PERGAMON

Journal of Steroid Biochemistry and Molecular Biology 70 (1999) 151–158

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Hormonal effects on hamster lacrimal gland female-specific major 20 kDa secretory protein and its immunological similarity with submandibular gland major male-specific proteins

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Received 16 September 1998; accepted 22 March 1999

Abstract

Hormonal regulation of a major 20 kDa protein of hamster exorbital lacrimal gland (LG) was studied by SDS-PAGE profile analysis and the purified protein's antisera was used to screen tissues of hamster and other species for crossreacting proteins. This protein was seen in female LG but not in males and late-pregnant or hCG-treated females. Low estrogen state in females after gonadectomy, prolonged light-deprivation, prolonged starvation or lactation increased its level several folds to ~20% of LG soluble proteins and similar levels were induced in males after gonadectomy (low androgen state). However, light-deprivation or melatonin treatment-induced low androgen state in males had no effect. In gonadectomized hamsters, this LG protein was obliterated on treatment with androgens, estrogens or thyroid hormones. Only estrogen inhibition of LG 20 kDa was prevented by simultaneous tamoxifen administration. Simultaneous treatment of gonadectomized hamsters with gonadotrophins and estrogen/androgen did not prevent the LG 20 kDa protein's inhibition. Relative potencies of estrogens (3.6 μg daily dose) were: estradiol-17β ≃ diethylstilbestrol > estrone > estradiol-17α, while estriol and chlorotrianisene had no effect. Dexamethasone, progesterone, prolactin, hypothyroid state or adrenalectomy had no effect on LG 20 kDa expression. Western blot studies confirmed the marked repression of LG 20 kDa by estrogen androgen and thyroid hormone and detected the protein in tears of females and gonadectomized hamsters but not in males. Interestingly, among other tissues tested, crossreaction was only seen with the estrogen-repressed 24 and 20.5 kDa major male-specific secretory proteins of hamster submandibular glands (SMG) which were previously reported by us. This strongly indicated that the LG and SMG proteins are products of the same or closely related genes. A possible role for these hamster sex-specific LG and SMG major secretory proteins in olfactory communication is suggested.

1. Introduction

We had reported the presence of a female-specific 20 kDa protein in hamster exorbital lacrimal gland (LG) [1]. Upon gonadectomy, females showed an increase in the levels of this protein and males showed a massive induction to gonadectomized female levels (~20% of soluble proteins). This protein was repressed by testosterone and estradiol and was possibly secretory [1].

In hamsters, extremely low sex hormone levels prevail during lactation [2,3] and starvation [4] in females and also after light-deprivation or melatonin treatment in both sexes [5–10] while estrogen levels are high during pregnancy [2,3]. If indeed, LG 20 kDa is regulated by estrogens and androgens, then the above states should affect its expression. Moreover, prolactin, thyroid hormones and gonadotrophins might also affect LG 20 kDa expression since they have known effects on another lacrimal gland of hamster, the Harderian gland (HG), where they regulate porphyrin biosynthesis [8,10–16]. Interestingly, regulation of porphyrin biosynthesis in HG has some similarities with that of LG 20 kDa. Porphyrin biosynthesis in HG is also female-specific and is massively induced in males after gonadectomy, this being also prevented by andro-

