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Interleukin 1-β Injected into the Testis Acutely Stimulates and Later Attenuates Testicular Steroidogenesis of the Immature Rat

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The effect of intratesticular administration of interleukin-1β (IL-1β) on steroidogenesis was studied in immature and adult rats. In 21-d-old animals local bilateral injection or unilateral administration of 0.1 µg/testis of IL-1β to hemicastrates resulted in a significant increase in basal testosterone secretion in vitro and serum testosterone concentration one day posttreatment. Six days after treatment the cytokine induced opposite effect in animals with two testes *in situ*, i.e., it suppressed steroidogenesis. When IL-1β was combined with hemicastration, IL-1β failed to alter the parameters studied. In adult animals subjected to bilateral treatment or to unilateral injection followed by hemicastration, IL-1β in doses of 1.5 µg/testis or 15 µg/testis did not influence steroidogenesis and serum testosterone concentration. No change in serum LH and FSH concentration could be observed in any experimental group. The data suggest that the proinflammatory cytokine IL-1β exerts a local action on testicular steroidogenesis, and the effect is age-dependent.

Key Words: Interleukin-1β; steroidogenesis; local control; age-dependent effect.

Introduction

Cytokines are regulatory proteins involved in the communication network of immune-competent cells *(1)*. Several cytokines including the proinflammatory cytokine family, the interleukin-1 (IL-1) system, have been implicated as local regulators of different bodily systems and organs, including the testis *(2–4)*. The family of IL-1 is composed of the two agonist isotypes IL-1α and IL-1β and the naturally occurring IL-1 receptor antagonist *(5,6)*. Two IL-1 receptors have been characterized: IL-1-type I and IL-1-type II receptors *(7)* that bind both IL-1α and IL-1β and the antagonist *(8)*. In the testis IL receptors are expressed in Sertoli, peritubular, and Leydig cells, pachytene spermatocytes, and early spermatids *(9)*. IL-1α is constitutively produced and secreted by Sertoli cells from postnatal d 20 *(10-15)*. There are some observations that spermatocytes and spermatids also synthesize IL-1α constitutively *(16)*. Several data indicate the physiological significance of IL-1α acting as a paracrine factor in the control of a wide range of testicular functions including differentiation of the organ, steroidogenesis, and spermatogenesis *(3,4)*. The effect of IL-1β on testicular physiology and pathology is not fully understood.

IL-1β is synthesized as an inactive 35 kDa precursor that is proteolytically processed to an active molecule (17 kDa) at the time of secretion *(17)*. By contrast to IL-1α, IL-1β does not seem to be constitutively synthesized in the healthy testis *(14,15,18)*. However, Leydig cells and testicular macrophages are able to express mRNA also for IL-1β *(13,19–23)* and secrete the protein *(24)* in response to proinflammatory stimuli. No detectable IL-1β production by Sertoli cell cultures could be demonstrated *(25)*.

IL-1β has been reported to affect Sertoli cell functions, spermatogenesis, and steroidogenesis. Petersen et al. *(26)* have demonstrated that in immature rat Sertoli cells both IL-1α and IL-1β increase 3H-thymidine incorporation, induce multiplication and morphological changes of the cultured cells. IL-1β proved to be less efficient than IL-1α in stimulation of Sertoli cell proliferation, but showed equal efficiency as FSH. Also, it was demonstrated that IL-1β significantly increases the capacity of Sertoli cell cultures (Sertoli cells isolated from 15-d-old mice) to secrete transferrin *(25)*.

Studies investigating the effect of IL-1β on testosterone secretion of cultured Leydig cells show conflicting results: IL-1β has been reported both to suppress *(27–30)* and stimulate steroidogenesis *(31, 32)*.

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Recent studies indicate that the effect of IL-1α and IL-1β on testicular steroidogenesis is age-dependent: in immature Leydig cells IL-1 stimulates, while in adult cultures it suppresses basal testosterone secretion. In both age groups IL-1 inhibited hCG-induced androgen formation *(33)*.

The purpose of the present study was to investigate the local, in vivo effect of IL-1β on testicular steroidogenesis in immature and adult rats.

