Differential effects of dexamethasone treatment on lipopolysaccharide-induced testicular inflammation and reproductive hormone inhibition in adult rats

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Abstract

A single intraperitoneal injection of lipopolysaccharide (LPS) causes a biphasic suppression of testicular steroidogenesis in adult rats, with inhibition at 6 h and 18–24 h after injection. The inhibition of steroidogenesis is independent of the reduction in circulating LH that also occurs after LPS treatment, indicating a direct effect of inflammation at the Leydig cell level. The relative contributions to this inhibition by intratesticular versus systemic responses to inflammation, including the adrenal glucocorticoids, was investigated in this study.

Adult male Wistar rats (eight/group) received injections of LPS (0.1 mg/kg i.p.), dexamethasone (DEX; 50 µg/kg i.p.), LPS and DEX, or saline only (controls), and were killed 6 h, 18 h and 72 h later. Treatment with LPS stimulated body temperature and serum corticosterone levels measured 6 h later. Administration of DEX had no effect on body temperature, but suppressed serum corticosterone levels. At the dose used in this study, DEX alone had no effect on serum LH or testosterone at any time-point. Expression of mRNA for interleukin-1β (IL-1β), the principal inflammatory cytokine, was increased in both testis and liver of LPS-treated rats. Serum LH and testosterone levels were considerably reduced at 6 h and 18 h after LPS treatment, and had not completely

recovered by 72 h. At 6 h after injection, DEX inhibited basal IL-1β expression and the LPS-induced increase of IL-1β mRNA levels in the liver, but had no effect on IL-1β in the testis. The effects of DEX on IL-1β levels in the liver were no longer evident by 18 h. In LPS-treated rats, DEX caused a significant reversal of the inhibition of serum LH and testosterone at 18 h, although not at 6 h or 72 h. Accordingly, DEX inhibited the systemic inflammatory response, but had no direct effect on either testicular steroidogenesis or intra-testicular inflammation, at the dose employed.

These data suggest that the inhibition of Leydig cell steroidogenesis at 6 h after LPS injection, which was not prevented by co-administration of DEX, is most likely due to direct actions of LPS at the testicular level. In contrast, the later Leydig cell inhibition (at 18 h) may be attributable to extra-testicular effects of LPS, such as increased circulating inflammatory mediators or the release of endogenous glucocorticoids, that were inhibited by DEX treatment. These data indicate that the early and late phases of Leydig cell inhibition following LPS administration are due to separate mechanisms.

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Introduction

Systemic inflammation due to infection or autoimmune diseases inhibits testicular steroidogenesis and spermatogenesis, leading to temporary or permanent fertility problems (Adamopoulos *et al.* 1978, Cutolo *et al.* 1988, Buch & Havlovec 1991). Several laboratories, including our own, have investigated this process using animal models of inflammation induced by injections of lipopolysaccharide (LPS), a bacterial outer cell wall molecule that activates the inflammatory functions of monocytes and macrophages

(Wallgren *et al.* 1993, Refojo *et al.* 1998, O'Bryan *et al.* 2000). Inhibition of both anterior pituitary secretion of luteinizing hormone (LH) and Leydig cell testosterone secretion have been observed in these models. Recently, we demonstrated that a single injection of even a relatively low dose of LPS (0.1 mg/kg) causes a biphasic decrease in serum and intratesticular testosterone concentrations in adult rats at 6 h (early phase) and at 18–24 h (late phase) after treatment, with an intervening period of partial recovery (O'Bryan *et al.* 2000). Although serum LH was also inhibited by higher doses of LPS, the decrease in

testosterone was independent of changes in LH, as confirmed by the inability of the Leydig cells to respond to a subsequent injection of exogenous human chorionic gonadotrophin. Systemic inflammation, therefore, has a direct inhibitory effect on testicular steroidogenesis, independent of the inhibition of gonadotrophin secretion at the hypothalamo–pituitary level.

