Prolactin Localization, Binding, and Effects on Peroxidase Release in Rat Exorbital Lacrimal Gland

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Prolactin immunoreactivity has been detected in human tears and in lacrimal glands, and it has been suggested that this hormone might be a modulator of lacrimal secretion as well as a component of lacrimal gland fluid. The present study was designed to confirm the immunocytochemical localization of prolactin in the rat lacrimal gland, to determine the source of the prolactin, and to evaluate the acute effects of prolactin on lacrimal secretory function. We have confirmed that prolactin-like immunoreactivity is present in secretory vesicles of acinar cells of male and female Sprague-Dawley rats. Prolactin message was present at detectable levels in RNA extracts of lacrimal glands from males, indicating that at least one component of the prolactin-like immunoreactivity was the product of synthesis within the lacrimal glands. Crude membrane fractions from acini isolated from males bound 43.1 ± 3.2 femtomoles prolactin/mg protein (mean ± standard error of the mean; n = 6), which was significantly more than comparable fractions from females (15.4 ± 2.4 fmoles/mg protein, n = 6). Preincubating membranes at 65° for 20 min to release endogenous ligands increased prolactin binding to 84.8 ± 20.8 fmoles/mg protein for males and 63.8 ± 17.4 fmoles/mg protein for females (P > 0.1), suggesting that, on average, similar numbers of receptors are expressed in acinar cells of male and female rats but a larger fraction of the receptors is occupied by endogenous prolactin-like peptides in females. Because prolactin binding triggers prolactin receptor internalization in various cell types, we propose that the prolactin-like immunoreactivity in lacrimal acinar cells of females has been accumulated from the circulation, while the immunoreactivity seen in males results, at least in part, from de novo synthesis. Ovine prolactin at concentrations of 10–20 ng/ml inhibited carbachol-induced peroxidase release by 19.6% ± 6.9% (n = 8, P < 0.02) but failed to alter peroxidase release in the absence of carbachol. These observations suggest that prolactin might function as an endocrine, paracrine, or autocrine modulator in the lacrimal gland. Invest Ophthalmol Vis Sci 33:641–650, 1992

The fluid secreted by the lacrimal glands contains a mixture of electrolytes, glycoproteins, nutrients, immunoglobulins, and growth factors. An adequate rate of lacrimal fluid production is essential for maintaining the integrity of the ocular surface. Insufficient fluid production leads to dry eye conditions, which make up one of the most frequently encountered categories of ocular morbidity.

There are a number of gender-related differences in lacrimal gland morphology, function, and susceptibility to disease. The acinar cells of lacrimal glands from the females of various species are smaller than those of the males. Female rat lacrimal glands express lower levels of secretory component and secrete smaller amounts of immunoglobulins than lacrimal glands of males. There is also a marked dimorphism in the incidence of Sjogren's syndrome, which is more than 10 times more prevalent in women. Sullivan and coworkers have presented abundant evidence that the gender-related differences in lacrimal structure and function depend on the hypothalamic-pituitary-gonadal axis. For example, castration of male rats decreases secretory component expression and IgA secretion. These effects can be reversed by administration of testosterone to otherwise intact rats but not to rats that have been hypophysectomized or that have had the pituitary transplanted to under the kidney capsule. Although there is no apparent difference between precorneal tear volumes of male and female rats, castration markedly increases the tear volume of male rats. On the other hand, hypophysectomizing intact male rats decreases the tear volume and the secretion of immunoglobulins and secretory component.
Very little is known about the specific hormonal basis of the lacrimal gland sexual dimorphisms. However, a wide variety of peptide hormones and neuropeptides have been found to influence lacrimal secretory function. Adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), cholecystokinin, and vasoactive intestinal peptide (VIP) stimulate secretion, while enkephalins inhibit VIP- and cholinergically-mediated secretion. Because an intact hypothalamic-pituitary-gonadal axis is required for synergizing with the effects of androgens, the effects of the pituitary peptides α-MSH, ACTH, and met-enkephalin are of potential interest.