Results

Observations in Immature Rats

Short-Term Study

One day after bilateral intratesticular injection of IL-1β (0.1 μg/testis) to 21-d-old rats a significant weight increase of the treated testes could be observed compared to animals who underwent saline administration (right control testis vs right treated testis: $p < 0.001$; left control testis vs left treated testis: $p < 0.01$) (Fig. 1, lower panel). Treatment resulted in a significant rise in both basal testosterone secretion in vitro ($p < 0.05$) (Fig. 1, middle panel) and serum testosterone concentration ($p < 0.05$) (Fig. 1, upper panel).

Unilateral local administration of the cytokine combined with the removal of the contralateral testis did not influence the weight increase of the remaining, treated testis (Fig. 2, lower panel). The treatment resulted in a significant rise in both basal testosterone secretion in vitro ($p < 0.05$) (Fig. 2, middle panel) and serum testosterone concentration ($p < 0.05$) (Fig. 2, upper panel).

Long-Term Study

Six days after treatment, bilateral administration of IL-1β induced no change in testicular weight (Fig. 3, lower panel) and serum testosterone concentration (Fig. 3, upper panel), but resulted in a significant decrease in steroidogenesis of the treated testes (right control testis vs right treated testis: $p < 0.01$; left control testis vs left treated testis: $p < 0.001$) (Fig. 3, middle panel). In hemicastrates, local treatment with IL-1β had no effect on testicular weight, steroidogenesis, and serum testosterone concentration (data not shown).

Systemic administration of IL-1β (0.1 μg/2 μL sc) either to hemicastrates or to rats with two testes did not alter the parameters studied 1 or 6 d posttreatment (data not shown).

Observations in Adult Animals

Six days posttreatment bilateral intratesticular injection of IL-1β (1.5 μg/30 μL or 15 μg/30 μL per testis) did not influence testis weight, testicular steroidogenesis, and serum testosterone concentration (Fig.4). In hemicastrated rats local treatment of the right testis with 15 μg/30 μL did not alter the parameters studied (data not shown).

IL-1β treatment did not affect serum LH and FSH concentration in any experimental group (data not shown).

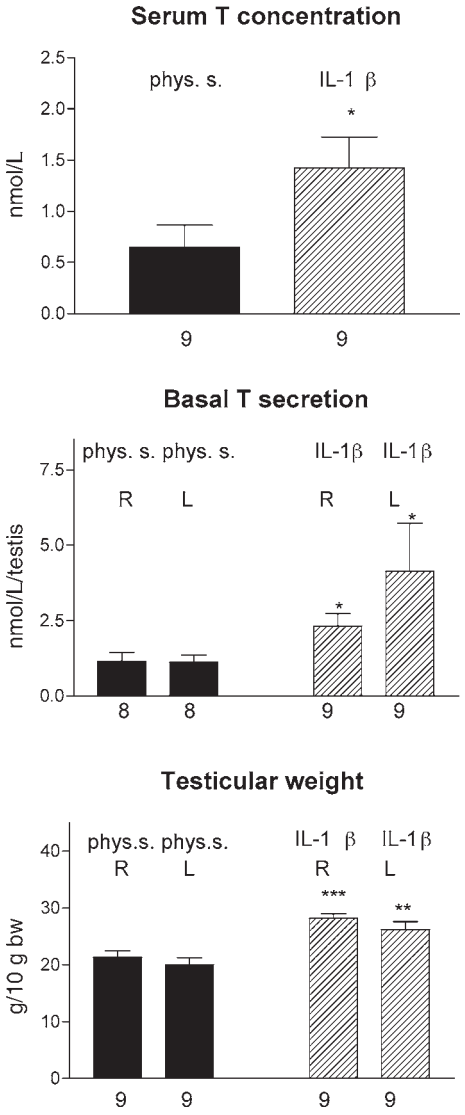

Fig. 1. The effect of bilateral intratesticular administration of 0.1 μg/2 μL IL-1β on serum testosterone concentration, on basal testosterone secretion in vitro, and on testicular weight in 21-d-old rats. Animals were sacrificed 1 d posttreatment. Values are mean ± SEM. Figures below the horizontal axis indicate the number of animals. L: left testis; phys.s.: physiological saline; R: right testis; T: testosterone. Asterisk indicates statistically significant difference (*$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$).

