The role of gonadectomy and testosterone replacement on thymic luteinizing hormone-releasing hormone production

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Abstract

We and others have identified luteinizing hormone-releasing hormone (LHRH) in cells of the immune system in both animals and humans. LHRH is an immunostimulant, and testosterone is an immunosuppressant. Because testosterone is known to modulate the concentrations of hypothalamic LHRH, we wondered whether testosterone might also alter the concentrations of rat thymic LHRH. Two weeks after castration or sham castration, adult male rats were implanted with either vehicle or testosterone capsules. All animals were killed 4 days after capsule implantation. Thymic LHRH concentration increased significantly in castrated animals. Testosterone replacement prevented this increase. The concentration of the LHRH precursor, proLHRH, decreased significantly, but testosterone replacement

prevented this decrease. Steady-state concentrations of LHRH mRNA were not changed by castration or by hormonal replacement. In contrast to the post-castration increase in thymic LHRH, LHRH content of the hypothalamus decreased significantly. Whereas concentrations of LHRH were lower in the thymus than in the hypothalamus, proLHRH concentrations were much greater in the thymus. These data suggest that gonadal manipulation modulates LHRH molecular processing and its tissue concentration in the thymus in addition to those in the hypothalamus, and that the regulation of LHRH molecular processing by testosterone in the hypothalamus is different from that in the thymus.

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Introduction

Recent reports have demonstrated that luteinizing hormone-releasing hormone (LHRH), in addition to having a pivotal role in regulating the hypothalamic–pituitary–gonadal axis, is an immunomodulator. Our laboratory and others have shown that LHRH is produced and processed in human and rat lymphocytes (Emanuele *et al.* 1990, Azad *et al.* 1991a,b, 1993). Moreover, many studies have demonstrated that LHRH has a significant stimulatory effect on T cell proliferative activity and other aspects of immune function (Marchetti *et al.* 1989a,b, Azad *et al.* 1990a, Batticane *et al.* 1991, Jacobson *et al.* 1994). Furthermore, the immunosuppressive effect of gonadal hormones, mainly testosterone, has been known for several decades (Wyle & Kent 1977, Grossman *et al.* 1983, Grossman 1984, Blagosklonny & Neckers 1994). The thymus gland, which is the major source of T lymphocyte production and has been shown to produce LHRH (Azad *et al.* 1991a), decreases in size and function after sexual maturation (Weksler *et al.* 1978, Makinodan & Kirokawa 1985). However, castration before puberty delays post-pubertal thymic involution and preserves T cell prolifer-

ative activity (Castro 1974, Weksler *et al.* 1978). The presence of testosterone (Kovacs & Olsen 1987) and dihydrotestosterone (Grossman *et al.* 1979, McCruden & Stimson 1981, Meikle *et al.* 1991) receptors, and the demonstration that gonadal hormones can be metabolized in thymic lymphocytes, further support an immunomodulator role of gonadal steroids (Weinstein *et al.* 1977, Sholiton *et al.* 1980).

Because gonadal steroids modify the concentrations of hypothalamic LHRH (Roselli *et al.* 1990), the following experiments were designed to study the effect of castration and testosterone replacement on the production and molecular processing of thymic LHRH, and to compare those results with the effect of the same manipulations on hypothalamic LHRH.

Materials and Methods

Animals

Castrated and sham-castrated male Sprague–Dawley rats, 60–70 days old, were obtained from Harlan Sprague–Dawley (Indianapolis, IN, USA). The rats were housed in

groups of two or three at a temperature of $22 \pm 2^\circ\text{C}$ and with a 12 h light/12 h darkness cycle. Food and water were available *ad libitum*.

Procedure

Gonadectomy was performed using standard methods. Male rats, 60 days old, were anesthetized with pentobarbital (1 mg/kg) i.p. The scrotal sac was cleaned with alcohol and a small incision of approximately 2 cm was made midsagittally at the scrotal septum. The spermatic cords were dissected, tied and cut. The testes were carefully removed from the scrotal sac. The incision was sutured. In sham operations, the scrotal incisions were immediately sutured and the gonadal system was not manipulated. The entire procedure was performed under aseptic conditions. Animals were allowed to recover from the anesthesia and, when they were able to eat and drink, they were returned to the animal quarters.

