Neuropeptides 52 (2015) 67–72

Contents lists available at ScienceDirect

Neuropeptides

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

Chronic heart failure alters orexin and melanin concentrating hormone but not corticotrophin releasing hormone-related gene expression in the brain of male Lewis rats

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ARTICLE INFO

Article history:
Received 1 December 2014
Accepted 3 June 2015
Available online 11 June 2015

Keywords:
Chronic heart failure
Hypothalamus
Orexin
Orexin receptors
Cardiac dysfunction

ABSTRACT

Objective: The aim of this study was to investigate the effect of chronic heart failure (HF; 16 weeks post left coronary artery ligation) on the brain's orexin (ORX) and related neuropeptide systems.
Methods: Indicators of cardiac function, including the percent fractional shortening (%FS) left ventricular posterior wall shortening velocity (LVPWSV) were assessed via echocardiography at 16 weeks post myocardial infarction or sham treatment in male Lewis rats (n = 5/group). Changes in gene expression in HF versus control (CON) groups were quantified by real-time PCR in the hypothalamus, amygdala and dorsal pons.
Results: HF significantly reduced both the %FS and LVPWSV when compared to CON animals ($P < 0.02$). In the hypothalamus ORX gene expression was significantly reduced in HF and correlated with changes in cardiac function when compared to CON ($P < 0.02$). No significant changes in hypothalamic ORX receptor (type 1 or type 2) gene expression were identified. Alternatively hypothalamic melanin concentrating hormone (MCH) gene expression was significantly upregulated in HF animals and negatively correlated with LVPWSV ($P < 0.006$). In both the amygdala and dorsal pons ORX type 2 receptor expression was significantly down-regulated in HF compared to CON. ORX receptor type 1, CRH and CRH type 1 and type 2 receptor expressions were unchanged by HF in all brain regions analyzed.
Conclusion: These observations support previous work demonstrating that cardiovascular disease modulates the ORX system and identify that in the case of chronic HF the ORX system is altered in parallel with changes in MCH expression but independent of any significant changes in the central CRH system. This raises the new possibility that ORX and MCH systems may play an important role in the pathophysiology of HF.

1. Introduction

Orexin (ORX) is a neuropeptide produced exclusively by neurons localized in the lateral hypothalamus. These neurons send efferent projections throughout the brain (Peyron et al., 1998) and have been shown to be involved in a variety of functions including feeding, locomotion, system arousal, cardiorespiratory reactivity to stress, and sleep–wake stability (Tsujino and Sakurai, 2013; Li and Nattie, 2014). The ORX system modulates these functions via the release of one of two peptides, ORX-A and ORX-B, cleaved from a common precursor, pre-proORX, (Scammell and Winrow, 2011). ORX-A and -B, in turn, modulate neuronal excitability by binding to one of two G-protein coupled receptors (ORX-R1 and ORX-R2). ORX-A has equal affinity for both ORX-R1 and ORX-R2 while ORX-B has a higher affinity for ORX-R2. Based on the ORX system's involvement in a wide variety of homeostatic responses, ORX has been hypothesized to be critical for the coordination of "survival-related processes (Chase, 2013)." Conversely, dysregulation of the ORX system has now been documented in several disease states, including narcolepsy (Sakurai, 2013), Parkinson's disease (Baumann et al., 2008), diabetes (Jöhren et al., 2006) and hypertension (Li et al., 2013; Lee et al., 2013), suggesting that understanding the relationship between the ORX system and disease may lead to new therapies.

Activation of the ORX system has been reported to be linked to another peptide system, to the brain's corticotrophin releasing hormone (CRH) system. ORXergic neurons for example are excited by CRH release via activation of CRH receptor type 1 (Winsky-Sommerer et al., 2005; Paneda et al., 2005). Alternatively, certain aspects of CRH-dependent systems, such as stress-induced release of adrenocorticotropic hormone, appear to involve ORX release via activation of ORX-R1s (Samson et al., 2007) and ORX-R2s (Chang et al., 2007). Moreover, brain regions with high concentrations of CRH receptors, such as the hippocampus, amygdala, paraventricular nucleus of the hypothalamus (PVN) and dorsal raphe (van Pett et al., 2000; Trivedi et al.,

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http://dx.doi.org/10.1016/j.npep.2015.06.001
0143-4179/

1998), also appear to be major target sites of ORXergic neurons, providing additional support to the notion that the two systems are interconnected.