Discussion

The results of the present study indicate that intratesticular injection of IL-1β affects steroidogenesis. However, local effect of the cytokine is not uniform; it depends on the age of the animals, on the time elapsed between treatment and sacrifice, and on whether the rats had two testes *in situ* or were hemicastrated.

IL-1β affected steroidogenesis in immature rats but was without effect in adults. The results of Svechnikov et al. *(33)* indicate that both isoforms of IL-1 had a stimulatory effect on testosterone production of purified immature Ley-

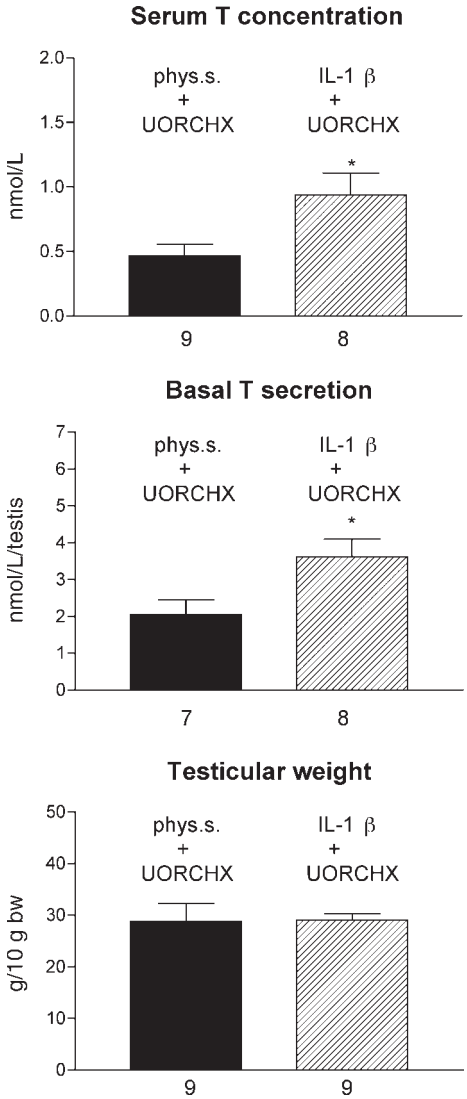

Fig. 2. The effect of unilateral administration of 0.1 μg/2 μL IL-1β in hemicastrates on serum testosterone concentration, on basal testosterone secretion in vitro, and on the weight of the remaining testis in 21-d-old rats. Animals were sacrificed 1 d posttreatment and hemicastration. Values are mean ± SEM. Figures below the horizontal axis indicate the number of animals. phys.s.: physiological saline; T: testosterone; UORCHX: unilateral orchidectomy. Asterisk indicates statistically significant difference (*$p <$ 0.05).

