (short, medium, long tick marks: $p < 0.05$, 0.01, 0.001, respectively, Linear Mixed Effects Model for Repeated Measures). B. Quantification of latency to sleep stage and sleep stage duration expressed as mean percent change relative to vehicle in the 4 h following almorexant treatment (*, **, ***, $p < 0.05$, 0.01, 0.001, population *t*-test).

relative to vehicle were observed between the first and last day of dosing (not shown).

4. Discussion

The demonstration of the somnolence promoting effects of MK-6096, a novel, structurally distinct DORA, conclusively validates orexin signaling mechanism as a specific and effective target for the treatment of insomnia and potentially other disorders in which sleep/wake dysregulation occurs. In particular the observation of translational efficacy across species and demonstrated proof of concept with other DORAs indicate high likelihood of clinical efficacy for MK-6096.

The lack of somnolence promoting effects of DORA-22 in mice lacking OX_1 and OX_2 receptors clearly demonstrates that the mechanism of action of this MK-6096 analog is mediated through orexin signaling. Both of these compounds have been engineered for their specific antagonism of these proteins, the only known biological receptors for OX-A and OX-B ligands, and these results provide further *in vivo* confirmation of that selectivity. While it is conceivable that DORA-22 had additional effects in these animals not detected by polysomnography, no obvious changes in behavior or physiology were overtly evident. In these studies in which orexin signaling is blocked pharmacologically, sleep promotion is more acute relative to that seen in constitutive mutants lacking orexin signaling which experience periodic, but not constant somnolence as well as intact circadian activity cycles (Chemelli et al., 1999; not shown), implying that additional pathways partially compensate for the genetic loss of orexin activity. The absence of any effects by a high dose of DORA-22 in $OX_{1/2}R$ knockout mice, however, indicates that the compound has no activity toward any compensatory mechanisms present in these animals. From a mechanism standpoint, these results underscore the importance of orexin signaling in the control of arousal and vigilance state, and also predict that antagonism of orexin receptor by DORAs have minimal efficacy during the inactive phase when orexin levels are normally at a minimum.

In multiple mammalian species, MK-6096 and DORA-22 exhibit similar binding and antagonist activities toward OX_1 and OX_2 receptors (see Table 1). These similarities are consistent with the high conservation of orexin receptors through evolution where even the most divergent sequence from rat shares 92 and 94% identical amino acid positions with human OX_1R and OX_2R, respectively (Table 2), with orexin peptides and receptors detected in vertebrates ranging from zebra fish (Kaslin et al., 2004), mice (Chemelli et al., 1999), rats (Gautvik et al., 1996), dogs (Lin et al., 1999) to humans (Sakurai et al., 1998). Compared to one another, the human receptors are 81% homologous and 69% identical, suggesting a basis for the ability of MK-6096 and DORA-22 to act on both receptors without appreciable activity on over 170 other known receptors and enzymes. Functionally, differences in OX_2R-mediated mechanisms have been suggested in dogs where either a truncation or point mutation of this receptor is associated with a narcoleptic phenotype that is similar to a complete loss of orexin signaling in humans or in mice harboring mutations in both receptors or the *prepro-orexin* gene (Hungs et al., 2001; Lin et al., 1999; Scammell et al., 2009; Willie et al., 2003). In the current studies, no salient differences were observed in the basal activity of dog orexin receptors relative to other mammalian receptors, nor did MK-6096 or DORA-22 exhibit any notable differences in binding or activity toward the canine receptors. These results indicate that differences in orexin signaling in dogs is not due to divergence in orexin receptor sequences, but may result from either altered receptor expression patterns or differences in the function of the mutant receptors expressed in these specific canine subjects (Boehmer et al., 2004).

Comparative biochemical and pharmacokinetic analysis of MK-6096 and DORA-22 relative to almorexant preformed in the present studies more clearly define the properties required to achieve appropriate *in vivo* efficacy. We detected clear somnolence promoting effects of almorexant at 30 mg/kg in both rats and mice consistent with the initial report of this compound (Brisbare-Roch et al., 2007). Although the *in vitro* potency of almorexant on OX_1R and OX_2R (16 and 15 nM, respectively) is comparable to MK-6096 (10 and 22 nM), significant *in vivo* efficacy with MK-6096 was observed at 3 mg/kg, owing to improved bioavailability and CSF/plasma ratio that results in a greater concentration of drug available in the brain to bind to the receptors. Differences between these compounds in canines is even more striking where significant increases in SWS II at the expense of active wake and decreased latency to persistent SWS II were observed at MK-6096 doses as low as 0.25 mg/kg while 100 mg/kg almorexant was