Prolactin is another anterior pituitary peptide that could participate in maintaining the sexual dimorphism of the lacrimal gland. Serum prolactin levels are approximately two times higher in females than in males. Frey et al have detected prolactin-like immunoreactivity in human tears and within epithelial cells and interstitial lymphocytes of human lacrimal glands. The purposes of this study were to determine whether prolactin-like immunoreactivity also is present within epithelial cells of rat lacrimal glands, to evaluate its subcellular localization and possible origins, and to determine the possible effects of acute exposure to prolactin on the secretory function of the lacrimal epithelium.

Methods

Materials

Rabbit antisera to ovine prolactin and ovine growth hormone were obtained from ICN Biochemicals (Lisle, IL). Fluoresceinated goat anti-rabbit second antibody was obtained from Zymed Laboratories (South San Francisco, CA). Purified collagenase was obtained from Sigma (St. Louis, MO). Rat prolactin and highly purified human growth hormone (HGH-I-1) was obtained from the National Hormone and Pituitary Program (Baltimore, MD). 125I was obtained from Amersham (Cambridge, IN). Other chemicals were reagent grade and were obtained from standard suppliers. cDNA for rat prolactin was obtained from Dr. Richard Maurer (University of Iowa).

Animals

Rats were used in accordance with the ARVO Resolution on the Use of Animals in Research. They were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The ages and genders of rats used in various experiments are given in the figure legends. For immunostaining experiments, they were killed with a lethal injection of sodium pentobarbital (Nembutal, Sigma, St. Louis, MO). For all other experiments, they were sacrificed by CO2 narcosis.

Prolactin Immunocytochemistry

Exorbital lacrimal glands and nearby parotid glands were removed from anesthetized animals and placed immediately in phosphate buffered 4% formaldehyde (generated from paraformaldehyde). Tissue was sliced into 1–2 mm3 fragments, left in fixative for 1–2 hr, rinsed in several changes of buffer, and stored overnight in fresh buffer. Blocks were infiltrated in acrylamide, frozen in OCT at liquid nitrogen temperature, and sectioned with an AO/Reichert cryostat at 6 μm in thickness. Sections were placed on gelatin-subbed slides and kept in a moist chamber for 1–24 hr prior to immunostaining. Nonspecific staining was blocked with 0.5 M NaCl in Tris-HCl buffer, pH 7.2. The primary antibody was rabbit anti-sheep prolactin reported by the vendor to be cross-reactive with rat prolactin. Sections were incubated overnight in a moist chamber with primary antibody at several dilutions (1:10, 1:25, 1:50, and 1:100), rinsed in Tris-HCl-buffered NaCl, incubated 2 hr in a 1:500 dilution of fluoresceinated anti-rabbit second antibody, rinsed in Tris-HCl-buffered NaCl, and mounted in a solution containing paraphenylenediamine to suppress quenching of fluorescein. All incubation and rinse solutions contained 0.5 M NaCl. Controls consisted of: (1) an irrelevant primary antibody at the same protein concentration as the primary antiserum; (2) parotid tissue embedded in the same block for direct comparison; (3) second antibody alone; (4) preabsorption of rat prolactin with the antiserum to ovine prolactin; and (5) antiserum to growth hormone instead of antiserum to prolactin as primary antibody. In addition, sections of anterior pituitary tissue were processed as positive controls. Sections were examined and photographed with an Olympus (Lake Success, NY) Vanox microscope equipped with fluorescence, phase, and bright-field optics.

Extraction, Fractionation, and Identification of mRNA

Lacrimal glands were excised from males and females immediately after they were killed, and the total RNA was immediately isolated according to the method of Chomczynski and Sacchi. Prolactin mRNA was separated by Northern analysis using formaldehyde-formamide agarose gels (1% agarose)
as described by Maniatis et al.20 The gel was blotted onto nitrocellulose filters for hybridization analysis. The prolactin cDNA probe was labeled with 32P using the multiprimer DNA-labeling technique.21 The blots were washed at high stringency (three times in 0.3 M NaCl, 0.03 M sodium citrate [2x SSC], pH 7.0, with 0.1% sodium dodecyl sulfate for 5 min each), then twice for 30 min each in 0.2x SSC with 0.1% sodium dodecyl sulfate at 65°C to ensure specificity of labeling. The hybridized filter then was exposed to x-ray film (Kodak XAR-5, Eastman Kodak Company, Rochester, NY) for 2 wk.