The mechanism for this action of LPS on Leydig cell function *in vivo* is poorly defined. It has been shown that high doses of LPS cause down-regulation of Leydig cell synthesis of steroidogenic acute regulatory protein, the cholesterol side-chain cleavage enzyme (P450_{scc}) and 17 α -hydroxysteroid/17–20-lyase (P450_{c17}) *in vivo* (Hales *et al.* 1992, Bosmann *et al.* 1996). Furthermore, several inflammatory mediators, including interleukin-1 β (IL-1 β), tumour necrosis factor- α (TNF α) and nitric oxide, inhibit Leydig cell steroidogenesis by similar mechanisms *in vitro* (Lin *et al.* 1991, 1998, Li *et al.* 1995, Del Punta *et al.* 1996, Mauduit *et al.* 1998). The increased production of these inflammatory mediators both within the testis and in the systemic circulation is likely to have a significant inhibitory effect on Leydig cell function. Indirect effects of inflammation on the testis have also been implicated, including interference with the testicular blood flow (Bergh & Söder 1990, Sharma *et al.* 1998), and inhibition mediated by inflammation-sensitive neural pathways from the central nervous system to the testis (Turnbull & Rivier 1997, Ogilvie *et al.* 1999).

Significantly, production of inflammatory cytokines also activates the hypothalamic–pituitary–adrenal axis resulting in increased production of glucocorticoids (reviewed by Imura *et al.* 1991). Glucocorticoids exert a negative feedback effect on the inflammatory response by reducing the production, secretion and actions of the principal inflammatory mediators, such as IL-1 β , and are widely used clinically as anti-inflammatory agents (Kapcala *et al.* 1995). However, glucocorticoids also inhibit testicular steroidogenesis by actions at the hypothalamus and pituitary (Bambino & Hsueh 1981), and by direct inhibition of P450_{scc}, 3 β -hydroxysteroid dehydrogenase and P450_{c17} levels (Sapolsky 1985, Hales & Payne 1989, Monder *et al.* 1994, Gao *et al.* 1996), acting through specific receptors on the Leydig cells (Stalker *et al.* 1989). Glucocorticoid levels are elevated for 4–6 h following LPS treatment in humans and experimental animals (Wolff 1973, Stenzel-Poore *et al.* 1993). As a consequence of these separate effects on both inflammation and Leydig cell function, the role of glucocorticoids in LPS-mediated inhibition of Leydig cell is difficult to anticipate. Glucocorticoids may mediate the suppression of testosterone concentrations in this model (Cumming *et al.* 1983). Conversely, production of glucocorticoids should inhibit the inflammatory response and inflammatory mediator production, and may limit the resulting effect on Leydig cell function and damage to the seminiferous epithelium. The aim of the present study, therefore, was to identify the

respective contributions of intratesticular and systemic inflammatory responses, and the adrenal glucocorticoids, in the inhibition of testicular steroidogenesis following LPS administration. This involved examining the effect of administration of the glucocorticoid, dexamethasone, at a dose that inhibits systemic inflammatory responses, on LPS-induced inhibition of the hypothalamo–pituitary–testis axis in adult rats. In this study, IL-1 β mRNA expression was used as a specific marker of tissue-specific inflammation, and the liver was used as a control tissue for systemic (i.e. extratesticular) inflammation.

Materials and Methods

Reagents

LPS (from *E. coli*, serotype 0127:B8) and testosterone were obtained from Sigma Chemical Co. (St Louis, MO, USA). Dexamethasone sodium phosphate (DEX) was obtained from David Bull Laboratories (Victoria, Australia). Reagents for the LH radioimmunoassay (RIA) were supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD, USA). The restriction enzymes, XbaI, EcoRI, HindIII, were obtained from Promega Corporations (Madison, WI, USA). The rat 1161 bp IL-1 β cDNA plasmid was generously provided by Dr D Nikolic-Paterson (Department of Nephrology, Monash Medical Centre, Clayton, Australia). [α ³²P]dCTP was obtained from Amersham Pharmacia Biotech (Amersham, Bucks, UK).

Animals and treatments

Outbred adult male Wistar rats (80–100 days old) weighing 300–330 g were housed three per cage, fed standard rat chow and water *ad libitum* and maintained at an ambient temperature of 22–25 °C under standard lighting regimens (12 h light:12 h darkness, lights on at 0700 h). Experimental procedures were approved by the Monash University Standing Committee on Ethics in Animal Experimentation and conformed to the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organisation/Australian Agricultural Council Code of Practice for the Care and Use of Animals for Experimental Purposes. For 7 days prior to the experiment, the rats were handled daily, for approximately 2–5 min, rectal temperatures were measured and body weights were recorded. This was undertaken in order to acclimatise the rats to their surrounding environment and human contact, and to decrease any hypothalamo–pituitary–adrenal axis responses to the handling involved in the subsequent experimental manipulations (Ma & Lightman 1998). Rats were randomly divided into four treatment groups, control ($n=8$), LPS treated ($n=8$), DEX treated ($n=8$), and LPS

