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Hormonal influence on the secretory immune system of the eye: endocrine interactions in the control of IgA and secretory component levels in tears of rats

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SUMMARY

Previous research from our laboratory has demonstrated that androgens regulate the ocular secretory immune system of the rat. The purpose of the present study was to determine whether other hormones might influence this androgen effect. Experiments involved the daily administration of saline or hormones to adult orchiectomized rats, the collection of tears 24 hr after the fourth hormone injection, and the measurement of free secretory component (SC), IgA and total protein levels in tears. Our first aim was to evaluate whether female sex steroids might antagonize androgen action on tear IgA and SC: orchiectomized rats were treated with combinations of saline, testosterone, oestradiol or progesterone. Testosterone induced a significant increase in the tear SC and IgA concentrations, as compared to those of saline-injected controls. This androgen effect was not inhibited by co-treatment with oestradiol or progesterone, nor duplicated by the administration of these hormones alone. Our second aim was to assess whether the absence of certain hormones might alter tear SC and IgA levels, or influence the ocular response to androgen exposure: rats underwent orchiectomies and specific endocrine organ ablations or appropriate sham-surgery. Absence of the pituitary gland, but not the thyroid, adrenal or pineal glands, resulted in a significant decrease in tear SC, IgA and total protein content. In addition, removal of the thyroid or adrenal glands did not prevent the testosterone-associated increase in tear SC and IgA, although thyroidectomy or adrenalectomy did diminish the magnitude of the androgen response. In contrast, hypophysectomy completely blocked the effect of testosterone on both tear SC and IgA. These results indicate that the hypothalamic–pituitary axis may regulate, or mediate, the action of androgens on ocular immunity in the rat.

INTRODUCTION

Recent research has demonstrated that androgens regulate the ocular secretory immune system of the rat (Sullivan & Allansmith, 1985; Sullivan, Bloch & Allansmith, 1984a, b). Thus, testosterone administration to orchiectomized rats results in a significant accumulation of both secretory component (SC) and IgA in tears. This hormone action appears to be due to the increased synthesis and/or secretion of SC and IgA by the lacrimal gland (Sullivan & Allansmith, 1985; Sullivan *et al.*, 1984b) which is the source of these tear proteins (Sullivan & Allansmith, 1984). In addition, this testosterone influence seems to account for the higher concentrations of SC and IgA antibodies in tear of male rats, as compared to those of female rats (Sullivan & Allansmith, 1985; Sullivan *et al.*, 1984a, b).

However, this sexual dimorphism in the mucosal immune system of the eye may not be entirely due to the effect of androgens. It has become increasingly clear in endocrine research that steroid hormone action on target tissues is often modulated by other hormones. For example, the effects of androgens may be significantly influenced by ovarian (Cavallero, 1967; Lauria & Porcelli, 1979), thyroid (Motwani, Unakar & Roy, 1980; Sica *et al.*, 1981; Chao & Margolis, 1983), adrenal (Steidler & Reade, 1982; Roy & Chaterjee, 1983); or pituitary (Cavallero, 1960; Roy, 1973) hormones. In fact, certain androgen actions on peripheral tissues may be mediated entirely through another endocrine system, such as the pituitary (Norstedt & Mode, 1982).

Given this information, the present study was designed to determine whether other hormones might modulate or control androgen effects on the ocular secretory immune system. As part of this research, we have also evaluated whether the absence of specific endocrine glands alters the tear SC and IgA profile. Our results indicate that the hypothalamic–pituitary axis may play an important role in hormonal regulation of ocular immunity.

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MATERIALS AND METHODS

Animals and surgical procedures

Adult male Sprague–Dawley rats (2–3 months old) were obtained from Zivic Miller Laboratories, Allison Park, PA and Charles River Breeding Laboratories, Wilmington, MA. Animals were maintained in constant temperature rooms with light/dark intervals of 12 hr length. When indicated, orchiectomies were performed 10–14 days prior to experimentation. Other surgical procedures, including adrenalectomy, thyroidectomy, hypophysectomy and combined orchiectomy and hypophysectomy, were performed by Zivic Miller Laboratories. These animals and their sham-operated controls were allowed to recover for 9–14 days before further experimental use. In order to compensate for endocrine gland ablation, as previously described (Sullivan & Allansmith, 1986), adrenalectomized rats were given a 0·15 M sodium chloride solution to offset the loss of mineralocorticoids. Thyroidectomized rats were given 0·1% calcium lactate in their water to counter possible damage to the parathyroid glands, which had been autografted to another site. Hypophysectomized animals were given an electrolyte solution containing sodium chloride (2·03 g/l), potassium chloride (0·083 g/l), magnesium chloride (0·017 g/l) and calcium chloride (0·035 g/l), as recommended by Zivic Miller Laboratories. At the time of kill, the renal environs, trachea and sella turcica were examined in randomly selected rats for adrenal, thyroid and pituitary remnants, respectively. In addition, serum from hypophysectomized rats was anslysed for thyroxine, according to a previously reported technique (Sullivan & Allansmith, 1986), to verify the absence of this hormone; serum thyroxine concentrations in sham-operated and intact controls were within the normal range. Lastly, sham-operated and pinealectomized rats were also obtained from Zivic Miller Laboratories. Tears were collected from these animals 11 weeks following surgery.