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PII: S0960-0760(99)00103-X

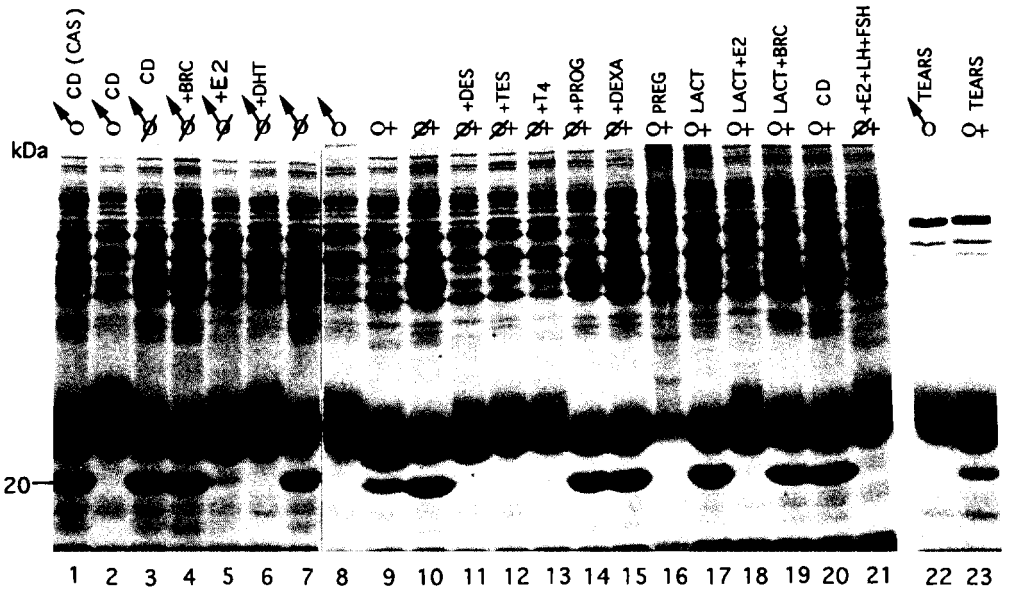

Fig. 1. Hormonal regulation of hamster LG 20 kDa major protein. SDS-PAGE profiles of LG extracts of intact (♂/♀), gonadectomized (⚥/⚥), 15 day lactating (LACT), 14 day pregnant (PREG) and constant darkness maintained (CD) hamsters with or without indicated additional treatments (+) with estradiol (E2), bromocriptine (BRC), dihydrotestosterone (DHT), diethylstilbestrol (DES), testosterone (TES), progesterone (PROG), dexamethasone (DEXA), T4, FSH and LH are shown in lanes 1–21 and lanes 22 and 23 are profiles of pooled tears. All gonadectomy indicated as ⚥ and ⚥ were for 45 days. In lane 1, CD treatment was for 105 days with a brief interruption for gonadectomy (CAS) after 75 days CD; in lane 2, CD was for 105 days; in lane 3, simultaneous CD and gonadectomy were done for 45 days; in lane 20, CD was for 70 days. See Results and Methods for other details of treatments.

gen treatment [8,10–16]. Additionally, light-deprivation or bromocriptine (prolactin release inhibitor) treatment prevents induction of HG porphyrin biosynthesis in gonadectomized males [8,10–16]. These treatments might also affect LG 20 kDa induction in gonadectomized males.

To obtain clues to the function of LG 20 kDa, it needs confirmation whether it is indeed secretory and whether a similar protein is present in other hamster tissues or in lacrimal glands of other species. In this context, we reported that hamster submandibular salivary gland (SMG) expresses male-specific 24 and 20.5 kDa secretory proteins, constituting ~40% of soluble proteins, which also show sex-hormonal repression [17]. However, any relationship between the LG and SMG proteins has not been investigated.

We reinvestigated here the expression of LG 20 kDa in different hormonal states/treatments. Since, effect of estrogens on lacrimal gland and presence of estrogen receptor in this tissue have not been detected in other species [1,18–25], we checked the inhibition of hamster LG 20 kDa by different estrogens and tested whether tamoxifen (an estrogen receptor antagonist) could prevent the estrogenic inhibition. Immunoblots using purified LG 20 kDa protein's antisera was used to confirm its expression pattern, presence in tears and search for crossreacting proteins in hamster SMG and other tissues.

2. Materials and methods

Tamoxifen, bromocriptine mesylate, methimazole, α-methylmannoside, chlorotrianisene and all hormones used were from Sigma (St. Louis, MO). Concanavalin A-Sepharose and protein A were from Pharmacia. Unless otherwise stated, Syrian (golden) hamsters (4–6 per cage) were maintained in 13:11 light:dark cycle (lights off at 1800 h) with food and water given ad libitum. All treatments were done on groups of hamsters (n = 4–6) with appropriate controls. Experiments were repeated twice and representative results are shown. Starved females (without food) and fed controls were housed individually. Light deprivation was started at ~60 day age by maintenance in a ventilated dark room or by blinding [14] and such hamsters with or without gonadectomy (in case of males) were killed at different times (see Results) with appropriate controls. Equine leutenizing hormone (LH) porcine follicle stimulating hormone (FSH), human chorionic gonadotrophin (hCG), sheep prolactin, thyroxine (T4), triodothyronine (T3) and melatonin were injected in saline. Steroidal hormones and nonsteroidal estrogens were dissolved in corn oil. Bromocriptine was suspended in corn oil [11]. Tamoxifen was dissolved in propylene glycol. Above compounds (doses in Results) were injected daily (0.15 ml; sc) between 1000 and 1100 h except for melatonin which was injected at

1600 h [6]. Initial studies showed no effect of the above vehicles on LG 20 kDa expression, so untreated controls were not vehicle injected. Bilateral gonadectomy, blinding and adrenalectomy were done under ether anesthesia. Except for ablation of regressed testes (gonadectomy) of 75 day light-deprived males (~135 day old), all surgeries were done on ~60 day old hamsters. Intact controls (if required) were left unoperated and killed along with treated (see below) or untreated operated hamsters. Treatments of 30 day gonadectomized hamsters with estrogens, androgens, progesterone, dexamethasone, T3, T4, tamoxifen, LH, FSH and hCG (separately or in combination as described in Results) were given for 15 days (20 days for prolactin) and then killed with appropriate untreated controls. Treatment of ~90 day old intact hamsters with hCG or tamoxifen was given for 15 days while prolactin was given for 20 days and then they were killed with untreated controls. In some experiments, ~60 day old hamsters were either gonadectomized or kept unoperated and they were injected from the day after surgery with bromocriptine for 45 days and then killed with appropriate controls.

From each hamster a pair of LG were carefully excised and weighed. Extracts (2.5% w/v) were prepared [1] by homogenizing in 20 mM potassium phosphate buffer (pH 8.0) and centrifuging at $28000 \times g$ for 30 min at 4°C. Supernatants (140 μl) containing ~600 μg protein were run in SDS-PAGE (10%) [26]. Gels were stained with 0.25% coomassie blue and were scanned using laser densitometer if required. Pooled tears were collected [22] and HG free porphyrins measured [8] as described earlier. Antisera against electroeluted pure LG 20 kDa was raised in rabbit. Western blots of LG extracts, other tissues and pooled tears were done as described earlier [27] using diluted antisera (1:100) and ^{125}I-protein A.

