Journal of Cellular Biochemistry 88:766–773 (2003)

Role of Testicular Interstitial Macrophages in Regulating Testosterone Release in Hyperprolactinemia

William J. Huang,[1,3] Jiun-Yih Yeh,[2] Shu-Fen Kan,[2] Luke S. Chang,[1,3] and Paulus S. Wang[2]*

[1]Department of Urology and Department of Physiology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, 11221, Republic of China
[2]Department of Physiology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, 11221, Republic of China
[3]Division of Urology, Department of Surgery, Taipei Veterans General Hospital, Taipei 11217, Taiwan, Republic of China

Abstract Hyperprolactinemia-induced hypogonadism has been linked to a dysfunction of the hypothalamus-pituitary-testis axis. The direct inhibitory effects of prolactin on the testicular release of testosterone have also been demonstrated, though their mechanisms remain unclear. Incubation of rat testicular interstitial cells (TICs) with prolactin stimulated the release of testosterone. TICs from rats with anterior pituitary-grafting-induced hyperprolactinemia release lower amounts of testosterone than controls. However, Leydig cells isolated from anterior pituitary-grafted rats release a greater amount of testosterone. These paradoxical observations have remained unexplained. This study examined the roles of testicular interstitial macrophages and of their product, tumor necrosis factor-α (TNF-α), in regulating Leydig cells under condition of hyperprolactinemia. Hyperprolactinemia was induced by grafting two anterior pituitary glands of rats under the renal capsule. Control animals were grafted with rat cortex tissue. The rats were sacrificed 6 weeks later. TICs and macrophages, and Leydig cells were isolated for in vitro incubation and drugs challenge. Testosterone released by testicular interstitial or Leydig cells was measured by radioimmunoassay. TNF-α concentration in the medium of TICs or macrophages was measured by enzyme-linked immunosorbent assay (ELISA). A dose-dependent stimulation of TNF-α secretion in the medium of TICs or macrophages by the prolactin challenge was observed. Higher amounts of TNF-α were released by TICs in the anterior pituitary-grafted rats than in the control group. In contrast, the release of TNF-α by testicular interstitial macrophages isolated from the anterior pituitary- and cortex-grafted groups was quantitatively similar. Challenge with human chorionic gonadotropin did not modify the TNF-α release by testicular interstitial macrophages in either group. Challenge of Leydig cells with TNF-α inhibited their release of testosterone stimulated by human chorionic gonadotropin, but not their basal testosterone release. These different patterns of testosterone release in TICs versus Leydig cells cultures in anterior pituitary-grafted rats may be due to the influence of testicular interstitial macrophages. These observations correlate with in vivo conditions, where prolactin increases the release of TNF-α by testicular interstitial macrophages, which, in turn, decreases the human chorionic gonadotropin-stimulated release of testosterone by Leydig cells. In summary, hyperprolactinemia-induced hypogonadism involves a mechanism of prolactin-originated, macrophage-mediated inhibitory regulation of testosterone release by Leydig cells. TNF-α, one of the cytokines secreted by macrophages, may play a key role in this mechanism. J. Cell. Biochem. 88: 766–773, 2003.

Key words: prolactin; hyperprolactinemia; Leydig cells; macrophage; TNF-α; testosterone; TIC

Grant sponsor: Taipei Veterans General Hospital; Grant numbers: VGH 89-092, VGH 90-104; Grant sponsor: National Science Council of ROC; Grant number: NSC 89-2314-B-075-104; Grant sponsor: Medical Research and Advancement Foundation.

*Correspondence to: Paulus S. Wang, PhD, Department of Physiology, National Yang-Ming University, Shih-Pai, Taipei 11221, Taiwan, Republic of China.
E-mail: pswang@ym.edu.tw

Received 3 September 2002; Accepted 10 September 2002
DOI 10.1002/jcb.10412

Prolactin (PRL) is a protein hormone released by the pituitary gland with broad physiologic functions in various kinds of cells [Molitch, 1992]. In the reproductive system, PRL plays dual roles. On one hand, it acts as a trophic hormone in the development of the genital tract. Prepubertal hypophysectomized rats supplemented only with PRL allow Leydig cells to differentiate and proliferate [Dombrowicz et al., 1992]. On the other hand, PRL has inhibitory effects on sex hormone secretion. Abnormally high serum PRL concentrations may cause a decrease in testosterone (T) release, which, in turn, predisposes to impotence or infertility [Bartke et al., 1986]. These opposite properties of PRL have been proposed as a mechanism of hyperprolactinemia (hyperPRL)-induced dysfunction of the hypothalamus, with decreased serum gonadotropin and T production as consequences [Bartke et al., 1986; deGreef et al., 1995].

