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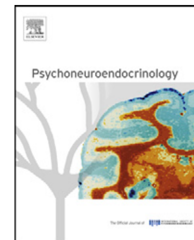

Examining the role of endogenous orexins in hypothalamus—pituitary—adrenal axis endocrine function using transient dual orexin receptor antagonism in the rat

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Summary The orexin neuropeptide system regulates wakefulness and contributes to physiological and behavioral stress responses. Moreover, a role for orexins in modulating hypothalamus—pituitary—adrenal (HPA) axis activity has been proposed. Brain penetrating dual orexin receptor (OXR) antagonists such as almorexant decrease vigilance and have emerged as a novel therapeutic class for the treatment of insomnia. Almorexant was used here as a pharmacological tool to examine the role of endogenous orexin signaling in HPA axis endocrine function under natural conditions. After confirming the expression of prepro-orexin and OXR-1 and OXR-2 mRNA in hypothalamus, pituitary and adrenal glands, the effects of systemic almorexant were investigated on peripheral HPA axis hormone release in the rat under baseline, stress and pharmacological challenge conditions. Almorexant did not alter basal or stress-induced corticosterone release despite affecting wake and sleep stages (detected by radiotelemetric electroencephalography/electromyography) during the stress exposure. Moreover, almorexant did not affect the release of adrenocorticotropin (ACTH) and corticosterone at different time points along the diurnal rhythm, nor corticotrophin-releasing hormone (CRH)- and ACTH-stimulated neuroendocrine responses, measured in vivo under stress-free conditions. These results illustrate that dual OXR antagonists, despite modulating stress-induced wakefulness, do not interfere with endocrine HPA axis function in the rat. They converge to suggest that endogenous orexin signaling plays a minor role in stress hormone release under basal conditions and under challenge.
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1. Introduction

The orexin neuropeptides orexin-A (OX-A) and orexin-B (OX-B) originate from the same precursor peptide, prepro-orexin (ppOX), and are synthesized by a small subpopulation of

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neurons in the lateral hypothalamus (Sakurai et al., 1998; de Lecea et al., 1998). They bind to two different excitatory G-protein coupled receptors, orexin receptor type 1 (OXR-1) and type 2 (OXR-2), which are widely distributed in the brain (Peyron et al., 1998). OX-A binds with high affinity to both orexin receptors, whereas OX-B selectively binds to OXR-2 (Carter et al., 2009). Besides the central nervous system, both OXRs are also present in a number of peripheral sites including endocrine glands, fat tissue, the gastrointestinal tract, and testis (Heinonen et al., 2008); the source of their ligands, OX-A and OX-B, in the periphery has, however, been discussed controversially (local production vs. release from brain) (Nishino and Mignot, 2002).

The pivotal role played by the orexin system in the regulation of vigilance and wakefulness is well recognized (Saper et al., 2005) and an involvement in the stress response has been proposed (Berridge et al., 2010). Indeed, orexin neurons receive input from several limbic regions involved in emotional stress processing, including the amygdala, infralimbic cortex, nucleus accumbens shell, lateral septum, and bed nucleus of the stria terminalis (Sakurai et al., 2005; Yoshida et al., 2006). Orexin neurons themselves project to stress output regions in the mid-/hindbrain area including the hypothalamus, the paraventricular thalamic nucleus, the locus coeruleus, the central gray, and the raphe nuclei, as well as to spinal thoracolumbar areas containing sympathetic preganglionic neurons (Peyron et al., 1998; Hagan et al., 1999; Date et al., 1999). Thus, orexin neurons are well placed at the interface of input and output stress signals to coordinate the stress response with preparation for action, which requires full alertness.