and DEX treated ($n=8$). Intraperitoneal injections of LPS at 0.1 mg/kg body weight in endotoxin-free saline and DEX at 50 µg/kg body weight were given to the appropriate rats at 1200 h, and rats were accordingly killed at 1800 h (6 h group), 0600 h (18 h group) and 1200 h (72 h group). Control groups received injections of saline and were included at each time-point because of significant circadian rhythms in testosterone and LH (Mock *et al.* 1978, O'Bryan *et al.* 2000). The dose of DEX used in this study was able to prevent death in adrenalectomised rats injected with 0.1 mg/kg LPS (our unpublished data), and was consistent with doses used to protect against the inflammatory actions of LPS in rats in previous studies (Nakano *et al.* 1987, Kapcala *et al.* 1995). This dose of DEX also significantly inhibits the adrenocorticotrophic hormone response to stress, and decreases plasma corticosteroid-binding globulin concentrations and thymic weight, which are established markers of integrated glucocorticoid exposure (Bradbury *et al.* 1994, Gomez *et al.* 1998).

Rats were anaesthetised with ether (BDH Laboratory Supplies, Poole, Dorset, UK) and a midline incision was made along the ventral surface. Blood (6–8 ml) was collected via cardiac puncture into uncoated tubes and the serum was separated by centrifugation at 3000 *g* for 10 min. The serum was stored frozen at -20°C until used for hormone analysis. One testis and a lobe of liver were collected for mRNA analysis. These tissues were snap frozen in dry ice and ethanol and stored at -70°C until needed.

Hormone assays

Serum LH concentrations were measured using a specific double-antibody RIA (Sun *et al.* 1990). Serum testosterone levels were measured using a direct double-antibody RIA as described previously (O'Donnell *et al.* 1994). Serum corticosterone concentrations were measured using a direct, double-antibody, ^{125}I RIA kit supplied by ICN Biomedicals (Costa Mesa, CA, USA) which has been validated for use with serum from rats and mice. All samples were measured in a single assay, which was conducted according to the manufacturer's instructions; the values of the quality control samples were within the recommended range.

Northern blot analysis

Total cellular RNA was extracted from control and treated testis and liver using the acid guanidinium thiocyanate-phenol-chloroform method of Chomczynski & Sacchi (1987). Samples of RNA (15 µg) were denatured in loading buffer (33% formamide, 14% formaldehyde, 0.05% bromophenol blue, 0.05% xylene cyanol and 80 µg ethidium bromide) and size fractionated on a 1.2% agarose-formaldehyde gel by electrophoresis in $1 \times 3\text{-}$

(N-morpholino)propanesulfonic acid buffer. The RNA was transferred onto Hybond N nylon membranes (Amersham Pharmacia Biotech) by capillary transfer, and the membranes were cross-linked using u.v. light at 125 mJ/m² (GS Gene Linker; Bio-Rad Laboratories, Richmond, CA, USA) (Church & Gilbert 1984, Khandjian & Meric 1986) and baked for 60 min at 80°C . The rat IL-1 β (1161 bp) cDNA probe was excised from a pMOSBlueT-vector using XbaI and EcoRI restriction enzymes and gel purified using QIAquick gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene to monitor RNA loading equality between samples and was prepared from a partial rat (650 bp) cDNA using EcoRI and HindIII. The cDNA probes were labelled by random priming using [$\alpha^{32}\text{P}$]dCTP in the RTS RadPrime DNA labelling system (Life Technologies, Gaithersburg, MD, USA). Unincorporated nucleotides were removed using ProbeQuantTM G-50 Micro Columns (Amersham Pharmacia Biotech). Blots were prehybridised for 2 h at 50°C with hybridisation buffer (50% formamide, 10% dextran sulfate, $5 \times \text{SSPE}$, $10 \times \text{Denhardt's}$, 100 µg/ml salmon sperm DNA, 1% sodium dodecyl sulfate). The blots were then hybridised overnight in fresh hybridisation buffer at 50°C with 10^6 c.p.m./ml of the labelled cDNA probe. The blots were washed to a maximum stringency of $0.1 \times \text{SSPE}$ for 15 min at 60°C . The blots were exposed to Kodak Biomax MR film (Kodak, Rochester, NY, USA) with an intensifying screen at -70°C for 2 h (GAPDH), and 70 h or 170 h (IL-1 β). Blots were quantified using an FLA 2000 Phosphorimager analyser (Fujix, Berthold, Australia) and analysed using Macintosh MacBAS version 2.4 software supplied by the manufacturer. Some samples with degraded RNA were excluded from the study, as indicated in the figure legends. After background subtraction, the density of each IL-1 β transcript band was normalised for loading differences as a ratio against the GAPDH band for the corresponding sample.