Isolation of Lacrimal Acini

The medium for isolation of acini and for several of the experimental procedures described below was a Tris-HEPES buffered saline (THBS), which consisted of NaCl, 120 mM; KCl, 6.2 mM; MgCl2, 1.6 mM; CaCl2, 1.0 mM; glucose, 10 mM; Tris, 10 mM; and HEPES, 10 mM; pH was adjusted to 7.4 with HCl. For all procedures, THBS was warmed to 37°C and saturated with 100% O2. Acini were isolated according to previously described procedures.22-24 Lacrimal glands were placed in 20 ml THBS, decapsulated, and minced into 1 mm3 fragments. The fragments were collected by centrifugation, then rinsed twice by resuspension in THBS and centrifugation. The washed fragments were resuspended in 30 ml digestion medium, which consisted of THBS supplemented with 1.3 mg/ml (173 U/ml) collagenase, and apportioned between two 50 ml polypropylene Erlenmeyer flasks (Nalge, Rochester, NY). The suspensions were incubated at 37° in a shaking water bath for 45 min, during which the atmosphere in the flasks was flushed with 100% O2. After the digestion period, the fragments were mechanically dispersed by passage through the tips of polypropylene pipettes. The resulting dispersions were filtered through 200-μm nylon mesh filters. The filtrates were layered over columns of THBS containing 2% bovine serum albumin, and acini were harvested by centrifugation for 4 min at 3.3 x g. They then were washed three times by resuspension in THBS and centrifugation.

Prolactin Binding

The assay for prolactin binding sites was based on the fact that HGH binds to the prolactin receptors of lower vertebrates with equal or greater affinity than prolactin.25 On the other hand, rat, ovine, and bovine growth hormones do not bind to prolactin receptors,22 and ovine prolactin will not displace growth hormone from growth hormone receptors. Therefore, as previously published,26 specific prolactin binding sites were determined from the ability of unlabeled prolactin to prevent binding of [125I]Iodo-HGH. Highly purified HGH was radiiodinated by the lactoperoxidase method,27 and [125I]Iodo-HGH was purified by chromatography on Bio-Gel P-60 (BioRad, Richmond, CA).26 Isolated acini were resuspended in phosphate buffered saline supplemented with 0.2 mM PMSF and 9 μg/ml aprotinin to suppress endogenous protease activity.

Membrane preparations were obtained from the acini isolated from both lacrimal glands from individual animals. After homogenization with a Tissumizer (Tekmar, Cincinnati, OH), crude membrane samples were sedimeted by centrifugation at 12,000 x g for 20 min. They were resuspended in phosphate buffered saline. After aliquots were removed for protein determinations, BSA was added to a final concentration of 1 mg/ml.

Total [125I]Iodo-HGH binding was assayed by incubation of 100-μl aliquots of the crude membrane sample in a total reaction volume of 250 μl containing 160,000 counts per minute (3 ng) of the radioiodinated hormone for 20 hr at 23°. Nonspecific binding was measured in the presence of 5 μg ovine prolactin. In general, non-specific binding accounted for less than 50% of the total bound [125I]Iodo-HGH. In experiments testing the effects of cyclosporine A, cyclosporine was added from 10-fold concentrated stocks in dimethylsulfoxide. The parallel control reactions also contained 10% dimethylsulfoxide. Bound and free hormones were separated by a 15-fold dilution and centrifugation at 1,500 x g for 60 min. For reasons of economy, nonsaturating concentrations of [125I]Iodo-HGH were used. Under these conditions, the numbers of prolactin binding sites are proportional to, but not identical with, the difference between the total binding and the nonspecific binding.28

