Interleukin-1 α stimulates steroidogenic acute regulatory protein expression via p38 MAP kinase in immature rat Leydig cells

K Svechnikov^{1,3}, D M Stocco² and O Söder¹

¹Department of Woman and Child Health, Pediatric Endocrinology Unit, Karolinska Institute and Hospital, S-17176 Stockholm, Sweden

²Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas 79430, USA

³Endocrinological Genetics Unit, Institute of Cytology and Genetics, 630090 Novosibirsk, Russia

(Requests for offprints should be addressed to K Svechnikov, Department of Woman and Child Health, Astrid Lindgren Children's Hospital, Pediatric Endocrinology Unit, Karolinska Institute and Hospital (Q2:08), SE-171 76 Stockholm, Sweden; Email: konstantin.svechnikov@kbh.ki.se)

Abstract

We have investigated the involvement of the steroidogenic acute regulatory (StAR) protein in interleukin-1 α (IL-1 α)-induced steroidogenesis in immature (40-day-old) and adult Leydig cells *in vitro*. Further, IL-1 α -mediated signaling pathway(s) controlling StAR expression in immature Leydig cells were also studied. IL-1 α stimulated both androgen production and StAR protein expression in a dose- and time-dependent manner in immature but not adult Leydig cells. These effects of IL-1 α were prevented by pretreatment of the cells with the specific inhibitors of the p38 MAP kinase, SB203580 and PD169316, suggesting that this kinase is an important part of IL-1 α signaling in the immature Leydig cell. The present results suggest that IL-1 α , which is constitutively produced by the rat testis from postnatal day 25, is an important paracrine regulator of postnatal Leydig cell maturation. Regulation of StAR protein expression is one of the possible mechanisms by which IL-1 α contributes to the differentiation of immature Leydig cells into adult cells.

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Introduction

Interleukin-1 α (IL-1 α) is a multifunctional cytokine induced in association with inflammation, infection and cancer (Dinarello 1991), and has also been found to be constitutively produced by normal tissues (Tovey *et al.* 1988) including the testis (Khan *et al.* 1987, Jonsson *et al.* 1999, Sultana *et al.* 2000). In the rat testis, IL-1 α mRNA and protein are highly expressed from postnatal day 25 and evidence indicates that IL-1 α is involved in the paracrine regulation of spermatogenesis (Pöllänen *et al.* 1989, Parvinen *et al.* 1991, Wahab-Wahlgren *et al.* 2000, Gustafsson *et al.* 2002) as well as Leydig cell steroidogenesis (Calkins *et al.* 1988, Moore & Moger 1991, Svechnikov *et al.* 2001). IL-1 α has been shown to inhibit luteinizing hormone (LH)/human chorionic gonadotrophin (hCG)- and cAMP-stimulated testosterone production by Leydig cells (Verhoeven *et al.* 1988, Moore &

Moger 1991, Svechnikov *et al.* 2001), with the major site of inhibition occurring at the level of the 17 α -hydroxylase/C17–20 lyase enzyme (Verhoeven *et al.* 1988, Svechnikov *et al.* 2001). Reports on IL-1 α action on basal testosterone production are conflicting, demonstrating either stimulatory (Verhoeven *et al.* 1988, Moore & Moger 1991, Svechnikov *et al.* 2001) or no effects (Calkins *et al.* 1988). Recently, we have found that the effect of IL-1 on steroidogenesis was dependent on the stage of maturation of the responding Leydig cells (Svechnikov *et al.* 2001). IL-1 isoforms were shown to stimulate basal testosterone production by immature but not adult Leydig cells and the effect was found to be IL-1 type I receptor (IL-1RI)-mediated. We hypothesized that the acute stimulatory effect of IL-1 α on steroidogenesis by immature (40-day-old) Leydig cells might be mediated by the stimulation of steroidogenic acute regulatory (StAR) protein expression, whose