3. Results

Representative SDS-PAGE profiles of LG from hamsters in different hormonal states are shown in Fig. 1. Descriptions below focus only on 20 kDa protein. Protein at 20 kDa was present in females (lane 9) but was undetectable in males (lane 8). Males gonadectomized for 45 days showed a massive induction of LG 20 kDa (lane 7). Females gonadectomized for 45 days showed a several fold increase in LG 20 kDa (lane 10). In both sexes, post-gonadectomy levels of LG 20 kDa were similar (~20% of soluble proteins) and at steady state [1]. Treatment of 30 day gonadectomized females with 5 μg diethylstilbestrol (lane 11), 50 μg testosterone (lane 12) or 60 μg T4 (lane 13) for 15 days almost obliterated the LG 20 kDa while 100 μg of progesterone or dexamethasone had no effect (lanes 14 and 15). Similar treatment of 30 day gonadectomized males with 5 μg estradiol or 50 μg dihydrotestosterone obliterated the LG 20 kDa (lanes 5 and 6). All above results were similar when gonadectomized hamsters of opposite sex were similarly treated for 15 days (not shown). In initial time-course studies using gonadectomized females (not shown), testosterone (50 μg), estradiol (3.6 μg) or T4 (60 μg) showed an almost complete inhibition of LG 20 kDa after 15 days treatment while 10 days treatment resulted in considerable but incomplete inhibition and 5 days treatment usually showed a very low inhibition. Gonadectomy is well known to increase circulating levels of LH and FSH which are decreased by sex hormone treatment. It is possible that sex hormones' effects are indirect and inhibition of LG 20 kDa is directly due to fall in levels of LH and/or FSH. However, 15 days treatment with heterologous LH (50 IU) and FSH (2 units) along with estradiol (5 μg) of 30 day gonadectomized females (lane 21) or similar treatment with these two gonadotrophins along with testosterone (50 μg) of 30 day gonadectomized males (not shown) did not prevent inhibition of LG 20 kDa. Moreover, 15 days administration of 50 IU hCG (which shares its receptor with LH and also increases gonadal steroidogenesis) to males or gonadectomized hamsters did not change the expression of LG 20 kDa and similar administration of hCG along with testosterone or estradiol treatment to gonadectomized hamsters did not prevent the inhibition of LG 20 kDa (not shown). However, LG 20 kDa was undetectable in hCG treated intact females (not shown) and also in 14 day pregnant females (gestation ~ 16 days; lane 16). It was also not detected in mothers 1 day after delivery (not shown). Interestingly, 15 day lactating mothers had gonadectomy-like high levels of LG 20 kDa (lane 17). Lactation in hamsters is a low estrogen and high prolactin state [2,3]. Daily post-parturn administration of estradiol (3.6 μg) but not bromocriptine (500 μg) for 15 days abolished this lactation-induced rise of LG 20 kDa (lanes 18 and 19). Levels of LG 20 kDa decreased and resembled normal female levels (not shown) by 25 day post-weaning (weaning on 22 day post-partum). Bromocriptine treatment (500 μg; 45 days) did not affect the induction of LG 20 kDa in gonadectomized males (lane 4) or gonadectomized females (not shown) or LG 20 kDa expression in intact males or females (not shown). Moreover, administration of prolactin (10 IU for 20 days) to intact or 30 day gonadectomized males or females did not affect their typical expression of LG 20 kDa (not shown). Female hamsters kept in constant darkness for 70 days had gonadectomy-like levels of LG 20 kDa (lane 20) but males kept for 45 (not shown), 70 (not shown) or 105 days in constant darkness showed no expression of LG 20 kDa (lane 2) although their testes and seminal

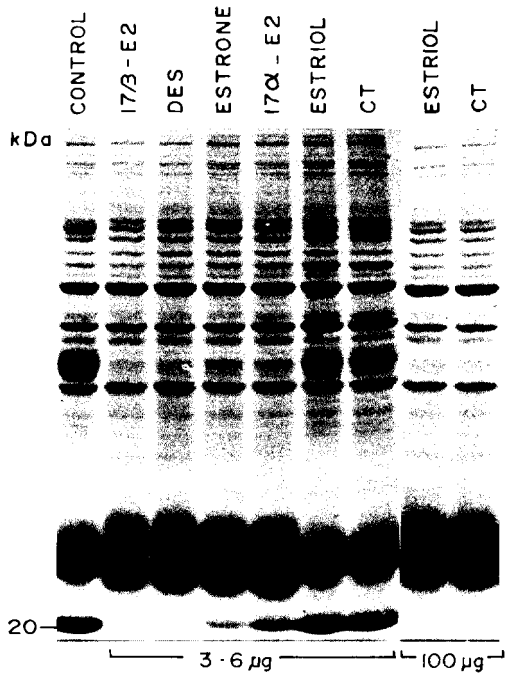

Fig. 2. Effect of different estrogens on LG 20 kDa protein. Gonadectomized females were treated daily for 15 days with indicated doses of estrogens. CT, chlorotrianisene.