In earlier studies in hyperPRL rats, we have demonstrated that testicular interstitial cells (TICs) release a lesser amount of human chorionic gonadotropin (hCG)-induced T and, from a malfunction of adenylate cyclase, produce less cAMP [Huang et al., 1999]. These results are consistent with those observed in vivo [Huang et al., 1999]. However, Leydig cells isolated from hyperPRL rats released considerably more T in response to hCG than corresponding cells in the control group. This rebound T release has also been noted with other types of challenges, such as forskolin (an adenylate cyclase activator), 8-Br-cAMP (a permeable cAMP), pregnenolone, and 25-OH-cholesterol (T precursors) [Huang et al., 2001]. Leydig cells isolated from hyperPRL rats seem to escape from a control which prevents the proper stimulation of TICs by hCG or other drugs. The mechanism behind this control remains unclear.

TICs are a cellular mixture, including a majority of Leydig cells, testicular interstitial macrophages (TIMs) and, in smaller numbers, fibroblasts and myoid cells. TIMs represent the second largest population, contributing up to 20% of TICs [Miller et al., 1983]. In vitro, TIMs are often present in TICs preparations, though are rarely observed in Leydig cells cultures [Niemi et al., 1986; Themmen et al., 1987; Hedger, 1997]. In the testicular interstitium, Leydig cells are in close physical contacts with neighboring TIMs [Miller et al., 1983]. This close stereological relationship suggests an intimate paracrine regulation between these two cell types [Gaytan et al., 1994]. On the other hand, TIMs are a major source of cytokines particularly of tumor necrosis factor-α (TNF-α) and IL-1 [Le and Vilcek, 1987]. Furthermore, through the actions of the cytokines, TIMs regulate the release of T by Leydig cells. Among macrophage-secreted cytokines, TNF-α is the strongest inhibitor of T release by Leydig cells [Mauduit et al., 1991].

This study tested the hypothesis that, through the intermediary action of TNF-α, TIMs regulate the release of T by Leydig cells under conditions of hyperPRL.

MATERIALS AND METHODS

Animal Model

Male Sprague–Dawley rats weighing 250–300 g were housed in a room at a controlled temperature of $22 \pm 1^{\circ}$C, artificially illuminated between 06:00 and 20:00 h. Food and water were available ad libitum. All animal experimentation was conducted humanely and in compliance with the policy statement of the Animal Experiment Review Committee of National Yang-Ming University.

Induction of Hyperprolactinemia

Under light ether anesthesia, an incision was made in the left pararenal region to expose the kidney. A small incision was made in the renal capsule to allow the implantation of two anterior pituitary (AP) glands in the underlying space [Everett, 1954; Huang et al., 1999]. An equivalent amount of brain cortex (CX) was similarly implanted in a control group of rats. Seven or eight rats were usually grafted with AP or CX in each experiment. The in vitro studies were performed after the 7th week of survival. The rats were decapitated, and the testes were aseptically removed and collected. Meanwhile, the kidney including the grafts was also examined. The extent of vascularization and size of the viable grafted pituitary tissue were confirmed grossly and, later, microscopically by a method described previously [Merchenthaler et al., 1995]. Animals whose grafts showed signs of survival and whose plasma PRL concentrations had increased by at least 50% above the mean control concentration were retained for further experimentation. The overall success rate of grafting in this experiment was 95%.

Preparation of TICs

The method used for the collagenase dispersion of TICs has been described elsewhere [Tsai et al., 1997]. Briefly, the medium (1% bovine serum albumin in Hank's balanced salt solution, with HEPES 25 mM, sodium bicarbonate 0.35 g/L, penicillin-G 100 IU/ml, streptomycin sulfate 50 mg/ml, heparin 2550 USP K U/L, pH 7.3) was aerated and saturated with 95% O_2 and 5% CO_2 before use. Collagenase (type IA, Sigma, St. Louis, MO), in a concentration of 700 μg/5ml, was used to disperse the TICs from the rat testis blocks. After filtration of the testicular tissue, the filtrate containing TICs was centrifuged, washed, and resuspended in the medium. TICs were plated into test tubes at a density of 1×10^6 cells/ml/tube. All tubes were arrayed in a 34°C water bath, with gentle shaking at a rate of 50 cycles/min. The water bath chamber was kept saturated with 95% O_2 and 5% CO_2. Each preparation contained approximately 20% Leydig cells [Lin et al., 1998].