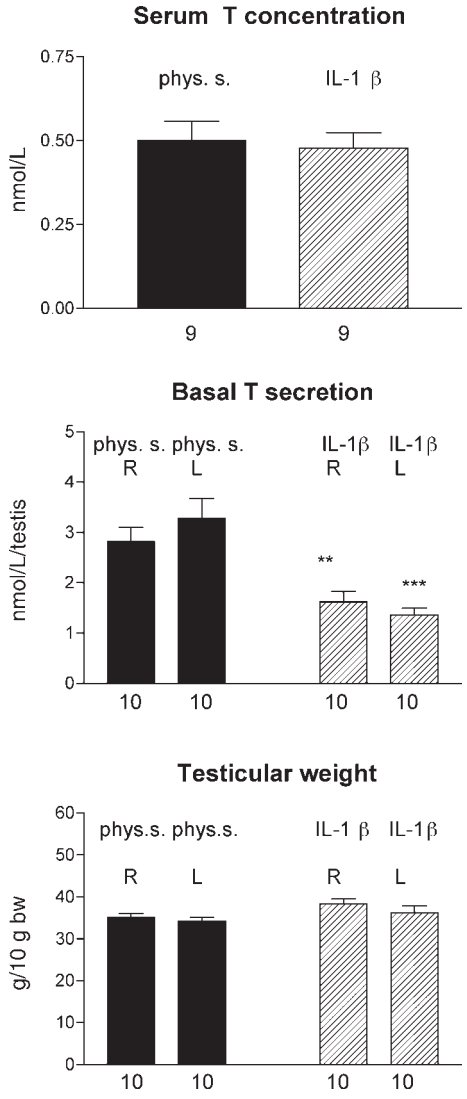

Fig. 3. The effect of bilateral intratesticular administration of 0.1 μg/2 μL IL-1β on serum testosterone concentration, on basal testosterone secretion in vitro, and on the weight of the remaining testis in 21-d-old rats. Animals were treated at 21 d of age and sacrificed 6 d later. Values are mean ± SEM. Figures below the horizontal axis indicate the number of animals. L: left testis; phys.s.: physiological saline; R: right testis; T: testosterone. Asterisk indicates statistically significant difference (**$p < 0.01$; *** $p < 0.001$).

dig cells and an inhibitory action on testosterone formation by adult Leydig cells in culture. The age-dependent effects of IL-1β are in agreement with previous observations. Tahri-Joutei and Pointis *(39)* have reported developmental changes in the steroidogenic responsiveness of purified Leydig cells to arginine-vasopressin and oxytocin. Moreover, our previous in vivo studies indicate that the local effect of somatostatin *(40)*, pituitary adenylate cyclase–activating peptide, vasoactive intestinal peptide *(41)*, or serotonin *(42)* on steroidogenesis is different in immature and in adult animals. A real explanation of the age-dependent effect cannot be offered at present.

As one possibility, the age-dependent effect of IL-1β on testicular steroidogenesis may be related to the type of Leydig cells present in the testis at the time of the treatment. In the rat, during the first two postnatal weeks, testes contain fetal-type Leydig cells *(35)*. Between the second and third postnatal week, the second generation of Leydig cells, the adult-type ones differentiate and gradually replace the fetal-type Leydig cells. One of the explanations for the age-dependent effect of IL-1β might be the difference in the expression of the two types of IL receptors *(33)*. A stronger expression of IL-1 receptor type I (active signaling receptor) was observed in immature Leydig cells,

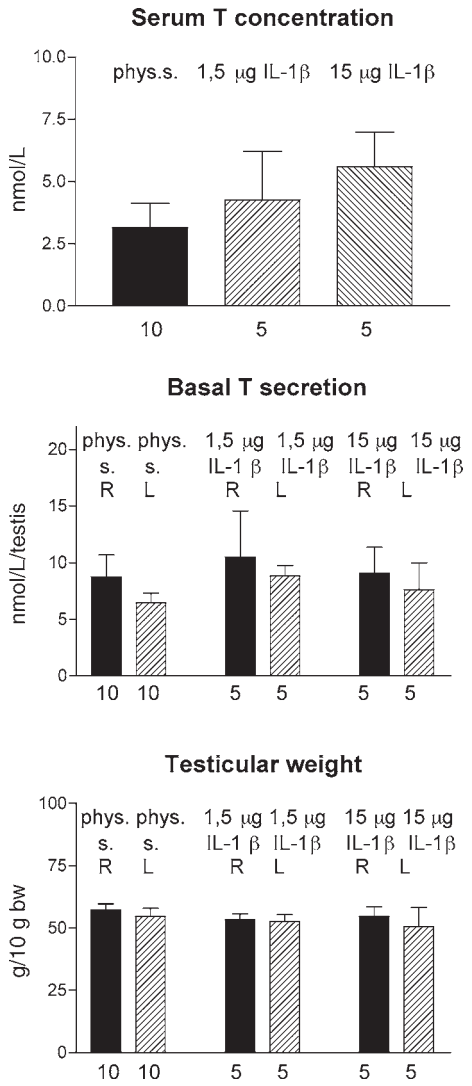

Fig. 4. The effect of bilateral intratesticular administration of 1.5 μg/30 μL or 15 μg/30 μL/testis IL-1β on serum testosterone concentration, on basal testosterone concentration in vitro and on testicular weight in adult rats. Animals were sacrificed 6 d after treatment. Values are mean ± SEM. Figures below the horizontal axis indicate the number of animals. L: left testis; phys.s.: physiological saline; R: right testis; T: testosterone.

