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SPECIES DIFFERENCES IN ANDROGEN RECEPTOR EXPRESSION IN THE MEDIAL PREOPTIC AND ANTERIOR HYPOTHALAMIC AREAS OF ADULT MALE AND FEMALE RODENTS

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Abstract—The medial preoptic and anterior hypothalamic areas (MPO/AH) are important androgen targets regulating homeostasis, neuroendocrinology and circadian rhythm as well as instinctive and sociosexual behaviors. Although species differences between rats and mice have been pointed out in terms of morphology and physiology, detailed distributions of androgen receptor (AR) have never been compared between the two rodents. In the present study, AR distribution was examined immunohistochemically in serial sections of the MPO/AH and compared for adult rats and mice. Western blotting and immunohistochemistry clearly demonstrated that AR expression in the brain was stronger in mice than in rats and was stronger in males than in females. In addition, we found (1) an ''obliquely elongated calbindin-ir cell island'' in mice medial preoptic nucleus (MPN) expressed AR intensely, as well as the sexually dimorphic nucleus in the MPN (SDN–MPN) in rats, strongly supporting a ''putative SDN–MPN'' previously proposed in mice; (2) AR expression in the suprachiasmatic nucleus (SCN) was much more prominent in mice than in rats and differed in localization between the two species; (3) a mouse-specific AR-ir cell cluster was newly identified as the ''tear drop nucleus (TDN)'', with male-dominant sexual dimorphism; and (4) two rat-specific AR-ir cell clusters were also newly identified as the ''rostral and caudal nebular islands'', with male-dominant sexual dimorphism. The present results may provide basic morphological evidence underlying species differences in androgen-modified psychological, physiological and endocrinergic responses. Above all, the findings of the mouse-specific TDN and differing AR expression in the SCN might explain not only species difference in gonadal modification of circadian rhythm, but also distinct structural bases in the context of transduction of SCN oscillation. The current study could also serve as a caution that data on androgen-sensitive functions obtained from one species should not always be directly applied to others among rodents.

Key words: androgen, sexually dimorphic nucleus, tear drop nucleus, rostral nebular island, caudal nebular island.

INTRODUCTION

Sex steroids, such as androgens, estrogens, and progestins are ubiquitous in all vertebrates and modulate a variety of brain activities, including cognition, emotion, decision-making, and behavioral programing as well as autonomic and neuroendocrinergic response (Vagell and McGinnis, 1998; Luine, 2008; Westberg and Eriksson, 2008). In particular, circulating androgen in male rodents primarily influences masculinization of the body and brain during development and exerts activational effects during adulthood on the organized neural circuits involved in sexual and reproductive functions (Goy, 1966; Shah et al., 2004), certain forms of aggressive behavior (Ohno et al., 1974; Meaney et al., 1983; Sato et al., 2004; Field et al., 2006; Raskin et al., 2009; Marie-Luce et al., 2013) and sociosexual behaviors (i.e., via pheromones) (Bodo and Rissman, 2007, 2008). Testosterone modulates the neural substrates underlying the above-mentioned activities either directly through the androgen receptor (AR) or indirectly through aromatization to estrogens via estrogen receptors, which are well characterized in many species of mammals (Shinoda, 1994; Shinoda et al., 1994; Wersinger et al., 1997; Zhao et al., 2007) and birds (Ball and Balthazart, 2004; Dulac and Kimchi, 2007). The studies using testicular feminization mutation (Tfm) rodent models suggest that AR contributes to masculinization of the brain and to masculine development in a wide range of behaviors (Ohno et al., 1974; Zuloaga et al., 2008b), at least, including anxiety-related behavior in male mice (Zuloaga et al., 2008a)

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Abbreviations: AH, anterior hypothalamic areas; AMPN, anterior medial preoptic nucleus; AR, androgen receptor; CNI, caudal nebular island; DAB, diaminobenzidine; DHT, dihydrotestosterone; DPAJ, dorsal preoptic and anterior hypothalmic junction area; FSH, follicle-stimulating hormone; ir, immunoreactive; KO, knockout; LH, luteinizing hormone; MPN, medial preoptic nucleus; MPO, medial preoptic area; NGS, normal goat serum; OCX, orchiectomy; OECI, obliquely elongated calbindin-ir cell island; OVLT, organum vasculosum of lamina terminalis; OVX, ovariectomy; PBS, phosphate-buffered saline; PBST-NGS, PBS containing 0.3% Triton X-100 and 0.05% NGS; PDPO, posterodorsal preoptic nucleus; Pe, periventricular zone; PeM, periventricular magnocellular nucleus; PVN, paraventricular nucleus; RNI, rostral nebular island; SCN, suprachiasmatic nucleus; SD, Sprague Dawley; SDN, sexually dimorphic nucleus; SDS, sodium dodecyl sulfate; SPVZ, subparaventricular zone; TBST, tris-buffered saline containing 0.1% Tween-20; TDN, tear drop nucleus; Tfm, testicular feminization mutation.

