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SUBSTANCE P AND THE NEUROKININ-1 RECEPTOR REGULATE ELECTROENCEPHALOGRAM NON-RAPID EYE MOVEMENT SLEEP SLOW-WAVE ACTIVITY LOCALLY

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Abstract—The neuropeptide substance P is an excitatory neurotransmitter produced by various cells including neurons and microglia that is involved in regulating inflammation and cerebral blood flow—functions that affect sleep and slow-wave activity (SWA). Substance P is the major ligand for the neurokinin-1 receptor (NK-1R), which is found throughout the brain including the cortex. The NK-1R is found on sleep-active cortical neurons expressing neuronal nitric oxide synthase whose activity is associated with SWA. We determined the effects of local cortical administration of a NK-1R agonist (substance P-fragment 1, 7) and a NK-1R antagonist (CP96345) on sleep and SWA in mice. The NK-1R agonist significantly enhanced SWA for several hours when applied locally to the cortex of the ipsilateral hemisphere as the electroencephalogram (EEG) electrode but not after application to the contralateral hemisphere when compared to saline vehicle control injections. In addition, a significant compensatory reduction in SWA was found after the NK-1R agonist-induced enhancements in SWA. Conversely, injections of the NK-1R antagonist into the cortex of the ipsilateral hemisphere of the EEG electrode attenuated SWA compared to vehicle injections but this effect was not found after injections of the NK-1R antagonist into contralateral hemisphere as the EEG electrode. Non-rapid eye movement sleep and rapid eye movement sleep duration responses after NK-1R agonist and antagonist injections were not significantly different from the responses to the vehicle. Our findings indicate that the substance P and the NK-1R are involved in regulating SWA locally.

Key words: neurokinin-1 receptor, substance P, local sleep, tachykinin, slow-wave activity, mice.

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Abbreviations: EEG, electroencephalogram; EMG, electromyogram; IL-1β, interleukin-1 beta; IL-6, interleukin-6; LPS, lipopolysaccharide; NK-1R, neurokinin-1 receptor; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NREMS, non rapid-eye movement sleep; P2X7R, purine type 2 X7 receptor; REMS, rapid-eye movement sleep; SWA, slow-wave activity; TNF-α, tumor necrosis factor-alpha; ZT, zeitgeber.

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INTRODUCTION

Substance P is a neuropeptide derived from the preprotachykinin A gene that is produced by many cell types including neurons and microglia (Marriott, 2004; Munoz and Covenas, 2014a,b). Substance P acts primarily through the neurokinin-1 receptor (NK-1R; also known as the tachykinin receptor 1) and is found throughout the body including the central nervous system (CNS), peripheral nervous system, pulmonary tissue, and immune and vascular endothelial cells (Steinhoff et al., 2014). Within the CNS, the NK-1R is distributed throughout the brain including regions highly involved in regulating sleep such as the hypothalamus, brainstem, and cortex (Dam et al., 1988; Brown et al., 2012; Allen Institute for Brain Science, 2014). The NK-1R couples to G protein-coupled receptors including $G_{q/11}$, $G_{\alpha s}$, and $G_{\alpha 0}$ proteins to activate phospholipase C beta leading to enhanced cytosolic calcium levels, arachidonic acid utilization, and cyclic adenosine monophosphate production (Harrison and Geppetti, 2001). Substance P and the NK-1R are implicated in many functions including the regulation of pain, anxiety, stress, neurogenesis, vasodilation, and inflammation (Munoz and Covenas, 2014a,b).

Non rapid-eye movement sleep (NREMS) electroencephalogram (EEG) delta power (~0.5–4.0 Hz frequency range) [also known as slow-wave activity (SWA)] is an indicator of sleep intensity (Achermann and Borbély, 2003). SWA is increased after acute enhanced waking activity that occurs during sleep deprivation in many species, including mice, rabbits, rats, and humans (Zielinski and Krueger, 2011). Enhanced brain activity from cognitive tasks also enhances SWA (Mölle et al., 2004; Harmony, 2013). Further, dysregulated SWA is prominent in sleep disorders, including insomnia and sleep apnea and many chronic inflammatory conditions such as type 2 diabetes, Alzheimer's disease, cancer, and cardiovascular disease (Zielinski and Krueger, 2011).

The exact mechanisms that regulate SWA are unknown, although the literature indicates that SWA is generated within cortico-thalamic loops (Steriade, 2006). However, a wide literature indicates that pro-inflammatory molecules, including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), enhance SWA (Imeri and Opp, 2009; Zielinski and Krueger, 2011). Injections of these substances centrally enhance NREMS and SWA. Enhanced waking activity induces increased expression of pro-inflammatory

molecules, such as IL-1β and TNF-α, in the brain including the cortex (Zielinski and Krueger, 2011). In addition, pathogens and related components, such as the gram-negative bacterial cell wall component lipopolysaccharide (LPS), enhance pro-inflammatory brain molecules and also enhance SWA (Zielinski and Krueger, 2011). Evidence indicates that LPS enhances substance P (Takeda et al., 2011), although the effects of LPS on substance P expression in the brain remains unknown. Inhibiting brain inflammatory molecules, including IL-1β and TNF-α using pharmacology, knockout mice or siRNA inhibits SWA or homeostatic sleep responses to sleep deprivation or somnogenic stimuli (Imeri and Opp, 2009; Zielinski and Krueger, 2011). Nevertheless, the role of the pro-inflammatory molecule substance P and the NK-1R effect on SWA remains unknown.