function is to translocate cholesterol from the outer to the inner mitochondrial membrane, the rate-limiting step in steroid hormone formation (Stocco 2001). Our attention was focused on IL-1 α -mediated signaling pathway(s) controlling StAR expression in immature Leydig cells. It is generally accepted that StAR gene expression is positively regulated by cAMP-dependent protein kinase A in steroidogenic cells (Christenson & Straus 2000), although other signaling systems have also been implicated in StAR gene expression (Wang *et al.* 2000, Gyles *et al.* 2001). The mitogen-activated protein (MAP) kinase family, which includes extracellular signal-regulated kinases (ERK2/1), c-Jun N-terminal kinases, and p38 kinases (p38 MAP kinase) could also be involved in the regulation of StAR gene expression. These enzymes are the terminal kinases in three distinct but interacting signal transduction cascades (Pearson *et al.* 2001) and are activated by IL-1 in different cell systems (Bankers-Fulbright *et al.* 1996). ERK2/1 was recently shown to regulate StAR gene expression by stimulating steroidogenic factor 1 phosphorylation in mouse adrenocortical cells (Gyles *et al.* 2001). To the best of our knowledge, virtually nothing is known about the role of p38 MAP kinase in steroidogenic cells. The purpose of the present study was to investigate whether StAR protein is involved in IL-1 α -induced stimulation of steroidogenesis in immature Leydig cells in culture. In addition, the IL-1 α -mediated signaling pathway(s) controlling StAR expression were also investigated. We found that IL-1 α is a potent stimulator of StAR protein expression in immature but not adult Leydig cells. IL-1 α -induced StAR expression is positively regulated by p38 MAP kinase in immature Leydig cells.

Materials and methods

Materials

Dulbecco's modified Eagle's medium (DMEM)–Ham's nutrient mixture F12, modified Eagle's medium, Hank's balanced salts solution (HBSS) without Ca²⁺ and Mg²⁺ and penicillin–streptomycin were obtained from Gibco/BRL (Life Technologies, Paisley, Strathclyde, UK). BSA (fraction V), Percoll, Hepes, collagenase type I, 22(R)-hydroxycholesterol (22R-OHC) and p38 MAP kinase antibodies (rabbit polyclonal IgG,

affinity purified) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Cytochrome P450 side chain cleavage enzyme (P450 scc) antibodies (rabbit polyclonal IgG, affinity purified) were obtained from Chemicon International Inc. (Temecula, CA, USA). Rat recombinant IL-1 α was purchased from RD Systems (Oxon, UK). PD169316 and SB203580 were obtained from Calbiochem (La Jolla, CA, USA). Phospho-specific p38 MAP kinase antibodies (rabbit polyclonal IgG, affinity purified) were purchased from New England Biolabs, Inc. (Beverly, MA, USA). The IL-1RI antibodies, mouse and rat reactive (rabbit polyclonal IgG, affinity purified) were obtained from Santa Cruz Biotech Inc. (Santa Cruz, CA, USA).

Animals

Male Sprague–Dawley rats (B&K Laboratories, Sollentuna, Sweden) aged 40 and 65–75 days were used as a source of immature and adult Leydig cells respectively (Ge *et al.* 1996, Mendis-Handagama & Ariyaratne 2001). Animals were fed a freely available standard pellet diet and water. Animal experiments were approved by the Northern Stockholm Animal Ethics Committee (registration no. N102/99).

Isolation and culture of Leydig cells

Leydig cells were prepared from immature (40-day-old) and adult rat testes by collagenase treatment as described earlier (Svechnikov *et al.* 2001). Briefly, decapsulated testes were incubated with collagenase (0.25 mg/ml) for 20 min at 37 °C. Crude interstitial cells were collected by centrifugation at 300 *g* for 10 min and then washed twice in 0.1% (w/v) BSA in HBSS. To obtain purified Leydig cells, the crude cell suspension was loaded on top of a discontinuous Percoll gradient (20, 40, 60 and 90% Percoll in HBSS) and centrifuged at 800 *g* for 20 min. The fractions enriched in Leydig cells were then centrifuged through a continuous self-generated 60% Percoll gradient at 20 000 *g* for 30 min at 4 °C. The purity of Leydig cells was 90%, as determined by histochemical 3 β -hydroxysteroid dehydrogenase staining (Payne *et al.* 1980). Cell viability was greater than 90% as assessed by trypan blue exclusion. Purified Leydig cells were washed twice with DMEM–F12 and

