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Blockade of orexin receptors attenuates the cardiovascular response to air-jet stress in spontaneously hypertensive rats

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ABSTRACT

This study tested the hypothesis that orexin plays a role in the elevated pressor response to acute stress in the spontaneously hypertensive rat (SHR). The pressor response to air jet stress (AJS) ($n = 11$/group) was 2.5 times greater in vehicle treated SHR versus Wistar (WIS) rats. Systemic delivery of 30 mg/kg of the dual orexin receptor antagonist almorexant did not significantly change resting mean arterial pressure (MAP) but did attenuate the pressor response elicited by AJS to a greater extent in the SHR compared to the Wistar rats (~65% versus ~33% reduction respectively; $n = 6$/group). Alternatively 100 mg/kg almorexant reduced resting MAP in the SHR (~25 mm Hg drop) and attenuated both the heart rate (HR; ~50% reduction) and MAP (~62% reduction) response to AJS in both strains ($n = 6$/group). Systemic application of SB-334867 (3 mg/kg), an orexin receptor type 1 antagonist ($n = 5$/group), selectively reduced resting MAP and attenuated the HR response to AJS in the SHR but had no effect on the pressor response in either strain. The potential role of endogenous orexin release in cardiovascular control in the SHR was linked to a significant increase in brain-derived neurotrophic factor mRNA expression in the hypothalamus and elevated orexin receptor expression (type 2 only) in the dorsal pons when compared to WIS ($n = 4$/group). These results demonstrate that the exaggerated pressor response in the SHR to stress is linked to increased orexin receptor activation and possibly altered orexin receptor expression in the dorsal pons and BDNF expression in the hypothalamus.

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1. Introduction

The hypocretin or orexin system consists of two distinct neuropeptides, orexin A and orexin B synthesized from the common precursor pre-pro-orexin by neurons located in the lateral hypothalamus (DeLecea et al., 1998; Sakurai et al., 1998). Both neuropeptides bind to two G-protein coupled receptors with varying affinity; orexin A binds with equal affinity to type 1 (Ox1R) and type 2 (Ox2R) orexin receptors while orexin B preferentially binds to Ox2R (Carrive, 2013; Scammell and Winrow, 2011). Activation of the orexin system has been shown to be involved in modulating a variety of behaviors linked to system arousal, including locomotion and cardiorespiratory responses to different forms of stress (Beig et al., 2015; Furlong et al., 2009) via projections throughout the brain targeting both noradrenergic neurons in the dorsal lateral pons (locus coeruleus) and sympathetic and respiratory nuclei in the medulla (Saper et al., 2005; Szymusiak and McGinty, 2008). In normotensive rats, for example, blockade of endogenous orexin activity can reduce baseline locomotor activity and associated changes in heart rate (HR) but does not appear to alter resting arterial pressure (AP)(Furlong et al., 2009). Furthermore, systemic administration of a selective Ox1R blocker also has no impact on baseline AP and HR in normotensive rats but can significantly attenuate the HR response to acute restraint stress or novelty stress (Beig et al., 2015; Rusyniak et al., 2012). Alternatively, Ox1R blockade has been reported to attenuate the pressor response triggered by hypercapnic stress independent of any changes in HR (Johnson et al., 2012b). Ox1R blockade has also been shown to significantly attenuate increases in HR and locomotor behavior triggered by drug-induced panic (Johnson et al., 2012a). On the other hand, dual orexin receptor blockade has been shown to selectively modulate HR and AP during exploration in a neutral context versus only attenuating AP during conditioned fear (Furlong et al., 2009). These observations currently suggest that orexin release and the associated changes in AP and HR are context specific.