Preparation of Leydig Cells

Following the procedure described above, the TICs were collected by centrifugation at 200*g* and 4°C, for 10 min. The cell pellet volume was recovered by the incubation medium to 5 ml, then gently added to the upper layer of a continuous gradient percoll. The continuous gradient percoll (25 ml/dispersion) was composed of 9 parts of percoll plus 11 parts of Medium 199 and 0.1% BSA before centrifugation at 20,000*g* and 4°C, for 60 min. The mixture of TICs with continuous gradient percoll was centrifuged at 800*g* and 4°C, for 20 min. The Leydig cells were located at 3–7 ml layers from the bottom. The Leydig cells layer was diluted to 5 ml, then centrifuged at 80*g* at room temperature for 8 min. After repeating the wash step, the cell pellet was resuspended to 10 ml in incubation medium. Cell concentration (1×10^5 cells/ml) and viability (>95%) were verified with a hemocytometer and by the trypan blue method, respectively. For the 1-h challenge experiments, the Leydig cells were plated into test tubes in a density of 1×10^5 cells/ml. The following procedures were similar to those of TICs incubation. For the 24-h challenge experiments, the Leydig cells were plated into 24-well culture dishes (Falcon, Franklin Lakes, NJ) in a density of 1×10^5 cells/ml. The culture dishes were incubated in a chamber kept aerated with 95% air and 5% CO_2 at 37°C. The 3β-hydroxysteroid dehydrogenase (3β-HSD) staining method was used to measure the abundance of Leydig cells in the preparation [Dirami et al., 1991; Krummen et al., 1994]. The cells (1×10^5 cells/ml) were incubated with a solution containing 0.2 mg/ml nitro blue tetrozolium (Sigma, St. Louis, MO), 0.12 mg/ml 5-androstane-3β-ol-one (Sigma), and 1 mg/ml NAD^+ (Sigma) in 0.05 M PBS, pH 7.4 at 34°C for 90 min. Upon development of the blue formazan deposit sites of 3β-HSD activity, the abundance of Leydig cells was determined with a hemocytometer. Our preparation contained approximately 87% Leydig cells.

Preparation of Testicular Interstitial Macrophages

The method to isolate TIMs was adopted from previous studies [Yee and Hutson, 1983; Xiong and Hales, 1993]. Briefly, the crude TICs suspension was plated in culture dishes and incubated for 30 min in a humidified atmosphere consisting of 95% air and 5% CO_2 at 37°C, allowing the macrophages to attach to the plastic culture dish. The non-adherent and loosely adherent cells were removed by aspirating the supernatant and twice rinsing the dishes with supplemented Medium 199. TIMs were then collected by trypsin digestion (1.25 mg/ml). The purity of TIMs was determined by flowcytometry, using an FITC-labeled monoclonal antibody to rat macrophage (ED1, IgG 1, Biosource, Camarillo, CA). Cell concentration (1×10^5 cells/ml) and viability (>95%) were verified with a hemocytometer and by the trypan blue method, respectively. TIMs were then plated into 24-well culture dishes in a density of 1×10^5 cells/ml, and incubated in a humidified atmosphere consisting of 95% air and 5% CO_2 at 37°C. The purity of macrophage in the TIMs preparation was 81%. The contamination of Leydig cells was determined by a 3β-HSD staining method. Leydig cells contamination was < 10%.

Treatment of Leydig Cells With TNF-α

The Leydig cells isolated from the CX- and AP-grafted rats (n = 6 in each group) were challenged with 200 μl of TNF-α solution in concentrations of 0.01, 0.1, 1, and 10 ng/ml. The cells were plated into culture dishes and

preincubated for 36 h. The medium was replaced, and incubated in a TNF-α solution for 24 h. At the end of incubation, the culture dishes were centrifuged to precipitate the cells and the supernatant was aspirated, frozen and stored for T measurement.

Treatment of TICs With *o*-Prolactin

TICs isolated from the CX- and AP-grafted rats (n = 6 in each group) were challenged with *o*-PRL in concentrations of 5 and 10 μg/ml. TICs, in a density of 1×10^6 cells/ml, were plated onto the culture dishes. After 36 h of preincubation with medium containing BSA, the medium was removed, the cells were twice washed with BSA-free medium, and incubated with *o*-PRL in BSA-free medium for 24 more hours. The incubation conditions were similar to those of the Leydig cells. At the end of incubation, the culture dishes were centrifuged to precipitate the cells and the supernatant was aspirated, frozen and stored for TNF-α measurement.

Treatment of Testicular Interstitial Macrophages With *o*-Prolactin

TIMs isolated from the CX- and AP-grafted rats (n = 6 in each group) were challenged with *o*-PRL in concentrations of 5 and 10 μg/ml. TIMs, in a density of 1×10^5 cells/ml, were plated onto the culture dishes. After 36 h of preincubation with medium containing BSA, the medium was removed, and the cells were twice washed with BSA-free medium, and challenged with *o*-PRL by incubation in BSA-free medium for 24 more hours. The incubation conditions were similar to those of the Leydig cells. At the end of incubation, the culture dishes were centrifuged to precipitate the cells, and the supernatant was aspirated, frozen, and stored for TNF-α measurement.

Radioimmunoassay of Testosterone and Prolactin

The concentration of T in medium was measured by radioimmunoassay (RIA) as described elsewhere [Wang et al., 1994]. The sensitivity of anti-T serum (W8) was 2 pg per assay tube. The intra- and interassay coefficients of variation (CV) were 4.1% (n = 6) and 4.7% (n = 10), respectively.