General and immunological procedures

Tears were obtained from the eyes of etherized rats, as previously described (Sullivan *et al.*, 1984a). Levels of IgA and SC in tear samples were measured by specific radioimmunoassays (Sullivan & Allansmith, 1984; Sullivan & Wira, 1983). The RIA for SC detected primarily free SC (Sullivan & Wira, 1983). Iodinated antigens in the assays were affinity-purified rat IgA (a gift from Dr J. P. Vaerman, Brussels, Belgium) and rat SC (a gift from Dr C. R. Wira, Hanover, NH, and prepared by Dr B. Underdown, Toronto, Canada). Unlabelled standards were a rat reference serum (Miles Laboratories, Elkhart, IN), containing known amounts of IgA, and purified rat SC. Antisera included goat anti-rat IgA (Miles Laboratories) and rabbit anti-rat SC (from Dr Wira, prepared by Dr Underdown) as first antibodies, and rabbit anti-goat IgG (Miles Laboratories) and goat anti-rabbit IgG (Miles Laboratories) as second antibodies. Total protein content in samples was determined by the Hartree (1972) method, utilizing bovine serum albumin as the standard. Statistical analysis of the data was performed by using the Student's *t*-test.

Steroid hormone preparations

Testosterone, progesterone and 17β-oestradiol were purchased from Calbiochem-Behring, La Jolla, CA. Testosterone and progesterone were suspended in a 0·9% sodium chloride solution (saline; NS) by glass–glass homogenization. Oestradiol was solubilized in absolute ethanol, evaporated and then resuspended in saline. Injections were administered subcutaneously, and dosages (200 μl volume) and injection schedules are described in the Results section. All control animals received saline.

RESULTS

Influence of female sex steroids on the androgen-induced accumulation of IgA and SC in tears

Progesterone and/or oestrogen have been shown to prevent a number of androgen effects on lacrimal gland structure and function (Cavallero, 1960, 1967; Lauria & Porcelli, 1979). In order to determine whether female sex steroids might also antagonize the influence of androgens on SC and IgA levels in tears, orchiectomized male rats (n = six/treatment group) were administered one of the following hormone combinations: (1)