whereas the inhibitory IL-1 receptor type II, that is considered an inhibitory decoy receptor *(36),* was detectable only in adult Leydig cells. In addition, IL-1β has been reported to induce proliferation of immature Leydig cells *(30)* and expression of several interleukins including IL-1α, IL-1β, and IL-16 in progenitor Leydig cells isolated from prepubertal but not adult rats *(37)*. The high potency of immature Leydig cells of secreting testosterone in response to IL-1β might be due to maturational difference in the steroidogenic pathway *(38)*. The age-dependent effect of IL-1β on steroidogenesis is consistent with the observations that indicate certain locally produced peptides exert different actions on Leydig cell steroid secretion during testicular ontogenesis. Another explanation of the lack of effect of IL-1β on steroidogenesis in adults could be that the dose of IL-1β given to adults was not adequate. Of course this possibility cannot be excluded, but does not seem to be very likely, because the adult rats were treated with two fairly different doses (1.5 or 15.0 μg).

In the present short-term study in immature rats local bilateral administration or unilateral injection of IL-1β in hemicastrates stimulated basal testosterone secretion in vitro and resulted in an increase in serum testosterone concentration. By contrast, in the long-term study the cytokine had an opposite effect in animals with two testes in situ: it suppressed steroidogenesis. However, when IL-1β treatment was combined with hemicastration, the cytokine failed to alter the parameters studied. These observations suggest an uniform effect of the cytokine both in animals with two testes and in hemicastrates in short-term experiments and different effect (lack of effect in hemicastrates) in the long-term study. This observation could be explained by assuming that hemicastration, i.e., a particular condition when the hypothalamo–pituitary axis is in a stimulated stage, might interfere with the inhibitory action of IL-1β on steroidogenesis. Interestingly enough, local treatment of the testis with different biologically active substances produced locally, such as somatostatin *(40)*, pituitary adenylate cyclase–activating peptide, vasoactive intestinal peptide *(41),* or serotonin *(42)* proved to induce a more profound effect in hemicastrates.

It should be mentioned that intratesticular injection of IL-1β, but not IL-1α or other substances studied (histamine, serotonin, bradykinin) induced inflammation-like changes in vascular permeability and morphology of the testis *(43)*. Similar studies in immature animals are not available.

The observation that systemic administration of IL-1β did not interfere with testicular steroidogenesis, serum testosterone, and LH concentration, strongly suggests that the site of action of the local treatment with cytokine is in the testis and its effect on steroid secretion is not mediated by altering LH secretion. This assumption is further supported by several observations that indicate that intracerebroventricular administration of IL-1, especially IL-1β, compared with IL-1α suppresses LH release through blockage of the hypothalamic (preoptic area, mediobasal hypothalamus) gonadotropin-releasing hormone while peripheral administration of IL-1 does not alter serum LH concentration *(44–51)*.

When taking into account that IL-1β is not produced constitutively by the testis, but its expression can be induced by proinflammatory stimuli *(13,19–24)* the data of the present in vivo experiments and those of the mentioned in vitro studies strongly suggest that IL-1β might exert an age-dependent local regulatory action on steroidogenesis.

Materials and Methods

Animals

Pregnant CFY (originally Sprague–Dawley) rats were housed in a light- (lights on for 12 h) and temperature- (22

± 2°C) controlled room with free access to rat chow and tap water. After delivery, 10 newborn male rats were placed with each lactating female.