Secretagogue-Induced Peroxidase Release

Peroxidase release was measured with a lacrimal gland fragment preparation similar to those used previously.29,30 A working stock of THBS supplemented with PMSF and aprotinin was placed in in a 37° water bath and gassed continuously with 100% O2. Glands were placed into an aliquot of THBS and minced into 1 mm3 fragments. The fragments were placed in baskets constructed from 15 ml polypropylene beakers with the bottoms removed and replaced with nylon mesh screens. The baskets were placed in 30 ml polypropylene beakers set in a manifold in a gently shaking 37° water bath. During an initial equilibration period of 50 min, the beakers contained 10 ml aliquots of THBS, which were replaced every 10 min. The equilibration period was followed by a 20 min incubation in a 5 ml aliquot of THBS, then by a 20
min incubation in a 5 ml aliquot of THBS containing carbachol. In several experiments, the carbachol concentration was 10 μM, in others it was 20 μM. This change had no significant effect on carbachol-induced peroxidase release, and results from the two groups of experiments were pooled. Samples to be treated with prolactin were incubated with prolactin throughout the equilibration and incubation periods. After the baskets containing the fragments were removed from the beakers, the media from the last two incubations were recovered and stored frozen until they could be analyzed for peroxidase activity. The fragments were resuspended in 5 ml aliquots of THBS and frozen until they could be analyzed for protein. Peroxidase was measured with the method of Herzog et al. After homogenization with a Tissumizer, protein was measured with the BioRad (Richmond, CA) assay kit with BSA as standard. Carbachol-induced peroxidase secretion was calculated as the difference between the amount of peroxidase released into the carbachol-containing medium and amount of peroxidase released into the medium from the final 20-min incubation prior to carbachol treatment. This value was normalized to the total amount of fragment protein.

Statistical Analysis

Student’s t-test was used to determine significant differences.

Results

Prolactin Immunoreactivity in Rat Lacrimal Glands

Figure 1 depicts the localization of prolactin-like immunoreactivity in lacrimal glands of 6-week-old males (Fig. 1A), 12-week-old males (Fig. 1C), 6-week-old females (Fig. 1E), and 12-week-old females (Fig. 1G). Twelve-week-old rats are sexually mature. Six-week-old animals are not fully mature, although in males sperm are present in the epididymis and in females vaginal opening has begun, as has normal adult estrus cycling. Prolactin-like immunoreactivity was detected in acinar cells of males and females at both ages. The pattern of staining was uniform in 12-week-old-females. That is, essentially all acini were positive. In contrast, males at both ages and 6-week-old females showed some acini with intense immunoreactivity, whereas others appeared to be stained only weakly, if at all. There were apparent differences between the amounts of staining in 6-week-old (Fig. 1A) and 12-week-old (Fig. 1C) males, and between 6-week-old (Fig. 1E) and 12-week-old (Fig. 1G) females, with more reactivity appearing in the glands from older animals. However, the essential features of the subcellular distribution pattern were the same in all cases, with the most intense staining occurring in association with secretory vesicles. From the phase contrast images, the acini and acinar cells are shown to be larger in the older males (Fig. 1D) than in the younger males (Fig. 1B) or in the females at either age (Fig. 1F and 1H). Lacrimal glands of rats at the ages studied contain relatively few infiltrating lymphocytes, and no staining of interstitial cells was noted.

Controls run in parallel with an irrelevant rabbit IgG antibody showed some nonspecific binding that outlined acinar cells (not seen in experimental samples) but showed no immunoreactivity of secretory vesicles (not shown). Preabsorption of the antisera to ovine prolactin with rat prolactin abolished staining in the lacrimal gland and pituitary (not shown). Furthermore, acinar cells showed no immunostaining with antiserum to ovine growth hormone. The positive controls, ie, sections of anterior pituitary gland, showed excellent staining of distinct cell populations with the prolactin and growth hormone antisera. On the other hand, sections of parotid glands run simultaneously with lacrimal gland had no prolactin-like immunoreactivity (not shown).