resuspended in DMEM-F12 supplemented with 15 mM Hepes (pH 7.4), 1 mg/ml BSA, 365 mg/l glutamine, 100 IU/ml penicillin and 100 μ g/ml streptomycin. The cells (1.5×10^5 cells/ml) were plated in a volume of 100 μ l in 96-well plates (Falcon, Franklin Lakes, NS, USA) and cultured for 24 h. The culture medium was replaced by fresh medium and the cells were pre-incubated with the specific inhibitors of the p38 MAP kinase, PD169316 and SB203580 (0.01–0.1 μ M) for 30 min, followed by incubation with IL-1 α (20 pg/ml) or 22R-OHC (1 μ M) for another 24 h. The range of concentrations of the inhibitors used was specific for inhibition of p38 MAP kinase without affecting activities of a number of other protein kinases (Davies *et al.* 2000). No changes in viability and the cell morphology after treatment with IL-1 α were found.

Testosterone determination

Culture media were collected and stored at -20°C until assayed for testosterone concentrations with a Coat-a-Count RIA kit (Diagnostic Products Corp., Los Angeles, CA, USA), according to the manufacturer's instructions.

Western blot analysis

The effects of IL-1 α and the inhibitors on p38 MAP kinase phosphorylation and StAR expression was analyzed by PAGE/Western blot. The cells were washed twice with PBS and then lysed and sonicated in a lysis buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 50 mM dithiothreitol and 10% glycerol. The cytosolic fraction was collected as a supernatant after centrifugation at 10 000 *g* for 6 min. Proteins from whole Leydig cells (30 μ g from each sample) were resolved on 10% SDS/polyacrylamide gels and electrophoretically transferred to Hybond-P polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Amersham, Bucks, UK), using 25 mM Tris, 185 mM glycine, pH 8.3, containing 20% methanol. Following transfer, the membrane was incubated in a blocking buffer (Tris-buffered saline (TBS) buffer containing 5% non-fat dry milk) for 1 h and washed three times with TBS/Tween 0.1% (3×10 min). Membranes were incubated with antibodies directed to phospho-p38 MAP kinase and total p38 MAP kinases according to the

manufacturer's specifications (Sigma and New England Biolabs). StAR polyclonal antiserum (Clark *et al.* 1994) and P450 scc antibodies were used for incubation at 1:4000 dilution in TBS containing 5% non-fat dry milk overnight at 4°C . The IL-1RI antibodies were used at 1:500 dilution at the same conditions as described for StAR protein. After washing, membranes were incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody (Amersham Pharmacia Biotech). Blots were incubated with ECL Plus Western blotting agent (Amersham Pharmacia Biotech) and exposed to X-ray film (Amersham Pharmacia Biotech).

Statistical analyses

The X-ray films were scanned using an HP ScanJet 5100C and HP PrecisionScan software (Hewlett Packard Sverige AB, Kista, Sweden). The images obtained were analyzed using NIH Image 1.57 software. Levels of StAR, P450 scc and IL-1RI were expressed as the fold difference (mean $\pm$ s.e) vs cultured untreated control cells or adult cells in the case of IL-1RI. The densitometry value of the control was set to 1. Statistical significance between groups was determined using Student's *t*-test. The results from dose-response experiments were analyzed by one-way ANOVA where appropriate and supplemented by the Dunnett *t*-test. $P < 0.05$ was considered as significant.

Results

IL-1RI is more highly expressed in immature than adult rat Leydig cells

We examined the IL-1RI protein level in immature and adult rat Leydig cells. Whole cell protein extract was prepared and analyzed by Western blotting. Leydig cell IL-1RI migrated with an apparent molecular mass of 80 kDa, and was expressed more strongly (2.2-fold, $P < 0.05$) in immature than in adult cells (Fig. 1).