Emerging evidence now suggests that the orexin system also plays a significant role in resting sympathetic drive in certain forms of hypertension. Specifically, selective blockade of either Ox1R or Ox2R receptors in the rostroventrolateral medulla (RVLM) has been shown to significantly reduce both resting AP and HR in an animal model of stress-induced hypertension, but had no effect in normotensive controls (Xiao et al., 2013). Over-activation of the orexin system in this model of hypertension was linked to both an increase in the number of orexin-A immunoreactive neurons in lateral hypothalamus and up-regulation of

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Ox1R protein expression in the RVLM. Alternatively, in the spontaneously hypertensive rat (SHR), a genetic model of hypertension, central blockade of Ox2R *but not Ox1R* has been reported to significantly reduce resting AP and HR (Lee et al., 2013) but has no effect on AP or HR in the normotensive control, the Wistar Kyoto (WKY). In the SHR dysregulation of the orexin system has been linked to a significant upregulation of orexin A mRNA and a significant downregulation of Ox2R protein in the RVLM when compared to the normotensive WKY (Lee et al., 2015; Li et al., 2013). Additionally two separate laboratories (Clifford et al., 2015; Lee et al., 2015) have recently identified that hypertension in the SHR is associated with an elevated number of orexin-A immunoreactive neurons in the lateral hypothalamus when compared to both the WKY and the normotensive Wistar (WIS). Interestingly, this increase in expression of orexin positive neurons in the SHR appears to be present at 4 weeks of age prior to onset of hypertension, suggesting that dysregulation of the orexin system may play a prominent role in both the development (Lee et al., 2015) and the maintenance of hypertension in this hypertensive model.

Many models of hypertension, including the SHR, have been reported to have an exaggerated cardiovascular response to acute stressor (Dickey et al., 2012; McDougall et al., 2000; Palmer and Printz, 1999, 2002; Porter and Hayward, 2011). To our knowledge, the role of the orexin system in the heightened autonomic response to stress has not been previously evaluated in an animal model of hypertension. Thus, the present study was undertaken to evaluate the role of the orexin system in the autonomic response to acute stress in the SHR. We hypothesized that orexin receptor blockade would attenuate the exaggerated autonomic response to stress to a greater extent and at a lower dose in the SHR when compared to a normotensive control (WIS). Ox1R and Ox2R mRNA expression differences in the SHR versus WIS were also analyzed in three critical brain regions involved in system arousal in response to stress, including the hypothalamus, amygdala, and dorsolateral pons (Bourtrel et al., 2010).

2. Methods

2.1. Animals

All experimental procedures were approved by the University of Florida Institutional Animal Care and Use Committee and followed the National Institutes of Health guidelines for animal use in research. Experiments were performed on 12–14 weeks old male SHR and WIS rats ($n = 26$/strain) that weighed between 300 and 400 g (Charles River Laboratories, Wilmington, MA). Animals were housed in a temperature controlled room (24 °C) that was maintained on a 12 h lights on/off cycle (lights on at 6 AM) with food and water available ad libitum. All animals were housed in pairs prior to surgery. Following surgery all animals were singly housed. All animals were euthanized at the end of the experiment with an overdose of sodium pentobarbital (200 mg/kg).

2.2. General surgical preparation

All animals assigned to undergo acute stress had surgery for placement of a femoral arterial catheter for measurement of arterial pressure as previously described (Hernandez and Hayward, 2014; Porter and Hayward, 2011). Briefly, animals were given a subcutaneous injection of Rimadyl (0.1 mg/kg) and buprenorphine (0.05 mg/kg) and then anesthetized to a surgical level (2–4% isoflurane in 100% O_2). While deeply anesthetized, an incision was made on the ventral surface of one hind-limb, the femoral artery was identified, isolated, tied distally and a heparinized saline (100 IU/ml) filled catheter (L-rat catheter; Braintree Scientific, Braintree, MA) was inserted into the artery and directed toward the abdominal aorta. The distal end of the catheter was plugged and tunneled subcutaneously to the nape of the neck where it was exteriorized and secured to the skin with suture. All incisions were closed, treated with triple antibiotic ointment, and the animals received a subcutaneous injection of saline (2–3 ml) for fluid replacement. Animals recovered on a heating pad and received an additional injection of buprenorphine (0.02–0.05 mg/kg) before returning their home cages. All animals were housed individually following surgery and were allowed 48–72 h to recover prior to experimentation.

