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Estrogen and androgen repression of two female specific lacrimal lipocalins in hamster: Pituitary independent and sex hormone receptor mediated action

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Abstract

Sexual dimorphism in lacrimal gland (LG) gene expression is believed to be due to direct inductive effects of androgens mediated by androgen receptors (AR) but hypophysectomy dramatically curtails these inductive effects. Since, functional estrogen receptors (ER) could not be detected in LG, estrogen effects on LG are believed to be indirectly mediated by changes in levels of pituitary hormones. We found that two lipocalins expressed in female hamster LG display an unusual and marked repression by both androgens and estrogens, which could be detected both at the level of transcripts and proteins. Here, we investigate whether these repressions, (i) require presence of pituitary and (ii) are mediated by androgen and estrogen receptors. Pituitary-ablation but not gonadectomy reduced LG weights in hamster. However, both pituitary-ablation and gonadectomy induced abundant expression of the LG lipocalins, which were markedly repressed by androgen or estrogen treatment. AR- and ER-antagonists prevented these repressions and only ER-α- but not ER-β-specific agonist could mimic the estrogenic repression. AR transcript and protein and ER-α transcript were also detected in hamster LG. Thus, pituitary factors are neither essential for the expression of these LG lipocalins nor for their estrogenic or androgenic repressions and these repressions are very likely mediated by functional ER and AR present in LG.

Keywords: Flutamide; Bicalutamide; Cyproterone acetate; Tamoxifen; Raloxifene; ICI-182780; Syrian hamster; Harderian gland; Dry eye

1. Introduction

Sex differences in lacrimal gland (LG) are reported in several mammals including humans (Gubits et al., 1984; Winderickx et al., 1994; Sullivan et al., 1998; Sullivan, 2004; Srikantan et al., 2005; Richards et al., 2006; Paliwal and De, 2006). Its hormonal basis has been investigated mainly in rat where higher levels of secretory component, IgA, α-2_U-globulin, etc., in male LG or tears were found to be due to inductive effects of androgens mediated by androgen receptors (AR) (Gubits et al., 1984; Sullivan and Allansmith, 1987; Sullivan et al., 1990; Winderickx et al., 1994; Lambert et al., 1994; Gao et al., 1995). However, hypophysectomy markedly reduces LG weight in rats and results in precipitous decline in expression of androgen-induced genes (including that of AR), which could not be restored by treatment with androgen indicating a critical requirement of pituitary factor(s) for maintenance of LG and androgen induction of LG genes (Ebling et al., 1975; Gubits et al., 1984; Sullivan, 1988; Rocha et al., 1993; Azzarolo et al., 1995; Sullivan et al., 1998). Moreover, in male hamster's Harderian gland (an accessory lacrimal gland), where porphyrin synthesis is negligible due to repression by androgens (and markedly induced after castration), hypophysectomy alone or in combination with castration did not result in castration-like induction of porphyrin synthesis, indicating a requirement of pituitary

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hormones for induction of Harderian gland porphyrin synthesis in an androgen-depleted state (Buzzell et al., 1989, 1992).

Many investigations had reported that estrogens have little or no effect on LG (Laine and Tenovuo, 1983; Winderickx et al., 1994; Sullivan et al., 1998; Sullivan, 2004) and specific estrogen binding sites were also not detected in rat LG (Laine and Tenovuo, 1983; Sullivan et al., 1996b; Suzuki et al., 2006). However, recent research in other species, including a very recent microarray analysis in mice revealed many inductive and also repressive effects of estrogens (and also androgens) on LG (Beauregard and Brandt, 2004; Srikantan et al., 2005; Richards et al., 2005; Suzuki et al., 2006; Paliwal and De, 2006, 2007). Nevertheless, it is suggested that estrogen effects on LG are indirectly mediated by pituitary hormones (Azzarolo et al., 1997; Sullivan et al., 1998; Sullivan, 2004; Suzuki et al., 2006). Interestingly, pituitary hormones are also known to modulate sex hormone-induced gene expression in liver (Kumar et al., 1969) and hypophysectomy strikingly affects tissue weight, morphology, AR levels and gene expression in many other tissues (Ebling et al., 1975; Bhatnagar, 1983).

Lipocalins are a family of lipophilic ligand binding proteins of Mr ~20-kDa, with conserved 3D structure but low sequence homology (Thavathiru et al., 1999; Redl, 2000; Srikantan et al., 2005). Tear lipocalin, an abundant lipid-binding protein in human tears, increases stability of tear film by binding lipids in tears (Glasgow et al., 1999; Redl, 2000). Fluctuations in its levels have been associated with dry eye disease (Glasgow et al., 1999; Redl, 2000; Evans et al., 2002), which is much more prevalent in women, particularly in altered endocrine states (Sullivan et al., 1998, 1999; Evans et al., 2002; Sullivan, 2004). We found, in LG of Syrian hamsters, female-specific expression of two closely related tear lipocalin genes, FLP and MSP, which display an unusual repression by both androgens and estrogens at the level of transcripts (Thavathiru et al., 1999; Srikantan et al., 2005); their female-specific expression being due to incomplete repression by endogenous estrogens (Srikantan et al., 2005). FLP (female lacrimal protein) has Mr of 20-kDa and being abundantly expressed, can be visualized in protein profiles of LG (Srikantan et al., 2005). MSP [male-specific protein; initially identified in salivary glands of males (Thavathiru et al., 1999)], is co-expressed in LG as a 20.5-kDa non-glycosylated species co-migrating with FLP and a 24-kDa *N*-glycosylated species detectable only by Western blotting (Srikantan et al., 2005). Other than a possible tear lipid-binding property of FLP/MSP, their close homology to odorant-/pheromone-binding lipocalins suggests that FLP/MSP might also bind pheromonal lipids in hamster tears (Srikantan et al., 2005).