Dose-dependent and time-dependent stimulation of StAR expression by IL-1 α in immature Leydig cells

Our previous findings have shown that IL-1 isoforms significantly stimulate steroidogenesis by

Figure 1 Immature Leydig cells express higher levels of IL-1RI protein than adult Leydig cells. Whole cell lysates were prepared from cultured Leydig cells and 50 μ g total protein were subjected to immunoblotting with anti-IL-1RI antibodies. Each IL-1RI-specific band was quantified using NIH Image software and expressed as the fold difference between immature and adult Leydig cells. The position of the molecular mass marker (80 kDa) is indicated. Top, representative blot. Bottom, data from four separate Leydig cell preparations are presented (means $\pm$ S.E.). * P <0.05 compared with adult cells.

immature but not adult Leydig cells (Svechnikov *et al.* 2001). As shown in Fig. 2, immature but not adult Leydig cells were capable of significant (7.6-fold over control, P <0.01) expression of StAR protein in response to IL-1 α . The basal testosterone secretion was 1.02 ± 0.30 ng/ml in immature cells and 3.80 ± 0.26 ng/ml in adult Leydig cells. Further, IL-1 α was found to stimulate StAR expression in cultured immature Leydig cells in a dose-dependent manner. A significant effect (3-fold over control, P <0.05) on StAR expression was observed at IL-1 α concentrations as low as 5 pg/ml, and maximal induction (9.5-fold over control, P <0.01) was found at IL-1 α concentrations of 20 pg/ml (Fig. 3A). Coincident with the increase in StAR protein levels, testosterone production by Leydig cells was increased (4-fold over control) at a concentration of 20 pg/ml IL-1 α (Fig. 3B). When the cells were cultured with 20 pg/ml rat IL-1 α for various periods of time, a time-dependent effect of IL-1 α on StAR expression was demonstrated. A significant stimulatory effect was evident within 3 h of incubation, reaching 8.5-fold increase over

Figure 2 Effect of IL-1 α on StAR protein expression in immature and adult rat Leydig cells. Cells were cultured for 24 h, fresh medium was added and the cultures were continued in the presence or absence of rat IL-1 α (20 pg/ml) for an additional 24 h. Whole cell extracts (30 μ g protein) from both immature (40 day) and adult Leydig cells were analyzed by Western blotting using antiserum specific for StAR. Each StAR-specific band was quantified using NIH Image software and expressed as the fold difference vs cultured untreated control cells. The position of the StAR protein is indicated. Top, representative blot. Bottom, data from three separate Leydig cell preparations are presented (means $\pm$ S.E.). ** P <0.01 compared with control from immature cells.

control (P <0.01) after 24 h (Fig. 4A). This effect was accompanied by an increase in androgen production by immature Leydig cells. The testosterone level was increased by 3 h, and was maximally elevated at 24 h (Fig. 4B).

IL-1 α induces phosphorylation of p38 MAP kinase in immature Leydig cells

Since IL-1 signaling in many cell systems occurs through activation of p38 MAP kinase, we investigated whether IL-1 α activates p38 MAP kinase in immature Leydig cells in culture. IL-1 α elicited phosphorylation of p38 MAP kinase in immature cells, and the specific inhibitor of the kinase, SB203580, markedly suppressed this effect of IL-1 α (Fig. 5).

Inhibition of p38 MAP kinase decreases IL-1 α -induced testosterone production by immature Leydig cells

To assay whether the activation of p38 MAP kinase is involved in IL-1 α -mediated Leydig cell steroidogenesis, the p38 MAP kinase-specific inhibitors SB203580 and PD169316 were employed. Both

Figure 3 Dose-dependent effect of IL-1 α on StAR protein expression and testosterone production by immature rat Leydig cells *in vitro*. Cells were cultured for 24 h, fresh medium was added and the cells were incubated with or without increasing concentrations of rat IL-1 α for an additional 24 h. (A) Whole cell lysates (30 μ g) were used to analyze StAR protein by Western blotting. Results are expressed as described in Fig. 2. The position of the StAR protein is indicated. Top, representative blot. Bottom, data from three separate Leydig cell preparations are presented (means $\pm$ s.e.). (B) Testosterone production in the medium was measured by RIA. The results are means $\pm$ s.e. of triplicate determinations. The results were confirmed in two additional experiments. * P <0.05, ** P <0.01 compared with control.

compounds significantly inhibited IL-1 α -induced steroidogenesis in Leydig cells at the highest concentration (0.1 μ M), resulting in about a 60% reduction of the IL-1 α effect (Fig. 6A). No effect on basal steroidogenesis was found (Fig. 6B).

