Regulation of activin A and inhibin B secretion by inflammatory mediators in adult rat Sertoli cell cultures

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Abstract

The regulation of Sertoli cell activin A and inhibin B secretion during inflammation was investigated in vitro. Adult rat Sertoli cells were incubated with the inflammatory mediators, lipopolysaccharide (LPS), interleukin-1β (IL-1β), IL-6 and the IL-1 receptor antagonist (IL-1ra) over 48h in culture. Activin A, inhibin B and IL-1α were measured in the culture medium by specific two-site ELISAs. Both IL-1β- and LPS-stimulated activin A and inhibited inhibin B secretion. LPS also stimulated the production of IL-1α in the cultures. In contrast to IL-1β, IL-6 had no effect on activin A, although it did have a significant inhibitory effect on inhibin B secretion. Ovine follicle-stimulating hormone (FSH) and the cAMP analogue dibutyryl cAMP opposed the actions of IL-1 and LPS by suppressing activin A and IL-1α secretion and by stimulating inhibin B. Blocking IL-1 activity in the cultures by addition of an excess of IL-1ra completely prevented the response of activin A to exogenous IL-1β, and reduced the response to LPS by 50%. In the presence of IL-1ra, basal secretion of inhibin B was increased, but IL-1ra was unable to reverse the suppression of inhibin B by LPS. These data indicate the importance of both IL-1 isoforms in regulating secretion of activin A and inhibin B by mature Sertoli cells during inflammation. The data also establish that inflammation exerts its effects on activin A and inhibin B secretion via other pathways in addition to those mediated by IL-1, and that hormonal stimulation by FSH and cAMP moderates the Sertoli cell response to inflammation. Interference with the complex interactions between these cytokines and hormones may contribute to the disruption of reproductive function that can accompany infection and illness in men.

Introduction


The actual mechanisms whereby inflammation inhibits spermatogenesis are uncertain. Although spermatogenesis is disrupted by heat, there is little direct evidence that an increase in temperature is the principal cause of testicular failure in febrile patients. In rats treated with LPS, spermatogenesis is disrupted even though this species is relatively resistant to the pyrogenic effects of this agent (Horan et al. 1989, O’Bryan et al. 2000). The effects of LPS-induced inflammation on spermatogenesis are also quite distinctly different in specificity and timing to the effects of androgen withdrawal (McLachlan et al. 1996, O’Bryan et al. 2000). In fact, even very high doses of LPS do not reduce intratesticular levels of testosterone in the rat much below 30% of normal, which is well above the threshold necessary to sustain spermatogenesis in this species (Sharpe et al. 1988, McLachlan et al. 1996). Altogether, the evidence suggests that inflammation exerts direct inhibitory effects on the seminiferous epithelium, and that disruption of normal regulatory networks may be involved (see review by Hedger & Meinhardt 2003).

Among the earliest events during inflammation is up-regulation of the pro-inflammatory cytokine, interleukin-1 (IL-1), which is produced as one of two isoforms with identical biological activity, IL-1α and IL-1β (Dinarello 1996). In turn, IL-1 stimulates production of the regulatory inflammatory cytokine, IL-6. In the normal testis, IL-1α is constitutively produced by Sertoli cells and IL-6 is produced by the Sertoli cell in response to IL-1α (Gérard et al. 1991, Syed et al. 1995). The production of...
both cytokines by the Sertoli cells is stimulated by the developing germ cells and residual bodies (Gérard et al. 1992, Syed et al. 1995, Jonsson et al. 1999), and both cytokines have been implicated in the control of spermatogonial and spermatocyte development (Parvinen et al. 1991, Söder et al. 1991, Hakovirta et al. 1995). Expression of IL-1β in the normal testis is relatively low, but this cytokine is up-regulated during inflammation due to production by testicular macrophages and Leydig cells (Gow et al. 2001, Jonsson et al. 2001, O’Bryan et al. 2005). Testicular production of IL-6 also increases during inflammation in adult rats (O’Bryan et al. 2005). The response of testicular IL-1α to inflammation is less clear. Production of this cytokine by cultured Sertoli cells is up-regulated by inflammatory stimuli (Gérard et al. 1992, Stéphan et al. 1997), but recent studies suggest that intratesticular IL-1α expression may actually decline during acute inflammation in vivo (Jonsson et al. 2001, O’Bryan et al. 2005).

