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Effects of aging on stress-related responses of serotonergic neurons in the dorsal raphe nucleus of male rats

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ABSTRACT

Responses to various stressors in the brain change with age. However, little is known about the neural mechanisms underlying age-dependent changes in stress responses. It is known that serotonin, a stress-related transmitter, is closely related with the regulation of stress responses in the brain and that serotonergic function is modulated by various factors, including estrogen, in both sexes. In the present study, to elucidate the effects of aging on stress responses in serotonergic neurons, we examined the expression levels of tryptophan hydroxylase (TPH; a marker of serotonergic neurons) in the dorsal, ventral and lateral parts of the dorsal raphe nucleus (DRN) in young and old intact male rats. In young males, repeated restraint stress significantly increased the number of TPH-positive cells in all subdivisions of the DRN. In contrast, the stress-induced increase in TPH expression was only observed in the ventral part of the DRN in old males. Pretreatment with an estrogen receptor β antagonist had no effect on the number of TPH-positive cells in the dorsal and lateral DRN in young stressed males, whereas the antagonist decreased the number of TPH-positive cells in all DRN subdivisions in old stressed males. Our results suggest that the effects of repeated stress exposure on the expression of TPH in serotonergic neurons in the DRN change with age and that estrogenic effects via estrogen receptor β on TPH expression in stressed old males differ from those in young males.

1. Introduction

Aging changes physiological and pathological states in humans and animals, including rodents. Responses to various stressors in the brain also change with age. Under basal conditions, the function of the hypothalamic-pituitary-adrenocortical (HPA) axis, as measured by adrenocorticotrophic hormones and cortisol/corticosterone secretions, seems to be unchanged or somewhat hyperactive in aged humans and rodents compared with younger adults (Cizza et al., 1995a, b; Lamberts et al., 1997). However, stress-induced reactions of the HPA axis in the aged are greater than those in younger adults, and tend to have long-lasting influences (Bazhanova et al., 2000; Cizza et al., 1995a; Sapolsky et al., 1986), raising the possibility of an age-dependent dysfunction of the negative feedback regulation in stress responses including HPA axis. In addition, the diurnal variation of adrenocorticotropin and cortisol secretion found in young adults flattens gradually with age (Rehman and Masson, 2001). These findings strongly suggest hyperactivation or impairment of adequate responses to stressors and increases in stress vulnerability in old age.

Stress responses are mediated by various neurotransmitters and neuropeptides, including serotonin, which is closely related with the regulation of physiological and psychological functions, such as anxiety and stress responses in the brain (Fuller, 1992; Handa and Weiser, 2014). Serotonergic neurons directly project to corticotropin-releasing factor-containing neurons in the paraventricular hypothalamic nucleus and control HPA function (Fuller, 1992; Weidenfeld et al., 2002). In the dorsal raphe nucleus (DRN), which is a major region of serotonin production in the midbrain and is composed of several subdivisions (Donner and Handa, 2009; Shikanai et al., 2012), innocuous stressors enhance neuronal activation in young male rats (Hale et al., 2008; Shikanai et al., 2012). In addition, the administration of a serotonin 1A receptor antagonist decreases the acute restraint stress-induced corticosterone response and neuronal activation in the paraventricular hypothalamic nucleus in adult rats (Goel et al., 2014). This evidence

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indicates the importance of the serotonergic system in stress responses in young rodents. The function of the serotonergic system changes with age (Miura et al., 2002; Rehman and Masson, 2001), and it has been proposed that such dysfunction of the serotonergic system is heavily involved with mood disorders including depression (Donner and Handa, 2009; Swaab et al., 2005). Therefore, it is highly possible that age-dependent changes in the serotonergic system contribute to the vulnerability to stress exposure in old age.

It has been reported that the serotonergic system is closely linked to estrogen (Barth et al., 2015; Donner and Handa, 2009). Estrogen modulates serotonergic function by the regulation of tryptophan hydroxylase (TPH) and the expression of serotonin transporters and receptors (Barth et al., 2015; Goel et al., 2014; Pecins-Thompson et al., 1996). It is well known that stress exposure increases estrogen levels in female rat brain (Liu et al., 2011), and estrogen in the brain is closely involved with the regulation of stress responses (Handa and Weiser, 2014). Estrogen exerts its action via the estrogen receptors (ER)-α and ER-β. It has been reported that ER-β mediates estrogenic effects in stress responses in young rodents (Liu et al., 2011; Lund et al., 2006), and ER-β is abundantly distributed in the DRN (Shughrue et al., 1997; Yamaguchi and Yuri, 2012), suggesting the possibility that regulatory function of serotonergic system under stress condition is partly due to an ER-β-mediated estrogenic effect.