vesicles were markedly regressed (testes weights after 105 days ~ 1/9th of controls; data not shown) indicating a low androgen state which is known to result after prolonged light-deprivation [5,6,8,10]. However, ablation of regressed testes after 75 days exposure to constant darkness markedly induced LG 20 kDa by another 30 days of continued exposure (lane 1). Alternatively, gonadectomized males maintained post-surgery in constant darkness for 45 days showed the usual gonadectomy-associated induction of LG 20 kDa (lane 3). All above results were essentially similar when hamsters were blinded instead of being maintained in constant darkness (not shown). A 20 kDa protein was seen in profiles of pooled tears from females but not from males (lanes 23 and 22).

Figure 2 shows the effect of different estrogens on LG 20 kDa in gonadectomized females. Estradiol-17β and diethylstilbestrol (nonsteroidal estrogen) were apparently equipotent and caused complete inhibition of LG 20 kDa at a low dose of 3.6 μg. When compared with control, estrone and estradiol-17α (at doses of 3.6 μg), showed ~85 and 65% inhibition respectively while estriol or chlorotrianisene (nonsteroidal estrogen) had no effect. However, 100 μg of estriol and chlorotrianisene showed complete inhibition. Interestingly, it is reported that both estriol and chlorotrianisene have extremely low uterotrophic activity in hamster and they also show very low binding with hamster uterine estrogen receptor [28]. Moreover, in a previous study by others on estrogenic inhibition of

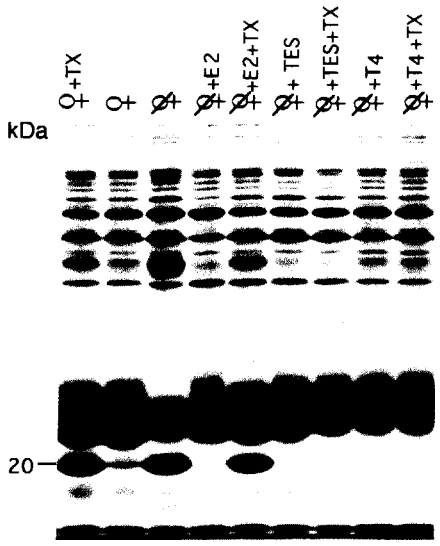

Fig. 3. Effect of tamoxifen on the inhibition of LG 20 kDa by estradiol, testosterone and thyroid hormone. Treatments were given daily for 15 days. TX, tamoxifen.

$\alpha 2_u$ globulin synthesis in livers of androgen treated gonadectomized female rats, estriol was found to be ineffective while estradiol-17α had considerable activity [29].

Figure 3 shows that when tamoxifen (500 μg) is administered simultaneously with either estradiol (3.6 μg), testosterone (50 μg) or T4 (60 μg) to gonadectomized females, it only (and completely) prevents estradiol's inhibition of LG 20 kDa. Tamoxifen injected to intact females results in a gonadectomy-like induction of LG 20 kDa which is likely to be due to its blocking of the inhibitory effect of endogenous estrogens. However, tamoxifen treatment of intact males (not shown) had no effect.

Fig. 4. Dose-dependent inhibition of LG 20 kDa by thyroid hormones. Treatments were given daily with indicated doses of T4 and T3.

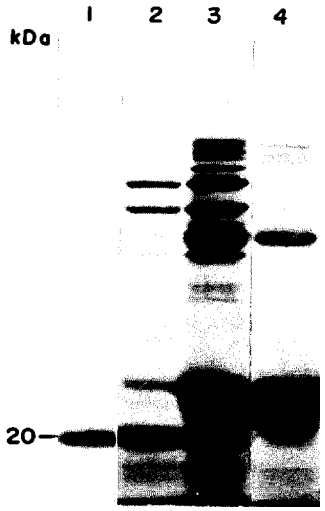

Fig. 5. Purification of the 20 kDa protein from LG extracts of gonadectomized females. Lane 1, pure LG 20 kDa electroeluted from the flowthrough fraction; lane 2, con A-Sepharose flowthrough; lane 3, LG extract of gonadectomized female; lane 4, con A-Sepharose bound fraction after elution from column.

Figure 4 shows the dose-dependent inhibition of LG 20 kDa by thyroid hormones in gonadectomized females. While 0.75 and 2.2 μg T4 had almost no effect, doses of 6.6, 20 and 60 μg caused ~60, 85% and complete inhibition respectively. T3 was more potent since 0.75 and 20 μg caused 65% and complete inhibition respectively. However, administration of 0.025% methimazole (antithyroid agent) in drinking water for 30 days did not affect LG 20 kDa expression in males or females (not shown) even though post-mortem examination showed hypertrophy of thyroid tissue indicative of a hypothyroid state.

Purification of nonglycosylated LG 20 kDa [1] is shown in Fig. 5. LG extract of gonadectomized females (lane 3) was fractionated on a concanavalin A-Sepharose column to separate the flowthrough (lane 2) containing all of the LG 20 kDa and the bound fraction (eluted with 0.5 M α-methylmannoside) which lacked this protein (lane 4). Pure LG 20 kDa (lane 1) was electroeluted from preparative gels of the flow-through fraction and was used for immunization.

