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EFFECTS OF HYPOCRETIN AND NOREPINEPHRINE INTERACTION IN BED NUCLEUS OF THE STRIA TERMINALIS ON ARTERIAL PRESSURE

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Abstract—Forebrain neuronal circuits containing hypocretin-1 (hcrt-1) and norepinephrine (NE) are important components of central arousal-related processes. Recently, these two systems have been shown to have an overlapping distribution within the bed nucleus of the stria terminalis (BST), a limbic structure activated by stressful challenges, and which functions to adjust arterial pressure (AP) and heart rate (HR) to the stressor. However, whether hcrt-1 and NE interact in BST to alter cardiovascular function is unknown. Experiments were done in urethane-α-chloralose anesthetized, paralyzed, and artificially ventilated male Wistar rats to investigate the effect of hcrt-1 and NE on the cardiovascular responses elicited by L-glutamate (Glu) stimulation of BST neurons. Microinjections of hcrt-1, NE or tyramine into BST attenuated the decrease in AP and HR to Glu stimulation of BST. Additionally, combined injections of hcrt-1 with NE or tyramine did not elicit a greater attenuation than either compound alone. Furthermore, injections into BST of the α_2-adrenergic receptor (α_2-AR) antagonist yohimbine, but not the α_1-AR antagonist 2-{[β-(4-hydroxyphenyl)ethyl]aminomethyl}-1-tetralone hydrochloride, blocked both the hcrt-1 and NE-induced inhibition of the BST cardiovascular depressors responses. Finally, injections into BST of the $GABA_A$ receptor antagonist bicuculline, but not the $GABA_B$ receptor antagonist phaclofen, blocked the hcrt-1 and NE attenuation of the BST Glu-induced depressor and bradycardia responses. These data suggest that hcrt-1 effects in BST are mediated by NE neurons, and hcrt-1 likely acts to facilitate the synaptic release of NE. NE neurons, acting through α_2-AR may activate Gabaergic neurons in BST, which in turn through the activation of $GABA_A$ receptors inhibit a BST sympathoinhibitory pathway. Taken together, these data suggest that hcrt-1 pathways to BST through their interaction with NE and Gabaergic neurons may function in the coordination of cardiovascular responses associated with different behavioral states.

Key words: orexin, adrenergic receptors, extended amygdala, GABA, stress, cardiovascular regulation.

INTRODUCTION

The bed nucleus of the stria terminalis (BST) is a limbic structure comprised of a group of subnuclei that form an interconnected complex surrounding the caudal anterior commissure (Dong and Swanson, 2003, 2004a,b, 2006a,b,c; Ju and Swanson, 1989; Ju et al., 1989; Dong et al., 2001b). BST is considered a component of the extended amgydala due to its extensive bidirectional connections with the central nucleus of the amygdala (Dong et al., 2001a). BST has been suggested to be involved in a variety of physiological functions associated with stress, fear, food intake, pain, and social behaviors (Lee and Davis, 1997; Gewirtz et al., 1998; Erb and Stewart, 1999; Crown et al., 2000; Delfs et al., 2000; Wang et al., 2001; Ciccocioppo et al., 2003; Sullivan et al., 2004; Dumont et al., 2005; Deyama et al., 2007, 2008, 2009; Sajdyk et al., 2008; Liu et al., 2009; Conrad et al., 2012; Lungwitz et al., 2012; Myers et al., 2013).

