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ANDROGENS AND ESTROGENS MARKEDLY INHIBIT EXPRESSION OF A 20-kDa MAJOR PROTEIN IN HAMSTER EXORBITAL LACRIMAL GLAND

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We report here for the first time a 20-kDa hamster lacrimal gland major protein whose expression is markedly inhibited by physiological levels of both androgens and estrogens. This novel protein was present in adult females but not in males. In females, its level was several fold elevated on ovariectomy to apparently 20% of total soluble proteins. Castration in males induced this 20-kDa protein from undetectable to ovariectomized female levels. Administration of only androgens or estrogens to gonadectomized hamsters of both sexes obliterated this major lacrimal protein and estrogens were more potent than androgens. The 20-kDa lacrimal protein was secreted only in female tears. This lacrimal 20-kDa protein of yet unknown function is a useful marker to study the hormonal regulation of the lacrimal gland and it provides a model system to study how both androgens and estrogens mediate inhibition of a specific protein's expression.

Lacrimal gland secretory activity is important for maintaining a healthy ocular mucosa (1,2). Additionally, lacrimal gland secretions may play a role in modulating sexual behaviour(3-6). Lacrimal glands in rodents (7,8) and other species(7,9) show histomorphological and biochemical sexual dimorphism. The exact hormonal basis has been investigated only in rat exorbital gland where the marked histomorphological sex differences (10) and higher levels of secretory component (11), IgA (12) and cystatin related protein (4) in male glands and tears have been attributed to the inductive effect of androgens on protein synthesis(1,4-7,10-14). These effects are believed to be mediated via androgen receptors present in lacrimal gland (15). Estrogens are believed to have no effect on the lacrimal gland (1,2,4,7,10-17) which lacks estrogen receptors (16).

In exorbital lacrimal gland of Syrian hamster, significant sex difference in levels of β-adrenergic receptors, melatonin and its biosynthetic enzymes have been shown (8,18). However, the hormonal regulation of this gland was not clear from these studies and we reinvestigated this by studying the protein profiles of this gland under different hormonal states. We show here that both androgens and estrogens have marked effects on this gland. Specifically, we report a 20 kDa lacrimal gland major protein which shows an unusual hormonal regulation in being markedly repressed by physiological levels of both androgens and estrogens.

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METHODS

Syrian hamsters were maintained (8) and pooled tears were collected (11) as previously described. Bilateral gonadectomy was performed on 2 month old animals. Groups of operated animals were sacrificed after 45 days along with intact controls. Different groups of gonadectomized animals, after 30 days post-operation, were separately injected (daily; sc in oil), with various hormones (Sigma), for a period of 15 days and then sacrificed. A pair of exorbital lacrimal glands from each animal was excised, weighed and homogenized (2.5% w/v) in 20 mM potassium phosphate buffer, pH 8.0 and centrifuged at 28,000 x g for 30 min at 4°C. Supernatants (140 µl) containing ~600 µg protein (19) were run in SDS-PAGE (10%) or in two-dimensional PAGE (IEF pI 3-10 / SDS-PAGE) as previously described (20,21). Gels were stained with Coomassie blue or for glycoproteins (22). Stained gels were scanned using laser densitometer. All groups contained 5-6 animals. Experiments were repeated twice. Representative results of each group are shown.

RESULTS

Fig.1 shows hormonal regulation of a 20 kDa protein monitored in gel profiles of hamster exorbital lacrimal gland. Descriptions below focus only on the 20 kDa protein. This protein was present as a distinct major protein in females (lane 7) but was undetectable in males (lane 6). In females, this protein's level was ~4 fold elevated on ovariectomy (lane 8) to apparently 20% of total soluble protein profile. Castration in males caused a dramatic induction of a 20 kDa protein (lane 5) to ovariectomized female levels. Treatment of gonadectomized hamsters of both sexes with low doses of 17β-estradiol (3.6 µg) or testosterone (50 µg) almost obliterated the 20 kDa protein (lanes 3, 10, 4 and 9). A dose of 3.6 µg diethylstilbestrol (a synthetic estrogen) or 50 µg dihydrotestosterone had the same effect (lanes 13 and 14). Progesterone (100 µg) or dexamethasone (100 µg) had no effect (lanes 11 and 12). All above