Detection of Prolactin Message

The presence of prolactin immunoreactivity in lacrimal acinar cells from male and female rats raised the question of whether it was derived from the circulation or from synthesis within the acinar cells. Northern blot analyses of lacrimal gland mRNA extracts were performed to determine whether this organ is able to synthesize prolactin. Hybridizations of rat prolactin cDNA to mRNA extracts of lacrimal glands from 12-week-old males and females are shown in Figure 2. Faint but discernible bands were visible in extracts of male glands but not in extracts of female glands. Extracts of pituitary glands from another group of males and females, used as positive controls, demonstrated dense bands in the same position as the faint bands from the male lacrimal gland extracts (not shown). Thus, it is clear that male lacrimal glands have the capacity to synthesize prolactin. That no message was detectable in extracts from female glands under these experimental conditions indicates that if the female lacrimal gland synthesizes prolactin at all, it must do so at a much lower level than the male lacrimal gland.

Prolactin Binding

Standard ligand binding studies were performed to determine whether lacrimal acinar cells contain binding sites for prolactin. As illustrated in Figure 3, acinar cell membrane samples from 6-week- to 8-week-old male rats specifically bound 43.1 ± 3.2
Fig. 1. Fluorescence and phase-contrast images of the same fields of lacrimal gland sections from 6-week and 12-week-old male and female rats after staining with anti-prolactin antibody. (A, B) Six-week male. (C, D) Twelve-week male. (E, F) Six-week female. (G, H) Twelve-week female. Both lacrimal glands from each of two animals at each age were examined, and representative sections are shown. All micrographs at ×290.
Fig. 2. Detection of prolactin message in mRNA extracts of lacrimal glands from 12-week rats. Message was present at detectable levels in extracts from males (M1, M2, and M3) but, under the conditions of these experiments, not from females (F1, F2, F3). Each sample represents the extract of four female or four male lacrimal glands. Twenty micrograms of mRNA was added to each lane of the gel. Pituitary extracts from males and females, run on parallel gels, exhibited intense bands in the same positions as seen in extracts from male lacrimal glands, whereas muscle extracts were negative (not shown).

fmoles/mg protein (mean ± standard error of the mean; n = 6) under the conditions described in Methods. Samples from female rats bound significantly less, ie, 15.4 ± 2.4 fmoles/mg protein (n = 6, P < 0.01).

Because the basal level of circulating prolactin is roughly twice as high in females as in males,16,17 the difference between the apparent numbers of prolactin binding sites in acinar cell membranes from males and females could have reflected a difference in the extent to which receptors were occupied by endogenous ligands. Alternatively, the difference in the number of binding sites could have reflected a difference in the number of receptors expressed per milligram of membrane protein, such as might have resulted from ligand-mediated receptor down-regulation. Treatment of membrane samples at 65°C for 20 min has been found to cause endogenous HCG to dissociate from its receptors.32 This treatment increased prolactin binding to 84.8 ± 20.8 fmoles/mg protein for males and to 63.8 ± 17.2 fmoles/mg protein for females (Fig. 3). The difference between these values was not statistically significant. Therefore, membranes from males and females appeared to contain similar numbers of prolactin receptors per mg protein, but a greater fraction of the receptors appeared to be occupied by endogenous ligands in membranes from females.

Recent work indicates that cyclosporine competitively inhibits binding of prolactin to receptors in T lymphocytes but not in mammary gland epithelial cells.36 Because clinical and laboratory observations have provided several indications that cyclosporine A can augment lacrimal secretion,37,38 determining whether cyclosporine A inhibited prolactin binding to acinar cell membrane samples was of interest. Rather than competitively inhibiting prolactin binding, cyclosporine at 6.6 μM, a concentration that enhances carbachol-induced release of peroxidase from male rat lacrimal gland fragments,38 increased prolactin binding to membrane samples from male and female rats by 20.6% ± 1.7% (n = 4) and 6.7% ± 4.7% (n = 3), respectively. Cyclosporine at a concentration of 1 mM increased prolactin binding to two separate samples from male rats by 68.5% and 82.5%. The effects of this cyclosporine concentration...
were not tested in membrane samples from female rats.