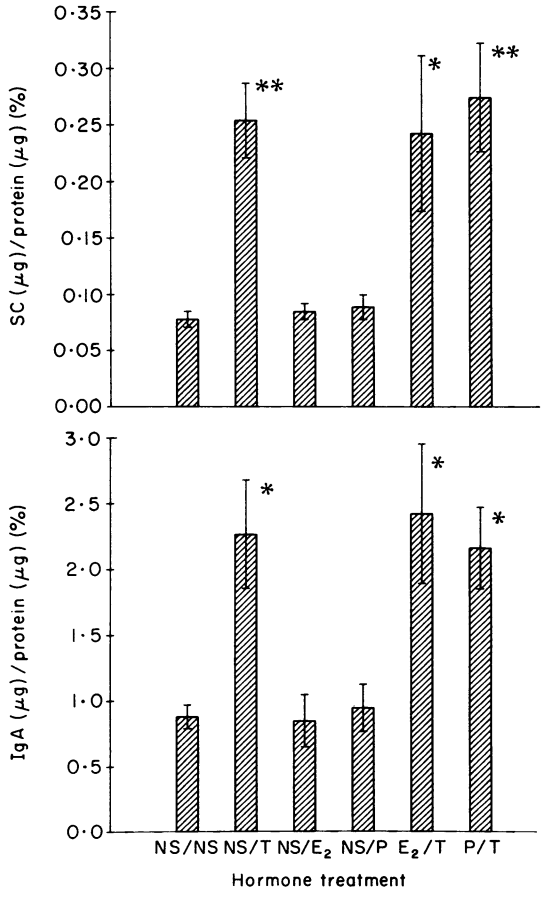

Figure 1. Effect of oestrogen or progesterone on the androgen-induced accumulation of IgA and SC in tears. Age-matched and orchiectomized rats (n = 6/treatment group) were administered one of the following hormone/vehicle combinations: (a) saline and saline (NS/NS; control); (b) saline and testosterone (NS/T; 2 mg T/day); (c) saline and oestradiol (NS/E_2; 5 μg E_2/day); (d) saline and progesterone (NS/P; 2 mg P/day); (e) oestradiol and testosterone (E_2/T; 5 μg E_2 and 2 mg T/day); and (f) progesterone and testosterone (P/T; 2 mg P and 2 mg T/day). Tears were collected 24 hr after the fourth daily hormone administration. Bars represent the mean ± SE. * Significantly ($P < 0·05$) greater than control value. ** Significantly ($P < 0·005$) greater than control value.

Table 1. Effect of endocrine gland ablation on IgA, SC and total protein levels in tears

Surgical group	No.	Tear volume (μl)	Total protein (μg)	Total IgA (ng)	Total free SC (ng)
Adrenalectomy					
Intact	13	4·6±0·5	168±14	2128±270	339±35
Sham-operated	12	5·6±0·6	188±19	2662±439	468±82
Adrenalectomized	14	5·9±0·6	251±31	2055±182	334±40
Thyroidectomy					
Intact	13	4·7±0·6	191±28	1824±207	326±36
Sham-operated	14	5·4±0·5	186±17	2052±267	333±36
Thyroidectomized	12	5·7±0·7	212±29	1799±324	285±27
Pinealectomy					
Sham-operated	7	6·3±1·9	161±61	1660±455	250±83
Pinealectomized	7	6·6±1·2	183±35	2267±569	322±55
Hypophysectomy					
Intact	13	5·4±0·7	198±26	2638±351	636±100
Sham-operated	14	6·1±1·0	248±35	2521±341	698±195
Hypophysectomized	14	3·4±0·5*†	110±20†	1171±152‡	134±11†

Surgical procedures were performed 9 days (thyroidectomy), 10 days (hypophysectomy), 14 days (adrenalectomy) and 11 weeks (pinealectomy) prior to single tear collections from individual rats. All animals within each experimental surgery group were age-matched. Numbers represent the mean ± SE.

* Value equals the mean tear volume in 14 rats. However, only 12 tear samples (tear volume = 3·9 ± 0·5) had sufficient volume (≥ 1·0 μl) for protein, IgA and SC measurements.

† Significantly ($P < 0·05$) less than value in sham-operated group.

‡ Significantly ($P < 0·005$) less than value in sham-operated group.

saline plus saline (control); (2) saline plus testosterone (2 mg/day); (3) saline plus oestradiol (5 μg/day); (4) saline plus progesterone (2 mg/day); (5) oestradiol (5 μg/day) plus testosterone (2 mg/day); or (6) progesterone (2 mg/day) plus testosterone (2 mg/day). Twenty-four hours after the fourth injection, tears were collected and analysed for SC, IgA and total protein content.

As shown in Fig. 1, testosterone exposure resulted in a significant accumulation of free SC and IgA in tears, as compared to levels measured in saline-treated controls. This androgen action was not prevented by the co-administration of oestradiol or progesterone with testosterone. In fact, neither female sex steroid appeared to influence the content of tear free SC or IgA, whether given alone or with androgen.

The results in Fig. 1 are expressed as a percentage of total tear protein. Similar significant findings are also obtained if results are expressed in terms of free SC and IgA concentration, or as a percentage of pretreatment levels. During the course of this study, hormone treatment had no effect on the total protein content of tears.

Effect of endocrine gland ablation on the levels of SC and IgA in tears

Orchiectomy has been shown to result in a significant decline in the levels of free SC and IgA in tears; this response is most probably due to the decrease in serum androgen levels (Sullivan & Allansmith, 1985; Sullivan *et al.*, 1984a). However, hormones from other glands are also known to influence lacrimal tissue (please refer to Sullivan & Allansmith, 1986). Therefore, to evaluate the possible effect of these hormones on the ocular secretory immune system, rats underwent one of the following surgical procedures: (1) adrenalectomy; (2) thyroidectomy; (3) pinealectomy; (4) hypophysectomy; or (5) appropriate sham-surgery. After designated animal recovery periods (see Table 1), tears were collected and processed for measurement of free SC, IgA and total protein.

As demonstrated in Table 1, tear levels of free SC and IgA were not altered in rats with adrenal ($n = 14$), thyroid ($n = 12$) or pineal ($n = 7$) gland ablation, as compared to those found in sham-operated controls ($n = 7$–14/surgical group). Similarly, removal of these endocrine tissues had no effect on the volume of, or total protein content in, tears, relative to values obtained in sham-operated animals.