The FSH-regulating hormone inhibin is a dimer of a common α subunit and either a βA or βB subunit, producing inhibin A or inhibin B, respectively (de Kretser & Robertson 1989). In the male, inhibin B is preferentially produced by the Sertoli cell. Activin A is a homodimer of the βA subunits of inhibin and is an endogenous stimulator of FSH release. In addition, activin A is a feedback regulator of IL-1 and IL-6 action in the liver and haematopoietic systems (Phillips et al. 2001). Activin A is stimulated by a number of inflammatory regulators, including LPS and IL-1, in various cell types (Shao et al. 1992, Abe et al. 2001, Okuma et al. 2005), and levels of activin A increase relatively rapidly in serum during inflammation (Phillips et al. 2001). Activin A is produced by the Sertoli cell and has effects on spermatogonial proliferation (Mather et al. 1990, Meehan et al. 2000). Thus, IL-1, IL-6 and activin A are all local regulators of spermatogonial proliferation and development, in addition to their immunoregulatory roles. In order to understand how inflammation might impact upon testicular regulation it is important to understand how these cytokines are regulated and how they interact during inflammation.

Previously, we have shown that Sertoli cells from immature (20 day old) rats respond to exogenous IL-1 by producing activin A, coinciding with a reduction in secretion of inhibin B (Okuma et al. 2005). This regulation is opposed in a reciprocal manner by FSH and cAMP. These data suggest that the Sertoli cell should respond to inflammatory stimuli by producing activin A, while reducing inhibin B secretion, and that endogenous IL-1α produced by the Sertoli cells would be involved in this pathway. This hypothesis was examined in Sertoli cell cultures from adult rats, since it has been shown that the Sertoli cell displays a developmentally-related increase in constitutive IL-1α production (Gérard et al. 1991, Jonsson et al. 1999).

Materials and Methods

Adult Sertoli cell cultures

Adult (80–100 days old) male Sprague–Dawley rats were obtained from Central Animal Services, Monash University, Victoria, Australia. Sertoli cells were prepared as previously described (Lampa et al. 1999), with some modifications. Briefly, a single testis was dissociated in 1 mg/ml trypsin (Sigma), 1 mg/ml collagenase (Type II, 300 U/ml; Worthington Biochemical, Lakewood, NJ, USA) and 0.5 mg/ml hyaluronidase (Type II; Sigma) in 0.01 M phosphate buffered saline (PBS) containing Ca2+ and Mg2+ by shaking (45 min, 34 °C) in an orbital mixer incubator (Ratek Instruments, Melbourne, Australia) at 90 cycles/min and 1 cm amplitude. The tubule fragments were washed into Ca2+- and Mg2+-free PBS and then cultured for a total of 5 days at 37 °C in 5% CO2/air (pH 7.2–7.3). Medium was replaced on day 3 of culture with fresh medium containing the appropriate test substances in triplicate wells: LPS (from E.Coli, serotype 0127:B8; Sigma), recombinant human (rh)IL-1β (200 000 U/µg) and rhIL-1α (160 000 U/µg) (R & D Systems, Minneapolis, MN, USA), rhIL-6 (PeproTech, Rocky Hill, NJ, USA), rhIL-1 receptor antagonist (IL-1ra; Amgen, Thousand Oaks, CA, USA), the cAMP analogue dibutyryl cAMP (dbcAMP; Sigma), and ovine FSH (NIH oFSH-20, 4453 IU/mg; NIDDK, Bethesda, MD, USA). Medium was collected 48 h later and stored at −20 °C prior to analysis. All experiments were repeated at least twice to confirm the reproducibility of the results.

The presence of peritubular cells was assessed by alkaline phosphatase staining (Chapin et al. 1987), and smooth muscle actin immunocytochemistry (Schlatt et al. 1996), as previously described. Peritubular cell contamination was routinely 5–10% as assessed by these procedures.

Activin A and inhibin B ELISAs

Activin A was measured using ELISA (Knight et al. 1996) according to the manufacturer’s instructions (Oxford
Bio-Innovations, Oxfordshire, UK) with some modifications. The recombinant activin A standard (Robertson et al. 1992) and samples were diluted in culture medium. A 6% sodium dodecyl sulphate solution in PBS was added (3% final concentration) followed by boiling for 3 min. The samples were allowed to cool before the addition of H<sub>2</sub>O<sub>2</sub> (2% final concentration) and subsequent 30 min incubation. Duplicates were added to the E4 (anti-β<sub>A</sub> subunit) monoclonal antibody coated plate and incubated overnight at room temperature. The plates were washed and the second detection antibody (biotinylated-E4) was added for 2 h at room temperature. After washing, alkaline phosphatase linked to streptavidin was added to the wells and incubated at room temperature for 1 h. After further washes, the alkaline phosphatase activity was detected using an amplification kit (ELISA Amplification System; Invitrogen, Carlsbad, CA, USA) whereby the substrate was incubated for 1 h at room temperature. This assay has an intra-plate coefficient of variation (CV) of 7-4% and an inter-plate CV of 6-3%, with an assay detection limit of 10 pg/ml (n=12 assays).

