HORMONAL INFLUENCES ON SYRIAN HAMSTER LACRIMAL GLAND

Marked Repression of a Major 20 kDa Secretory Protein by Estrogens, Androgens, and Thyroid Hormones

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1. INTRODUCTION

Lacrimal gland secretory activity is important for maintaining a healthy ocular mucosa.1,2 Lacrimal glands in rodents and other species including humans show histomorphological and biochemical sexual dimorphisms.3-6 Moreover, in humans, lacrimal gland secretory insufficiency leading to dry eyes (keratoconjunctivitis sicca) is known to predominantly afflict women.5 The hormonal regulation of this gland and its secretory activity has been investigated mainly in the rat, where marked histomorphological sex differences and higher levels of secretory component and IgA in male gland and tears have been attributed to the inductive effect of androgens on protein synthesis.7 Such effects are likely to be mediated via androgen receptors in the lacrimal gland.7 Unlike androgens, estrogens are believed to have no effect on the lacrimal gland.7

Prolactin has been shown to have some trophic effects on the rat lacrimal gland,8 although it had no effect on IgA or secretory component levels.9 Moreover, thyroidectomy did not affect IgA or secretory component levels in rat tears.10

We recently investigated the sex-hormonal effects on the Syrian hamster lacrimal gland by examining protein profiles of lacrimal glands taken from hamsters in different hormonal states.6 The expression of a hamster lacrimal 20 kDa major protein was markedly inhibited by both androgens and estrogens.6 We report here our further studies in which we investigated the expression of this protein in hamsters during pregnancy and lactation. The effect of tamoxifen (an estrogen receptor antagonist) administration on the inhibitory effect of estrogen and the effect of thyroid hormones and bromocriptine (a prolactin release inhibitor) on the expression of this protein were studied. The hormonal regu-
lation of the lacrimal 20 kDa protein was also checked in Western blots using the purified protein’s antisera. Some properties of the purified protein are also described.

2. MATERIALS AND METHODS

Syrian hamsters of both sexes were bilaterally gonadectomized at 2 months of age. After 30 days, different groups were separately injected daily (sc) with various hormones (Sigma) for 15 days, and then sacrificed along with vehicle-injected gonadectomized and intact controls. Groups of intact and gonadectomized hamsters of both sexes were injected with 500 μg bromocriptine for 45 days and then sacrificed along with controls. All investigations were done on groups of animals (n=5–6). After sacrifice, lacrimal glands were excised, weighed, and homogenized (2.5% w/v), and supernatants were prepared as described.

Equal volumes of supernatants (140 μl) were run in SDS-PAGE and gels stained as described. Western blot of lacrimal extracts was done using rabbit antisera against hamster lacrimal 20 kDa protein. Lacrimal 20 kDa protein was purified from supernatants of lacrimal glands taken from ovariectomized hamsters. Supernatant was passed consecutively through columns of Biogel-HTP and concanavalin A-Sepharose. The unbound proteins in the final flowthrough in which the 20 kDa protein was present were concentrated and then resolved in a Sephadex G-75 column and fractions collected. Several fractions that were clearly homogeneous for the 20 kDa protein were pooled and used for immunization and characterization studies.

3. RESULTS

A 20 kDa major protein was seen in SDS-PAGE profiles of lacrimal glands of female hamsters but not in males or 15-day-pregnant females. Females ovariectomized for 45 days and 15-day-lactating females had several-fold higher levels of this lacrimal 20 kDa protein, which then constituted ~ 20% of total soluble proteins. Males gonadectomized for 45 days had high levels of this lacrimal 20 kDa protein similar to ovariectomized females. Daily administration for 15 days of either estradiol (3.6 μg), testosterone (50 μg), diethylstilbestrol (3.6 μg), dihydrotestosterone (50 μg), or thyroxine (60 μg), but not progesterone (100 μg) or dexamethasone (100 μg), to 30-day-gonadectomized males or females obliterated this lacrimal 20 kDa protein. Bromocriptine (500 μg) administered for 45 days to gonadectomized or intact hamsters of either sex had no effect on the levels of this lacrimal 20 kDa protein when compared with vehicle-treated controls. Daily postpartum administration of estradiol (3.6 μg), but not bromocriptine (500 μg), to lactating hamsters for 15 days obliterated the lacrimal 20 kDa protein. The post-gonadectomy increase of lacrimal 20 kDa protein was time-dependent, and similar maximum levels were found in males and females by 30 days and 10 days respectively. Daily administration of different doses of estradiol or testosterone for 15 days to 30-day-orchiectomized males showed that although a minimum of 3.6 μg estradiol could cause complete inhibition of lacrimal 20 kDa protein, it required 50 μg testosterone for the same effect. Daily administration of different doses of thyroid hormones to ovariectomized females for 15 days showed no inhibition of lacrimal 20 kDa protein with a dose of 2.2 μg thyroxine, whereas doses of 20 μg and 60 μg showed ~ 80% and complete inhibition respectively. Triiodothyronine was more potent than thyroxine and showed ~ 80% inhibition at 0.75 μg dose. Daily administration of 500 μg tamoxifen, along with complete inhibitory doses (see