**Prolactin Effects on Lacrimal Peroxidase Release**

Because the immunocytochemical and ligand binding experiments summarized above indicated that lacrimal acinar cells contained endogenous prolactin and prolactin-binding sites, it was of interest to determine whether prolactin might influence lacrimal secretion. As illustrated in Figure 4, ovine prolactin at concentrations of 10 and 20 ng/ml had no significant effect on the basal rate of peroxidase release from fragments obtained from 6-week- to 8-week-old males. On the other hand, these concentrations of ovine prolactin inhibited carbachol-induced peroxidase release. There was no significant difference between the two prolactin concentrations, and the mean inhibition was 19.6% ± 6.9% (P < 0.02, n = 8).

**Discussion**

The experiments summarized above confirm that prolactin-like immunoreactivity is present in the lacrimal gland, that lacrimal acinar cells possess receptors for prolactin and the capacity to synthesize prolactin, and that acute exposure to exogenous prolactin inhibits carbachol-induced secretion. We believe these observations have a number of interesting potential implications.

![Graph](image)

**Fig. 4.** Prolactin inhibition of carbachol-induced peroxidase release by rat exorbital lacrimal gland fragments. Values presented are means ± SEM. Six 6-week to 8-week rats were used for each experiment. The prolactin concentration was 20 ng/ml in seven of the experiments. A concentration of 10 ng/ml was used in the remaining experiment; the results were within the range of the other experiments, and results from all eight experiments were pooled. Paired analyses of carbachol-induced peroxidase release in the presence and absence of prolactin indicated that prolactin decreased the response to carbachol by 19.6% ± 6.9% (*P < 0.02).

In view of the low (or possibly absent) level of prolactin message in RNA extracts from females, two observations suggest that lacrimal acinar cells in females accumulate prolactin from the circulation: (1) that acinar cells in lacrimal glands from females contain prolactin-like immunoreactivity and (2) that a large proportion of the prolactin binding sites in membrane preparations from females are occupied by endogenous ligands. In several cell types in which this phenomenon has been studied previously, prolactin was found to be taken up by receptor-mediated endocytosis. Recent studies with the covalent membrane surface labeling reagent, sulfo-NHS-biotin, indicate there is an extensive traffic of membrane constituents between the basal-lateral membrane and an intracellular pool and that the Golgi complex is an important compartment of the intracellular pool. Therefore, it seems reasonable to suggest the working hypothesis that prolactin is internalized after binding to receptors expressed in the basal-lateral membranes, that it dissociates from its receptor at some point along the internalization pathway, and that once it reaches the Golgi complex, it is packaged into secretory products with the cell's own biosynthetic products.

The observation that lacrimal glands from male rats contain demonstrable levels of prolactin mRNA suggests they have the capacity to synthesize prolactin. In this regard, of possible interest is that the eccrine sweat gland—which, like the lacrimal gland, delivers an aqueous secretion to the external surface of the body—also can synthesize prolactin. If prolactin message exists at all in lacrimal glands from females, it must be at a much lower level than in males. This relationship leads us to propose the hypothesis that some mechanism regulates the acinar cell's prolactin content. When the amount of prolactin available to be taken up from the circulation is inadequate, the acinar cell would be induced to synthesize prolactin. When an adequate amount of prolactin is available from the circulation, lacrimal prolactin synthesis would be suppressed.

The observation that the prolactin-like immunoreactivity within lacrimal acinar cells is specifically localized to secretory vesicles supports the suggestion that the lacrimal gland represents at least one pathway for prolactin-like immunoreactivity to enter the tear film. That prolactin is in the tear film and that there is some mechanism for maintaining its level suggests this hormone plays some role in the physiology of the cornea, the conjunctiva, or the conjunctival-associated lymphoid tissue.