Inhibin B was measured using a specific ELISA (Groome et al. 1996) according to the manufacturer’s instructions (Oxford Bio-Innovations, Oxfordshire, UK) with some modifications. The standard used was WHO 96/784 inhibin B reference standard (NIBSC, Potter’s Bar, UK). Standards and samples were diluted in unconditioned culture medium and treated as per the manufacturer’s protocol. Duplicates were added to the plates and incubated overnight at room temperature. The plates were washed and the second detection antibody (R1 coupled to alkaline phosphatase) was added for 3 h at room temperature. After washing, the alkaline phosphatase activity was detected using an amplification kit (ELISA Amplification System; Invitrogen) whereby the substrate was incubated for 2 h at room temperature. This assay has an intra-plate CV of 4-1% and an inter-plate CV of 6-6%, with an assay detection limit of 5 pg/ml (n=7 assays).

**Rat IL-1α ELISA**

Secreted IL-1α was measured in the culture medium using a commercial two-site rat IL-1α ELISA ( Biosource International, Camarillo, CA, USA) which employed recombinant rat IL-1α as standard. Cross-reactivities with rat IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-10, IFN-γ and TNFα were <0-1%, according to the manufacturer’s specifications. Samples were assayed in a single plate assay with a sensitivity of 4 pg/ml and an intra-assay CV of 8-4%.

**Statistical analyses**

All data were analysed using one-way or two-way ANOVA following appropriate transformations to normalize data and equalize variance, where necessary. Mean values were compared using either Student–Newman–Keuls multiple range test or Dunnett’s test for multiple comparisons with a control (Sigmasstat version 1.0; Jandel Scientific Software, San Rafael, CA, USA). Differences between responses were considered statistically significant at the *P*<0.05 level.

**Results**

In adult Sertoli cell cultures, both IL-1α and IL-1β stimulated activin A secretion in an identical dose-dependent manner (Fig. 1A). All subsequent data are from studies using exogenous IL-1β. Basal production of activin A over 48 h varied between 55–160 pg/ml in 8 cultures (mean ± s.e.m.: 103 ± 39 pg/ml). In the same cultures, 40U/ml IL-1β stimulated secretion by an average of 2.1 ± 0.7 fold (range 1.4–3.6). Although a consistently effective dose, it should be noted that 40 U/ml IL-1β was not a maximum stimulatory dose for activin A secretion. IL-1β had a relatively minor inhibitory effect on inhibin B in some experiments (Fig. 1B), but this was not consistently observed (cf. Figs. 2B, 4C and 7B). In contrast to the IL-1 isoforms, FSH and dbcAMP generally had no effect on activin A (data not shown) over a range of doses that consistently stimulated inhibin B secretion (Fig. 1C and 1D). However, a small stimulatory effect on activin A secretion by supramaximal doses of dbcAMP was occasionally observed in the cultures (cf. Fig. 5A). In contrast to IL-1β, a relatively high concentration of IL-6 had no effect on activin A secretion, but had a significant inhibitory effect on inhibin B secretion (Fig. 2).

At doses up to 500 µg/ml, LPS stimulated activin A and IL-1α secretion, and inhibited inhibin B secretion by adult Sertoli cell cultures (Fig. 3). The data suggested the existence of two phases of response to LPS, with a smaller plateau of effect at doses below 25 µg/ml, and a much greater effect above this dose. A maximum stimulatory effect was observed at a dose of approximately 250 µg/ml, with an average increase over basal secretion of 5.2 ± 1.8 fold (mean ± s.e.m.; n=4 cultures). Higher doses of LPS (>500 µg/ml) were toxic to the Sertoli cells, as indicated by the loss of cellular processes, rounding and detachment of the cells in culture (data not shown). Although the data are not presented, very similar effects of LPS on activin A, IL-1α and inhibin B were observed using Sertoli cells from immature (20 day old) rats.