In contrast, hypophysectomy resulted in a significant decrease in the content of tear free SC and IgA, compared to levels measured in sham-operated controls (Table 1). These reductions were also associated with a decline in the total protein content in, and volume of, tears in hypophysectomized animals.

In the above studies, no significant differences were found between sham-operated and intact control animals ($n = 13$/group) with respect to any tear parameter (Table 1).

Effect of adrenal gland absence on the testosterone-induced increase in tear IgA and SC levels in orchiectomized rats

Certain androgen effects depend upon the co-operative action between adrenal and testicular hormones (Steidler & Reade,

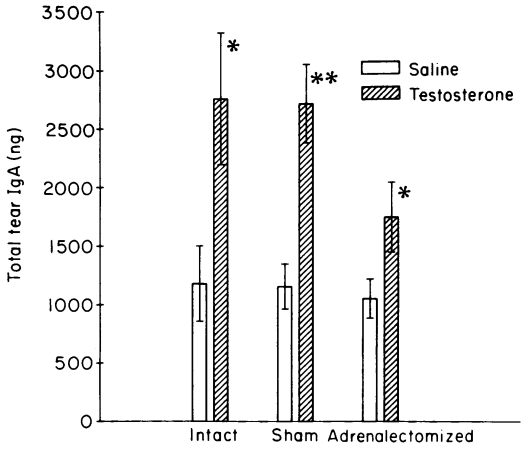

Figure 2. Influence of testosterone on tear IgA levels in adrenalectomized rats. Age-matched rats underwent one of the following surgical protocols: (a) adrenalectomy; (b) sham-adrenalectomy; (c) no surgery (intact). Fourteen days later, animals were orchiectomized, and after a 10-day recovery period, were injected with saline (NS; $n=6$/group) or testosterone (T; 2 mg T/day; $n=6$–7/group). Tears were obtained 24 hr following the fourth daily hormone treatment. Bars represent the mean ±SE. *Significantly ($P<0.05$) greater than saline-treated group. **Significantly ($P<0.005$) greater than saline-treated group.

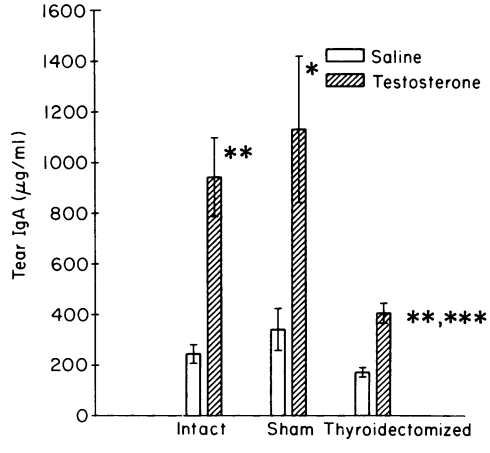

Figure 3. Effect of testosterone on tear IgA levels in thyroidectomized rats. Age-matched rats underwent one of the following surgical protocols: (a) thyroidectomy; (b) sham-surgery; and (c) no surgery (intact). Nine days later, animals were orchiectomized, and after an 11-day recovery period, were injected with saline (NS; n=6–7/group) or testosterone (T; 2 mg T/day; $n=6$–7/group). Tears were collected 24 hr following the fourth daily androgen treatment. Bars represent the mean ±SE. *Significantly ($P<0.05$) greater than saline-injected group. **Significantly ($P<0.005$) greater than saline-treated group. ***Significantly ($P<0.05$) less than level in testosterone-treated and 'sham-operated' group.

1982; Roy & Chaterjee, 1983). In order to evaluate whether the adrenal gland might play a role in the testosterone-induced increase in tear IgA and SC levels, orchiectomized rats were adrenalectomized, sham-adrenalectomized or not exposed to further surgery ('intact'). After a recovery period, animals ($n=6$–7/treatment group) were administered either saline or testosterone (2 mg/day) for 4 days. Tears were collected 24 hr after the last injection.

As shown in Fig. 2, absence of the adrenal gland did not prevent the testosterone-associated increase in tear IgA levels. However, the magnitude of this elevation in adrenalectomized rats was somewhat, but significantly ($P<0.05$), less than that of sham-operated controls. With regard to SC content, androgen administration resulted in a significant ($P<0.05$) rise in tear free SC concentrations of all groups. There was no significant difference between the tear SC response to testosterone of adrenalectomized rats (NS = 25·4 ± 2·2 μg/ml; T = 59·1 ± 7·8 μg/ml) and sham-adrenalectomized controls (NS = 31·8 ± 4·7 μg/ml; T = 85·8 ± 14·1 μg/ml). Further, androgen treatment did not increase the levels of total tear protein in any surgical group, relative to those of saline-injected animals.