Two weeks after castration, castrated rats were implanted s.c. with a silastic capsule containing either vehicle or testosterone and the sham-castrated rats were implanted with vehicle capsules only. Testosterone (CIBA, Summit, NJ, USA) capsules were made using Silastic tubing (inside diameter 0.062 mm; outside diameter 0.125 mm) purchased from Dow-Corning (Midland, MI, USA). The animals were killed 96 h after the implantation of the silastic capsules (Azad *et al.* 1990b).

The data presented were pooled from several experiments, and do not contain equal numbers of animals in each case presented. Data were not arbitrarily discarded. Some thymuses were analyzed for LHRH mRNA by reverse transcription (RT)-PCR; some were used for LHRH assay and some were used to assay the precursor of LHRH, proLHRH. Because of shortages of proLHRH antibody at the time of the experiment, we were not able to analyze all of the thymuses for proLHRH.

Processing of tissues

The rats were killed by decapitation, and the hypothalamus and thymus were quickly dissected out. Trunk blood was collected, and serum stored at -20°C . The tissues used to assay proLHRH RNA were immediately placed in liquid nitrogen, then stored at -70°C .

To process the tissues for RIA, the thymuses were immediately weighed and homogenized in chilled 0.01 M PBS, pH 7.4, at a concentration of 25 mg/ml, using a Tekmar tissue mixer. They then were centrifuged at 2000 r.p.m. (1720 g) for 30 min in a Sorvall RC-5B refrigerated centrifuge, and the supernatant kept at -20°C until required for RIA. On the day of the RIA, the supernatant was thawed and filtered using a 25-mm syringe filter unit (PGC Scientific, Gaithersburg, MD, USA). After removal from the animals, the hypothalami were immediately frozen in liquid nitrogen and kept at -70°C . On the day

of the RIA, the hypothalami were thawed and homogenized with a Tekmar tissue mixer in chilled 0.01 M PBS, pH 7.4, at a concentration of 1 hypothalamus/ml, centrifuged in the same way as thymuses, and supernatant taken for RIA the same day. All the tissue processing was performed on ice.

On the basis of experience gained in our laboratory, this method of tissue processing for LHRH and proLHRH RIA was demonstrated to be much simpler, yet yielded the same amount of peptides in RIA compared with other methods of tissue preparation for LHRH and proLHRH.

LHRH and proLHRH RIA

The LHRH RIA was conducted, as described previously (Azad *et al.* 1993), using antiserum (CRR11B73) graciously supplied by Dr Victor Ramirez of the University of Illinois (Champaign-Urbana, IL, USA). The assay sensitivity was 15.6 pg/ml (3.1 pg/tube), and the intra- and interassay coefficients of variation were 5.1% and 15% respectively.

ProLHRH RIA was performed using antiserum and proLHRH for standard and iodination kindly supplied by Dr John P Adelman, Oregon Health Sciences University (Portland, OR, USA) as previously described (Azad *et al.* 1993). ARK2 antiserum specifically recognizes the proLHRH molecule and was directed against amino acid residues 6–16, which span the cleavage site between LHRH and LHRH-related peptide in the proLHRH molecule. This antiserum has virtually no cross reactivity with the mature LHRH peptide (Roselli *et al.* 1990). The sensitivity of the assay was 78 pg/ml, and the intra- and interassay coefficients of variation were 4% and 12% respectively.

Testosterone RIA

Testosterone RIA was performed using a kit purchased from Pantex (Pantex, Santa Monica, CA, USA). The sensitivity of the assay was 2 pg, equivalent to 0.1 ng/ml in serum, and the intra- and interassay coefficients of variation were 8% and 12% respectively.

Reverse transcription-polymerase chain reaction

The following primer pairs were used in these experiments: LHRH (exon 2) 5'-GCCAATTCAAAAAGCTCTAGCTGGCC-3', LHRH (exon 4) 5'-ATCTTCTTCTGCCAGTTTCCTCTT-3'. The expected product of 375 bp was produced after PCR amplification. The histone H3.3 primers produced a PCR product of 213 bp.