In cultures of Sertoli cells stimulated with IL-1β, both FSH and dbcAMP antagonised activin A secretion (Fig. 4A and 4B). However, at the highest doses used, dbcAMP induced a greater and more consistent inhibition than FSH. Stimulation of inhibin B secretion by dbcAMP was not affected by the presence of IL-1β (Fig. 4C). Stimulation of both activin A and IL-1α secretion by LPS was inhibited by the co-administration of dbcAMP (Fig. 5A and 5B).
Blocking of endogenous IL-1α activity in the cultures with excess doses of IL-1ra had no effect on basal activin A secretion (data not shown), but increased basal inhibin B secretion in a dose-dependent manner (Fig. 6). IL-1ra completely blocked the stimulatory action of IL-1β on activin A (Fig. 7A) but had no additional effect on inhibin B in the presence of exogenous IL-1β (Fig. 7B). IL-1ra also reduced LPS-stimulated activin A secretion, but even a high dose could not reduce this below 50% (Fig. 7C). In contrast, IL-1ra had no effect on the inhibition of inhibin B secretion by LPS (Fig. 7D).

Discussion

Treatment of adult rats with an inflammatory stimulus (i.e. LPS) in vivo disrupts both Leydig cell function and spermatogenesis, and is accompanied by a local inflammatory cytokine response (O’Bryan et al. 2000, O’Bryan et al. 2005). However, the intensity of the inflammatory cytokine response in the testis is substantially reduced compared with that observed in other tissues, such as the liver, indicating that testicular inflammatory responses are uniquely modulated by local mechanisms. The present study establishes that LPS alters activin A and inhibin B secretion by Sertoli cells from adult rats. LPS causes an increase in activin A secretion by the Sertoli cells, which is partially mediated by endogenous IL-1α but not by IL-6, and a decrease in basal and FSH/cAMP-stimulated secretion of inhibin B, mediated in part by IL-1α and possibly, IL-6. These data confirm our previous observation that IL-1 plays a role in the control of the production of these important testicular regulators (Okuma et al. 2005), and further indicate that other components of the inflammatory cascade also must be involved. FSH, acting through its intermediate cAMP, reverses the effects of inflammation on activin A, which has important implications in vivo since serum FSH levels are directly inhibited by systemic inflammation as well (Refojo et al. 1998). The observation that these regulatory responses occur in Sertoli cells from both adult and immature rats indicates that these responses are a property of the mature testis. It is concluded that alterations in activin A and inhibin B production by the Sertoli cell may play an important role in the
pathogenesis of inflammation-induced infertility, and also in controlling the severity of testicular inflammation responses.

In a previous study using cultured Sertoli cells from immature rats, IL-1 and FSH/cAMP exerted a reciprocal regulation of activin A and inhibin B secretion (Okuma et al. 2005). Although IL-1 also stimulated production of activin A by Sertoli cells from adult rats in the present study, IL-1 was not a particularly effective inhibitor of inhibin B production over the same range of doses in these cultures. Reciprocal regulation of the IL-1-mediated responses by FSH/cAMP was observed in the adult Sertoli cell cultures. IL-6 had no effect on activin A, but had a significant inhibitory effect on inhibin B secretion by adult Sertoli cells, in contrast to the lack of effect of IL-6 in immature Sertoli cell cultures (Okuma et al. 2005).

Figure 2 Secretion of activin A (A) and inhibin B (B) by adult rat Sertoli cells after 48 h in culture with no addition (control), IL-1β (IL-1; 40 U/ml) alone, IL-6 (50 ng/ml) alone (control), or IL-1β and IL-6 in combination. All values are mean ± S.E.M, n = 3 wells. Values with same letter superscript are not significantly different (P>0.05). *significantly different at P<0.05; "not significantly different (P>0.05).

Figure 3 Secretion of activin A (A), inhibin B (B) and IL-1α (C) by adult rat Sertoli cells after 48 h in culture with lipopolysaccharide (LPS). All values are mean ± S.E.M, n = 3 wells. *significantly different from control (0 µg/ml) at P<0.05; **P<0.01; ***P<0.001; "not significantly different from control (P>0.05).
The inflammatory mediator LPS also stimulated activin A and blocked inhibin B production quite effectively. This latter effect appeared to be partially mediated via regulation of endogenous Sertoli cell production of IL-1α, as it could be reduced by administration of excess IL-1ra. These data indicate that local production of IL-1α and IL-6 by the adult Sertoli cell appears to be involved in autocrine or paracrine regulation of activin A and inhibit B secretion within the testis, and that activation of inflammatory responses affect activin A and inhibit B secretion via these pathways.