Experiments, Treatments

Experiments were carried out in immature (21-d-old) and adult animals (weighing 220 ± 10 g). In both immature and adult rats IL-1β or physiological saline was injected into both testes or IL-1β or saline was administered into one testis and at the same time the other gonad was removed. Additional groups included animals treated systemically with IL-1β (the same dose that was used for local treatment, i.e., 0.1 μg sc). Intratesticular injections and unilateral gonadectomy were made under ether anaesthesia. Immature animals were sacrificed 1 d or 6 d and adult rats 6 d after the interventions. The experimental procedures were approved by the Local Animal Care and Use Committee in accordance with NIH Guidelines for the Care and Use of Laboratory Animals.

Experiments in Immature Animals

In 21-d-old rats the testes were exposed and 0.1 μg/2 μL per testis of human recombinant IL-1β (Sigma Chemical Co., St. Louis, MO) dissolved in physiological saline was injected bilaterally into the testes using a Hamilton microsyringe. Control animals were treated locally with the corresponding volume of physiological saline. In additional groups of animals immediately after injection of IL-1β or the vehicle into the right testis, the left gonad was removed. Animals of the groups were sacrificed 1 or 6 d posttreatment.

Experiments in Adult Animals

IL-1β (1.5 μg or 15 μg/30 μL per testis) was injected bilaterally into the testes. In another group of animals following the administration of 15 μg/30 μL IL-1β into the right testis, the left gonad was removed. In control groups the same volume of physiological saline was given intratesticularly. Animals were sacrificed 6 d after interventions.

The doses applied and the time elapsed between treatment and sacrifice were chosen on the basis of several preliminary studies.

Blood Collection

Animals were sacrificed by decapitation, and trunk blood was collected. Blood was allowed to clot, and serum was separated by centrifugation and was stored at –20°C until assayed for testosterone, LH, and FSH.

Testicular Incubation

When animals were killed, testes were immediately removed, decapsulated, and incubated in medium 199 containing 25 m*M* Hepes for 3 h, in a metabolic shaker at 35°C. In the case of adult testes the medium also contained 0.1% bovine serum albumin/mL. The volume of medium was 1 mL for testes for 22- and 27-d-old rats at sacrifice and 3 mL for adult animals. The incubation time for immature testes was 3 h, while for adult gonads it was 2 h. After the incubation, media were transferred to tubes and stored at –20°C until assayed for testosterone. The results are expressed as testosterone produced per testis (nmol/L/testis) during the incubation period.

Assay

Testosterone concentrations of serum or tissue culture medium (Medium) were determined by RIA as previously described in detail *(34)*. Briefly, 20 μL serum was extracted with 2 ml ether. The dried extract was dissolved in 500 μL assay buffer (ASB, 0.5 *M* PBS with 1 g/L gelatin, pH 7.4). For standard, Calbiochem Testosterone for HPLC standard was used in a nine-step series ranging 3.5 to 1000 fmol/tube. Direct determination was made from 20 μL Medium samples. In this case, to each standard tube 20 μL unused Medium was added. The RIA tubes contained the samples (in duplicates) or standards, 7 nL/tube antibody (CV-RT 17, 1:100 000 final dilution) and 12,000 cpm ^{3}H-labeled testosterone (100 fmol, The Radiochemical Center, Amersham) in a total volume of 0.7 mL ASB. The cross reactions of the antibody were: 5α-DHT 45%, 5β-DHT 9%, and androstendione 2%. With 27 other natural and synthetic steroids examined, the antibody showed less than 0.05% cross reaction. After an overnight incubation at 4°C, the bound and free steroids were separated by dextran-coated charcoal. The radioactivity was measured in a two-phase liquid scintillation system. The sensitivity limit of the assay is 3 fmol/tube. The inter- and intraassay coefficients for variation were 9.8% and 5.9.8%, respectively.

LH and FSH levels from rat sera were determined by RIA utilizing National Hormone and Pituitary Program kit. For reference, rLH-RP-3 and rFSH-RP-2 preparations were used. The inter- and intraassay coefficients of variation were 7–9% and 4–6%, respectively.

Statistical Analysis

Results were analyzed by ANOVA followed by Student–Newman–Keuls multiple comparison methods. When only two means were compared, Student's *t* test was performed. Results were considered significant if $p < 0.05$.

Acknowledgments

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