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ScienceDirect
The Veterinary Journal 181 (2009) 193–199
The Veterinary Journal
www.elsevier.com/locate/tvjl

Distribution of the androgen receptor in the diencephalon and the pituitary gland in goats: Co-localisation with corticotrophin releasing hormone, arginine vasopressin and corticotrophs

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Accepted 13 February 2008

Abstract

Previously it has been shown that androgen suppresses transportation-induced increases in plasma adrenocorticotropic hormone (ACTH), possibly by suppressing the secretion of corticotrophin releasing hormone (CRH) or arginine vasopressin (AVP) from the hypothalamus, or secretion of ACTH from the pituitary gland. The aim of the present study was to examine androgen target sites in the caprine diencephalon and pituitary gland using immunohistochemical methods. The androgen receptor (AR) was expressed strongly in the bed nucleus of the stria terminalis, the medial preoptic area, the arcuate nucleus, the ventromedial hypothalamic nucleus and the suprachiasmatic nucleus in the diencephalon. Between 8% and 11% of CRH and AVP neurons in the paraventricular hypothalamic nucleus (PVN) expressed AR. In the pituitary gland, 7.1% of corticotrophs expressed AR. The results are consistent with the proposal that androgen acts directly and indirectly on CRH and/or AVP neurons in the PVN. The possibility of a direct action of androgen on the corticotrophs in the pituitary gland was also considered.

Keywords: Adrenocorticotropic hormone (ACTH); Androgen receptors; Arginine vasopressin (AVP); Corticotrophin releasing hormone (CRH); Goat

Introduction

Exposure to stressful stimuli is associated with activation of the hypothalamic-pituitary–adrenal (HPA) axis when the hypothalamus releases corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP). CRH and AVP induce the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland, and this in turn induces the secretion of glucocorticoids from the adrenal cortex (Whitnall, 1993). In many species of mammal, including goats, the cells that produce CRH are in the paraventricular hypothalamic nucleus (PVN) (Kikusui et al., 1997). AVP, which is released from the PVN, is also an important factor in activation of the HPA axis, because the peptide also facilitates the secretion of ACTH from corticotrophs in the anterior pituitary gland (Familari et al., 1989). CRH producing cells have been found to be localised in the inner region of the PVN, while AVP producing cells are localised in the outer region of the PVN in goats (Kikusui et al., 1997).

In recent years, attention has been drawn to the effects of gonadal hormones on the response of the HPA axis. For example, after exposure to a novel open field, plasma ACTH concentration was higher in female rats than in males (Handa et al., 1994a). Gonadal hormones have been found to play important roles in governing sex differences in the HPA response (Handa et al., 1994a). When rats were

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doi:10.1016/j.tvjl.2008.02.021

exposed to novel environmental stress, estrogen facilitated the secretion of ACTH and corticosterone whereas androgen suppressed the secretion of ACTH and corticosterone (Handa et al., 1994b). In sheep, estrogen facilitated the activity of HPA axis by increasing AVP and CRH concentrations in the brain (Wood et al., 2001) whereas androgen treatment significantly reduced ACTH and cortisol responses to metabolic stress in sheep (Dawood et al., 2005).

In castrated male goats, androgen decreased cortisol secretion by approximately 30% when induced by transportation stress, but no effect was found when the goats were treated with estrogen (Aoyama et al., 2003). Previous work has indicated that the effects of estrogen on the response of the HPA axis to stress varies between species. It facilitates the response of the HPA axis to stress in rats, sheep and humans (Kirshbaum et al., 1996) but apparently not in goats, although androgen appears to suppress the response of the HPA axis to stress in all species including the goat.

Maejima et al. (2006) demonstrated that androgen treatment inhibited the increase in plasma cortisol concentration induced by ACTH administration by up to 42% of control (non-androgen treated castrated male goats) values. This extent of the suppression did not differ from that induced by transportation (37%) as reported by Aoyama et al. (2003). The suppression of transportation-induced increases in cortisol secretion by androgen seems to be mainly as a result of the suppression of the response of the adrenal cortex to ACTH by androgen. However, in goats, transportation-induced increases in plasma ACTH are also reduced by androgen treatment, which may explain the decrease in ACTH secretion from the pituitary gland and/or CRH or AVP secretion from the PVN by androgen (Aoyama et al., 2005).