In the present study, to elucidate the effects of aging on repeated stress-induced changes in the expression of serotonergic neurons in the DRN of male rats, we examined the expression levels of TPH, the rate-limiting enzyme in serotonin synthesis, as a marker of serotonergic neurons, in the dorsal, ventral and lateral parts of the DRN in young and old male rats. In addition, to reveal whether the role of endogenous estrogen via ER-β in serotonergic responses under stress condition change with age, we examined the effect of the ER-β blockade on TPH expression in the DRN in young and old male stressed rats. Furthermore, we examined the effects of stress experience in young adulthood on stress-related TPH expression in aged male rats. Finally, we tried to reveal whether stress-responsive TPH expression in old males differs from those in old females.

2. Materials and methods

2.1. Animals

Young male (7 weeks, n = 12), old male (20 months, n = 16) and old female (20 months, n = 8) Wistar/ST rats (body weight, 250–270 g, 560–615 g and 340–360 g, respectively) were used. All rats were purchased from SLC (Shizuoka, Japan) at the age of 4 weeks and were maintained in our animal facilities under a controlled light and temperature environment (14:10 h light:dark cycle, lights on at 6:00 a.m., 23 °C), with food and water provided *ad libitum*. Both male and female rats were gonadally intact when they were used in all experiments, because we attempted to examine the effects of stress exposure under normal physiological conditions and the role of endogenous estrogen under stress condition. Old female rats were checked for their oestrus cycle stage by the vaginal smear method for 10 days before the start of the experiment, and showed constant diestrus. Our experimental procedures were reviewed and approved by Kochi University, and all rats were treated in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by Kochi University.

2.2. Experimental design

Table 1 summarizes the experimental groups used in this study (n = 4 in each group). All rats were assigned to each experimental group at the age of 4 weeks (n = 8 in each group). To analyze the data from rats with no obvious abnormality such as cancer or abnormal behavior, the number of rats was eventually culled to 4 in each group. The rats of the same experimental group were housed in each cage (two rats per cage).

Table 1
Summary of experimental groups.

Group	7-week		20-month		Sex
	RS	Treatment	RS	Treatment	
1	(−)	Vehicle			Male
2	(+)	Vehicle			Male
3	(+)	PHTPP			Male
4	(−)	(−)	(−)	Vehicle	Male
5	(−)	(−)	(+)	Vehicle	Male
6	(−)	(−)	(+)	PHTPP	Male
7	(+)	(−)	(+)	Vehicle	Male
8	(−)	(−)	(−)	Vehicle	Female
9	(−)	(−)	(+)	Vehicle	Female

RS, restraint stress.

In the young male group, rats were used in the experiments at the age of 7 weeks, and there were three subgroups: vehicle-treated non-stressed young males (Group 1), vehicle-treated stressed young males (Group 2) and ER-β antagonist-treated stressed young males (Group 3). In the old male group, rats were used in the experiments at the age of 20 months, except for one subgroup (Group 7), and there were four subgroups: vehicle-treated non-stressed old males (Group 4), vehicle-treated stressed old males (Group 5), ER-β antagonist-treated stressed old males (Group 6) and vehicle-treated stressed old males that were exposed to repeated restraint stress in young adulthood (Group 7). In the old female group, rats were used in the experiments at the age of 20 months, and there were two subgroups: vehicle-treated non-stressed old females (Group 8) and vehicle-treated stressed old females (Group 9).

2.3. ER-β antagonist administration

A selective ER-β antagonist, 4-[2-phenyl-5,7-*bis*(trifluoromethyl) pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP; Tocris Bioscience, Minneapolis, MN, USA), was dissolved in dimethyl sulfoxide (1.0 mg/0.2 ml). PHTPP or vehicle was administered intraperitoneally in a volume of 0.2 ml/kg body weight at 60 min before restraint stress in 5 consecutive exposures. The dose of PHTPP was determined based on previous studies showing the effects of PHTPP administration on nitric oxide production in the paraventricular hypothalamic nucleus (Grassi et al., 2013) and food intake (Santollo et al., 2010). To minimize the number of rats used in the experiments, all rats in the groups other than the PHTPP-treated groups were administered vehicle intraperitoneally.

2.4. Restraint stress

At the age of 7 weeks (young groups) or 20 months (old groups), rats in the stressed groups were exposed to repeated restraint stress. Stress exposure was performed according to our previous report (Yamaguchi et al., 2010) with slight modifications. They were retained in an acrylic rodent restrainer (KN-325, Natsume, Tokyo, Japan; type C-4 [63-mm diameter × 216 mm] for young males and old females; type C-5 [89-mm diameter × 228 mm] for old males) for 1 h per day. The duration of stress exposure was decided based on previous studies showing the HPA responses to acute restraint stress (Figueiredo et al., 2002; Liu et al., 2011) and our previous study (Yamaguchi et al., 2010). It has been reported that repeated