In Western blots, using rabbit antiserum against LG 20 kDa (Fig. 6), pure LG 20 kDa showed a single crossreaction (lane 13) confirming its purity. Male LG extract showed no crossreaction (lane 5) while cross-reaction at 20 kDa was seen for female LG (lane 6). LG of gonadectomized females and males (lanes 7 and 8) showed higher levels of crossreaction at 20 kDa while gonadectomized females treated with T3 or estradiol showed no crossreaction (lanes 3 and 4). Pooled tears from females and gonadectomized males (lanes 9 and 11) showed crossreaction at 20 kDa but not tears from males or testosterone treated gonadectomized males (lanes 10 and 12). Minor crossreaction at ~24 kDa was seen in all LG and tear samples having the major 20 kDa crossreaction. Interestingly, male hamster SMG (lane 1) showed 2 major crossreacting proteins at ~24 and ~20.5 kDa and a minor one at

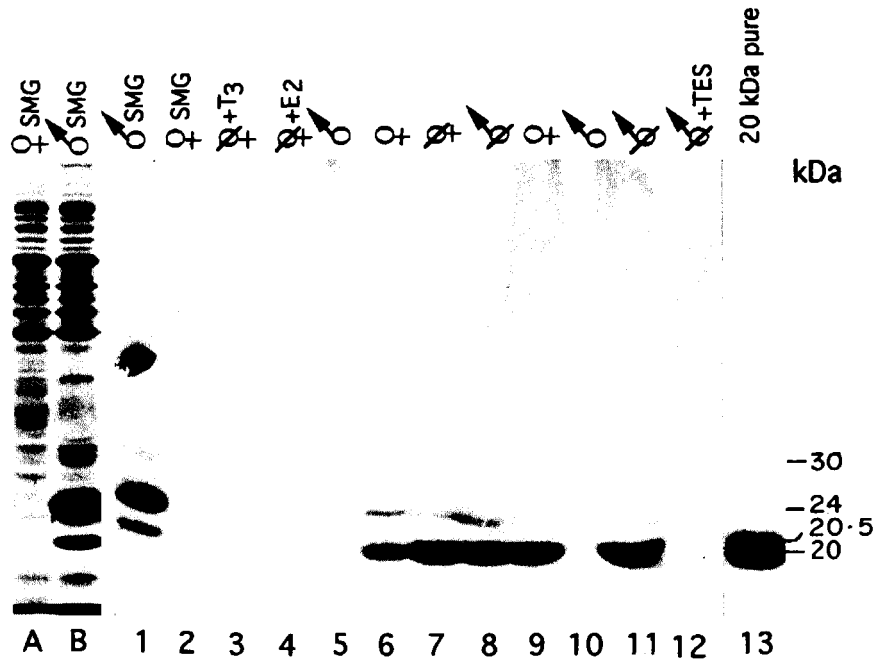

Fig. 6. Western blots of LG, SMG and pooled tears of hamsters using antisera against LG 20 kDa. Lanes 1–13 are Western blots and lanes A and B are coomassie stained profiles. Lanes A, B, 1 and 2 are SMG extracts; lanes 3–8 are LG extracts; lanes 9–12 are pooled tears; lane 13 is pure LG 20 kDa. Sex, gonadal status and treatments (+) if any are indicated on top of each lane.

~30 kDa while female SMG (lane 2) showed no cross-reaction. The major crossreactions in male SMG obviously represent the major male-specific 24 and 20.5 kDa proteins previously reported by us [17] to be present in coomassie stained profiles of male SMG (lane B) but not in female SMG (lane A). Moreover, antisera previously raised by us against the electroeluted male SMG 20.5 kDa protein [27] crossreacted with LG 20 kDa (not shown) as well as with 24, 20.5 and 30 kDa SMG proteins [27]. LG of long-term light-deprived males and many other hamster tissues including HG, parotid, sublingual and flank glands, liver, kidney, spleen and brain from both sexes and prostate, seminal vesicles, testis, lactating mammary, milk, uterus and vaginal discharge showed no crossreaction (not shown). Surprisingly, LG, SMG and saliva of rat, mouse and guinea pig and human tears and saliva from both sexes also showed no crossreaction (not shown).

Our other observations for which data are not shown were as follows: Comparison of profiles of equal volumes of LG extracts from 13 day starved females and fed controls showed a several fold higher level of LG 20 kDa in starved profiles which were similar to profiles seen after gonadectomy. Average ratio for uterine wt:body wt. of 13 day starved and 70 day light-deprived females were respectively 31 and 23% of their controls which indicated their known low estrogen state [4,6,7,10]. The hCG treated intact females had distinctly higher uterine vasculation and ~1.5 fold higher average uterine weight than untreated controls. These indirectly indicated a higher estrogen state, well known to result after such treatment. Administration of melatonin (25 μg) to ~60 day old male hamsters for 60 days caused marked regression of testes and seminal vesicles which is known to result after such treatment [6] but did not induce LG 20 kDa and LG profiles were indistinguishable from those of untreated males. Adrenalectomy and maintenance for 30 days with 1% saline (instead of drinking water) did not affect expression of LG 20 kDa in males or females.