To investigate the site of steroidogenesis affected by p38 MAP kinase inhibitors, the effect of SB203580 on androgen precursor-induced testosterone production by immature Leydig cells in culture was studied. Co-treatment of the cells with SB203580 and 22R-OHC completely reversed the

Figure 4 Time-dependent effect of IL-1 α on StAR protein expression and testosterone production by immature rat Leydig cells *in vitro*. Cells were cultured for 24 h, fresh medium was added and the cultures were continued in the presence or absence of rat IL-1 α (20 pg/ml) for various periods of time. (A) Whole cell lysates (30 μ g) were used to analyze StAR protein by Western blotting. Results are expressed as described in Fig. 2. The position of the StAR protein is indicated. Top, representative blot. Bottom, data from three separate Leydig cell preparations are presented (means $\pm$ s.e.). (B) testosterone production in the medium was measured by RIA. The results were confirmed in two additional experiments. * P <0.05, ** P <0.01 compared with control.

inhibition of steroidogenesis induced by the inhibitor (Fig. 7), suggesting that the expression of StAR protein might be regulated by p38 MAP kinase.

Effect of p38 MAP kinase inhibitor SB203580 on IL-1 α -induced StAR expression by immature Leydig cells

Since the p38 MAP kinase inhibitors used inhibited androgen production stimulated by IL-1 α , we investigated whether SB203580 may affect IL-1 α -induced StAR expression by Leydig cells. IL-1 α

Figure 5 SB203580 inhibits IL-1 α -induced phosphorylation of p38 MAP kinase in immature Leydig cells *in vitro*. The cultured cells were pretreated with 1 μ M SB203580 (lane 3 and 4) or vehicle for 30 min, and then stimulated by rat IL-1 α (1 ng/ml) (lane 2 and 4) or vehicle (lane 1 and 3) for 15 min. The cells were lysed and 30 μ g total protein were used to analyze phosphorylated and total p38 MAP kinases by Western blotting. Upper panel, antibodies specific for phosphorylated p38 MAP kinase. Lower panel, anti-p38 MAP kinase antibodies. The results were confirmed in two additional experiments.

significantly (7-fold over control, $P < 0.01$) stimulated StAR protein expression, while SB203580 markedly (50% of the value with IL-1 α alone) suppressed this stimulation (Fig. 8). In a separate experiment the effect of SB203580 (1 μ M) on P450 scc expression was investigated. No significant changes in the P450 scc level after treatment with SB203580 (1 μ M) were found (1.000 ± 0.030 -fold in IL-1 α -treated cells vs 1.180 ± 0.035 -fold in IL-1 α +SB203580-treated cells, $P = 0.34$).

Discussion

The present study is the first to demonstrate that IL-1 α is a potent stimulator of StAR protein expression in immature but not adult Leydig cells. We provide evidence that this process is positively regulated by p38 MAP kinase. A stimulatory effect of IL-1 on basal testosterone production by rat Leydig cells in culture has previously been found in both prepubertal (Verhoeven *et al.* 1988) and adult animals (Moore & Moger 1991) but the regulatory mechanisms of the process have been unclear. The present data have confirmed our previous suggestion that acute steroidogenesis induced by IL-1 α in immature Leydig cells is mediated by StAR protein (Svechnikov *et al.* 2001). In our experiments a close correlation between StAR protein expression activated by IL-1 α and testosterone production by Leydig cells was found. In contrast, no correlation between StAR protein expression and corticosteroid production after acute short-term stimulation