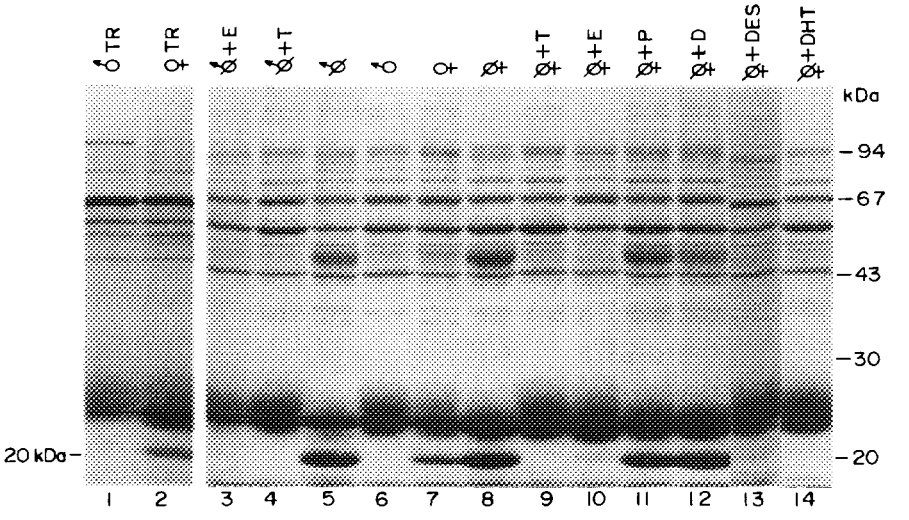
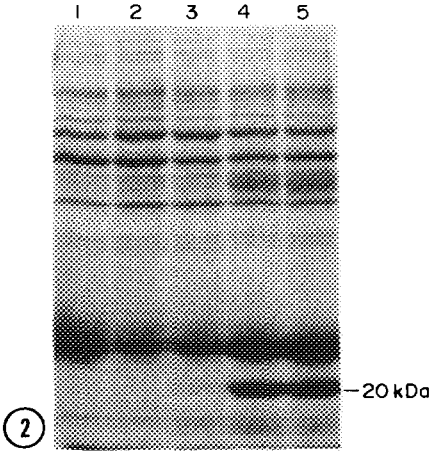


Fig.1. Hormonal regulation of 20-kDa lacrimal gland major protein. SDS-PAGE profiles of lacrimal gland extracts of intact adult (♂/♀) and gonadectomized (♂/♀) male and female hamsters and tears (TR) are shown. Gonadectomized hamsters were treated (+) with either testosterone (T), estradiol (E), progesterone (P), dexamethasone (D), dihydrotestosterone (DHT) or diethylstilbestrol (DES) for 15 days (doses in text). Positions of molecular weight markers are shown.

results were similar in 10-20% gradient gels (not shown). Interestingly, a 20 kDa major protein was present in female tears (lane 2) and in higher levels in tears of gonadectomized hamsters of both sexes (not shown) but not in male tears (lane 1) or in tears of androgen or estrogen treated gonadectomized animals (not shown). The 20 kDa lacrimal and tear proteins did not stain for glycoprotein and did not bind concanavalin-A-Sepharose (results not shown) indicating that they were not glycosylated. Major protein(s) at 24-27 kDa were however glycosylated. In males, marked increase in lacrimal 20 kDa protein occurred within 28 days of castration after which the levels were essentially unchanged (Fig.2). Ovariectomy induced increase in levels of 20 kDa protein was complete within 7-10 days (data not shown). Fig.3 shows the dose-dependent inhibition of 20 kDa protein of castrated males by testosterone and estradiol. A daily dose of 50 µg testosterone caused complete inhibition in 15 days while only 3.6 µg estradiol had the same effect. Results were essentially similar when ovariectomized females were treated (not shown). In two-dimensional gels (Fig. 4) the lacrimal 20 kDa proteins of gonadectomized males and females resolved as single spots at relatively similar regions. Such a protein was absent in intact males.

Other observations for which data are not presented were as follows: Addition of a number of proteolytic inhibitors during extract preparation did not affect SDS-PAGE protein profiles of lacrimal glands. There was no significant variation in lacrimal gland weights among intact, gonadectomized or hormone treated male or female hamsters. Extracts of 14 hamster tissues (including liver, brain, kidney, lungs, spleen, intestine, etc.) showed no detectable sex or gonadectomy related differences in their protein profiles and no protein at 20 kDa was discernible in stained gels.



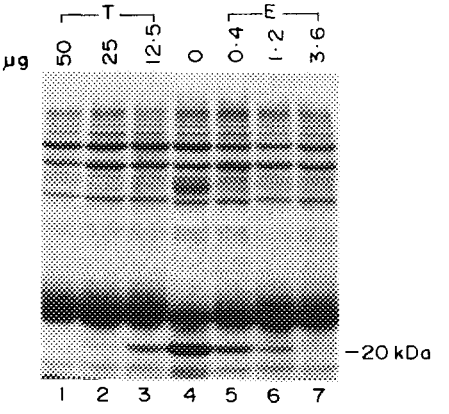


Fig.2. Time-dependent increase of 20-kDa lacrimal protein in castrated males. SDS-PAGE protein profiles of lacrimal gland extracts from males castrated for 7, 14, 28 and 56 days are shown in lanes 2, 3, 4 and 5, respectively. Profile of 56 days unoperated control is in lane 1.