As was observed in cultures of Sertoli cells from immature testes (Okuma et al. 2005), there was a large variation in basal secretion of activin A by the adult Sertoli cell cultures. This did not appear to be due to the activity

Figure 4	Secretion of activin A and inhibin B by adult rat Sertoli cells after 48 h in culture. A: no addition (control), IL-1β (40 U/ml) alone, ovine FSH (500 ng/ml) alone (control) or IL-1β and FSH in combination. B and C: no addition (control), IL-1β (40 U/ml) alone, dbcAMP (1000 μg/ml) alone (control) or IL-1β and dbcAMP in combination. All values are mean ± S.E.M, n=3 wells. Values with same letter superscript are not significantly different (P>0.05).

Figure 5	Secretion of activin A (A) and IL-1α (B) by adult rat Sertoli cells after 48 h in culture with no addition (control), LPS (125 μg/ml) alone, dbcAMP (1000 μg/ml) alone (control) or LPS and dbcAMP in combination. All values are mean ± S.E.M, n=3 wells. Values with same letter superscript are not significantly different (P>0.05).
of endogenous IL-1α because addition of IL-1ra had no effect on basal production of activin A by these cells. Presumably, other autocrine factors were responsible. On the other hand, the effect of exogenous IL-1β on inhibin B in the adult cultures was less consistent than in the immature cultures. This difference in sensitivity was most likely attributable to the increased levels of endogenous IL-1α in the adult Sertoli cell cultures compared with those from immature rats (Gérard et al. 1991). Accordingly, blockade of IL-1 action by IL-1ra significantly enhanced basal levels of inhibin B in adult cultures, which also indicates that endogenous IL-1α is a negative regulator for inhibin B production by Sertoli cells. The data in the present study suggest that IL-6 is also a negative regulator of inhibin B secretion in adult Sertoli cells, and that its effects are separate and additive with those of IL-1, although it has no corresponding effect on activin A secretion.

As expected, FSH and a potent cAMP analogue stimulated inhibin B secretion, but had little effect on basal activin A (Lampa et al. 1999, Okuma et al. 2005).

**Figure 6** Secretion of inhibin B by adult rat Sertoli cells after 48 h in culture with IL-1ra. *significantly different from control (no addition) at *P*<0.05; **P*<0.01.

**Figure 7** Secretion of activin A and inhibin B by adult rat Sertoli cells after 48 h in culture with no addition (control), and IL-1β (40 U/ml)(A, B) or LPS (125 μg/ml)(C, D) in the presence of increasing doses of IL-1ra. All values are mean ± S.E.M., n=3 wells. Values with same letter superscript are not significantly different (*P*>0.05).
A maximal dose of dbcAMP did affect basal activin A in some experiments but, unexpectedly, this was a stimulation. These responses in the adult Sertoli cell cultures were quite distinct from the response of basal activin A secretion by immature Sertoli cells, which was inhibited by FSH, but not by dbcAMP. These age-related differences did not appear to be explained by interference by endogenous Sertoli cell IL-1α, as blocking endogenous IL-1 by IL-1ra did not have a significant effect on basal activin A. However, cultured mouse Sertoli cells have been shown to produce IL-1α under basal conditions, and endogenous production of this cytokine in the cultures might obscure small responses to exogenous IL-1α (Zeyse et al. 2000). It also should be noted that adult Sertoli cells are less responsive to FSH stimulation than immature Sertoli cells (Monn et al. 1972, Steinberger et al. 1978, Lampa et al. 1999). In both immature and adult Sertoli cells, however, dbcAMP and FSH mostly inhibited the action of IL-1 on activin A secretion. The discrepancies between responses seen with FSH and dbcAMP, both in the present study and in the previous study (Okuma et al. 2005), also indicate that FSH exerts some effects on activin A and inhibit B secretion via pathways that do not involve cAMP, such as the cAMP-independent calcium-mediated pathway (Grasso & Reichert 1990, Gorczynska & Handelsman 1991).