The nature of the prolactin receptor will need to be documented in future work. Also important will be to validate the provisional assumption that the binding sites detected in our ligand-binding study are identical...
to the receptor(s) coupled to prolactin uptake and to acute inhibition of carbachol-induced secretion. Because cyclosporine A enhanced, rather than inhibited, prolactin binding, the lacrimal acinar prolactin receptor can be predicted to resemble the prolactin receptor of the mammary epithelial cell rather than that of T lymphocytes.\textsuperscript{33,34,35} Accounting for cyclosporine's ability to increase prolactin binding is difficult. Cyclosporine is highly lipophilic, so its effect on binding could be mediated via nonspecific increases in membrane fluidity. Such a phenomenon apparently accounts for increases in prolactin binding by membranes from ventral prostate induced by aliphatic alcohols.\textsuperscript{4} However, cyclosporine does not significantly increase prolactin binding by membranes from mammary gland.\textsuperscript{36} Regardless, the previously noted ability of cyclosporine to enhance carbachol-induced peroxidase secretion\textsuperscript{38} is not likely the direct consequence of its ability to increase prolactin binding, because prolactin acutely inhibits peroxidase secretion.

The ability of ovine prolactin to partially inhibit carbachol-induced peroxidase secretion suggests that it will be useful to investigate whether prolactin is an additional element in the complex of signals that lacrimal acinar cells must integrate in performing their secretory functions. From the perspective of the short-term regulation of lacrimal secretion, the secretomotor input mediated by the parasympathetic transmitters, acetylcholine and VIP,\textsuperscript{13,14,22,29} and possibly by the sympathetic transmitter, norepinephrine,\textsuperscript{45-49} can be seen to be superimposed on a background of additional stimulatory and inhibitory signals. The likely stimulatory signals include circulating catecholamines and VIP, as well as \( \alpha \)-MSH and ACTH.\textsuperscript{10,11} The list of likely inhibitory signals is small, consisting of met-enkephalin\textsuperscript{15} and, now, prolactin. Regarding the long-term regulation of the lacrimal gland, we believe it reasonable to predict that chronic exposure to prolactin leads to responses that are distinct from its acute, inhibitory effects. The precedents for this prediction include, most notably, the evidence that prolactin influences the pattern of gene expression in mammary epithelial cells, where it stimulates synthesis of casein, \( \beta \)-adrenergic receptors,\textsuperscript{50,51} class II histocompatibility antigens,\textsuperscript{52} and a secretory protein characteristic of gross cystic breast disease.\textsuperscript{33,34}

We can envision at least three distinct pathways via which prolactin might, in principle, act on the lacrimal acinar cell. We believe all three merit investigation. The first is the classical endocrine pathway, through the circulation from the pituitary. The second is an autocrine pathway. The third is a paracrine pathway, to lacrimal acinar cells from infiltrating lymphocytes. This last possibility is especially intriguing in view of recent reports that prolactin and prolactin-like peptides are not only released by activated T cells but also contribute to T cell activation.\textsuperscript{35,55,56} Thus, prolactin might mediate a two-way communication between lacrimal acinar cells and the lymphocytes that accumulate in the lacrimal interstitium during aging,\textsuperscript{57} Sjögren's syndrome,\textsuperscript{58,59,60} and Graves' ophthalmopathy.\textsuperscript{61}

The possibility that pituitary prolactin mediates short- and long-term regulation of lacrimal function is particularly interesting considering the lacrimal gland's sexual dimorphisms. As noted above, basal circulating prolactin levels are generally two times higher in females than in males. Testosterone inhibits prolactin synthesis in the pituitary,\textsuperscript{62} and castration increases serum prolactin levels in male rats.\textsuperscript{63} Placing the pituitary under the kidney capsule increases serum prolactin levels by freeing lactotrophs from inhibitory hypothalamic input.\textsuperscript{64} The simplest hypothesis that might be formed based on these observations is that increasing circulating prolactin promotes the characteristics typical of female lacrimal glands. However, the observations that (1) hypophysectomizing intact males decreases lacrimal immunoglobulin and secretory component secretion,\textsuperscript{9} and (2) hypophysectomizing castrated males impairs the ability of testosterone administration to restore male-like lacrimal characteristics\textsuperscript{7} indicate that this hypothesis is, at best, an over-simplification. For this reason, it will be extremely interesting in future work to explore the hormonal regulation of lacrimal prolactin synthesis and to determine whether there are circumstances in which lacrimal prolactin functions as an autocrine mediator.

Key words: tear film, fluid secretion, immunocytochemistry, Northern blot analysis, exocrine secretion, neuro-immunomodulation

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