Effect of thyroid gland absence on the testosterone-induced increase in tear IgA and SC levels in orchiectomized rats

Thyroid hormones have been shown to enhance the responsiveness of target cells to androgens (Motwani *et al.*, 1980; Sica *et al.*, 1981; Chao & Margolis, 1983), as well as to affect directly the lacrimal gland (Nover, 1954; Carriere, 1964). Therefore, to assess whether the thyroid gland might be required for androgen influence on the ocular secretory immune system, orchiectomized rats were thyroidectomized, sham-operated or not further operated ('intact'). Animals ($n=6$–7/treatment group) were administered saline or testosterone (2 mg/day) for 4 days, and tears were obtained 24 hr after the fourth injection.

The effect of thyroid gland absence on the tear IgA response to androgens is demonstrated in Fig. 3. Testosterone treatment significantly ($P<0.005$) increased the tear IgA concentration in thyroidectomized rats. However, the extent of this increase was significantly ($P<0.05$) diminished relative to that of sham-thyroidectomized rats. With respect to tear free SC levels, these were significantly ($P<0.001$) elevated following androgen treatment of thyroidectomized rats (39·9 ± 3·6 μg/ml), as compared to free SC concentrations measured in saline-injected animals (16·8 ± 2·6 μg/ml). Nevertheless, the extent of this increase was significantly ($P<0.05$) less than that found in testosterone-treated and sham-operated (75·5 ± 14·8 μg/ml) or 'intact' (88·7 ± 12·1 μg/ml) rats.

No elevations in the tear levels of total protein were observed in any of the experimental groups following androgen treatment. Moreover, amounts of total tear protein were similar in both thyroidectomized and sham-thyroidectomized rats.

Effect of pituitary gland ablation on the testosterone-induced increase in tear IgA and SC levels in orchiectomized rats

Pituitary hormones have been demonstrated to control or modulate androgen action in a variety of tissues, including the lacrimal gland (Cavallero, 1960; Roy, 1973; Norstedt & Mode, 1982). In order to determine whether pituitary gland influence extends as well to the testosterone regulation of tear SC and IgA levels, the following experiment was performed. Rats were orchiectomized and either sham-hypophysectomized or hypophysectomized. After a recovery period, animals ($n=15$–17/treatment group) were treated with saline or testosterone (2 mg/

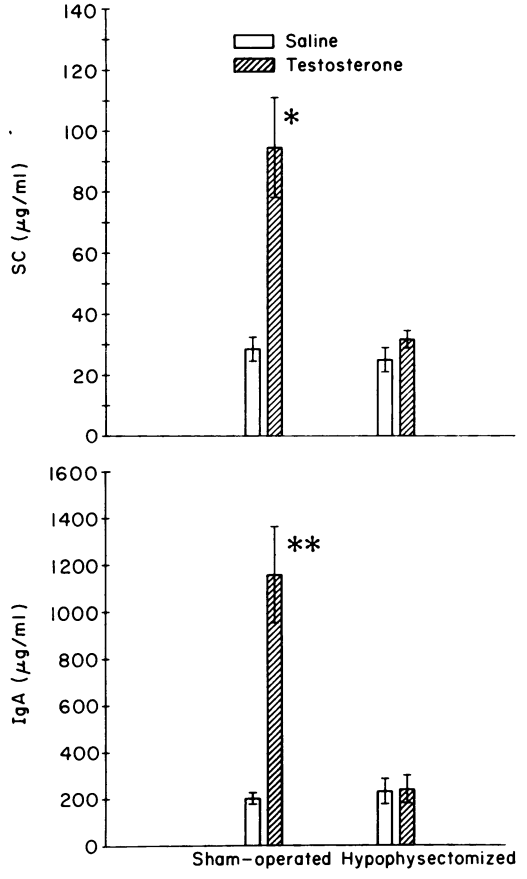

Figure 4. Influence of testosterone on tear IgA and SC levels in hypophysectomized rats. Age-matched rats were orchiectomized and either sham-hypophysectomized or hypophysectomized. After a recovery period, animals were administered saline (NS; $n = 15$–17/surgical group) or testosterone (T; 2 mg T/day; $n = 15$/surgical group) for 4 days. Twenty-four hours later, tears were collected. Data represent the combined results of two separate experiments, which were similarly conducted and yielded the same findings. Bars represent the mean ± SE. *Significantly ($P < 0.05$) greater than saline-injected group. **Significantly ($P < 0.005$) greater than saline-treated group.

day) for 4 days and their tears were collected 24 hr after the fourth hormone administration.