Statistical analysis

Data were analysed using SPSS for Windows (version 7; SPSS Inc., Cary, NC, USA). All analyses were performed using a general factorial two-way analysis of variance (ANOVA), with treatment with LPS and DEX as the main factors. The data were analysed for homogeneity of variance using Levene's test, and square root or log-transformation was undertaken when necessary. When significant interactions between the main factors occurred, differences between individual means were calculated using Fisher's least significant difference test. Results are expressed as means $\pm$ s.e.m. and significance was defined as $P < 0.05$.

Figure 1 The effect of LPS and DEX on serum corticosterone (B) concentrations 6 h after treatment in rats (means $\pm$ S.E.M., $n=8$ /group) injected with saline or LPS (0.1 mg/kg i.p.) in combination with saline (open bars) or DEX (solid bars, 50 μ g/kg i.p.). * $P<0.05$ LPS vs saline treatment; ** $P<0.01$ DEX vs saline treatment.

Results

General observations

Rats that received LPS showed physical signs of general inflammation, including piloerection and shivering. The extent of these signs appeared to be less in the rats treated with both LPS and DEX, when compared with those receiving LPS alone, although these effects were not quantified. Any physical signs had resolved by 12–18 h after LPS administration. Rectal temperatures measured 6 h after treatment were elevated in rats receiving LPS alone (38.2 ± 0.2 °C), when compared with vehicle-treated rats (37.6 ± 0.1 °C, $P<0.05$). This LPS-induced increase in rectal temperature was prevented by the co-administration of DEX (37.9 ± 0.1 , $P>0.05$ vs control), although DEX alone had no effect on rectal temperature (37.5 ± 0.2 °C, $P>0.05$ vs control). At 18 and 72 h after injection, no treatment had any significant effect on rectal temperature.

In order to confirm the action of LPS and DEX in modulating the hypothalamo–pituitary–adrenal axis, the concentration of corticosterone in the serum was measured in those animals killed 6 h after treatment. Corticosterone concentrations were increased in the LPS-treated animals ($P<0.05$), while administration of DEX significantly decreased ($P<0.01$) the secretion of the endogenous glucocorticoid (Fig. 1).

Serum LH and testosterone concentrations

The effects of LPS and DEX treatment on serum LH and testosterone concentrations are shown in Figs 2 and 3. At 6 h following injection, LPS caused a significant decrease in serum LH ($P<0.01$) and testosterone ($P<0.01$) to less than 50% of control levels, while the administration of DEX was without effect on hormone concentrations. By 18 h following injection there was a significant statistical

Figure 2 The effect of LPS and DEX on serum LH concentrations 6 h (upper panel), 18 h (middle panel) or 72 h (lower panel) after treatment in rats (means $\pm$ S.E.M., $n=8$ /group) treated with saline or LPS (0.1 mg/kg i.p.) in combination with saline (open bars) or DEX (solid bars, 50 μ g/kg i.p.). Upper and lower panels: * $P<0.01$ LPS vs saline treatment; middle panel: bars with different lower case letters are significantly ($P<0.05$) different from each other.

interaction ($P<0.05$) between the effect of LPS and DEX on serum LH and testosterone concentrations. When compared with vehicle-treated controls, the concentrations of both hormones remained decreased ($P<0.05$) in the animals treated with LPS alone, while the concomitant administration of DEX was able to significantly ($P<0.05$) attenuate the LPS-induced decrease in the concentrations of both LH and testosterone. The concentrations of both hormones in the animals receiving both LPS and DEX were not different from those obtained in the vehicle-treated animals, while DEX, when given alone, had no significant effect on hormone concentrations. At 72 h following treatment, the concentrations of both LH and testosterone were significantly decreased ($P<0.01$) in the

Figure 3 The effect of LPS and DEX on serum testosterone concentrations 6 h (upper panel), 18 h (middle panel) or 72 h (lower panel) after treatment in rats (means $\pm$ S.E.M., $n=8$ /group) treated with saline or LPS (0.1 mg/kg i.p.) in combination with saline (open bars) or DEX (solid bars, 50 μ g/kg i.p.). Upper and lower panels: * $P<0.01$ LPS vs saline treatment; middle panel: bars with different lower case letters are significantly ($P<0.05$) different from each other.

animals treated with LPS, whereas DEX was without significant effect.