The concentration of PRL in plasma was determined by RIA as described elsewhere [Tang et al., 1986]. The rat PRL RIA kit was provided by the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK), the National Institute of Child Health and Human Development, and the U.S. Department of Agriculture. Rat PRL-I-5 was used for iodination. Rat PRL-RP-3 served as standards. The intra- and interassay CV were 3.8 and 3.2%, respectively. The sensitivity was 30 pg/tube.

Enzyme-Linked Immunosorbent Assay (ELISA) of TNF-α

The concentration of TNF-α in culture medium was determined by ELISA with a commercially available kit (R & D systems, Minneapolis, MN). The sensitivity was <5 pg/ml.

Statistical Analyses

All values are expressed as mean ± SE of the mean (SEM). For multiple comparisons, the means from different treatments were tested for homogeneity by two-way analysis of variance, and the difference between specific means was tested for significance by Duncan's multiple-range test [Steel and Torrie, 1960]. A difference between two means was considered statistically significant when $P < 0.05$.

RESULTS

Plasma Prolactin Levels in Anterior Pituitary- and Cortex-Grafted Rats

The plasma sample was collected at the time of decapitation. Plasma PRL levels were significantly higher in the AP-grafted than in the CX-grafted rats ($P < 0.01$, Fig. 1).

Effects of TNF-α on Testosterone Release by Leydig Cells With or Without Human Chorionic Gonadotropin Challenge

TNF-α alone (0.01–10 ng/ml) had nonsignificant effects on the basal release of T,

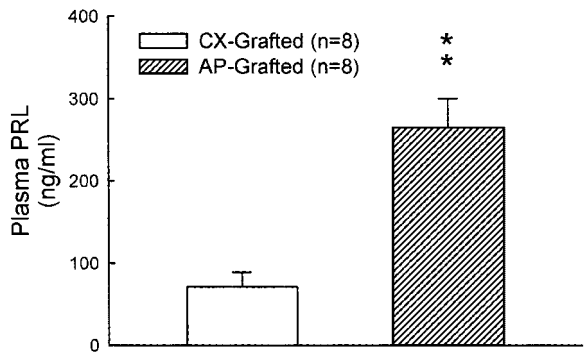

Fig. 1. Plasma prolactin (PRL) concentrations in AP- and CX-grafted rats. $^{**}P < 0.01$ vs. the CX-grafted rats.

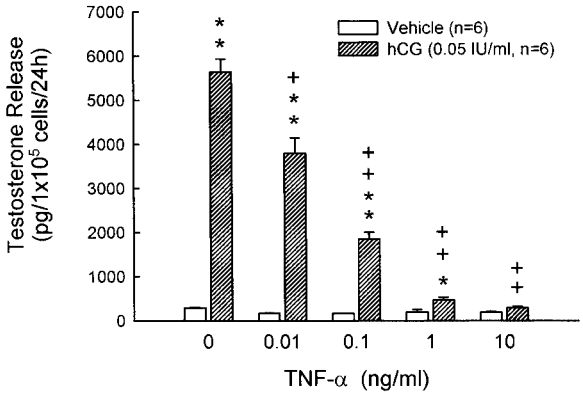

Fig. 2. Testosterone release by rat Leydig cells 1×10^5 cells/24 h in response to tumor necrosis factor-α (TNF-α) in presence, versus absence of hCG, 0.05 IU/ml. $^*P < 0.05$, and $^{**}P < 0.01$ vs. the group treated with the vehicle only. $+P < 0.05$, and $++P < 0.01$ vs. the group untreated with TNF-α.

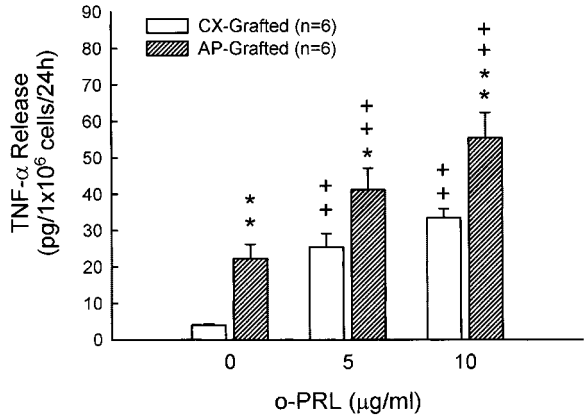

Fig. 3. Tumor necrosis factor-α (TNF-α) release by rat testicular interstitial cells (TICs) 1×10^6 cells/24 h in response to *o*-PRL. $^*P < 0.05$, and $^{**}P < 0.01$ vs. the CX-grafted group. $++P < 0.01$ vs. TNF-α release at *o*-PRL = 0 μg/ml.

however inhibited the hCG-stimulated T release by Leydig cells, in both CX- (data not shown) and AP-grafted rats (Fig. 2). In a dose of 10 ng/ml, TNF-α completely blocked the effect of hCG.