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and some aspects of playful aggression in male rats (Field et al., 2006). Additionally, a study using the AR^{NesCre} mouse (a conditional knockout in which only neurons lack AR) has suggested that AR is required for the expression of inter-male aggressive behavior in mice (Raskin et al., 2009; Marie-Luce et al., 2013), and Sato et al. (2004) reported that AR is also involved in male-typical sexual behavior. Regarding these functions, the region containing the medial preoptic and anterior hypothalamic areas (MPO/AH) is one of the most important androgen-responsive regions regulating homeostatic and allostatic responses that employ the humoral/neuroendocrinergic, viscerosensory/autonomic and instinctive behavioral systems (Mathieson et al., 2000). Lesions of the MPO abolish male sexual behavior (Edwards et al., 1996; Rhees et al., 1999), and androgen plays a pivotal role in the regulation of male sexual behaviors and certain forms of aggressive behavior at least via AR in the MPO/AH (Harding and McGinnis, 2004; Swaney et al., 2012).

While the two species, rats and mice are closely related and belong to the same family of mammals (rodents), it varies considerably across species how far and how critically androgen can influence sexual differentiation, development and regulation of the related brain functions (Lieberburg et al., 1980; Young et al., 1989). For example, androgen is not critical for the establishment of partner preference behavior in male rats (Hamson et al., 2009), whereas defects in AR function crucially affect partner preference behavior and bedding investigatory behavior in male mice (Bodo and Rissman, 2007; Marie-Luce et al., 2013). In male rats, acquisition of copulatory potential is dependent on circulating levels of androgen (Vagell and McGinnis, 1998; Harding and McGinnis, 2003, 2004), whereas in male mice, AR is not necessarily essential for the expression of coital behavior (mounts and thrusts) (Bodo and Rissman, 2007; Zuloaga et al., 2008b). Thus, rats and mice might have different strategies for androgen-dependent maturation of sociosexual behaviors, and such species differences (even strain difference) in AR function can sometimes compromise the direct comparison and application of their data across species, particularly among normal rats and KO mice (Creutz and Kritzer, 2004; Merchenthaler et al., 2004; Sheng et al., 2004; Routh et al., 2009).

Distribution of AR-immunoreactive (-ir) neurons in the rat brain has been reported previously (Sar et al., 1990; Simerly et al., 1990; Bingaman et al., 1994; Williamson and Viau, 2007; Zhao et al., 2007; Kritzer and Creutz, 2008). In general, the distribution pattern of AR is well-conserved across diverse mammalian species (Sar et al., 1990; Simerly et al., 1990); AR is widely expressed throughout the neocortex, allocortex, hippocampus, amygdala and subcortical limbic and hypothalamic regions (Kashon et al., 1996; Wood and Newman, 1999; Fernandez-Guasti et al., 2000). Compared to the rat, however, clear elucidation of AR expression in the normal mouse brain as determined by immunohistochemistry remains incomplete (Sheng et al., 2004; Karatsoreos et al., 2011); in spite of that mice have become an increasingly valuable species in terms of availability for transgenic or KO models (Zuloaga et al., 2008b; Raskin et al., 2009; Marie-Luce et al., 2013). Relevant information concerning AR distribution underlying functional species differences has yet to be obtained for these two rodents.

In the present study, primarily focusing on the MPO/AH, detailed AR distribution patterns were clarified immunohistochemically in complete serial sections and compared between rats [Wistar, Sprague–Dawley (SD)] and mice (C57BL/6J, BALB/c and DBA/2J) of both sexes through light and fluorescence microscopy as well as through Western blotting. Consequently, the current precise histological analysis for each species has provided clear new findings on species differences in brain AR expression and has successfully identified three distinct species-specific AR-ir cell clusters with male-dominant sexual dimorphism, the ‘‘tear drop nucleus (TDN)’’ and the ‘‘rostral and caudal nebular islands (RNI and CNI)’’.

EXPERIMENTAL PROCEDURES

Primary antibodies

A rabbit polyclonal anti-AR antibody [AR (N-20)], raised against a peptide corresponding to amino acids 2–21 of the N-terminus of Human AR, was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A mouse monoclonal anti-calbindin-D-28K antibody was purchased from Sigma–Aldrich (Saint Louis, MO, USA), derived from the CB-955 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with purified bovine kidney calbindin-D-28K. We also used a rabbit polyclonal anti-oxytocin antibody as well as a rabbit polyclonal anti-vasopressin antibody, both obtained from Millipore (USA), the former of which was produced using synthetic oxytocin conjugated to thyroglobulin as immunogen and the latter of which was made using arginine vasopressin conjugated to thyroglobulin as immunogen.

Animals

Two strains of adult rat (Wistar and SD; 9 weeks old; $n = 20$ for male and $n = 10$ for female of each strain) and three strains of adult mouse (C57BL/6J, BALB/c and DBA/2J; 9 weeks old; $n = 20$ for male and $n = 10$ for female of each strain) were used in this study to clarify the regional distribution of AR. Wistar rats and C57BL/6J mice were obtained from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan), and SD rats and BALB/c and DBA/2J mice were purchased from CLEA Japan Inc. (Higashiyama, Tokyo, Japan). They were group-housed (3–4 rats or mice/cage) at a constant temperature (22 °C) with a 12:12-h light–dark cycle (lights on 08:00–20:00), and provided food and water *ad libitum*. All experimental protocols were approved by the Committee on the Ethics of Animal Experimentation at Yamaguchi University School of Medicine and conducted according to the guidelines for Animal Research of Yamaguchi University School of Medicine and the Law (No. 105) and Notification (No. 6) of the Japanese Government. All efforts were made to minimize the number of rats and mice used and their sufferings.