RNA isolation, reverse transcription-polymerase chain reaction

The methodology for semiquantitative or comparative RT-PCR has been previously described (Chomczynski &

Sacchi 1987, Azad *et al.* 1993, Kelley *et al.* 1993). Briefly, total RNA was extracted from the cells according to the procedure of Chomczynski & Sacchi (1987) as previously described. This procedure results in RNA isolation without genomic DNA contamination. Three micrograms of total RNA were added to a reaction tube containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 2 mM of each deoxynucleotide triphosphate (dNTP), 10 U RNasin (RNase inhibitor, Promega, Madison, WI, USA), 100 pmol oligo-(dT) primers (Bethesda Research Laboratories, Gaithersburg, MD, USA), and RNase-free deionized distilled water to a final volume of 19 µl. This mixture was heated for 10 min at 65 °C and quenched on ice, then 200 U Moloney murine leukemia virus RNase-H reverse transcriptase (Superscript, Bethesda Research Laboratories) in 1 µl were added to give a total reaction volume of 20 µl. This mixture was incubated at room temperature for 10 min, and then at 42 °C for 1 h. The reaction was terminated by heating at 95 °C for 5 min and quenching on ice. RNase-H (2 U) was then added to the reaction and incubated for 20 min at 37 °C. Five microliters of the reverse transcription reaction from each sample were diluted into a final volume of 100 µl in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 µM each of deoxynucleotide triphosphate, 0.01% gelatin, 0.01% Tween-20, 0.01% Nonidet P-40, 2 µM of each oligonucleotide, and 2 U *Taq*-thermostable polymerase (Epicentre, Madison, WI, USA). The polymerase amplification was carried out using an MJ Research programmable heating/cooling dry block for 23 cycles of amplification (94 °C, 30 s; 60 °C, 1 min; 72 °C, 2 min), followed by 10 min at 72 °C. Negative controls were run in parallel, and consisted of a reaction performed as described above, but without the addition of reverse transcriptase. PCR products labeled with phosphorus-32 were transferred to nytram paper, then exposed to radiographic film. The density of the PCR product on film was measured by densitometer and expressed as arbitrary densitometry units (ADU).

Statistical analysis

A one-way ANOVA was applied to analyze the effect of gonadal manipulation on hypothalamic and thymic LHRH and proLHRH. Tukey's test was used for multiple comparisons between the three experimental groups: sham-castrated, castrated, and castrated replaced with testosterone.

Results

To confirm the effect of gonadal manipulation on serum testosterone, sera of several randomly selected rats were studied. The overall effect of gonadal manipulation was

significant by ANOVA ($P < 0.001$). The serum concentration of testosterone decreased from 3.78 ± 0.6 ng/ml ($n=5$) in sham-castrated animals, to 1.05 ± 0.2 ng/ml ($n=5$) in castrated animals ($P < 0.01$). The serum testosterone concentration in testosterone-replaced animals was 3.72 ± 0.3 ng/ml ($n=4$), essentially identical to that in the sham-operated group and significantly greater than that seen in the castrated group ($P < 0.01$).

The effects of gonadectomy and testosterone replacement on thymic LHRH concentration (the amount of LHRH per mg of tissue) and molecular processing from proLHRH to LHRH were studied. Two weeks after surgery, sham-castrated rats were implanted with vehicle capsules and castrated rats were implanted with testosterone or vehicle capsules; animals were killed 96 h later.

The overall effect of gonadal manipulation on thymic LHRH concentration was significant ($P < 0.0026$ by ANOVA). The thymic LHRH concentration increased from 3.1 ± 0.3 pg/mg tissue ($n=23$) in sham-castrated animals to 6 ± 0.95 pg/mg tissue ($n=18$) 2 weeks after castration ($P=0.05$). Thymic LHRH concentration returned to that observed in the sham-operated rats (3.1 ± 0.4 pg/mg tissue; $n=10$) when gonadectomized rats were treated with testosterone (Fig. 1, upper panel). This value was not different from that in sham-castrated rats, but it was significantly lower than the LHRH concentration seen in gonadectomized, non-testosterone-replaced animals ($P=0.05$).

