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Sex differences in serotonin (5-HT) 1A receptor regulation of HPA axis and dorsal raphe responses to acute restraint

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Summary The serotonin (5-HT) 1A receptor subtype has been implicated as an important mediator for the stimulatory influence of serotonin on stress hypothalamic—pituitary—adrenal (HPA) activity, at least in males. Females show greater HPA axis responses to stress compared to males. To determine the nature by which the 5-HT 1A receptor contributes to the sex difference in stress, we examined neuroendocrine and cellular (Fos) responses in male and female rats receiving systemic injections of the 5-HT 1A receptor antagonist, WAY 100635, prior to acute restraint exposure. WAY decreased the corticosterone response in males, but not in females. In the paraventricular nucleus of the hypothalamus (PVH), WAY produced similar decrements in the restraint-induced activation (Fos) of neuroendocrine neurons in males and females. In contrast to the PVH, WAY administration increased total Fos activation in the dorsal raphe nucleus, but only in males. WAY also provoked higher Fos responses within subsets of dorsal raphe cells identified as serotonergic (tryptophan hydroxylase-, TPH-ir) in both males and females. These data provide evidence to suggest a differential influence of presynaptic 5-HT 1A receptors to regulate the stress-induced recruitment of non-serotonergic dorsal raphe neurons in males and females. At present, we cannot rule out a possible role for estrogen in females to alter 5-HT outflow to the HPA axis. There was a negative correlation between estrogen and Fos responses within TPH-positive cells in the dorsal raphe of WAY-administered females, whereas a positive correlation was found between estrogen and 5-HT 1A mRNA expression localized to the region of the zona incerta in close proximity to the PVH. As the raphe complex and 5-HT system impinge on several central autonomic, behavioral and neuroendocrine control systems, the current findings provide an important framework for future studies directed at sex differences in adaptive homeostatic responses.

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1. Introduction

The prevalence of several stress-related psychiatric disorders (major depression, generalized anxiety disorder, post-traumatic stress disorder) is greater in women than in men, with approximately a two-fold disparity (Kessler et al., 1993, 1995, 2005). In animal models, the stress reactivity of the hypothalamic—pituitary—adrenal (HPA) axis is consistently greater in females compared to males (Goel and Bale, 2008; Handa et al., 1994; Viau et al., 2005), which may be relevant to the sex disparity in these disorders. However, the neurobiological underpinnings of the sex differences in disease prevalence remain largely unknown, which creates challenges in developing new and effective treatments.

Activation of the HPA axis is a necessary component of an organism's response to homeostatic threat (i.e. stress). The release of glucocorticoids from the adrenal cortex plays an important role in meeting energy requirements and coordinating behavioral responses in order to adapt to the stressor. However, efficient regulation is required to return stress hormone levels to baseline, as excessive stimulation or reactivity of the HPA axis can trigger a long-lasting state of distress, dysfunction in the limbic system, as well as increased risk for psychiatric disorders (de Kloet et al., 2005).

Understanding the mechanisms underlying the sex difference in stress HPA axis activity provides an important source of insight into the etiology of these disorders. Gonadal hormones are at least partly responsible, as androgen administration decreases adrenocorticotropic hormone (ACTH) and corticosterone (CORT) secretion, while estrogens increase these measures, leading to greater stress responses in females (Lund et al., 2004; Viau and Meaney, 1991). Additional sex differences have been identified, such as greater corticotropin-releasing hormone (CRH) and arginine vasopressin, as well as lower glucocorticoid receptor gene expression in the paraventricular nucleus of the hypothalamus (PVH) in females (Seale et al., 2004; Viau et al., 2005). Thus, sex differences in the HPA axis may involve mechanisms of biosynthetic drive and glucocorticoid-mediated negative feedback regulation of PVH motor neurons.