Effects of Hyperprolactinemia on TNF-α Release by TICs in Response to Prolactin Challenge

TICs from both CX- and AP-grafted rats (n = 6 in each group) were challenged with *o*-PRL for 24 h. The TNF-α concentrations, measured by ELISA, were significantly higher in the AP-grafted than in CX-grafted animals, at baseline ($P < 0.01$) as well as following *o*-PRL challenge ($P < 0.05$ or <0.01). Likewise, *o*-PRL, 5–10 μg/ml, had dose-dependent stimulatory effects on the TNF-α release by TICs in both CX- and AP-grafted rats (Fig. 3).

Effects of Hyperprolactinemia on TNF-α Release by Testicular Interstiatial Macrophages in Response to Prolactin With or Without Human Chorionic Gonadotropin

TIMs, isolated from CX- and AP-grafted rats (n = 6 in each group), were challenged with *o*-PRL for 24 h. TNF-α concentrations, measured by ELISA, were consistent with the amount of *o*-PRL administered. However, there was no difference in TNF-α concentrations between CX- and AP-grafted rats. Administration of hCG, 0.05 IU/ml, to the TIMs incubations did not modify the release of TNF-α (Fig. 4).

DISCUSSION

HyperPRL-induced hypogonadism has been attributed to a dysfunction of hypothalamic GnRH neurons, which, in turn, decrease the amount and frequency of GnRH release. As a result, the AP gland responds by decreasing its secretion of gonadotropins, and T release by the testes is decreased [Bartke et al., 1986; deGreef et al., 1995]. However, a hyperPRL-related decrease in gonadotropin concentrations is not uncommonly associated with unchanged serum T concentrations [Boyar et al., 1974; Bartke and Dalterio, 1976; McNeilly et al., 1978], and a hyperPRL-induced suppression of T secretion may sometimes be associated with unchanged

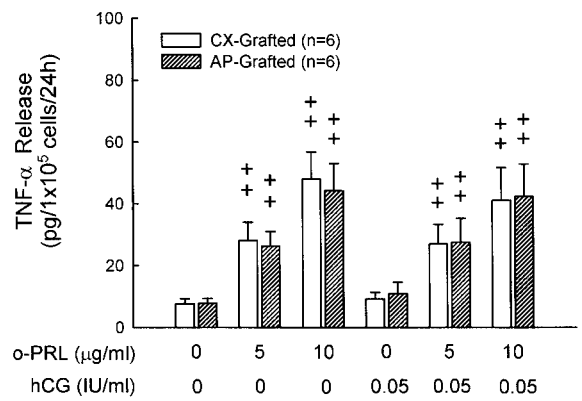

Fig. 4. Tumor necrosis factor-α (TNF-α) release by rat testicular interstitial macrophages (TIMs) 1×10^5 cells/24 h in response to *o*-PRL with or without hCG. $++P < 0.01$ vs. corresponding baseline release of TNF-α.

[Saidi et al., 1977], or even significantly increased gonadotropin concentrations [Klemcke and Bartke, 1981]. These observations suggest that the underlying mechanism of hyperPRL-induced hypogonadism utilize pathways other than those of the hypothalamus-pituitary-testis axis.

From our previous studies, TICs isolated from AP-grafted hyperPRL rats had a decreased T release response after challenges with hCG, *o*-PRL, T precursors, or 8-Br-cAMP [Huang et al., 1999]. However, under similar conditions, the Leydig cells purified from TICs caused a paradoxically more prominent release of T in AP-grafted rats than in the control group [Huang et al., 2001].

When comparing the TICs and Leydig cells incubations, we noticed that differences in cell compositions between the two preparations were most significant. In the Leydig cells preparations, nearly all the TIMs were removed. Since TIMs are the second largest cell population among TICs, the importance of their role in the process of T biosynthesis cannot be overemphasized [Hayes et al., 1996]. In the testis, Leydig cells are in close physical contact with neighboring TIMs [Miller et al., 1983; Hutson, 1992]. Therefore, the hypothesis of an intimate paracrine regulation between these two cell types is plausible [Yee and Hutson, 1985; Gaytan et al., 1994]. Resting TIMs in coculture with rat Leydig cells inhibit the basal and hCG-or LH-stimulated T production [Sun et al., 1993; Wang et al., 1995]. Therefore, TIMs are negative regulators of T release by Leydig cells.

In addition, TIMs are a major source of cytokine production, particularly TNF-α and IL-1, which modulate the production of T by the Leydig cells [Le and Vilcek, 1987; Hales, 1992; Mauduit et al., 1992, Xiong and Hales, 1993]. Among macrophage-secreted cytokines, TNF-α is the strongest inhibitor of T release by Leydig cells [Mauduit et al., 1991]. Hutson has shown the TIMs medium has inhibitory effects on T release from Leydig cells, and that the effects of TIMs on Leydig cells are mediated by TNF-α [Hutson, 1993].