LPS is a potent inflammatory stimulus, which up-regulates IL-1α and IL-6 production in Sertoli cells (Khan et al. 1987, Gérard et al. 1991, Syed et al. 1995). LPS increased activin A secretion and inhibited inhibit B secretion much more effectively than did either IL-1 isoform in the adult Sertoli cell cultures. LPS also increased endogenous IL-1α secretion, and the action of LPS on both activin A and IL-1α was effectively reduced by cAMP, suggesting the LPS was acting, at least partially, through increased IL-1α. This was confirmed when IL-1ra completely blocked the response of activin A to exogenous IL-1β, but only blocked the response to LPS by 50%, indicating that other factors are also involved. IL-1ra, however, was unable to affect the suppression of inhibit B by LPS. Clearly, although IL-1 and IL-6 are involved in the response of activin A and inhibit B to LPS in cultured Sertoli cells, other cytokine-mediated or inflammatory pathways activated by LPS are involved as well.

Binding of bacterial LPS to its receptor, Toll-like receptor 4, or binding of IL-1α or IL-1β to the IL-1 receptor leads to interaction with the adaptor molecule MyD88 (Akira et al. 2001, Medzhitov 2001). Subsequent signaling via MyD88 through IL-1 receptor associated kinases and tumour necrosis factor receptor activated factors leads to degradation of the NF-κB repressor protein IκB, and activation of mitogen activated protein (MAP) kinases. In turn, the MAP kinases activate multiple downstream events, including production of the transcription factor AP-1 via the c-jun N-terminal kinase, Jnk. The activin βA subunit promoter in the human (Tanimoto et al. 1996), rat (Ardekani et al. 1998), bovine (Thompson et al. 1994) and mouse (Yoshida et al. 1998) contains multiple AP-1 responsive elements, indicating that the stimulation of activin A synthesis by both LPS and IL-1 involves this transcription factor. In fact, the regulation may be even more complex than this since IL-1, and to a lesser degree LPS, also signals via bioactive lipid intermediates produced through the action of sphingomyelinase (the ceramide pathway), phospholipase A2 (the arachidonic acid metabolite pathway) and phosphoinositide-specific phospholipase C (the diacylglycerol and inositol trisphosphate pathways) (Dinarello 1996). Moreover, both LPS and IL-1 stimulate the endogenous production of other inflammatory cytokines leading to the engagement of parallel and overlapping signalling pathways. In contrast to the βA subunit, regulation of the α and βB subunits principally involves cAMP response elements (CRE) and the transcription factors SP1, AP-2 and GATA (Feng et al. 1989, Dykema & Mayo 1994, Thompson et al. 1994, Feng et al. 2000). In the rat and mouse, the βA subunit promoter also contains a CRE or CRE-like sequence (Ardekani et al. 1998, Yoshida et al. 1998), which may account for the small stimulatory effect of maximal doses of dbcAMP on baseline activin A production in some experiments. Nonetheless, while differences in the promoters partially help to understand the differential regulation of activin A and inhibit B by LPS/IL-1 and FSH/cAMP, it is clear that the complexity of this interaction requires considerably more study.

Finally, it is important to keep in mind that the responses of the Sertoli cells to LPS were modulated by hormonal regulation via FSH/cAMP, and that LPS suppresses the production of both FSH and its endogenous regulators: inhibit B and testosterone. These observations suggest that the activin A and inhibit B responses to inflammation in vivo (i.e. in the presence of an intact pituitary-testicular axis) may be somewhat more complicated than is indicated by the in vitro model described here. Moreover, the earlier studies using immature Sertoli cells indicated that there is a delay of at least 6 hours between the initial increase in activin A mRNA expression and the secretion of the activin A protein following stimulation with IL-1 (Okuma et al. 2005). This is consistent with the observation that testicular activin A protein levels were unaltered up to 6 hours after acute LPS stimulation in intact male rats (O’Bryan et al. 2005), in contrast to the rapid responses of both intratesticular IL-1β and circulating activin A levels, which reached a peak at 3 hours then declined. Similarly, testicular IL-1α expression is not acutely up-regulated by LPS in vivo, at least not within the first 3 hours after stimulation (Jonsson et al. 2001, O’Bryan et al. 2005). Altogether, these data indicate that the secretion of activin A by the Sertoli cell in response to inflammation occurs over a much longer time course to that of most other LPS-induced responses. This would suggest that the production of activin A by the Sertoli cell
is not part of the acute response to inflammation, but may be a more chronic response subsequent to the initial inflammation events. This response pattern is consistent with our hypothesis that locally produced activin A may play a role in the amelioration and/or resolution of inflammation in the adult tests, and is entirely consistent with the reduced in vivo response to inflammation in this organ (O’Bryant et al. 2005). It appears that the tests and the spermatogenic cells in particular are protected to some extent from the potentially deleterious effects of inflammation, but that inflammation exerts effects on testicular function nonetheless.

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