In responses to stressful challenges, BST also contributes to the activation of a neuronal system that adjusts the levels of arterial pressure (AP) and heart rate (HR) appropriate for the stressor thus either activating or inhibiting the sympathetic nervous system (SNS) (Ciriello and Janssen, 1993; Roder and Ciriello, 1993; Dunn and Williams, 1995; Alves et al., 2007, 2010; Crestani et al., 2009; Zhang et al., 2009; Kuwaki, 2011). Focal chemical and electrical stimulation of BST has been shown to elicit either decreases (Ciriello and Janssen, 1993; Gelsema et al., 1993; Giancola et al., 1993; Dunn and Williams, 1995, 1998; Hatam and Nasimi, 2007) or increases (Dunn and Williams, 1995, 1998) in AP with a concomitant decrease in HR in the anesthetized rat. Additionally, activation of muscarinic (Alves et al., 2007) or adrenergic (AR) (Crestani et al., 2007) receptors within BST have been shown to elicit increases in AP followed by a reflex bradycardia in the

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Abbreviations: A1/A2/A5, brainstem noradrenergic/norepinephrine cell groups; ac, anterior commissure; AMB, nucleus ambiguus; AMG, amygdala; AP, arterial pressure; AR, adrenergic receptor; BIC, $GABA_A$-receptor antagonist bicuculline methiodide; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CVLM, caudal ventrolateral medulla; fx, fornix; Glu, L-glutamate; hcrt-1, hypocretin-1; HEAT hydrochloride, 2-{[β-(4-hydroxyphenyl)ethyl]aminome thyl}-1-tetralone hydrochloride; HR, heart rate; IML, intermediolateral cell column; int, internal capsule; LH, lateral hypothalamus; LPO, lateral preoptic area; LS, lateral septum; MAP, mean arterial pressure; MPN, medial preoptic nucleus; MS, medial septum; NE, norepinephrine/noradrenaline; oc, optic chiasma; RVLM, rostral ventrolateral medulla; SF, septofimbrial nucleus; SI, substantia innominata; SNS, sympathetic nervous system; st, stria terminalis; V3, 3rd ventricle; VL, lateral ventricle; VN, vagus nerve.

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unanesthetized rat. Furthermore, data exist suggesting that BST is involved in modulating the arterial baroreceptor reflex (Crestani et al., 2008a; Alves et al., 2011; McKitrick et al., 1992; Nasimi and Hatam, 2011). Finally, BST has been shown to play an important role in the chronic increases in AP associated with one-kidney, one-clip renovascular hypertension (Earle and Pittman, 1995) and deoxycorticosterone acetate-salt hypertension (Ciriello, 1988).

There are considerable data suggesting that the hypothalamic hypocretin system acts as an essential modulator in central circuits not only involved in reward processing and addictive behaviors involving BST (Aston-Jones et al., 2010; Conrad et al., 2012; Laorden et al., 2012; Lungwitz et al., 2012), but also in the control of the autonomic functions associated with these behaviors mediated by BST (Kuwaki, 2011). Hypocretin knock-out mice do not show the cardiorespiratory changes associated with application of a stressor, and stimulation of BST evoked long-lasting increases in AP and respiration in wild-type mice, but not in the hypocretin knock-out mice (Kuwaki, 2011). Hypocretins are neuropeptides made almost exclusively within neurons of the lateral hypothalamus (de Lecea et al., 1998; Sakurai et al., 1998; Taheri and Bloom, 2001). However, the hypocretin containing axons emanating from these neurons have an extensive distribution throughout the central nervous system, including projections to BST (Peyron et al., 1998; Sakurai et al., 1998; Cutler et al., 1999; Date et al., 1999; Horvath et al., 1999; Mondal et al., 1999; Nambu et al., 1999; Taheri et al., 1999; Sutcliffe and de Lecea, 2000; Taheri and Bloom, 2001; Kukkonen et al., 2002; Nixon and Smale, 2007). Furthermore, within BST a group of hypocretin-2 (hcrt-2) producing neurons has also been identified (Ciriello et al., 2003b). Hypocretins have been implicated in a variety of physiological functions including feeding, neuroendocrine regulation, sleep-wakefulness, sleep disorders, analgesia, drinking, motor control, thermoregulation, energy balance and cardiovascular regulation (Date et al., 1999; de Lecea and Sutcliffe, 1999; Kunii et al., 1999; Shirasaka et al., 1999; Tamura et al., 1999; Chen et al., 2000; Jaszberenyi et al., 2000; Sutcliffe and de Lecea, 2000; Antunes et al., 2001; Bingham et al., 2001; Gerashchenko et al., 2001; Hwang et al., 2001; Matsumura et al., 2001; Yoshimichi and Sakata, 2001; de Lecea et al., 2002; Kukkonen et al., 2002; Lin et al., 2002; Mileykovskiy et al., 2002; Smith et al., 2002; Ciriello and de Oliveira, 2003; Ciriello et al., 2003a; de Oliveira et al., 2003a,b; Kayaba et al., 2003; Berthoud et al., 2005; Samson et al., 2007; Zhang et al., 2009; Badami et al., 2010; Gao and Horvath, 2011; Parise et al., 2011; Wu et al., 2011; Dalal et al., 2013; Inutsuka and Yamanaka, 2013; Miranda et al., 2013).