In the present study, TNF-α suppressed the hCG-stimulated T release by Leydig cells in a dose-dependent manner. In absence of hCG, TNF-α in amounts up to 10 ng/ml, had no effects on the T release by Leydig cells. The inhibitory activity of TNF-α on the hCG-stimulated T release may be upon the pathway between LH receptors and the downstream processes of steroidogenesis. TNF-α receptors are present in Leydig cells [Mauduit et al., 1991, 1992], and TNF-α has an inhibitory effect on their steroidogenesis via a decrease in steroidogenic acute regulatory protein expression. This inhibitory action is probably mediated by TNF-α receptor p55 expressed in the testicular cells [Mauduit et al., 1998].

In the present study, TNF-α was detected in the medium of TICs and TIMs preparations. In the TICs, the addition of *o*-PRL caused a dose-dependent increase in TNF-α release, an increase which was significantly greater in AP-grafted than in CX-grafted rats. While in the TIMs cultures, *o*-PRL also caused a dose-dependent release of TNF-α, no difference was observed in the amount of TNF-α released between CX- and AP-grafted rats. These observations suggest that the presence of Leydig cells is necessary to express a difference in TNF-α release in TIMs between the AP- and CX-grafted groups. In the in vivo model, TIMs, in the AP-grafted rats, are exposed to a significantly higher amount of serum PRL. As a result, a more abundant amount of TNF-α is expected to be released from the stimulated TIMs. On the other hand, hCG seems not to influence the release of TNF-α by TIMs. No change in TNF-α release was noted in TIMs incubated in presence versus absence of hCG. These results indicate that there is probably no LH/hCG receptor on TIMs.

The inhibitory effect of TNF-α on T production has been studied in several laboratories. TNF-α may interfere with the transport of cholesterol into mitochondria [Mauduit et al., 1998], and with the expression of cytochrome enzymes, such as P450scc or $P450c_{17}$ [Xiong and Hales, 1993; Lin et al., 1994; Li et al., 1995; Stocco and Clark, 1996]. TNF-α also blocks the normal binding of LH with its receptors and adenylate cyclase function [Mauduit et al., 1991]. A study using 8-Br-cAMP to bypass the limitations of LH receptor-adenylate cyclase malfunction failed to restore the normal production of T [Huang et al., 1999]. These observations suggest other mechanism(s) of TNF-α related inhibition operating upon processes distal to the action of cAMP.

The role of IL-1 on the inhibition of LH-induced T release by Leydig cells has also been proposed [Calkins et al., 1988; Fauser et al.,

1989; Lin et al., 1991a; Mauduit et al., 1992; Sun et al., 1993]. The consensus is that IL-1 enhances the inhibitory effect of TNF-α [Calkins et al., 1990b]. However, this inhibitory effect usually required higher concentrations of IL-1 in in vitro studies [Calkins et al., 1990a]. Subsequently, it was associated with the blockade of $P450c_{17}$ and P450scc [Lin et al., 1991b; Hales, 1992; Hales et al., 1992].

The use of Fc receptors test by flowcytometry allowed to measure the number and percentage of macrophages present among TICs. The numbers and percentages of TIMs remained constant regardless of the prior performance of AP- or CX-grafting (80% vs. 82%). This indicates that hyperPRL induced by AP-grafting does not modify the number of TIMs. Therefore, the differences in T or TNF-α release in response to PRL or other to challenges between the AP- or CX-grafted rats is not explained by a change in the number of TIMs.

HyperPRL induced by AP-grafting also changed the characteristics of Leydig cells, for instance by increasing the responsiveness to LH/hCG challenge, and enhanced function of the steroidogenic process. On the other hand, these cells may also be more sensitive to the action of TNF-α, by which the process of steroidogenesis is slowed.

In summary, hyperPRL-induced hypogonadism involves a mechanism of PRL-originated, macrophage-mediated inhibitory regulation of T release by the Leydig cells. This is a new mechanism, which adds to our understanding of the inhibition of reproductive functions by PRL. We conclude that TNF-α, one of the macrophage-secreted cytokines, plays an active role in this mechanism.

ACKNOWLEDGMENTS

This study was supported by Grant VGH 89-092, VGH 90-104 from the Taipei Veterans General Hospital and Grant NSC 89-2314-B-075-104 from National Science Council of ROC both awarded to W.J. Huang, MD, PhD. It was also supported by awards from the Medical Research and Advancement Foundation in memory of Dr. Chi-Shuen Tsou, ROC, to P.S. Wang, PhD. The rat PRL RIA kit was kindly supplied by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and The U.S. Department of Agriculture, USA.

REFERENCES

Bartke A, Dalterio S. 1976. Effect of prolactin on the sensitivity of the testis to LH. Biol Reprod 15:90–93.

Bartke A, Klemcke H, Matt K. 1986. Effects of physiological and abnormally elevated prolactin levels on the pituitary-testicular axis. Med Biol 63:264–272.