Previous work has identified increased CRH expression in the amygdala of the spontaneously hypertensive rat (SHR) (Porter and Hayward, 2011) and elevated CRH expression in the PVN has been documented in hypertensive humans (Goncharuk et al., 2007). These observations are paralleled by new evidence of dysregulation of the ORX system in the SHR (Li and Nattie, 2014). Specifically, in a recently study, significantly more ORX positive neurons were identified in the medial hypothalamus of the adult male SHR compared to two normotensive strains(Clifford et al., 2015). Another study documented a significant reduction in ORX-R2 gene expression in the brainstem of the SHR. These changes have been linked to both elevated activation of ORX-R2s and heightened sympathoexcitation in this animal model (Lee et al., 2013). Additionally, in an animal model of stress-induced hypertension, upregulation of ORX protein expression in the hypothalamus has been reported and was coupled to an increase in ORX-R1 expression in the brainstem (Xiao et al., 2013). These observations suggest that cardiovascular disease in general may be linked to dysregulation of the ORX/CRH interactions.

To our knowledge the impact of other forms of cardiovascular disease, such as heart failure (HF), on ORX and CRH expression in the brain is largely unexplored. Thus, the present study was undertaken to test the hypothesis that chronic HF induces parallel changes in ORX and CRH receptor gene expression in select regions of the brain and these changes correlate with severity of disease. Three regions of interest were evaluated, including the hypothalamus (Kang et al., 2011), the amgydala (Nemeroff and Goldschmidt-Clermont, 2012), and the dorsal pons, a region of the brain which contains both the locus coeruleus, a region known for its function in anxiety and depression (Itoi et al., 2011), and the parabrachial nucleus, which is linked to baroreflex regulation, sympathetic drive, and volume regulation (Callera et al., 2005). Additionally, the impact of chronic HF on melanin concentrating hormone (MCH) gene expression in the hypothalamus and CRH-R2 expression in myocardial tissue were evaluated due to the potential role of MCH in modifying the actions of ORX (Li et al., 2014; Konadhode et al., 2013; Burdakov et al., 2013) and the putative role of CRH-R2 in modulating cardiac and vascular function in HF, respectively (Adão et al., 2015; Rademaker et al., 2005).

2. Methods

All procedures followed NIH guidelines for the ethical treatment of animals and procedures were preapproved by the University of Florida Animal Care and Use Committee. Ten adult normotensive male Lewis rats were studied. Lewis rats were chosen as a background strain based on evidence that Lewis rats have lower mortality following relatively large infarcts compared to Sprague Dawley rats (Empinado et al., 2014; Liu et al., 1997). At 8–10 weeks of age, rats were assigned to one of two groups, HF or healthy controls (CON). In the HF group, myocardial infarction was induced via ligation of the left main coronary artery while rats were deeply anesthetized (isoflurane-O_2), intubated, and mechanically ventilated as previously described (Empinado et al., 2014). Prior to recovery from anesthesia, all animals received an appropriate analgesic agents bupivacaine (1.5 mg kg^{-1}) and buprenorphine (0.01–0.05 mg kg^{-1} I.M.). CON rats underwent sham operations, including visualization of the left coronary main artery. Rats were monitored daily for 3 days post-operatively and received appropriate supportive care and analgesics.