The overall effect of gonadal manipulation on thymic proLHRH concentration was significant by ANOVA ($P < 0.024$). Thymic proLHRH concentration decreased from 77.4 ± 10.3 pg/mg tissue ($n=12$) in sham-castrated rats to 43 ± 4 pg/mg tissue ($n=11$) in castrated animals ($P=0.05$). Testosterone replacement restored thymic proLHRH to 80.4 ± 16.8 pg/mg tissue ($n=7$; $P=0.05$ compared with castrated, vehicle-replaced rats), which did not differ from the concentration in sham-castrated rats (Fig. 1, lower panel). Thymic proLHRH concentration changed in a direction opposite to that observed for LHRH.

The overall effect of gonadal manipulation on thymic weight was highly significant by ANOVA ($P < 0.0001$). As expected, thymic weight increased (from 440 ± 19 mg ($n=15$) in sham-castrated rats to 793 ± 26 mg ($n=12$) in castrated animals, $P < 0.001$; Fig. 2). Testosterone replacement reduced thymus weight to 662 ± 22 mg ($n=14$), which was significantly lower than that in castrated animals ($P < 0.001$), but still higher than that in sham-castrated animals (Fig. 2).

The overall effect of gonadal manipulation on total thymic LHRH content (total amount of LHRH in whole thymus) in this experiment was significant by ANOVA ($P < 0.0025$). Total thymic LHRH content increased from 1537 ± 133 pg ($n=14$) in sham-castrated animals to 3832 ± 826 pg ($n=12$) in castrated animals ($P < 0.01$).

Figure 1 Upper panel: Effect of castration and testosterone replacement on thymic LHRH concentration. Lower panel: Effect of castration and testosterone replacement on thymic proLHRH concentration. Sham, sham-castrated; GX, castrated.

Figure 2 Effect of castration and testosterone replacement on thymus weight. Sham, sham-castrated; GX, castrated.

Ninety-six hours after testosterone replacement, the thymic content of LHRH in castrated animals decreased to 1564 ± 147 pg ($n=10$; $P<0.01$ compared with castrated, non-testosterone-replaced animals; Fig. 3, upper panel). Changes in thymic proLHRH content in the various experimental groups were in the same direction as those in thymic proLHRH concentration, but did not reach statistical significance (Fig. 3, lower panel).

Figure 3 Upper panel: Effect of castration and testosterone replacement on total thymic LHRH content. Lower panel: Effect of castration and testosterone replacement on thymic proLHRH content. Sham, sham-castrated; GX, castrated.

We also examined the effect of castration and testosterone replacement on LHRH mRNA expression in the thymus (Fig. 4). Because of large differences in LHRH mRNA expression in individual animals, the effect of gonadal manipulation did not reach statistical significance. The density of PCR product increased from 469 ± 23 ADU in intact rats ($n=4$) to 605 ± 204 ADU ($n=3$) in gonadectomized rats. Testosterone treatment restored this value to 483 ± 54 ADU ($n=4$).

The overall effect of gonadal manipulation on hypothalamic LHRH was significant ($P<0.0024$ by ANOVA). Total hypothalamic LHRH content decreased from 2809 ± 37 pg/hypothalamus ($n=10$) in sham-operated rats, to 1949 ± 197 pg/hypothalamus ($n=7$) in castrated animals ($P=0.05$). Testosterone replacement increased hypothalamic LHRH to 2487 ± 140 pg/hypothalamus ($n=5$; $P=0.05$ compared with castrated, non-testosterone-replaced rats), which did not differ from the value in sham-castrated rats (Fig. 5 upper panel). The hypothalamic content of proLHRH was not significantly changed by experimental manipulation: it was 580 ± 69 pg/hypothalamus ($n=14$) in sham-operated animals,

Figure 4 RT-PCR analysis of the effect of gonadectomy and testosterone replacement on steady state concentrations of LHRH mRNA. Each lane represents the thymus from one animal. GX, castrated; Sham, sham-castrated; GXT, castrated and replaced with testosterone. Histone 3·3 (H3·3) was used as a control for loading and amplification.

558 ± 96 pg/hypothalamus ($n=7$) in rats 2 weeks after castration, and 406 ± 16 pg/hypothalamus ($n=8$) in castrated animals treated with testosterone (Fig. 5, lower panel).

Figure 5 Upper panel: Effect of castration and testosterone replacement on hypothalamic LHRH content. Lower panel: Effect of castration and testosterone replacement on hypothalamic proLHRH content. Sham, Sham-castrated; GX, castrated.