Orexin neurons are activated by a variety of stressors (Ida et al., 2000; Sakamoto et al., 2004; Winsky-Sommerer et al., 2004; Chang et al., 2007), albeit to a different extent, with psychological stressors inducing greater activation than physical stressors (Furlong et al., 2009). Orexins increase arousal (de Lecea et al., 1998), activate the "fight and flight" reaction in rodents (Kayaba et al., 2003), contribute to fear-potentiated startle reactions (Steiner et al., 2012a), and stimulate autonomous nervous system responses to stress (Kayaba et al., 2003; Zhang et al., 2006; Furlong et al., 2009). The involvement of the orexin system in the neuroendocrine stress response has also been proposed. Anatomically, orexin neuropeptides and OXRs are found both at central hypothalamus—pituitary—adrenal (HPA) axis-regulating brain structures such as the hippocampus, amygdala, and the paraventricular nucleus of the hypothalamus (PVN) (Trivedi et al., 1998; Date et al., 1999; Marcus et al., 2001) as well as at peripheral HPA axis sites including the pituitary (Date et al., 2000; Johren et al., 2001) and adrenal glands (Randeva et al., 2001; Ziolkowska et al., 2005; Johren et al., 2006). Functionally, when orexins are infused centrally into the cerebral ventricle, the HPA axis is activated, resulting in increased corticotrophin releasing hormone (CRH) production in the hypothalamus (Al-Barazanji et al., 2001; Sakamoto et al., 2004), and a consequent release of adrenocorticotropin (ACTH) from the pituitary and corticosterone from the adrenal glands (Jaszberenyi et al., 2000; Kuru et al., 2000; Russell et al., 2001). Despite this evidence for HPA axis stimulation upon local administration of exogenous orexins at supra-physiological concentrations into the brain, investigations on the role of the endogenous orexin system in endocrine stress processing under natural conditions are missing.

The OXR antagonist almorexant has equimolar potencies at human OXR-1 (IC50 = 16 nM) and OXR-2 (IC50 = 15 nM) (Brisbare-Roch et al., 2007). It penetrates well into the brain and reaches more than 90% of native OXR-2 occupancy in an OXR-2 transgenic rat model at total plasma concentration of about 2 μM at steady state (Winrow et al., 2011b). These concentrations are achieved with an oral dose of 100 mg/kg, which promotes sleep in rats when given during the active night phase (Brisbare-Roch et al., 2007). Almorexant was used here to examine the function of endogenous orexins in stress hormone release. First, the mRNA expression of ppOX and OXRs at the different sites of the HPA axis (i.e., hypothalamus, pituitary, adrenal) in Wistar rats was investigated. Second, the endocrine HPA axis function under basal, stress and pharmacological challenge conditions was explored after transient pharmacological OXR blockade with almorexant. To ascertain orexin engagement during stress, the effect of almorexant on stress-induced changes in wake and sleep stages was also studied by electrophysiologically measuring brain and muscle activity via radiotelemetric electroencephalography (EEG) and electromyography (EMG).

2. Methods

2.1. Animals

Male Wistar rats (Harlan, Horst, Netherlands) were purchased at an age of 7—8 weeks. Upon arrival to Actelion's animal facility they were housed in groups of four under controlled temperature and humidity conditions with a 12 h light-dark cycle (lights on 0600 h) and food and water ad libitum. Following at least 2 weeks of acclimation, rats were housed individually and entered the experiments, which were conducted during the light phase unless otherwise specified. All experimental procedures were approved by the local Veterinary Office, and strictly adhered to Swiss federal regulations on animal experimentation.

2.2. Drugs and formulation

Almorexant hydrochloride (ACT-078573A; Actelion Pharmaceuticals Ltd.) was formulated in polyethylene glycol 400 or in 0.25% methylcellulose (MC) for oral gavage (PO) at 5 mL/kg. Doses of almorexant were calculated as free base and were selected based on previous publications showing pharmacodynamic efficacy: sleep promotion in Wistar rats during the dark phase under non-stimulating conditions (home cage) is achieved within 30 min of treatment for both the 100 and 300 mg/kg oral doses, and sleep is maintained for at least 6 h at the 100 mg/kg dose and for at least 12 h at the 300 mg/kg dose (Brisbare-Roch et al., 2007); similarly, autonomous nervous system responses to stress (Furlong et al., 2009) and fear-potentiated startle responses (Steiner et al., 2012a) are reduced by both doses with a pre-treatment time of 1 h or 2 h and 30 min. For experiments conducted with jugular vein catheterized rats a 2 h pretreatment time was employed for practical reasons, according to a previous study with almorexant using the same pre-treatment time (Steiner et al., 2012b).

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