In addition to hypocretin, BST is also known to receive a dense innervation from noradrenergic axons (Woulfe et al., 1990; Phelix et al., 1992; Kozicz, 2002; Antonopoulos et al., 2004; Park et al., 2009, 2012). It is well established that central norepinephrine (NE) containing systems also participate in arousal, attention and behavioral states, and autonomic nervous system regulation (Foote et al., 1980; Foote and Morrison, 1987; Fendt et al., 2003; Conrad et al., 2012; Hott et al., 2012). With regard to AP control, injections of NE into BST have been reported to elicit increases in AP mediated by the activation of α_1-AR and α_2-AR (Crestani et al., 2007, 2008a,b). Also, microinjection into BST of tyramine, an indirectly acting sympathomimetic amine without agonist activity, has been shown to elicit cardiovascular responses similar to NE (Crestani et al., 2007).

Interestingly, Baldo et al. (2003) demonstrated that BST contained the densest concentrations of hypocretin-1 (hcrt-1) and dopamine beta-hydroxylase co-mingled immunoreactive processes in the entire brain (Baldo et al., 2003). The region of BST with both hcrt-1 and dopamine beta-hydroxylase immunoreactive processes also coincided with those regions where receptor mRNA for hcrt-1, hcrt-2, and α-AR and β-AR exist (Asanuma et al., 1991; Rosin et al., 1996; Talley et al., 1996; Trivedi et al., 1998; Marcus et al., 2001). This latter finding suggests that hcrt-1 and NE may interact within BST to alter behavioral and/or autonomic function. In support of this suggestion, administration of an α_2-AR antagonist into BST has been shown to depress excitatory transmission within the nucleus, and to impair the extinction of cocaine place-preference through an hcrt-1-dependent process (Conrad et al., 2012). However, whether hcrt-1 and NE interact within BST to alter AP is not known. In this study, the effect of microinjection of hcrt-1, hcrt-1 in combination with NE and its receptor antagonists, and hcrt-1 in combination with GABA receptor antagonists (Hatam et al., 2009; Kuwaki, 2011) on the cardiovascular responses to L-glutamate (Glu) activation of BST neurons was investigated in the anesthetized rat.

EXPERIMENTAL PROCEDURES

General procedures

All animals were housed under controlled conditions with a 12-h light/dark cycle. Food and water were available *ad libitum*. All experimental procedures were done in accordance with the guidelines on the use and care of laboratory animals as set by the Canadian Council on Animal Care and approved by the Animal Care Committee at the University of Western Ontario.

Experiments were done in adult male Wistar rats (250–325 g; Charles River Canada, St. Constant, Canada, $n = 41$), anesthetized with urethane (1.5 g/kg, ip) and α-chloralose (80 mg/kg, iv, initially and supplemented by additional doses of 10–20 mg/kg every 2–3 h) mixture, after induction with equithesin (0.3 ml/100 g). The number of animals used within each study was calculated to be the minimum required for appropriate statistical analysis of the data.

The trachea was cannulated and the animals were artificially ventilated using a small rodent ventilator