Discussion

Several lines of evidence support an immunostimulatory role for LHRH. Immunoreactive and bioactive LHRH has been demonstrated to be present in rat and human T lymphocytes (Emanuele *et al.* 1990, Azad *et al.* 1991a,b, 1993). In addition, demonstration of the presence of proLHRH mRNA in these tissues confirms local synthesis (Azad *et al.* 1991, 1993). The presence of LHRH receptor sites on lymphocytes has been reported in several species (Marchetti *et al.* 1989a, Standaert *et al.* 1992). Immunologic stimulation with phytohemagglutinin or anti-receptor antibody significantly increased T cell LHRH production in human and rat T cells (Azad *et al.* 1990a, 1993 and unpublished observations). This upregulation is PKC- and tyrosine kinase-dependent (Mohagheghpour *et al.* 1995). It has been shown that LHRH agonist increased T cell proliferative activity of rat splenic and thymic lymphocytes (Emanuele *et al.* 1990, Azad *et al.* 1991a,b, 1993). Chronic treatment with an LHRH agonist regenerated the involuted thymus gland in aged rats and improved the compromised T cell proliferative activity in aged female and male rats (Marchetti *et al.* 1989b). Furthermore, LHRH agonist treatment increased interleukin-2 receptor expression in resting and concanavalin-A-treated thymic and splenic lymphocytes (Batticane *et al.* 1991).

It has also been demonstrated that LHRH has an important role in an immunostimulated state. LHRH antagonist administration to lupus-prone mice decreased antibody production and hematuria, and significantly increased the animals' life spans (Jacobson *et al.* 1994), suggesting that this autoimmune state is, in part, dependent upon excessive expression of immune cell LHRH.

Testosterone, a testicular androgen, had been shown to be an immunosuppressor (Grossman 1984). Castration of

prepubertal male rats delayed involution of the thymus gland (Castro 1974, Weksler *et al.* 1978). In addition, castration during senescence reversed the age-related loss of cell-mediated immunity (Chiodi 1976), but administration of testosterone prevented this process (Ablyn *et al.* 1974, Chiodi 1976, Grossman 1984, Kelley *et al.* 1988). Dihydrotestosterone (DHT), the active metabolite of testosterone, and testosterone itself had inhibitory effects on the production of various antibodies and retarded the progression of autoimmune disorders in N2B/NZW mice (Steinberg *et al.* 1979). A recent study demonstrated that testosterone and DHT reverse the castration-induced expansion of pre-B cells in bone marrow, and that this effect was independent of the thymus gland (Kovacs *et al.* 1996).

We have shown for the first time that testosterone modulated thymic proLHRH and LHRH concentrations. The concentration of LHRH in the thymus gland increased after castration, whereas proLHRH decreased. These effects were totally prevented by replacement with testosterone (Fig. 1). Similarly, LHRH content increased after castration, an effect again totally prevented by testosterone replacement (Fig. 3). The decreased proLHRH and increased LHRH, coupled with unchanged LHRH mRNA in castrated rats, indicate that testosterone acted post-translationally to inhibit processing of LHRH from its precursor molecule, proLHRH.

Our studies, in agreement with the findings of many previous investigations (Grossman 1984, Marchetti *et al.* 1989b), showed significant enlargement in the size of the thymus gland after castration and partial inhibition of this increase in thymic size with testosterone replacement (Fig. 2). Considering the known immunostimulatory effect of LHRH on T cells, our data are consistent with the hypothesis that the increased concentrations of LHRH in the thymus gland of castrated rats might have played a significant part in thymus growth and T cell development of these animals.

The inhibitory effect of testosterone on thymic LHRH concentrations, coupled with the well-known immunosuppressive effect of testosterone, suggest that the mechanism of the inhibitory role of testosterone on the immune system may be, in part, achieved by its diminishing the concentrations of LHRH in lymphocytes.

The second objective of this study was to compare the effect of castration and testosterone replacement on hypothalamic LHRH with their effect on thymic LHRH. In accordance with the observations of others (Roselli *et al.* 1990), our data showed that castration significantly decreased hypothalamic LHRH content, by 31%, and that testosterone replacement restored hypothalamic LHRH to sham control values (Fig. 5). This is in direct contrast to the effect on the thymus, indicating markedly different, organ-specific regulatory patterns.