In addition to these intrinsic elements of HPA axis regulation, sex differences in neuroendocrine responses may also arise from neurotransmitters and brain regions that innervate the PVH and regulate ACTH release. One candidate is the serotonin (5-HT; 5-hydroxytryptamine) system, known for its involvement in mood disorders (Cryan and Leonard, 2000; Owens and Nemeroff, 1994), but also its marked stimulatory effect on stress HPA axis responses (Fuller, 1981). Of the numerous 5-HT receptor subtypes, the 5-HT 1A receptor plays a pivotal role in stress-related depressive disorders, and is a crucial determinant of the antidepressant response (Blier and Ward, 2003; Cryan and Leonard, 2000). Presynaptic 5-HT 1A (somatodendritic) receptors diminish excitability of dorsal raphe neurons to reduce serotonin release (Bonvento et al., 1992; Sprouse and Aghajanian, 1987). Postsynaptic 5-HT 1A receptors mediate 5-HT transmission to various forebrain regions, including cortex, septum, hippocampus, the ventromedial hypothalamic nucleus, and the perinuclear zone of the PVH (Chalmers and Watson, 1991). Activation of the 5-HT 1A receptor via systemic administration of selective agonists stimulates the HPA axis, whereas 5-HT 1A antagonists reduce acute stress-induced elevations in ACTH and CORT (Jorgensen et al., 2001; Vicentic et al., 1998).

In consideration of the opposing influences of 5-HT 1A somatodendritic and heteroreceptors to mediate 5-HT signal flow (Blier and Ward, 2003), the magnitude of the HPA responses to stress may reflect the balance of pre- and postsynaptic 5-HT 1A receptor activation. Where this balance is struck to explain the sex difference in the stress-induced activation of the HPA axis has not been examined. Building on the capacity of WAY 100635, a selective 5-HT 1A antagonist, to unmask endogenous requirements for 5-HT 1A receptor utilization (Commons, 2008), here we examined HPA axis output responses and patterns of Fos induction in male and female rats receiving an injection of WAY 100635 prior to acute restraint exposure. Finally, we also examined relative levels of 5-HT 1A receptor mRNA to determine whether the sex difference in stress HPA axis activity may be related to variations in receptor expression.

2. Methods

2.1. Animals and treatment

Adult male and female Sprague-Dawley rats (Charles River, Canada) were 60 days of age at arrival. Rats were pair-housed under controlled temperature ($23 \pm 2\ ^{\circ}C$) and lighting conditions (12:12-h light:dark cycle, lights on at 07.00 h) with food and water available ad libitum. Commencing 15 days after arrival (age 75 days), animals were exposed to a single 30 min episode of restraint (onset between 09.00 and 10.00 h) or were similarly handled, but never restrained. An age-matched separate control group was used for 5-HT 1A mRNA analysis. All experimental protocols were approved by the University of British Columbia Animal Care Committee.

2.2. Blood sampling and hormone assays

The 5-HT 1A receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635; 0.5 mg/ml/kg sc; Sigma—Aldrich, St. Louis, MO) or vehicle (0.9% saline) was administered 30 min in advance of restraint exposure or to unstressed controls. The dose and route of drug administration chosen was based on pilot studies, and those previously shown to inhibit corticosterone release (McLaughlin et al., 2009). Blood samples (300 μl) taken from the lateral tail vein were obtained immediately after placement into Plexiglass restrainers (time 0) and at the termination of restraint (30 min), collected into ice-chilled, EDTA- and aprotinin-treated tubes, centrifuged at 4 °C, and plasma stored at −80 °C.

To survey drug effects on stress HPA output responses, plasma ACTH and CORT concentrations were measured using commercial RIA kits (MP Biomedicals, Solon, OH). Sample group sizes were too small to make comparisons attributed to different phases of the estrous cycle in females. Nonetheless, to explore direct relationships between gonadal status and parameters of interest, plasma testosterone concentrations were measured in males (MP Biomedicals) and estradiol in females (Siemens Medical Solutions, Malvern, PA). Intra- and interassay coefficients of variation for these assays ranged