Fig.3. Dose-dependent inhibition of 20-kDa lacrimal gland protein by testosterone and estradiol. SDS-PAGE profiles of extracts of lacrimal glands of castrated males treated daily for 15 days

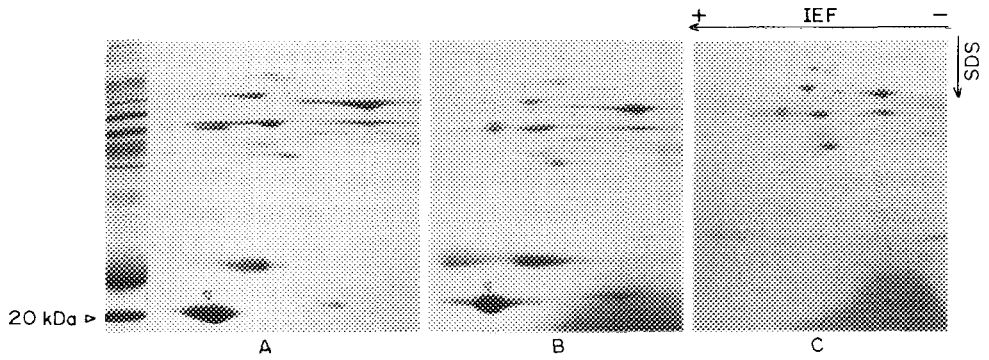


Fig.4. Two-dimensional gels of lacrimal gland extracts. A, castrated male; B, ovariectomized female; C, intact male. Arrows indicate directions of IEF and SDS-PAGE. SDS-PAGE profile of castrated male lacrimal extract is shown in left of A. Open arrowhead indicates 20-kDa protein.

DISCUSSION

Sex hormone-mediated induction of synthesis of specific proteins is known (23-25) but sex hormone-mediated repression of specific protein synthesis is relatively rare (17,25-27). Direct visualization of such effects of sex hormones are rarely possible in crude protein profiles and require intricate detection methods. To the best of our knowledge there is no report of a major protein whose expression is inhibited by both androgens and estrogens. Our experiments involving examination of protein profiles of lacrimal glands, from hamsters in various hormonal states, show that expression of a 20 kDa major protein was markedly inhibited in both sexes by androgens and estrogens. Given the way androgens and estrogens are known to act (25), this inhibition is probably at the level of transcription (resulting in inhibition of synthesis of this protein) although unusual post-transcriptional effects could also be possible (23-25). Recently, androgen receptors were reported in lacrimal glands of rat and hamsters (15). On the other hand, estrogen receptors are absent at least in rat lacrimal glands (16) and it is generally believed that estrogens have no effect on the lacrimal gland (1,4,7,10-17). Our findings contradict this view and indicate a species difference in this regard. Recently, androgen inhibition of gene expression in rat lacrimal gland has been demonstrated, although once again estrogens had no effect (17). Thus, the hamster lacrimal 20 kDa protein is a promising candidate to study at the molecular level how androgens and estrogens inhibit expression of the same protein and to what extent common mechanisms are involved.

Similar resolution in two-dimensional PAGE of lacrimal 20 kDa proteins from gonadectomized hamsters of both sexes and their similar hormonal regulation strongly indicates that they are identical proteins in both sexes. Although testosterone was found to be at least 15 fold less potent inhibitor of this protein than estradiol, the absence of this protein in intact males and not in females was due to its complete inhibition by physiological levels of testosterone in males and incomplete inhibition by endogenous levels of estradiol in females. This is possible because testosterone levels in males are about 500 fold higher than estradiol levels in females (28,29) which results in a net higher inhibition in males. In addition to its novel regulation, the

level of expression of this 20 kDa protein in the lacrimal gland was regulated in a tissue specific manner since any 20 kDa protein was either undetectable or if present, only in trace levels, in many tissues including seminal vesicles and uterus from intact or gonadectomized hamsters (results not shown).

Tears are secretions of the lacrimal gland. The similar hormonal regulation and lack of glycosylation in both lacrimal gland and tear 20 kDa proteins strongly indicates that they are identical proteins. Thus, the hamster 20 kDa lacrimal protein was secreted in tears and had a distinctly different hormonal regulation from all other hormonally regulated tear proteins known in other species, which are all androgen induced and present in higher levels in males (4,6,11,12,30).

Lacrimal glands of rodents have been proposed to function as a scent gland (3-6) although no secretory product has been examined in this respect. Hamsters sniff (and possibly come in contact with) eye secretions on encounter with each other probably to determine sex and age (3-5). Recently, a major 17 kDa protein of hamster vaginal discharge has been purified and shown to possess aphrodisiac property (31,32). Any such related function of the female hamster 20 kDa tear protein needs investigation.

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