As shown in Fig. 4, testosterone treatment significantly ($P < 0.005$) increased the concentrations of free SC and IgA in tears of sham-hypophysectomized rats. In contrast, absence of the pituitary gland completely blocked androgen action on both tear free SC and IgA. These results could not be accounted for by variations in tear volume between experimental groups.

DISCUSSION

During the past four decades, researchers have reported that distinct, gender-related differences exist in the structure and function of the lacrimal gland (see Cornell-Bell, Sullivan & Allansmith, 1985; Sullivan & Allansmith, 1986). These differences appear to be due to the effect of androgens (Cornell-Bell *et al.*, 1985; Sullivan & Allansmith, 1986). One possible mechanism underlying these hormone actions may involve androgen binding to specific lacrimal gland receptors (Ota, Kyakumoto & Nemoto, 1985), which then initiate an appropriate hormone response. However, another possibility, as demonstrated in this study, is that androgen influence on the lacrimal gland and its immune system may require either the cooperative support of, or mediation by, other hormones.

Our initial experiments examined the potential antagonism by female sex steroids of androgen action on the ocular secretory immune system. These studies were conducted because oestrogen administration has been shown to inhibit testosterone-induced effects on lacrimal gland morphology (Cavallero, 1960, 1967; Lauria & Porcelli, 1979). Moreover, oestrogen and/or progestin interference could explain why tear SC levels in females do not increase during the pro-oestrous stage of the oestrous cycle (Sullivan *et al.*, 1984a): at this stage, ovarian secretion of androgens is highest (Rush & Blake, 1982), but serum concentrations of oestrogen are also maximal and are soon followed by rising levels of progesterone (Nequin, Alvarez & Schwartz, 1979). Our findings, however, demonstrated that female sex steroids had no effect on the testosterone-associated increase in tear free SC and IgA content. This lack of susceptibility to oestrogen or progesterone competition indicates that the androgenic processes controlling the lacrimal immune system may be different from those regulating lacrimal tissue morphology.

We also explored whether the absence of the adrenal gland might alter the influence of testosterone on tear SC and IgA. Adrenal hormones are known to support androgen actions on various target tissues (Motwani *et al.*, 1980; Steidler & Reade, 1982; Roy & Chaterjee, 1983). Our results showed that adrenal absence lessened, but did not prevent, the tear IgA response to testosterone exposure. Furthermore, the androgen-associated rise in tear SC levels was not affected by adrenalectomy. Consequently, these findings suggest that adrenal hormones assist, but are not required for, the effect of testosterone on the lacrimal immune system. Of interest, adrenalectomy itself causes a reflex increase in the circulating levels of ACTH, and this peptide is apparently a potent suppressor of antibody production by splenic lymphocytes (Johnson *et al.*, 1982). Our studies, though, found no evidence to indicate that IgA antibody content in tears declines following adrenalectomy.

Our experiments involving the thyroid gland were prompted by the reports of others that thyroid hormones: (i) act in concert with androgens to promote cellular growth (Carriere & Buschke, 1978; Aloe & Levi-Montalcini, 1980; Chao & Margolis, 1983); (ii) enhance responsiveness of cells to androgens (Sica *et al.*, 1981); (iii) modulate androgen-induced protein synthesis (Motwani *et al.*, 1980; Roy & Chaterjee, 1983); (iv) may regulate steroid hormone receptor levels (Barbanel & Assenmacher, 1982); and (v) influence lacrimal gland growth, development and function (Nover, 1954; Carriere, 1964; Crowe *et al.*, 1980). The present findings showed that the presence of an intact thyroid gland supports, but is not essential for, testosterone expression on the ocular secretory immune system. Nevertheless, given the diminished tear free SC and IgA response to androgens in thyroidectomized rats, it may be that thyroid hormones act to create a suitable environment for androgen action on the lacrimal gland.

Experiments were also performed to evaluate whether the pineal gland may influence the levels of IgA and SC in tears. This gland affects pituitary and gonadal function, and is, in turn, modulated by androgens (Preslock, 1984). Ablation of this

gland, though, did not elicit an alteration in tear IgA or free SC content.