Figure 6 Inhibition of p38 MAP kinase abolishes IL-1 α -driven steroidogenesis in Leydig cells. Cells were cultured for 24 h, fresh medium was added and the cells were pretreated with or without increasing concentrations of PD169316 or SB203580 for 30 min, and then incubated with rat IL-1 α (20 pg/ml) (A) or vehicle (B) for an additional 24 h. Testosterone production in the medium was measured by RIA. Results are means $\pm$ S.E. of triplicate determinations. Each experiment was performed four times with the same results. * $P < 0.05$, ** $P < 0.01$ compared with the value of IL-1 α alone.

of adrenal cells with adrenocorticotropin (ACTH) has been shown, since only a small fraction of newly synthesized StAR was active (Artemenko *et al.* 2001). Differences in cell systems used (adrenocortical vs Leydig cells), time of treatment (short-term vs long-term stimulation) and types of activators used (ACTH vs IL-1 α) might explain this discrepancy.

We show here that IL-1 α stimulates phosphorylation of p38 MAP kinase, suggesting that this enzyme is activated in the responding Leydig cell. The involvement of p38 MAP kinase in the regulation of StAR protein expression is supported

Figure 7 Site of action of SB203580 on IL-1 α -stimulated testosterone production by immature Leydig cells *in vitro*. Cells were cultured for 24 h, fresh medium was added and the cells were pretreated with or without increasing concentrations of SB203580 for 30 min, and then incubated with rat IL-1 α (20 pg/ml) and 22R-OHC (1 μ M) or vehicle for an additional 24 h. Testosterone production in the medium was measured by RIA. Each experiment was performed four times with the same results. ** $P < 0.01$ compared with the effect of IL-1 α alone.

Figure 8 Inhibition of p38 MAP kinase decreases StAR expression induced by IL-1 α in immature Leydig cells *in vitro*. Cells were cultured for 24 h, fresh medium was added and the cells were pretreated with SB203580 (1 μ M) or vehicle for 30 min, and then incubated with rat IL-1 α (20 pg/ml) or vehicle for an additional 24 h. Whole cell extracts (30 μ g) were analyzed by Western blotting using antiserum specific for StAR. Results are expressed as described in Fig. 2. The position of the StAR protein is indicated. Top, representative blot. Bottom, data from four separate Leydig cell preparations are presented (means $\pm$ s.e.). ^a $P < 0.01$ compared with control, ^b $P < 0.05$ compared with the value of IL-1 α alone.

by several observations. First, IL-1 α -stimulated testosterone production was shown to be inhibited by both SB203580 and PD169316, specific inhibitors of p38 MAP kinase (Pearson *et al.* 2001), and this effect was abolished by 22R-OHC, a cell-permeable form of cholesterol. These findings give evidence that both inhibitors suppress Leydig cell steroidogenesis at the level of StAR protein, and that p38 MAP kinase seems to be involved in this process. Indeed, in further experiments SB203580 was found to significantly inhibit IL-1 α -induced StAR expression. Moreover, we used low concentrations of p38 MAP kinase inhibitors (up to 1 μ M), which were shown to specifically suppress activity of p38 MAP kinase without affecting activities of other kinases (Davies *et al.* 2000), adding to the relevance of our findings. One possible mechanism by which p38 MAP kinase activated by IL-1 α could promote StAR expression in Leydig cells is activation of the nuclear transcription factor C/EBP β (NF-IL6 or LAP). This factor belongs to the family of CCAAT/enhancer-binding proteins and has been reported to be an IL-1-inducible factor (Isshiki *et al.* 1990). The expression of C/EBP β is activated by several inflammatory agents, such as IL-6, lipopolysaccharide and tumor necrosis factor- α (Metz & Ziff 1991), and is one of the targets of p38 MAP kinase (Baldassare *et al.* 1999). Interestingly, C/EBP β is expressed in steroidogenic cells, including Leydig cells (Nalbant *et al.* 1998) and ovarian granulosa cells (Sirois & Richards 1993) and two putative C/EBP-binding sites in the StAR promoter have been identified (Reinhart *et al.* 1999, Silverman *et al.* 1999). Another transcriptional factor that is known to be one of the targets for p38 MAP kinase and was shown recently to be involved in StAR expression is a cAMP response-element binding protein (CREB). Transient expression of wild-type CREB in MA-10 mouse Leydig tumor cells was found to increase the levels of (Bu)₂cAMP-induced StAR promoter activity, StAR mRNA and StAR protein expression. These responses were significantly inhibited by transfection with a dominant-negative CREB, or with a CREB mutant that cannot be phosphorylated (Manna *et al.* 2002). It can be hypothesized that p38 MAP kinase might regulate CREB phosphorylation and StAR gene expression through activation of the ribosomal S6 kinase (RSK) family member, RSK-B. This kinase is a p38 MAP kinase substrate and