The 1,7 fragment of substance P is produced by the enzymatic hydrolyzing of the phenylalanine–phenylalanine bond of substance P in the extracellular space and binds to the NK-1R (Zhou et al., 2000). Intracerebroventricular injections of substance P fragment 1,7 enhances the expression of N-methyl-D-aspartate (NMDA) receptors in the brain (Zhou et al., 2000)—receptors that are known to regulate sleep and the EEG (Brown et al., 2012). Herein, we examined the effects of local cortical hemispheric injections of the NK-1R agonist substance P fragment 1,7 and the NK-1R antagonist CP96345 on sleep and SWA in mice.

EXPERIMENTAL PROCEDURES

Animals

Two-month-old male C57BL/6J mice ($N = 8$ per treatment group) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and used for the present experiments. Mice were kept in individual cages in a sound attenuated room maintained at 22 ± 3 °C. Mice were kept on a 12:12-h light/dark cycle and had *ad libitum* access to food and water at all times. All experimental protocols were approved by Harvard University and Veteran Affairs Boston Healthcare system Animal Care and Use Committee and were in compliance with the National Institutes of Health guidelines.

Polysomnography surgery and recording

Mice were anesthetized with a ketamine and xylazine cocktail (80 and 10 mg/kg, respectively) for surgical procedures. Mice were implanted with EEG electrodes over the left somatosensory cortex (1 mm posterior to bregma and 1 mm lateral to the midline) and a reference electrode over the cerebellum (0.5 mm posterior to lambda placed centrally) (Paxinos and Franklin, 2001). Additionally, a cannula was placed into the ipsilateral hemisphere as the EEG electrode (1.5 mm posterior to bregma and 1 mm lateral to the midline; injection syringe dorsal −0.5 mm) and into the contralateral hemisphere as the EEG electrode (1.5 mm posterior to bregma and 1 mm lateral to the midline) for the administration of pharmacological substances. Two electromyogram (EMG) electrodes were implanted into the nuchal muscles to assess muscle activity. The EEG and EMG electrodes were secured to the skull and a pedestal with dental cement. Mice were tethered to wireless transponders (F20-EET transponders; Data Sciences International, St. Paul, MN, USA) using a system (Neurotargeting Systems, Inc., Chestnut Hill, MA, USA) that allows mice to move freely as previously described (Zielinski et al., 2013a). Mice were placed in standard mouse caging on top of receiver plates (PhysioTel receiver RPC-1; Data Sciences International, St. Paul, MN, USA) that detected the FM signals of the transponders. EEG and EMG signals were amplified and recorded.

Pharmaceutical substances and injections

Mice were allowed at least 10 days to recover from the surgical procedure and were acclimated to the tethered system for 2 days prior to experimental treatments. Mice were injected with 0.2 μL of 0.9% NaCl (i.e., saline) into the ipsilateral or contralateral hemisphere as the EEG electrode at light onset [zeitgeber (ZT) 0] 24 h prior to each pharmacological injection. Sleep was recorded for 24 h after the saline injection, which served as a baseline (experiment 1). Thereafter, 5000 nM, 500 nM, or 50 nM diluted in 0.2 μL of saline concentrations of the NK-1R agonist substance P fragment 1–7 (Sigma–Aldrich, St. Louis, MO, USA) (experiment 2) or the NK-1R antagonist CP96345 (Sigma–Aldrich, St. Louis, MO, USA) (experiment 3) were injected into the ipsilateral hemisphere as the EEG electrode in counter balance order of dosage concentrations at light onset (ZT 0). Sleep was then recorded for 24 h. In addition, the NK-1R agonist substance P fragment 1,7 (500 nM diluted in 0.2 μL of saline) and the NK-1R antagonist CP96345 (500 nM diluted in 0.2 μL of saline) were given in the contralateral hemisphere of the EEG electrode and sleep was then recorded for 24 h. 0.2 μL of the saline vehicle control was injected prior to each dosage of the pharmaceutical that was applied.

Polysomnography analysis

Sleep states [NREMS, rapid-eye movement sleep (REMS), and wake] were determined manually off-line in 10-s epochs as previously described (Zielinski et al., 2013b). Sleep state durations were calculated across 2-h time periods. Sleep state episode durations and episode frequencies were determined in 12-h time periods after injections of the vehicle. EEG signal power spectra were determined after vehicle and pharmaceutical injections. Fast Fourier transformation of the EEG signals (μV^2) within the 0.5–20-Hz frequency range in 0.5-Hz bins was made for each epoch over 24-h periods after vehicle and pharmaceutical injections. Additionally, NREMS EEG SWA (0.5–4 Hz frequency range) was determined in 2-h time bins across 24-h periods after vehicle and pharmaceutical injections. SWA data for each 2-h time bin were normalized to mean vehicle injection data over 24-h periods for each individual mouse as previously described (Zielinski et al., 2013a). After vehicle injections, EEG power spectra (0.5–20 Hz) during the dark period (ZT 12–0) were normalized to EEG power spectra (0.5–20 Hz) during the