We found that removal of the pituitary gland resulted in a significant decline in the volume (Sullivan & Allansmith, 1986) of, and IgA, free SC and total protein level in, tears. Moreover, testosterone action on tear SC and IgA content was completely blocked in hypophysectomized animals. These results may in part be due to the lacrimal gland atrophy and cellular degeneration that occurs following hypophysectomy (Martinazzi, 1962). Another possibility, though, is that androgen action on ocular immunity may be regulated by, or mediated through, pituitary hormones. The pituitary gland, a known target organ for androgens, has a marked effect of the lacrimal gland (Matinazzi & Baroni, 1963; Ebling *et al.*, 1975; Jahn *et al.*, 1982). Furthermore, this gland has a tremendous impact on mucosal, as well as cell-mediated and humoral, immunity (Johnson *et al.*, 1982; Grossman & Roselle, 1983; Nagy, Berczi & Freisen, 1983; Weisz-Carrington, Emancipator & Lamm, 1984; Besedovsky, del Rey & Sorkin, 1985; Blalock, Harbour-McMenamin & Smith, 1985). Additional studies are required to determine which pituitary hormones might be involved with androgen effects on tear SC and IgA.

Overall, our results indicate that other hormones, but especially those from the hypothalamic–pituitary axis, may play an integral role in the androgen regulation of the rat ocular secretory immune system. In this regard, it is of interest that the endocrine control of the hypothalamus and pituitary varies between males and females (Bhatnagar, 1983). Further, gender appears to determine which pituitary hormones affect the lacrimal gland (Martinazzi & Baroni, 1963). Thus, gender-related differences in the regulation of, and output from, the hypothalamic–pituitary axis may be the critical factors underlying the sexual dimorphism in ocular mucosal immunity.

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REFERENCES

Aloe L. & Levi-Montalcini R. (1980) Comparative studies on testosterone and L-thyroxine effects on the synthesis of nerve growth factor in mouse submaxillary glands. *Exp. Cell Res.* **125,** 15.

Barbanel G. & Assenmacher I. (1982) Effects of thyroid hormones on the ontogeny of oestradiol-binding sites in the rat. *Mol. Cell Endocr.* **28,** 247.

Besedovsy H.E., del Rey A.E. & Sorkin E. (1985) Immune-neuroendocrine interactions. *J. Immunol.* **135,** 750s.

Bhatnagar A.S. (ed.) (1983) *The Anterior Pituitary Gland.* Raven Press, New York.

Blalock J.E., Harbour-McMenamin D. & Smith E.M. (1985) Peptide hormones shared by the neuroendocrine and immunologic systems. *J. Immunol.* **135,** 858s.

Carriere R. (1964) The influence of the thyroid gland on polyploid cell formation in the external orbital gland on the rat. *Am. J. Anat.* **115,** 1.

Carriere R.M. & Buschke M. (1978) The influence of thyroid and testicular hormones in globule leucocytes in the rat duodenal crypt epithelium. *Anat. Rec.* **192,** 407.

Cavallero C. (1960) The influence of various steroids on the Lowenthal lachrymal glands of the rat. *Acta Endocr. (Kbh),* Suppl. 51, 861.

Cavallero C. (1967) Relative effectiveness of various steroids in an androgen assay using the exorbital lacrimal gland on the castrated rat. *Acta Endocr.* **55,** 119.

Chao J. & Margolis H. (1983) Differential effects of testosterone, thyroxine and cortisol on rat submandibular gland versus renal kallikrien. *Endocrinology,* **113,** 2221.

Cornell-Bell A.H., Sullivan D.A. & Allansmith M.R. (1985) Gender-related differences in the morphology of the lacrimal gland. *Invest. Ophthalmol.* **26,** 1170.

Crowe J.P., Christensen E., Butler J., Wheeler P., Doniach D., Keenan J. & Williams R. (1980) Primary biliary cirrhosis: the prevalence of hypothyroidism and its relationship to thyroid autoantibodies and sicca syndrome. *Gastroenterology,* **78,** 1437.

Ebling F.J., Ebling E., Randall V. & Skinner J. (1975) The synergistic action of alpha-melanocyte stimulating hormone and testosterone on the sebaceous, prostate, preputial, harderian and lachrymal glands, seminal vesicles and brown adipose tissue in the hypophysectomized-castrated rat. *J. Endocr.* **66,** 407.

Grossman C.J. & Roselle G.A. (1983) The interrelationship of the HPG-thymic axis and immune system regulation. *J. Steroid Biochem.* **19,** 461.

Hartree E.F. (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Analyt. Biochem.* **48,** 422.

Johnson H.M., Smith E.M., Torres B.A. & Blalock J.E. (1982) Regulation of the *in vitro* antibody response by neuroendocrine hormones. *Proc. natl. Acad. Sci. U.S.A.* **79,** 4171.

Jahn R., Padel U., Porsch P.H. & Soling H.D. (1982) Adrenocorticotrophic hormone and alpha-melanocyte stimulating hormone induce secretion and protein phosphorylation in the rat lacrimal gland by activation of a cAMP-dependent pathway. *Eur. J. Biochem.* **126,** 623.