Boyar RM, Kapen S, Finkelstrin JW, Perlow M, Sassin JF, Fukushima DK, Weitzman ED, Hellman L. 1974. Hypothalamic-pituitary function in diverse hyperprolactinaemic states. J Clin Invest 53:1588–1598.

Calkins JH, Sigel MM, Nankin HR, Lin T. 1988. Interleukin-1 inhibits Leydig cell steroidogenesis in primary culture. Endocrinology 123:1605–1610.

Calkins JH, Guo H, Sigel MM, Lin T. 1990a. Differential effects of recombinant interleukin-1α and β on Leydig cell function. Biochem Bioph Res Co 167:548–553.

Calkins JH, Guo H, Sigel MM, Lin T. 1990b. Tumor necrosis factor-α enhances inhibitory effects of interleukin-1β on Leydig cell steroidogenesis. Biochem Bioph Res Co 166:1313–1318.

deGreef WJ, Ooms MP, Vreeburg JTM, Weber RFA. 1995. Plasma levels of luteinizing hormone during hyperprolactinemia: Response to central administration of antagonists of corticotropin-releasing factor. Neuroendocrinology 61:19–26.

Dirami G, Poulter LW, Cooke BA. 1991. Separation and characterization of Leydig cells and macrophages from rat testes. J Endocrinol 130:357–365.

Dombrowicz D, Sente B, Closset J, Hennen G. 1992. Dose-dependent effects of human prolactin on the immature hypophysectomized rat testis. Endocrinology 130:695–700.

Everett JW. 1954. Luteotropic function of autografts of the rat hypophysis. Endocrinology 54:685–690.

Fauser BCJM, Galway AB, Hsueh AJW. 1989. Inhibitory actions of interleukin-1β on steroidogenesis in primary cultures of neonatal rat testicular cells. Acta Endocrinol 120:401–408.

Gaytan F, Romero JL, Bellido C, Morales C, Reymundo C, Aguilar E. 1994. Effects of growth hormone and prolactin on testicular macrophages in long-term hypophysectomized rats. J Reprod Immunol 27:73–84.

Hales DB. 1992. Interleukin-1 inhibits Leydig cell steroidogenesis primarily by decreasing 17α-hydroxylase/C17-20 lyase cytochrome P450 expression. Endocrinology 131: 2165–2172.

Hales DB, Xiong Y, Tur-Kaspa I. 1992. The role of cytokines in the regulation of Leydig cell p450c17 gene expression. J Steroid Biochem Mol Biol 43:907–914.

Hayes R, Chalmers SA, Nikolic-Paterson DJ, Atkins RC, Hedger MP. 1996. Secretion of bioactive interleukin 1 by rat testicular macrophages in vitro. J Androl 17: 41–49.

Hedger MP. 1997. Testicular leukocytes: What are they doing? Rev Reprod 2:38–47.

Huang WJ, Yeh JY, Tsai SC, Lin H, Chiao YC, Chen JJ, Lu CC, Hwang SW, Wang SW, Chang LS, Wang PS. 1999. Regulation of testosterone secretion by prolactin in male rats. J Cell Biochem 74:111–118.

Huang WJ, Yeh JY, Kan SF, Chang LK, Wand PS. 2001. Effects of hyperprolactinemia on testosterone production in rat Leydig cells. J Cell Biochem 80:313–320.

Hutson JC. 1992. Development of interdigitations between Leydig cells and macrophages. Cell Tissue Res 267:385–389.

Hutson JC. 1993. Secretion of tumor necrosis factor alpha by testicular macrophages. J Reprod Immunol 23:63–72.

Klemcke HG, Bartke A. 1981. Effects of chronic hyperprolactinemia in mice on plasma gonadotropin concentrations and testicular human chorionic gonadotropin binding sites. Endocrinology 108:1763–1768.

Krummen LA, Moore A, Woodruff TK, Covello R, Taylor R, Working P, Mather JP. 1994. Localization of inhibin and activin binding sites in the testis during development by in situ ligand binding. Biol Reprod 50:734–744.

Le J, Vilcek J. 1987. Tumor necrosis factor and interleukin 1: Cytokines with multiple overlapping biological activities. Lab Invest 56:234–244.

Li X, Youngblood GL, Payng AH, Hales DB. 1995. Tumor necrosis factor-α inhibition of 17α-hydroxylase/C17-20 lyase gene (cyp17) expression. Endocrinology 136:3519–3526.

Lin T, Guo H, Calkins JH, Wang D, Chi R. 1991a. Recombinant monocyte-derived interleukin-1 receptor antagonist reverses inhibitory effects of interleukin-1 on Leydig cell steroidogenesis. Mol Cell Endocrinol 78: 205–209.

Lin T, Wang D, Nagpal ML, Calkins JH, Chang W, Chi R. 1991b. Interleukin-1 inhibits cholesterol side-chain cleavage cytochrome P450 expression in primary cultures of Leydig cells. Endocrinology 129:1305–1311.