4. Discussion

Protein profile analysis and immunoblots show that LG 20 kDa is markedly inhibited by androgens, estrogens and thyroid hormones and is secreted in tears. The sex hormones obviously inhibit at physiological levels but this is unclear for thyroid hormones since no induction of LG 20 kDa was seen in hypothyroid hamsters. Our results do not indicate any direct effect of gonadotrophins or prolactin on LG 20 kDa expression. The heterologous gonadotrophins and prolactin used by us are however otherwise physiologically active in the hamster [3,5,6,15]. The gonadectomy-like expression of LG 20 kDa in females after starvation, light-deprivation or lactation was likely to be due to their low estrogen states [2–4,6,7,9,10]. Our uterine weight measurements and results of estrogen and bromocriptine treatment of lactating hamsters strongly support this view. Conversely, absence of LG 20 kDa in hCG treated females and in late-pregnancy could be due to their known high estrogen states [2,3]. The well known (and thus expected) fall in endogenous levels of prolactin, gonadotrophins, thyroid hormones and other endocrine changes in our light-deprived males [5,6,9,10] and the expected depression of prolactin levels in bromocriptine treated males and females [10,11,15,16] did not prevent the induction of LG 20 kDa when such hamsters were also gonadectomized. Although, LG 20 kDa and HG porphyrin biosynthesis are both androgen repressed in males and are both markedly induced after gonadectomy [1,8,10,12–16], the above mentioned endocrine changes due to light-deprivation or bromocriptine treatment are however known to prevent the gonadectomy-induced increase of HG porphyrins in males [8,10–16]. Our measurements of HG porphyrin levels (data not shown) confirmed this and indirectly indicated that the known endocrine changes were indeed occurring in our treated hamsters. The lack of expression of LG 20 kDa in light-deprived intact males (despite their low androgen state) must be due to effective inhibition by low levels of androgens still being secreted by their regressed testis [30] since their ablation results in induction. The lack of LG 20 kDa induction in melatonin treated males (which also had regressed testis) might have the same explanation. Although, LG 20 kDa expression appears to be overwhelmingly dependent only on endogenous sex hormone levels, the effects of other pituitary hormones and hypophysectomy with and without gonadectomy need investigation [16,31].

Androgen repression of hamster LG 20 kDa is unusual since other known hormonally regulated tear proteins are all androgen induced [1,18–23,32]. This repression might be mediated by androgen receptors demonstrated in hamster LG [20,21]. Inhibition of LG 20 kDa by several estrogens and prevention of only estrogen's inhibition by tamoxifen indicate that estrogen's inhibition requires estrogen receptors.

Hormonal repression of gene expression is rare and its mechanism poorly understood when compared with induction [1,12,17–19,25,28,29,32–35]. Moreover, changes in gene expression can rarely be visualized in protein profiles as it is possible for LG 20 kDa or the SMG male-specific 24 and 20.5 kDa secretory proteins of hamster [17]. Male expression of these SMG proteins is not androgen dependent, but is markedly repressed by exogenous estrogens [17]. Gonadectomized females show male-like expression of these

SMG proteins which are obliterated by estrogens and surprisingly also by androgen treatment [17]. The LG and SMG proteins thus have similarities and dissimilarities in their sex-hormonal repression [1,17] and could be excellent models to study molecular mechanism of gene repression by estrogens and androgens.

In spite of differences in their Mr [17] and hormonal regulation, the immunological similarity between the LG and SMG secretory proteins and the lack of cross-reaction in other hamster tissues strongly suggests that they are tissue-specific products of same or closely related genes with a similar function which might be related to their common secretory nature. The LG 20 kDa and SMG 24 and 20.5 kDa proteins might differ in their post-translational modification and the minor crossreaction at 24 kDa (LG) and 30 kDa (SMG) could be precursors of the major proteins. The function of the LG and SMG proteins must also be related to their sex-specific expression. Moreover, it is intriguing that immunologically similar protein was not detected in other species.

On encounter, hamsters sniff and come in contact with eye, nasal and salivary secretions which are well known sources of species-specific olfactory cues (pheromones) [11,18,31,36–42]. Pheromone and odorant binding proteins in tears, saliva, nasal mucus and urine of different species [40,42–46] and a pheromonal protein in hamster vaginal discharge [40,47] are known and they all belong to a family of lipophilic ligand binding proteins (lipocalins) which are mostly abundant secretory proteins of Mr $\sim$ 20 kDa with low sequence homology but considerable structural similarity [40,42–49]. The female-specific LG 20 kDa and male-specific SMG 24 and 20.5 kDa major secretory proteins of hamster might be lipocalins and function as pheromone or odorant binding proteins or they themselves could have pheromone activity.

Acknowledgements

Authors are grateful to Mr. P. Jayaraman for performing most of the animal surgeries and his advise and help in animal handling.