phosphorylates CREB and c-Fos proteins (Pierrat *et al.* 1998).

Activation of StAR expression by IL-1 α in immature Leydig cells can itself promote Leydig cell maturation. Immature Leydig cells expressing a higher level of StAR protein seem to have a significant capacity for producing androgens, which may be an important transition phase involved in the onset of adult Leydig cell differentiation. This hypothesis is consistent with the notion that androgens are required for the maturational acquisition of steroidogenic enzymes in later steps of Leydig cell postnatal maturation (Mendis-Handagama & Ariyaratne 2001). Immature Leydig cells have been found to express the highest levels of both androgen receptor mRNA and protein (Buzek & Sanborn 1988), suggesting that androgens are needed in the differentiation of these cells into adult Leydig cells. This suggestion is in agreement with the observation that administration of androgens restored Leydig cell morphology and function in LH-releasing hormone antagonist-treated immature rats (Misro *et al.* 1993).

Our findings demonstrating that IL-1RI expression is dependent on the maturational stage of Leydig cell development suggest that the amount of IL-1RI expressed might explain, at least in part, the different capacity of immature and adult Leydig cells to express StAR in response to IL-1 α . The present data with IL-1RI protein confirm our previous finding of a stronger expression of IL-1RI mRNA in immature than in adult Leydig cells as revealed by RT-PCR analysis (Svechnikov *et al.* 2001). Moreover, we have previously shown that IL-1 receptor type II (IL-1RII) mRNA expression is detectable only in adult Leydig cells (Svechnikov *et al.* 2001). Since IL-1RII is a scavenger receptor neutralizing IL-1 action (Neumann *et al.* 2000), this may also contribute to the low response of adult cells to IL-1 action.

Collectively, our previous (Svechnikov *et al.* 2001) and present data form the basis of a hypothesis that IL-1 α is an important paracrine factor involved in the onset of final Leydig cell maturation. This suggestion is supported by several observations. First, we have recently shown that IL-1 α has biological action on Leydig cell function by activating steroidogenesis in immature Leydig cells (Svechnikov *et al.* 2001). This effect was receptor-mediated and blockade of IL-1RI reversed IL-1 α -mediated effects. Secondly, IL-1 α is detect-

able in the rat testis from postnatal day 20 and reaches high concentrations from day 25 (Jonsson *et al.* 1999, Wahab-Wahlgren *et al.* 2000). Moreover, the intratesticular concentration of IL-1 α protein in the rat testis is in the same bioactive range as those used in our present Leydig cell cultures *in vitro* (Gustafsson *et al.* 1988, 2002, Wahab-Wahlgren *et al.* 2000, Jonsson *et al.* 2001), adding to the potential physiological relevance of our findings.

In conclusion, the results of the current study show clearly and for the first time that IL-1 α is a potent stimulator of StAR protein expression in immature but not adult Leydig cells. IL-1 α -induced StAR expression is positively regulated by p38 MAP kinase in immature Leydig cells. The data presented here suggest that IL-1 α is an important paracrine factor regulating postnatal Leydig cell maturation and IL-1 α -mediated StAR protein expression is one of the mechanisms that might contribute to differentiation of immature Leydig cells into adult cells.

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