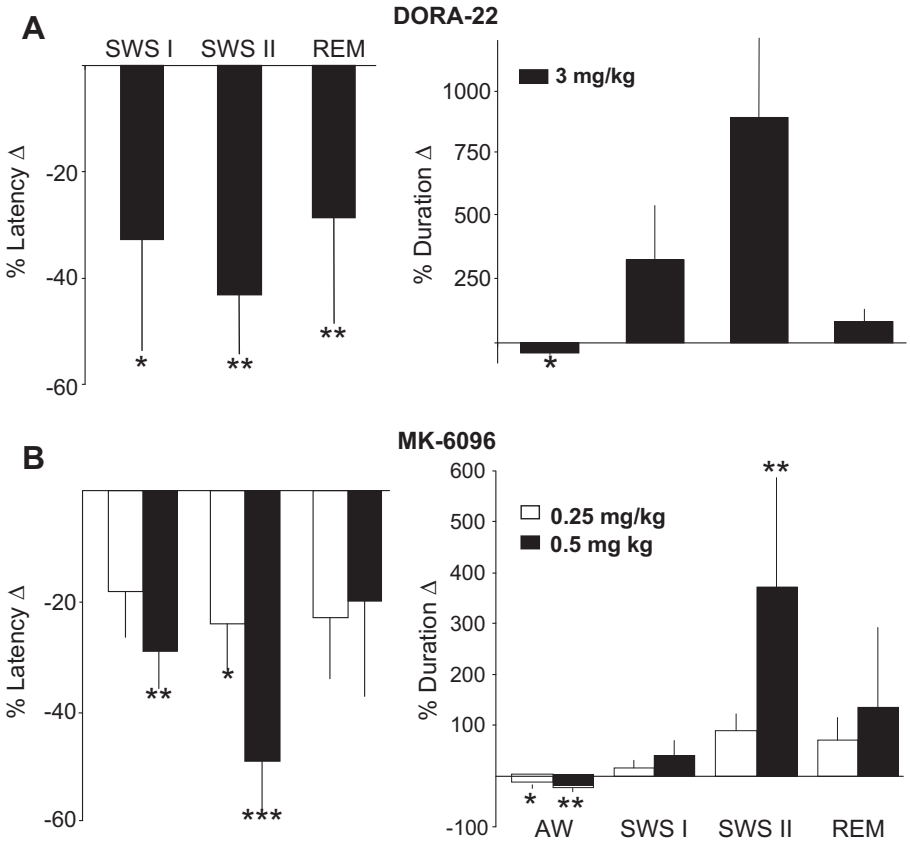

Fig. 6. Comparison of sleep promoting effects of DORA-22 (A) and MK-6096 (B) in dogs. Quantification of latency to sleep stage and mean sleep stage duration expressed as mean percent change relative to the vehicle (20% Vitamin E TPGS, p.o.) condition in the 3 h following 3 mg/kg DORA-22 or 0.25 and 0.5 mg/kg MK-6096 treatment are shown (*, **, ***, $p < 0.05$, 0.01, 0.001, population *t*-test).

required for significant induction of somnolence promoting effects in dogs (Brisbare-Roch et al., 2007). This superior efficacy may be due to greater bioavailability (49%) and exposure (AUC at 0.25 mg/kg = 0.32 μM h). Still another difference is the relatively faster binding kinetics exhibited by MK-6096 which are at least 2-fold more rapid in both human OX_2R binding and dissociation relative to almorexant. This difference may actually be an underestimate since, unlike MK-6096, almorexant was applied at concentrations 3-fold above its K_d in order to measure its kinetics alongside MK-6096. The delayed kinetics of almorexant may be expected to contribute to substantial delays in the appearance and dissipation of somnolence promoting effects relative to the time course of pharmacokinetic exposure. The *in vivo* effects of MK-6096, on the other hand, are expected to more closely track with changing exposure levels.

The current standard of care for the treatment of insomnia includes $GABA_A$ receptor modulators, eszopiclone and zolpidem. These drugs interact with this receptor allosterically at the benzodiazepine binding site to enhance GABA-mediated chloride conductance and inhibitory CNS efficacy (Costa and Guidotti, 1979). Given the wide expression of these receptors, the action of these modulators results in a general suppression of neuronal activity impacting a number of pathways including those associated with arousal, anxiety, cognition and psychomotor tone (Ashton, 1994; Hoque and Chesson, 2009). The somnolence promoting effects of $GABA_A$ receptor modulators are distinct from that of observed in response to orexin antagonism. Although these drugs do reduce latency to persistent sleep and promote NREM sleep, unlike DORAs they also suppress REM and slow wave components of normal sleep (Lancel, 1999). In rats for example, the most salient effect of

Table 2
Similarity of mammalian orexin receptor protein sequences.