References

[1] V. Ranganathan, P.K. De, Androgens and estrogens markedly inhibit expression of a 20 kDa major protein in hamster exorbital lacrimal gland, Biochem. Biophys. Res. Commun. 208 (1995) 412–417.
[2] R. Baranczuk, G.S. Greenwald, Plasma levels of oestrogen and progesterone in pregnant and lactating hamsters, J. Endocrinol. 63 (1974) 125–135.
[3] G.S. Greenwald, Endocrinology of the pregnant hamster, in: H.I. Siegel (Ed.), The Hamster, Reproduction and Behaviour, Plenum Press, New York, 1985, p. 53.
[4] R.H. Printz, G.S. Greenwald, Effects of starvation on follicular development in the cyclic hamster, Endocrinology 80 (1970) 290–295.
[5] A. Bartke, Male hamster reproductive endocrinology, in: H.I. Siegel (Ed.), The Hamster, Reproduction and Behaviour, Plenum Press, New York, 1985, p. 73.
[6] R.J. Reiter, Pineal-reproductive interactions, in: H.I. Siegel (Ed.), The Hamster, Reproduction and Behaviour, Plenum Press, New York, 1985, p. 99.
[7] J.S. Massa, D.E. Blask, Depressed prolactin cell activity in long-term blind-pinealectomized female hamsters is due to loss of estrous cyclicity, Proc. Soc. Exp. Biol. Med. 195 (1990) 119–123.
[8] R.A. Hoffman, Influence of some endocrine glands, hormones and blinding on the histology and porphyrins of the Harderian glands of golden hamsters, Am. J. Anat. 132 (1971) 463–478.
[9] J. Vriend, F.D. Bertalanffy, T.A. Ralcewicz, The effects of melatonin and hypothyroidism on estradiol and gonadotrophin levels in female Syrian hamsters, Biol. Reprod. 36 (1987) 719–728.
[10] G.R. Buzzell, Sexual dimorphism in the Harderian gland of the Syrian hamster is controlled and maintained by hormones, despite seasonal fluctuations in hormone levels: functional implications, Micros. Res. Tech. 34 (1996) 133–138.
[11] G.R. Buzzeil, A. Menendez-Pelaez, T. Porkka-Heiskanes, A. Pangerl, B. Pangerl, M.K. Vaughan, R.J. Reiter, Bromocriptine prevents the castration-induced rise in porphyrin concentration in Harderian glands of the male Syrian hamster, *Mesocricetus auratus*, J. Exp. Zool. 249 (1989) 172–176.
[12] A. Menendez-Pelaez, C. Rodriguez, P. Dominguez, 5-Aminolevulinate synthase mRNA levels in the Harderian gland of Syrian hamsters: correlation with porphyrin concentrations and regulation by androgens and melatonin, Mol. Cell. Endocrinol. 80 (1991) 177–182.
[13] C. Rodriguez, M. Kotler, L. Antolin, R.M. Sainz, A. Menendez-Pelaez, Regulation of the aminolevulinate synthase gene in the Syrian hamster Harderian gland: Changes during development and circadian rhythm and role of some hormones, Micros. Res. Tech. 34 (1996) 65–70.
[14] J.W. Clabough, J.E. Norvell, Effects of castration, blinding and the pineal gland on the Harderian glands of the male golden hamster, Neuroendocrinol. 12 (1973) 344–353.
[15] F.A. Marr, S.W. Shah, J. McGadey, M.R. Moore, T. Houston, A.P. Payne, The effects of bromocriptine and prolactin on porphyrin biosynthesis in the Harderian gland of the male hamster, *Mesocricetus auratus*, J. Comp. Physiol. B 164 (1995) 524–529.
[16] G.R. Buzzell, R.A. Hoffman, M.K. Vaughan, R.J. Reiter, Hypophysectomy prevents the castration-induced increase in porphyrin concentrations in the Harderian glands of the male golden hamster: a possible role for prolactin, J. Endocrinol. 133 (1992) 29–35.
[17] P.K. De, Sex-hormonal regulation of 20.5 and 24 kDa major male-specific proteins in Syrian hamster submandibular gland, J. Steroid Biochem. Molec. Biol. 58 (1996) 183–187.
[18] J. Winderickx, I. Vercaeren, G. Verhoeven, W. Heyns, Androgen-dependent expression of a cystatin-related protein (CRP) in exorbital lacrimal gland of rat, J. Steroid Biochem. Mol. Biol. 48 (1994) 165–170.
[19] D.A. Sullivan, Hormonal influence on the secretory immune system of the eye, in: S. Freier (Ed.), The Neuroendocrine-Immune Network, CRC Press, Boca Raton, FL, 1990, p. 199.
[20] D.A. Sullivan, J.A. Edwards, A. Wickham, J.D.O. Pena, J. Gao, M. Ono, R.S. Kelleher, Identification and endocrine con-