Previous studies have reported the effects of gonadal hormones on the stress response within the central nervous system (CNS) in rats. Lund et al. (2004) suggested that the inhibitory effect of androgens on the reactivity of the HPA axis was the result of decreased activation of both CRH- and AVP-expressing neurons in the PVN. In addition, androgen inhibited increases in CRH content and the number of CRH immunoreactive cells (Bingaman et al., 1994b), regardless of the absence of AR in CRH and AVP neurons (Bingaman et al., 1994a). These data suggest that androgen may not act on CRH and/or AVP neurons in the PVN directly, but on other neurons in the PVN or other regions in the brain, so suppressing the secretion of CRH and AVP.

The suppressive effects of androgen on the HPA response within the CNS and the pituitary gland of goats are not clearly understood. In order to clarify the direct or indirect effects of androgen on CRH or AVP neurons in the PVN or corticotrophs in the pituitary gland in this species, the localisation of the AR in the diencephalon and the pituitary gland and their co-localisation with the CRH or AVP neurons or the corticotrophs were investigated by immunohistochemical methods. We have previously demonstrated the expression of c-fos, a marker of neural activation, in the bed nucleus of the stria terminalis (BNST), the medial preoptic area (MPOA), the suprachiasmatic nucleus (SCN) and the arcuate nucleus (ARC) of the caprine diencephalon after transportation (Maejima et al., 2005). These areas have an anatomical connection with the PVN in rats (Herman et al., 1994; Baker and Herkenham, 1995; Pacak et al., 1995; Bell et al., 2000) and are thought to regulate the function of the PVN. Androgen may act on the CRH or AVP neurons via these regions. In the present study, the distribution of AR in other areas in the goat diencephalon was also investigated.

Materials and methods

Experimental work was conducted from September to December, 2004. All procedures involving animals were carried out according to the Guide for Care and Use of Laboratory Animals at Utsunomiya University.

Animals

Three entire healthy male Shiba goats were obtained from the experimental station of the University of Tokyo. The animals were housed in the research farm of the Faculty of Agriculture, Utsunomiya University. Their ages ranged from 3 to 4 years, and their bodyweights ranged from 25 to 35 kg.

Sampling of brain and pituitary gland

The animals were deeply anaesthetised with pentobarbital (40 mg/kg BW; Dainippon Pharmaceutical Company) at 1100 h and then perfused transcardially with 4 L of Ringer's solution followed by 4 L of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). The brain and the pituitary gland were removed immediately after perfusion, and post-fixed with the same fixative for 2 days. The diencephalon, which was removed from the cerebral cortex, and the pituitary gland were embedded in paraffin wax.

Coronal sections of the diencephalon, 8 μm thick, taken between +34 and +22 mm of the rostral interaural (IA) line based on the stereotaxic atlas for the Shiba goat brain (Zuccolilli et al., 1995), were cut using a microtome. From the pituitary gland, 6 μm saggital sections were cut using a microtome from the regions containing the anterior lobe (AL), the intermediate lobe (IL) and the posterior lobe (PL). These sections from the three goats were used for single labelling of AR, double labelling of AR and CRH or AVP in the diencephalon, or double labelling of AR and ACTH in the pituitary gland.

Single labelling of AR in the diencephalon

Sections of the diencephalon, at 600 μm intervals, from the series cut through between +34 and +22 mm IA line, were stained using a peroxidase- anti-peroxidase (PAP) immunohistochemical method. The sections were placed on APS-coated slide glasses (S-8215, Matsunami Glass), and deparaffinised with xylene and dried for 2–3 days. The slides were then soaked in a beaker filled with citrate buffer (pH 6.0, 0.1 M), and heated in an autoclave at 121 °C for 20 min to retrieve antigenicity (Shi et al., 1993). The sections were cooled and rinsed with phosphate buffered saline (PBS) (pH 7.4, 0.01 M). To reduce background staining, the sections were pre-incubated with 3% H_2O_2 in methanol followed by 10% normal goat serum in PBS, each for 30 min.

Rabbit polyclonal anti-AR antibody, PG-21 (Upstate), was used for the AR's immunostaining. There are two types of AR, with molecular