Following recovery, all animals singly housed and remained in their home cages for another 16 weeks and were allowed free access to water and standard rodent diet (Teklad; www.harlan.com). A 16 week recovery period following coronary ligation was allowed due to our interest in chronic adaptations that occur beyond the first 4–6 weeks when initial compensatory mechanisms are more active (Krzemiński et al., 2008). At 16 weeks post-surgery transthoracic echocardiography was performed under gaseous isoflurane anesthesia (3% for induction, 1.5–2.5% for maintenance) using an ultrasound system (Aplio XV, Toshiba Medical Systems, Tokyo, Japan) equipped with a 6.5 MHz transducer. Two-dimensional (2D) ultrasound images and M-mode tracings of the left ventricle (LV) were obtained in the parasternal long- and short-axis view. The following measurements were performed using the leading edge to leading edge technique: LV diameter during end-diastole (LVEDD) and end-systole (LVEDS), LV posterior wall thickness during diastole (LVPWThD) and systole (LVPWThS), and left ventricular posterior wall shortening velocity (LVPWSV). Fractional shortening (%FS), an index used to assess LV systolic function, was calculated as (LVEDD − LVEDS) / LVEDD × 100%. Measurements of diameters and a calculation of cardiac cycle length for determination of heart rate (HR) were performed on 3 to 5 cardiac cycles and averaged for each rat. Animals were euthanized between 12 and 1 PM and brain and right heart tissue were harvested and rapidly stored at −80 °C. The size of the infarct in the left ventricle was quantified by measuring the total endocardial area and the infarcted area and calculating the percent of the total area via planimetry (Finsen et al., 2005). Tissue samples were coded and coding was not disclosed until after completion of tissue analysis.

Brain samples were sectioned at −20 °C into ~8 mm^3 bilateral blocks of regions interest, including the hypothalamus (−2.2 to −3.3 mm caudal from bregma, ±2.5 mm from midline, 2 mm above ventral surface), amygdala (−2.2 to −3.3 mm caudal from bregma, ±3.0 to 7.0 lateral mm from midline, 2 mm above ventral surface), and dorsal pons (−8.9 to 9.9 mm caudal from bregma, ±4 mm from midline, 6.5 mm and 8.5 mm below dorsal surface). A small region of the right ventricle was also isolated for testing. mRNA was extracted from tissue samples using standard procedures including tissue homogenization with Trizol in a Tissuelyzer with a single metal bead (Qiagen). Following extraction the mRNA concentration was measured using a Nanodrop Spectrophotometer and then purified according to manufacturer's instructions (using the RNeasy product from Qiagen). Purified mRNA quality was quantified by the Interdisciplinary Center for Biotechnology Research of the University of Florida. All samples had RNA integrity numbers in the range of 8.0–9.1. Next, total mRNA (3 μg) was converted to cDNA using a high-capacity cDNA archive kit and standard procedures (Applied Biosystems, Foster City, CA). RT-PCR was used to quantify gene expression using LifeTechnologies Taqman probes, include CRH (Rn01462137_m1), CRH-R1(Rn00578611_m1), CRH-R2 (Rn00575617_m1), ORX-R1 (Rn00565032_m1) and ORX-R2 (Rn00565155_m1), and pre-pro melanin concentrating hormone (MCH; Rn00561766_g1). Pre-proORX expression was determined using Syber Green detection (Syber Green Master Mix, Applied Biosystems) with forward 5′-CATCCTCACTCTGGGAAA G-3′ and reverse primers—5′-AGGGATATGGCTCTAGCT C-3′ (Geno-Mechanix, Alachua, FL).

Each gene's expression level was quantified relative to the expression of 18s ribosomal mRNA in the sample (ΔCT). The average ΔCT for the control samples was then calculated and the HF samples were quantified relative to the CON average (ΔΔCT). The fold change relative to the CON average was calculated for all samples ($2^{-\Delta\Delta CT}$). A two-tailed independent t-test was used to determine statistical differences between CON and HF (statistical analysis was performed on ΔCT values). Pearson product–moment correlation coefficients were calculated to determine the linear relationship between changes in cardiac function that were significantly altered by HF (LVEDD, LVESD, %FS and LVPWSV) and gene expression. All statistical analyses were performed using GraphPad software (6.0; www.graphpad.com). P values less than or equal to 0.05 were accepted as significant.

3. Results

The survival rate for those rats undergoing coronary ligation was 78% (Empinado et al., 2014). At the time of sacrifice all rats weighed between 380 and 444 g and there was no significant difference in body