Finally, this is the first report examining the relative contents of LHRH in the hypothalamus and immune

tissue. Our data showed comparable concentrations of LHRH in the two tissues, but a much greater content of proLHRH in the thymus. It is thus tempting to speculate that neuroendocrine and immune tissues may possess marked differences in their rates of processing of proLHRH to LHRH. Future investigations are required to examine this issue.

In summary, castration increased the thymic concentration of LHRH, and decreased thymic proLHRH, whereas testosterone treatment prevented these effects. In contrast, the hypothalamic concentration of LHRH was significantly decreased in castrated rats, but normalized with testosterone treatment. In addition, the thymus contains much more proLHRH, but less LHRH, than the hypothalamus. These data suggest that gonadal manipulation modulates thymic LHRH molecular processing and its tissue concentration, in addition to that in the hypothalamus, and that the regulation of LHRH molecular processing by testosterone is different in the hypothalamus and in the thymus.

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References

- Ablyn RJ, Bruns GR, Guinan P & Bush IM 1974 Antiandrogenic suppression of lymphocytic blastogenesis: *in vitro* and *in vivo* observations. *Experientia* **30** 1351–1353.
- Azad N, Emanuele NV, Duffner L, Kirsteins L & Lawrence AM 1990a Further studies on lymphocyte luteinizing hormone releasing hormone (LHRH). Program of the 72nd Annual Meeting of the Endocrine Society, p 361 (Abstract).
- Azad N, Duffner L, Paloyan EB, Reda D, Kirsteins L, Emanuele NV & Lawrence AM 1990b Hypothalamic prolactin stimulates the release of luteinizing hormone-releasing hormone from male rat hypothalamus. *Endocrinology* **127** 1928–1933.
- Azad N, Jurgens J, Young MR, Reda D, Duffner L, Kirsteins L, Emanuele NV & Lawrence AM 1991a Presence of luteinizing hormone-releasing hormone in rat thymus. *Progress in NeuroEndocrin-Immunology* **4** 113–120.
- Azad N, Emanuele NV, Halloran MM, Tentler J & Kelley MR 1991b Presence of luteinizing hormone-releasing hormone (LHRH) mRNA in rat spleen lymphocytes. *Endocrinology* **128** 1679–1681.
- Azad N, LaPaglia N, Abel K, Jurgens J, Kirsteins L, Emanuele NV, Kelley MR, Lawrence AM & Mohagheghpour N 1993 Immunoactivation enhances the concentration of luteinizing hormone-releasing hormone peptide and its gene expression in human peripheral T-lymphocytes. *Endocrinology* **133** 215–223.
- Batticane N, Morale MC, Gallo F, Farinella Z & Marchetti B 1991 Luteinizing hormone-releasing hormone signaling at the lymphocyte involves stimulation of interleukin-2 receptor expression. *Endocrinology* **129** 277–286.
- Blagosklonny MV & Neckers LM 1994 Cytostatic and cytotoxic activity of sex steroids against human leukemia cell lines. *Cancer Letters* **76** 81–86.
- Castro JE 1974 Orchidectomy and the immune response I. Effect of orchidectomy on lymphoid tissue of mice. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **1985** 425–436.