Species	Accession #	OX_1R comparisons to human[a]		Accession #	OX_2R comparisons to human[a]	
		Identity[b]	Similarity[b]		Identity[b]	Similarity[b]
Rhesus	XP_001099090.1	98%	99%	XP_001109616.1	98%	99%
Chimp	XP_524646.2	94%	96%	XP_5185522	99%	99%
Canine	XP_544446.2	94%	95%	NP_001002933.1	98%	99%
Rabbit	XP_002720686.1	94%	95%	XP_002714559.1	97%	98%
Mouse	NP_945197.2	92%	94%	NP_945200.1	94%	97%
Rat	NP_037196.1	92%	94%	NP_037206.1	94%	96%

[a] Comparisons were performed using the BLASTP algorithm at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST) using default parameters applied to the protein sequences to NP_001516.2 and NP_001517.2 for OX_1 and OX_2 receptor sequences, respectively. Compared to one another, human OX_1R and OX_2R share 69% identity and 81% similarity.

[b] Identity and similarity scores refer to identical and homologous amino acid positions as given by Identities and Positives obtained from BLASTP outputs, respectively, within un-gapped core regions of these proteins.

zolpidem is the suppression of both the number of REM sleep bouts and the mean time spent in REM sleep, with comparatively slight reductions in active wake time (Renger et al., 2004). Whether these effects on REM suppression or the myriad pathways affected by $GABA_A$ receptor activity are responsible for the rare adverse effects reported for zolpidem is a matter of debate (Hoque and Chesson, 2009). However, the relatively dedicated role of orexin signaling in the control of arousal and vigilance state suggests that OXR antagonism represents a more selective mechanism for the maintenance of normal sleep. "Z-drugs" such as eszopiclone and zolpidem also exhibit low to moderate risk of dependence, particularly in substance abusers and psychiatric patients (Zammit, 2009). On the other hand, preclinical evidence indicates that OXR antagonists are well tolerated (current studies) and do not exhibit dependence. In fact, orexin receptors are expressed in nuclei associated with reward pathways including the ventral tegmental area and nucleus accumbens (Marcus et al., 2001; Trivedi et al., 1998), and genetic or pharmacological disruption of orexin signaling is documented to alleviate drug withdrawal and drug seeking behavior (Borgland et al., 2006; Georgescu et al., 2003; Harris et al., 2005).

Preclinical characterization of suvorexant, a DORA currently under clinical evaluation, has recently been described (Winrow et al., 2011). The *in vitro* and *in vivo* profile of this compound is similar to MK-6096. Suvorexant exhibits equivalent potency with affinities for orexin receptors across species (OX_1R Ki = 0.54 nM–2.1 nM, OX_2R Ki = 0.34 nM–0.68 nM), the kinetics of this binding ($T_{1/2}$ ON = 80.1 min, $T_{1/2}$ OFF = 89.4 min) being similar to that of MK-6096 and faster than almorexant. Suvorexant also exhibits dose-dependent receptor occupancy (Occ_{90} = 1.1 μM) and significantly increases sleep in rats (10 mg/kg, (p.o.), plasma C_{max} = 1.6 μM and AUC (0–24 h) = 12 μM h), dogs (1 mg/kg, (p.o.), plasma C_{max} = 0.32 μM and AUC (0–24 h) = 1.7 μM h) and rhesus monkeys (10 mg/kg, (p.o.), plasma C_{max} = 1.44 μM and AUC (0–24 h) = 8.8 μM h). With several orexin receptor antagonists currently in late stage development, comparisons of the clinical safety and efficacy of these compounds will more fully establish the translational value of differences seen between compounds in animal studies and their effectiveness in insomnia patients will become more evident. Ultimately, analysis of the three dimensional structures of potent and selective OXR antagonists from divergent classes, including MK-6096, will prove invaluable toward understanding both orexin ligand binding and the optimal steric properties required for antagonists to disrupt it.

5. Conclusions

The structurally distinct OXR antagonist, MK-6096, shows favorable *in vitro* potency, on/off-rate kinetics, *ex vivo* occupancy, *in vivo* pharmacokinetics and pharmacodynamic efficacy, further validating the mechanism of targeting orexin signaling for promoting normal sleep. It arises from a piperidine-series of antagonists, and demonstrates higher preclinical *in vitro* and *in vivo* potency than any publicly disclosed DORAs to date. DORA-22, an analog of MK-6096, exhibits similar sleep promoting properties that are absent $OX_{1/2}R$ double knockouts, demonstrating the mechanism of action and selectivity of these effects. Comparison of the pharmacokinetic and biochemical properties of these compounds relative to almorexant more clearly define the properties (e.g. OX_2R occupancy, oral bioavailability, CSF/plasma exposure, $T_{1/2}$, On/Off rate) required for OXR antagonists to promote sleep. Selection of MK-6096 as a clinical development candidate was based on this favorable preclinical profile, including a clear demonstration of efficacious sleep induction in multiple species. Given the functional role for orexin signaling in arousal and the demonstration of clinical proof of concept for DORAs in insomnia, MK-6096 potentially represents a novel therapeutic treatment for this disorder as well as others that are secondary to sleep/wake dysregulation.

Acknowledgments

The authors would like to acknowledge Jacquelyn Binns, Joe Bruno, John Majercak and Wei Lemaire, Alan Savitz and Merck's Laboratory Animal Resources Staff for their contributions to this work.

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