Here we investigate whether the abundant expression and unusual estrogenic/androgenic repressions of FLP and MSP lipocalins in hamster LG require an intact pituitary. Additionally, employing a variety of AR- and ER-antagonists we investigate whether functional ER and AR mediate the sex-hormonal repressions of these lipocalins. The presence of AR and ER transcripts and proteins in hamster LG is also investigated.

2. Materials and methods

2.1. Sex hormones, their receptor antagonists and agonists

Estradiol-17β, dihydrotestosterone, flutamide, cyproterone acetate and tamoxifen were from Sigma; bicalutamide and raloxifene hydrochloride were from Dr. Reddy's Laboratory, India. ICI-182780 (Tocris) was gifted by Dr. Rajeshwar Tekmal. Propyl-pyrazole-triol (PPT) and diarylpropionitrile (DPN) were kindly provided by Dr. John Katzenellenbogen.

2.2. Animal experimentation

Our institutional animal experimentation ethics committee approved all experiments involving animals. Syrian hamsters were maintained in 14:10::light:dark cycle. Adult (~60-day-old) hamsters were hypophysectomized via the parapharyngeal route (Alvarez-Buylla et al., 1991) or bilaterally gonadectomized. All experiments were done on groups of animals, each containing 4–8 hamsters. Groups of 30-day hypophysectomized males and females were injected daily (sc) for 15 days with androgen (dihydrotestosterone; 400 μg/kg body weight) and estrogen (estradiol-17β; 40 μg/kg), respectively, and sacrificed along with untreated, hypophysectomized, gonadectomized, sham operated and intact control hamsters. Groups of gonadectomized hamsters, 15 days after surgery, were injected (sc) daily for 15 days with dihydrotestosterone alone or along with different AR-antagonists (flutamide, bicalutamide or cyproterone acetate; all 24 mg/kg). Similarly, groups of intact males were also treated with AR-antagonists alone. Other groups of gonadectomized females were injected (sc) daily for 15 days with estradiol alone or along with different ER-antagonists (tamoxifen or raloxifene at a dose of 8 mg/kg or ICI-182780 at 2 mg/kg). Two-month-old intact female hamsters were similarly treated with only tamoxifen or raloxifene. Additionally, gonadectomized females were injected daily for 15 days with PPT or DPN (both 6.4 mg/kg). The day after the last injection, all hamsters including controls were sacrificed, pairs of exorbital lacrimal glands (LG) were carefully excised, cleaned of extraneous tissues and wet weights taken. The reproductive tissues of hamsters were examined and tissues like testis, ovaries, uterus and seminal vesicles were excised and weighed. Hypophysectomy was confirmed by autopsy.

2.3. Tissue extract preparation, SDS–PAGE and Western analysis

Tissues were homogenized (2.5% w/v) in chilled 20 mM Tris–HCl, pH 8.0 (for FLP/MSP detection) or in RIPA buffer containing protease inhibitor cocktail and PMSF (Sigma) (for AR/ER detection) and centrifuged at 30,000*g* for 30 min. MCF-7 cell extracts were prepared using RIPA buffer. SDS–polyacrylamide slab gels (Laemmli, 1970) were prepared using 30% acrylamide (w/v) (29:1::acrylamide:bisacrylamide) stock solution. For detection and comparison of FLP/MSP expression by Coomassie stain and Western blotting, equal volumes of soluble extracts of LG were loaded in 10.5% gels (Ranganathan and De, 1995; Srikantan et al., 2005) and for detection of AR and ER-α by Western blotting, samples were loaded in 8% gels. All samples were loaded after boiling with sample buffer containing β-mercaptoethanol, as described earlier (Laemmli, 1970). Gels were run overnight at constant current and either stained with Coomassie R-250 (Sigma) or the resolved proteins were electro-blotted onto nitrocellulose membranes (Hybond C; Amersham) for Western analysis (Ranganathan and De, 1996; Thavathiru et al., 1999). Blots were probed with MSP or FLP antisera, (each crossreacts readily with both lipocalins (Thavathiru et al., 1999; Srikantan et al., 2005)). When required, ER-α specific antibody (sc-542; Santa Cruz) or AR specific antibody (PG-21; Upstate) was used to probe the blots. Crossreaction with antibody was