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Low cerebrospinal fluid and plasma orexin-A (hypocretin-1) concentrations in combat-related posttraumatic stress disorder

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Summary The hypothalamic neuropeptide, orexin-A has a number of regulatory effects in humans and pre-clinical evidence suggests a link to neuroendocrine systems known to be pathophysiologically related to posttraumatic stress disorder (PTSD). However, there are no reports of central nervous system (CNS) or peripheral orexin-A concentrations in patients with PTSD, or any anxiety disorder. Cerebrospinal fluid (CSF) and plasma levels of orexin-A were serially determined in patients with PTSD and healthy comparison subjects to characterize the relationships between orexin-A (in the CNS and peripheral circulation) and central indices of monoaminergic neurotransmission and to determine the degree to which CNS orexin-A concentrations reflect those in the circulating blood. CSF and plasma samples were obtained serially over a 6-h period in 10 male combat veterans with chronic PTSD and 10 healthy male subjects through an indwelling subarachnoid catheter. Orexin-A concentrations were determined in plasma and CSF and CSF levels of the serotonin metabolite, 5-hydroxyindolacetic acid (5-HIAA), and the dopamine metabolite, homovanillic acid (HVA), were determined over the sampling period. CSF and plasma orexin-A concentrations were significantly lower in the patients with PTSD as compared with healthy comparison subjects at all time points. In addition, CSF orexin-A

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concentrations strongly and negatively correlated with PTSD severity as measured by the Clinician-Administered PTSD Scale (CAPS) in patients with PTSD. Peripheral and CNS concentrations of orexin-A were correlated in the healthy comparison subjects and peripheral orexin-A also correlated with CNS serotonergic tone. These findings suggest low central and peripheral orexin-A activity in patients with chronic PTSD are related to symptom severity and raise the possibility that orexin-A is part of the pathophysiological mechanisms of combat-related PTSD.

1. Introduction

Just over a decade ago, two groups independently identified a new class of hypothalamic neuropeptides by searching for the endogenous ligands for orphan G protein-coupled receptors (Sakurai et al., 1998) and by subtractive PCR in hypothalamic extracts (de Lecea et al., 1998). This class includes orexin-A and -B (or hypocretin 1 and 2), a 33-amino acid peptide (3562 Da), and a 28-amino acid peptide (2937 Da), which are produced from proteolytic processing of the pre-pro-orexin peptide (Nishino et al., 2006) and bind to orexin-1 and orexin-2 receptors (G-protein-coupled receptors) (Nishino et al., 2006). These orexin receptors are expressed throughout the brain though they are extensively localized within the hypothalamus, hippocampus, raphe nuclei, basal ganglia, locus coeruleus and cortex (Nishino, 2007; Blanco et al., 2001; Mazzocchi et al., 2001).

Until recently, this peptide has been studied predominately in human narcolepsy and in animal models of the condition. In fact, cerebrospinal fluid (CSF) orexin-A concentrations have been consistently noted to be low (or even undetectable) in narcoleptic patients (Mignot et al., 2002; Ripley et al., 2001). However, the system is also linked to stress and arousal. For example, in rodent studies, increased hypothalamic expression of orexin-A is linked to acute stress including insulin-induced hypoglycemia (Griffond et al., 1999) immobilization and cold stress (Ida et al., 2000). Further, the stress of neonatal maternal deprivation in rodents increases both orexin-A and frontal cortical orexin-1 receptors (Feng et al., 2007). In addition, pre-clinical and cellular evidence suggest that orexin-A or orexin afferents may modulate noradrenergic, serotonergic, dopaminergic and GABAergic (gamma-aminobutyric acid) systems (Singareddy et al., 2006; Korotkova et al., 2006; Bubser et al., 2005) and CSF concentrations of this peptide are correlated with corticotropin releasing hormone levels in humans (Sarchielli et al., 2008). Interestingly, these neuroendocrine and neurochemical systems have also been implicated in the pathophysiology of anxiety or anxiety disorders, including posttraumatic stress disorder (PTSD) (Bremner et al., 1997; Southwick et al., 1997; Baker et al., 1999; Geracioti et al., 2001, 2008; Strawn and Geracioti, 2008). Also, some (but not all) reports of orexin administration in lower-animal models of anxiety have found orexin-A to be anxiogenic (Zhu et al., 2002; Suzuki et al., 2005; Singareddy et al., 2006). Thus, pre-clinical evidence that suggests a possible role for this arousal-regulating neuropeptide in the modulation of anxiety and fear also raises the possibility that orexin-A may be involved in the pathophysiology of PTSD, an anxiety disorder characterized by hyperarousal symptoms which occur in concert with intrusive and avoidant symptoms (Geracioti et al., 2009).

To date, there have been few clinical studies of the orexin system in psychiatric illnesses. CSF orexin-A levels are reportedly normal in patients with schizophrenia (Nishino et al., 2002), low in recent suicide attempters with major depression as compared with suicide attempters with adjustment disorder or dysthymia (Brundin et al., 2007a) and negatively correlated with severity of depressive illness (Brundin et al., 2007b). However, some studies have found orexin-A concentrations to be normal in patients with major depression (Salomon et al., 2003) although in the this group, there was a trend toward higher levels which decreased significantly following treatment with the selective serotonin-reuptake inhibitor (SSRI), sertraline but not with the norepinephrine and dopamine reuptake-inhibiting bupropion, suggesting that orexin-A may be influenced by serotonergic tone (Salomon et al., 2003).

To our knowledge, there are no reports of central or peripheral orexin-A concentrations in any anxiety disorder and the degree to which peripheral concentrations of orexin reflect central orexin concentrations has not been reported until now. Moreover the relationship between serotonergic and dopaminergic systems — which are often pharmacotherapeutic targets in PTSD — remains to be elucidated.

We used serial CSF sampling over several hours through an indwelling subarachnoid catheter to test the hypothesis that CSF orexin-A concentrations are increased in patients, with chronic, combat-related PTSD. Further, the use of an indwelling subarachnoid catheter permitted us to wait for the stress of the lumbar puncture procedure and spinal canal catheterization to resolve before sampling CSF (Hill et al., 1999). In addition, we measured serotonin and dopamine metabolites to determine the relationship between the orexin system and these indices of monoaminergic neurotransmission.

2. Patients and methods

2.1. Patients

The study was approved by the Institutional Review Board of the University of Cincinnati Medical Center and the Research Committee of the Cincinnati Veterans Affairs (VA) Medical Center. Written, informed consent was obtained from each patient or volunteer before their participation. The serial CSF sampling studies were performed in the Psychoneuroendocrinology Research Suite at the Cincinnati VA Medical Center.

We studied 10 male combat veterans with chronic PTSD and 10 healthy subjects. The healthy men were carefully screened using the Structured Clinical Interview for *DSM-IV* (SCID) and unstructured exploratory clinical interviews to exclude those with current or past psychiatric disorder or a history of psychiatric disorder in first-degree relatives as well