Lauria M. & Porcelli F. (1979) Leucine aminopeptidase (LAP) activity and sexual dimorphism in rat exorbital lacrimal gland. *Basic Appl. Histochem.* **23,** 171.

Martinazzi M. (1962) Effetti dell'ipofisectomia sulla ghiandola lacrimale extraorbitale del ratto. *Folia Endocrinologica (Pisa),* **150,** 120.

Martinazzi M. & Baroni C. (1963) Controllo ormonale delle ghiandola lacrimale extraorbitale nel topo con nanismo ipofisario. *Folia Endocrinologica (Roma),* **16,** 123.

Motwani N.M., Unakar N.J. & Roy A.K. (1980) Multiple hormone requirement for the synthesis of $\alpha 2\mu$-globulin by monolayers of rat hepatocytes in long term primary cultures. *Endocrinology,* **107,** 1606.

Nagy E., Berczi I., & Freisen H.G. (1983) Regulation of immunity in rats by lactogenic and growth hormones. *Acta Endocrinol. (Copenh.),* **102,** 351.

Nequin L.G., Alvarez J. & Schwartz N.B. (1979) Measurement of serum steroid and gonadotropin levels and uterine and ovarian variables throughout 4-day and 5-day estrous cycles in the rat. *Biol. Reprod.* **20,** 659.

Norstedt G. & Mode A. (1982) On the primary site of action of estrogens and androgens in the regulation of hepatic prolactin receptors. *Endocrinology,* **111,** 645.

Nover A. (1954) Tierexperimentelle untersuchungen uber die regenerations und transplantation-sfahig keit der tranendruse uter lokalem und allgemeinem hormonreiz (thyrozin). *Graefes Archiv fur Ophthalmol., Bd.* **156,** 177.

Ota M., Kyakumoto S. & Nemoto T. (1985) Demonstration and characterization of cytosol androgen receptor in rat exorbital lacrimal gland. *Biochem. Internat.* **10,** 129.

Preslock J.P. (1984) The pineal gland: basic implications and clinical correlations. *Endocrine Rev.* **5,** 282.

RUSH M.E. & BLAKE C.A. (1982) Serum testosterone concentrations during the 4-day estrous cycle in normal and adrenalectomized rats. *Proc. Soc. exp. Biol. Med.* **169,** 216.

ROY A.K. (1973) Androgen-dependent synthesis of $\alpha 2\mu$-globulin in the rat: role of the pituitary gland. *J. Endocr.* **56,** 295.

ROY A.K. & CHATERJEE B. (1983) Sexual dimorphism in the liver. *Am. Rev. Physiol.* **45,** 37.

SICA G., NATOLI C., MARCHETTI P. & IACOBELLI S. (1981) Effect of thyroid hormones on androgen responsiveness in a mammary tumor cell line. *J. Steroid Biochem.* **15,** 415.

STEIDLER N.E. & READE P.C. (1982) An immunohistochemical and histological study of the influence of the testes and adrenal glands on epidermal growth factor-containing cells in the submandibular salivary glands of male mice. *J. Anat.* **135,** 413.

SULLIVAN D.A. & ALLANSMITH M.R. (1984) Source of IgA in tears of rats. *Immunology*, **53,** 791.

SULLIVAN D.A. & ALLANSMITH M.R. (1985) Hormonal influence on the secretory immune system of the eye: androgen modulation of IgA levels in tears of rats. *J. Immunol.* **134,** 2978.

SULLIVAN D.A. & ALLANSMITH M.R. (1986) Hormonal modulation of tear volume in the rat. *Exp. Eye Res.* **42,** 131.

SULLIVAN D.A., BLOCH K.J. & ALLANSMITH M.R. (1984a) Hormonal influence on the secretory immune system of the eye: androgen regulation of secretory component levels in rat tears. *J. Immunol.* **132,** 1130.

SULLIVAN D.A., BLOCH K.J. & ALLANSMITH M.R. (1984b) Hormonal influence on the secretory immune system of the eye: androgen control of secretory component production by the rat exorbital gland. *Immunology*, **52,** 234.

SULLIVAN D.A. & WIRA C.R. (1983) Variations in free secretory component levels in mucosal secretions of the rat. *J. Immunol.* **130,** 1330.

WEISZ-CARRINGTON P., EMANCIPATOR S. & LAMM M.E. (1984) Binding and uptake of immunoglobulins by mouse and mammary gland epithelial cells in hormone-treated cultures. *J. Reprod. Immunol.* **6,** 63.