2.3. Testing procedure for evaluating the effect of orexin receptor blockade on the cardiovascular response to air-jet stress (AJS)

On the day of the experiment the animal was brought to the lab and randomly assigned to a treatment group. The animal was then briefly restrained while the exteriorized catheter was connected to additional PE 50 tubing filled with heparinized saline (50–100 IU/ml) and connected to a calibrated pressure transducer (Stoelting Inc., Wood Dale, IL) for continuous measurement of pulsatile and mean AP (MAP). The animal then received a single intraperitoneal (i.p.) injection of drug or vehicle.

Drugs tested included the selective dual receptor orexin antagonist almorexant (Actelion Pharm Ltd. Gewerbestrasse, Allschwil, Switzerland; (Malherbe et al., 2009)), a selective Ox1R antagonist, SB-334867 (Tocris Bioscience, Minneapolis, MN), or vehicle. Almorexant was generously supplied by Actelion. Each animal received a single injection. All drugs were prepared just prior to injection. Almorexant was delivered at a dose of either 30 or 100 mg/kg (Beig et al., 2015; Morairty et al., 2012) and was dissolved in 20% cyclodextrin (Sigma Aldrich, St Louis, MO) in sterile saline (volumes between 0.7 and 1.5 ml were dose dependent (Beig et al., 2015)). SB-334867 was tested at 3 and 10 mg/kg and was dissolved in mixture of 15 μl DMSO, 15 μl 1 M HCl and 700 μl 10% cyclodextrin dissolved in sterile saline (Rusyniak et al., 2012). Vehicle for each drug was prepared similarly but without the addition of the drug.

Immediately following the i.p. injection, the animal was placed in the testing chamber (Hernandez and Hayward, 2014; Porter and Hayward, 2011) which was a 15 in. high × 10.5 in. diameter circular container (MTANK, Instech Laboratories, Inc., Plymouth, MA) with three tubes (~0.5 cm diameter) inserted into the wall; one tube every ~30 cm (equidistant around the circumference) at a height of approximately ~5 cm from the bottom of the chamber. The tubes were connected to a three-way valve manifold for regulation of the air stream through each tube. A small video camera was placed at the top of the chamber for monitoring animal behavior. MAP and AP, and video were simultaneously sampled by a computer A/D converter (500 Hz, CED 1401, Spike2 software, version7; Cambridge Electronics Design, Cambridge, England).

Once the animal was in the chamber, white noise was created by the release of pressurized air at 20 psi near the experimental chamber and the animal was left undisturbed to acclimate to the chamber. White noise was used to mask other noise in the room and to acclimate the animals to the noise associated with the AJS protocol (Hernandez and Hayward, 2014; Porter and Hayward, 2011), since the sudden onset of noise alone can elicit a stress response (Kaehler et al., 2004). Based on data from previous investigators suggesting that the effects of i.p. delivery of (Li et al., 2013; Morairty et al., 2012) almorexant or SB-334867 (Rusyniak et al., 2011, 2012) on autonomic control can occur within a minimum of 30–60 min following injection, all animals were left undisturbed for 60 min prior to being exposed to AJS. AJS was then induced by suddenly redirecting air pressure into the chamber through one of three valves. For the next 20 min the direction and duration of the air jet was changed randomly to generate AJS. After 20 min, the air was then redirected away from the experimental chamber (white noise) followed by a 20–30 min recovery period.

2.4. Orexin related gene expression in unstressed rats

A separate group of rats (un-instrumented) were removed from their home cages at 11 AM and given an intraperitoneal injection of pentobarbital (200 mg/kg). Once deeply anesthetized the animal was

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