- Chiodi H 1976 Thymus hypertrophy induced by castration in old male rats and mice. *Federation Proceedings* **35** 277A.
- Chomczynski P & Sacchi N 1987 Single-step method of RNA isolation by guanidinium thiocyanate–phenol–chloroform extraction. *Annals of Biochemistry* **162** 156–159.
- Emanuele NV, Emanuele MA, Tentler J, Kirsteins L, Azad N & Lawrence AM 1990 Rat spleen lymphocytes contain an immunoactive and bioactive luteinizing hormone-releasing hormone. *Endocrinology* **126** 2482–2486.
- Grossman CJ 1984 Regulation of the immune system by sex steroids. *Endocrinology Reviews* **5** 435–455.
- Grossman CJ, Nathan P, Taylor BB & Sholiton LJ 1979 Rat thymic dihydrotestosterone receptor: preparation, location and physicochemical properties. *Steroids* **34** 539–553.
- Grossman CJ, Sholiton LJ & Roselle GA 1983 Dihydrotestosterone regulation of thymocyte function in the rat – mediation by serum factors. *Journal of Steroid Biochemistry* **19** 1459–1467.
- Jacobson JD, Nisula BC & Steinberg AD 1994 Modulation of the expression of murine lupus by gonadotropin-releasing hormone analogs. *Endocrinology* **134** 2516–2523.
- Kelley KW, Davila DR, Brief S, Sumon J & Arkins S 1988 A pituitary thymus connection during aging. *Annals of the New York Academy of Sciences* **521** 88–98.
- Kelley MR, Jurgens JK, Tentler J, Emanuele NV, Blutt SA & Emanuele MA 1993 Coupled reverse transcription-polymerase chain reaction (RT-PCR) technique is quantitative and rapid: uses in alcohol research involving low abundance mRNA species. *Alcohol* **10** 185–189.
- Kovacs WJ & Olsen NJ 1987 Androgen receptors in human thymocytes. *American Association of Immunology* **139** 490–493.
- Kovacs WJ, Viselli SM, Reese K, Fan J & Olsen NJ 1996 Androgens influence B cell development by thymic-independent mechanisms. Program of the 10th International Congress of Endocrinology, p 108 (Abstract), San Francisco, USA
- McCrudden AB & Stimson WH 1981 Androgen binding cytosol receptors in the rat thymus: physicochemical properties, specificity and localisation. *Thymus* **3** 105–117.
- Makinodan T & Kirokawa K 1985 Normal aging of the immune system. In *Relations between Normal Aging and Disease*, p 117. Ed HA Johnson. New York: Raven Press.
- Marchetti B, Guarcello V, Morale MC, Bartoloni G, Farinella Z, Cordaro S & Scapagnini U 1989a Luteinizing hormone-releasing hormone-binding sites in the rat thymus: characteristics and biological function. *Endocrinology* **125** 1025–1036.
- Marchetti B, Guarcello V, Morale MC, Bartoloni G & Scapagnini U 1989b Luteinizing hormone-releasing hormone (LHRH) agonist restoration of age-associated decline of thymus weight, thymic LHRH receptors, and thymocyte proliferative capacity. *Endocrinology* **125** 1037–1045.
- Meikle AW, Dorchuck RW, Araneo BA, Stringham JD, Evans TG, Spruance SL & Daynes RA 1991 Dehydroepiandrosterone specific receptor and interleukin-2 stimulation in murine T-cells. Program of the 73rd Annual Meeting of the Endocrine Society, p 191 (Abstract).
- Mohagheghpour N, Abel K, LaPaglia N, Emanuele NV & Azad N 1995 Signal requirements for production of luteinizing hormone-releasing hormone by human T cells. *Cellular Immunology* **163** 280–288.
- Roselli CE, Kelly MJ & Ronnekleiv OK 1990 Testosterone regulates progadotropin-releasing hormone levels in the preoptic area and basal hypothalamus of the male rat. *Endocrinology* **126** 1080–1086.
- Sholiton LJ, Grossman CJ & Taylor BB 1980 Rat thymic homogenates convert testosterone to androgenic metabolites. *Journal of Steroid Biochemistry* **13** 1365–1367.
- Standaert FE, Chew BP, DeAvila D & Reeves JJ 1992 Presence of luteinizing hormone-releasing hormone binding sites in cultured porcine lymphocytes. *Biology of Reproduction* **46** 997–1000.
- Steinberg AD, Melez KA, Raveche ES, Reeves JP, Boegel WA, Smathers PA, Taurog JD, Weinlein L & Duvic M 1979 Approach to the study of the role of sex hormones in autoimmunity. *Arthritis and Rheumatism* **22** 1170–1176.
- Weinstein Y, Lindner HR & Eckstein B 1977 Thymus metabolises progesterone – possible enzymatic marker for T lymphocytes. *Nature* **266** 632–633.
- Weksler ME, Innes JB & Goldstein G 1978 Immunological studies of aging IV. The contribution of thymic involution to the immune deficiencies of aging mice and reversal with thymopoietin. *Journal of Experimental Medicine* **148** 996–1006.
- Wyle RA & Kent JR 1977 Immunosuppression by sex steroid hormones the effect upon PHA- and PPD-stimulated lymphocytes. *Cellular Immunology* **27** 407–415.

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