trol of sex steroid binding sites in the lacrimal gland, Curr. Eye Res. 15 (1995) 279–291.
[21] F.J. Rocha, A. Wickham, J.D.O. Pena, J. Gao, M. Ono, R.W. Lambert, R.S. Kelleher, D.A. Sullivan, Influence of gender and the endocrine environment on the distribution of androgen receptors in the lacrimal gland, J. Steroid Biochem. Mol. Biol. 46 (1993) 737–749.
[22] D.A. Sullivan, K.J. Block, M.R. Allansmith, Hormonal influence on the secretory immune system of the eye: Androgen regulation of secretory component levels in rat tears, J. Immunol. 132 (1984) 1130–1135.
[23] J. Gao, R.W. Lambert, A. Wickham, G. Banting, D.A. Sullivan, Androgen control of secretory component mRNA levels in the rat lacrimal gland, J. Steroid Biochem. Mol. Biol. 52 (1995) 239–249.
[24] Y. Myal, B. Iwasiow, A. Yarmill, E. Harrison, J.A. Paterson, R.P.C. Shiu, Tissue-specific androgen-inhibited gene expression of a submaxillary gland protein, a rodent homolog of the human prolactin-inducible protein/GCDFP-15 gene, Endocrinology 135 (1994) 1605–1610.
[25] P.K. De, Tissue distribution of constitutive and induced soluble peroxidase in rat: purification and characterization from lacrimal gland, Eur. J. Biochem. 206 (1992) 59–67.
[26] U.K. Laemmii, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[27] V. Ranganathan, P.K. De, Western blot of proteins from coomassie stained polyacrylamide gels, Anal. Biochem. 234 (1996) 102–104.
[28] W.W. Leavitt, T.J. Chen, T.C. Alien, Regulation of progesterone receptor formation by estrogen action, Ann. NY Acad. Sci. 286 (1977) 210–225.
[29] A.K. Roy, D.M. McMinn, N.M. Biswas, Estrogenic inhibition of hepatic synthesis of $\alpha 2_u$ globulin in rat, Endocrinology 97 (1975) 1501–1508.
[30] V. Chandrashekar, A. Bartke, The influence of short photoperiod on testicular and circulating levels of testosterone precursors in the adult golden hamster, Biol. Reprod. 40 (1989) 300–306.
[31] W. Chen, M.A. Kelly, X. Opitz-Araya, R.E. Thomas, M.J. Low, R.D. Cone, Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides, Cell 91 (1997) 789–798.
[32] P.H. Shaw, W.A. Held, N.D. Hastie, The gene family for major urinary proteins: expression in several secretory tissues of the mouse, Cell 32 (1983) 755–761.
[33] M. Beato, Gene regulation by steroid hormones, Cell 56 (1989) 335–344.
[34] C.W. Bardin, J.F. Catterall, Testosterone: a major determinant of extragenital sexual dimorphism, Science 211 (1981) 1285–1294.
[35] C.M. Rudnick, S.B. Dowton, Serum amyloid P (female protein) of the Syrian hamster, J. Biol. Chem. 268 (1993) 21760–21769.
[36] F. Dieterlen, Das Verhalten des syrischen Goldhamsters (*Mesocricetus auratus* Waterhouse), Z. Tierpsychol. 16 (1959) 47–103.
[37] B. Gray, R.B. Fischer, G.F. Meunier, Preferences for salivary odor cues by female hamsters, Horm. Behav. 18 (1984) 451–456.
[38] R.E. Johnston, Communication, in: H.I. Siegel (Ed.), The Hamster, Reproduction and Behaviour, Plenum Press, New York, 1985, p. 121.
[39] P. Dominguez, Cloning of a Syrian hamster cDNA related to sexual dimorphism: establishment of a new family of proteins, FEBS Lett. 376 (1995) 257–261.
[40] A.G. Singer, A chemistry of mammalian pheromones, J. Steroid Biochem. Mol. Biol. 39 (1991) 627–632.
[41] J.D. Hahn, Effect of cyproterone acetate on sexual dimorphism of the exorbital lacrimal gland in rats, J. Endocrinol. 45 (1969) 421–424.
[42] S. Marchese, D. Pes, A. Scaloni, V. Carbone, P. Pelosi, Lipocalins of boar salivary glands binding odours and pheromones, Eur. J. Biochem. 252 (1998) 563–568.
[43] P. Pelosi, Odorant binding proteins, Crit. Rev. Biochem. Mol. Biol. 29 (1994) 199–228.
[44] D. Pes, P. Pelosi, Odorant binding proteins of the mouse, Comp. Biochem. Physiol. 112 (1995) 471–479.
[45] A. Bacchini, E. Gaetani, A. Cavaggioni, Pheromone-binding proteins in the mouse *mus musculus*, Experimentia 48 (1992) 419–421.
[46] A. Miyawaki, F. Matsushita, Y. Ryo, K. Mikoshiba, Possible pheromone-carrier function of two lipocalin proteins in the vomeronasal organ, EMBO J. 13 (1994) 5835–5842.
[47] M. Kruhoffer, A. Bub, A. Cieslak, K. Adermann, I. Kunstyr, W.G. Forssmann, H.J. Magert, Gene expression of aphrodisin in female hamster genital tract segments, Cell Tissue Res. 287 (1997) 153–160.
[48] D.R. Flower, The lipocalin protein family: structure and function, Biochem. J. 318 (1996) 1–14.
[49] B.J. Glasgow, A.R. Abduragimov, Z.T. Farahbakhsh, K.F. Faull, W.L. Hubbell, Tear lipocalins bind a broad array of lipid ligands, Curr. Eye Res. 14 (1995) 363–372.