Lin T, Wang D, Nagpal ML, Chang W. 1994. Recombinant murine tumor necrosis factor-alpha inhibits cholesterol side-chain cleavage cytochrome P450 and insulin-like growth factor-I gene expression in rat Leydig cells. Mol Cell Endocrinol 101:111–119.

Lin H, Wang SW, Tsai SC, Chen JJ, Chiao YC, Lu CC, Huang WJS, Wang GJ, Chen CF, Wang PS. 1998. Inhibitory effect of digoxin on testosterone secretion through mechanisms involving decrease of cyclic AMP production and cytochrom P450scc activity in rat testicular interstitial cells. Br J Pharmacol 125:1635–1640.

Mauduit C, Hartmann DJ, Chauvin MA, Revol A, Morera AM, Benahmed M. 1991. Tumor necrosis factor α inhibits gonadotropin action in cultured porcine Leydig cells: Site(s) of action. Endocrinology 129:2933–2940.

Mauduit C, Chauvin MA, Hartmann DJ, Revol A, Morera AM, Benahmed M. 1992. Interleukin-1α as a potent inhibitor of gonadotropin action in porcine Leydig cells: Site(s) of action. Biol Reprod 46:1119–1126.

Mauduit C, Gasnier F, Rey C, Chauvin M-A, Stocco DM, Louisot P, Benahmed M. 1998. Tumor necrosis factor-α inhibits Leydig cell steroidogenesis through a decrease in steroidogenic acute regulatory protein expression. Endocrinology 139:2863–2868.

McNeilly AS, Sharpe RM, Davidson DW, Fraser HM. 1978. Inhibition of gonadotrophin secretion by induced hyperprolactinemia in the male rat. J Endocrinol 79:59–68.

Merchenthaler I, Lennard DE, Cianchetta P, Merchenthaler A, Bronstein D. 1995. Induction of proenkephalin in tuberoinfundibular dopaminergic neurons by hyperprolactinemia: The role of sex steroids. Endocrinology 136: 2442–2450.

Miller SC, Bowman BM, Rowland HG. 1983. Structure, cytochemistry, endocytic activity, and immunoglobulin (Fc) receptors of rat testicular interstitial-tissue macrophages. Am J Anat 168:1–13.

Molitch ME. 1992. Pathologic hyperprolactinemia. Endocrin Metab Clin N Am 21:877–901.

Niemi M, Sharpe RM, Brown WRA. 1986. Macrophages in the interstitial tissue of the rat testis. Cell Tissue Res 243:337–344.

Saidi K, Wenn RV, Sharif F. 1977. Bromocriptine for male infertility. Lancet 1 (8005):250–251.

Steel RGD, Torrie JH. 1960. Principles and procedures of statistics. New York: McGraw-Hill.

Stocco DM, Clark BJ. 1996. Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 17:221.

Sun XR, Hedger MPM, Risbridger GP. 1993. The effect of testicular macrophages and interleukin-1 on testosterone production by purified adult rat Leydig cells cultured under in vitro maintenance conditions. Endocrinology 132:186–192.

Tang TK, Wang SW, Wang PS. 1986. Effects of thyroidectomy and thyroxine replacement on the responsiveness of the anterior pituitaries from male rats to thyrotropin-releasing hormone in vitro. Experientia 42:1031–1034.

Themmen APN, Molenaar R, Visser WJ, Jongkind JF, Rommerts FFG, van der Molen HJ. 1987. Comparison of the cellular composition and steroidogenic properties of preparations of interstitial cells isolated from immature and mature rat testis. J Endocrinol 112:361–366.

Tsai SC, Chen JJ, Chiao YC, Lu CC, Lin H, Yeh JY, Lo MJ, Kau MM, Wang SW, Wang PS. 1997. The role of cyclic AMP production, calcium channel activation and enzyme activities in the inhibition of testosterone secretion by amphetamine. Br J Pharmacol 122:949–955.

Wang PS, Tsai SC, Hwang GS, Wang SW, Lu CC, Chen JJ, Liu SR, Lee KY, Chien EJ, Chien CH, Lee HY, Lau CP, Tsai CL. 1994. Calcitonin inhibits testosterone and luteinizing hormone secretion through a mechanism involving an increase in cAMP production in rats. J Bone Miner Res 9:1583–1590.

Wang D, Nagpal ML, Shimasaki S, Ling N, Lin T. 1995. Interleukin-1 induces-like growth factor binding protein-3 gene expression and protein production by Leydig cells. Endocrinology 136:4049–4055.

Xiong Y, Hales DB. 1993. The role of tumor necrosis factor-α in the regulation of mouse Leydig cell steroidogenesis. Endocrinology 132:2438–2444.

Yee JB, Hutson JC. 1983. Testicular macrophages: Isolation, characterization and hormonal responsiveness. Biol Reprod 29:1319.

Yee JB, Hutson JC. 1985. Effects of testicular macrophage-conditioned medium on Leydig cells in culture. Endocrinology 116:2682–2684.