IL-1 β mRNA levels in the liver and testis

Representative examples of Northern blot analyses of the relative amounts of IL-1 β and GAPDH mRNAs in the liver and testis following 6 h and 18 h of the various treatments are shown in Figs 4 and 5. Barely detectable levels of IL-1 β were observed in testis or liver samples from saline-treated control rats after 70 h of film exposure (Fig. 4), but were clearly evident at a longer exposure time (170 h, Fig. 5). The analysis revealed a transcript of 1.3 kb, consistent with the previously published size of rat IL-1 β mRNA (Zuckerman *et al.* 1989). Six hours after administration, LPS caused a significant increase ($P<0.01$) in IL-1 β mRNA in both the testis and liver (Fig. 4). Administration of DEX inhibited ($P<0.05$) expression of IL-1 β in the livers of saline- and LPS-treated animals, but

had no significant effect on expression of IL-1 β in the testes (Fig. 4). By 18 h after treatment, DEX was without significant effect on IL-1 β mRNA expression in either the liver or testis, whereas LPS-stimulated IL-1 β expression remained significantly elevated ($P<0.01$) in both organs (Fig. 5).

Discussion

Previously, we have shown that intraperitoneal injection of LPS causes a biphasic suppression of testosterone secretion, through direct inhibition of Leydig cell function, with phases of inhibition at 6 h and at 18–24 h after LPS administration (O'Bryan *et al.* 2000). In the present study, it has been shown that these effects were accompanied by an increase in IL-1 β mRNA in the testis and liver, indicating both a local testicular and a systemic inflammatory response to LPS. Co-administration of the anti-inflammatory glucocorticoid, DEX, inhibited the systemic inflammatory response to LPS, as indicated by liver IL-1 β expression and endogenous corticosteroid levels, for at least the first 6 h. On the other hand, DEX did not prevent the local inflammatory response (i.e. increased IL-1 β production) in the testis. Significantly, DEX neither enhanced nor prevented the inhibition of testosterone levels at 6 h after LPS treatment, but reversed the effects of LPS on serum testosterone concentrations at the later time-point (18 h). These data indicate that inhibiting systemic cytokine responses does not reverse the early down-regulation of Leydig cell function after LPS administration. Consequently, this phase of inhibition of the Leydig cell is most likely the result of intratesticular inflammatory events stimulated by LPS, which were not affected by DEX treatment, such as the local up-regulation of pro-inflammatory cytokines including IL-1 β and TNF α , or even a direct effect of LPS on the Leydig cell itself (Hales *et al.* 1992, Lin *et al.* 1993, Bosmann *et al.* 1996). However, the longer-term inhibition of Leydig cell steroidogenesis observed at 18–24 h after LPS treatment appears to be due to effects of LPS that were inhibited by DEX, such as systemic up-regulation of inflammatory mediators or adrenal glucocorticoids. These data indicate that the early and late phases of Leydig cell inhibition following LPS administration are due to separate mechanisms, and this should be considered in all future investigations of inflammation-induced inhibition of testicular function.

In the present study, serum LH levels were reduced at all experimental time-points following LPS treatment. This was in contrast to one of our previous studies, where LH was largely unaffected using the same LPS batch and dose, and was only inhibited at higher doses of LPS (O'Bryan *et al.* 2000). Since Wistar rats were used in the present study, while Sprague–Dawley rats were used in the earlier study, these data may be attributable to

Figure 4 The effect of LPS and DEX on IL-1 β mRNA in the testis and liver 6 h after treatment. Upper panels: representative duplicate examples of a Northern blot of IL-1 β and GAPDH mRNAs in the testis (left panel) and liver (right panel) 6 h after treatment with saline or LPS (0.1 mg/kg i.p.) in combination with saline or DEX (50 μ g/kg i.p.). Lower panels: relative expression of IL-1 β mRNA in the testis (left panel) and liver (right panel) of rats (means $\pm$ S.E.M., $n=7-8$ /group) 6 h after treatment with saline or LPS (0.1 mg/kg i.p.) in combination with saline (open bars) or DEX (solid bars, 50 μ g/kg i.p.). ** $P<0.01$ DEX vs saline treatment; *** $P<0.001$ LPS vs saline treatment.

differences in sensitivity to LPS in the periphery between the two rat strains. Alternatively, since stress minimisation was not employed in the previous study, there may have been a reduction in experimental sensitivity, due to handling stress-induced reduction in endogenous LH levels (Rivier *et al.* 1986). In any case, the data underline the importance of measuring both testosterone and LH in any inflammatory model of testicular function.

Regardless of the pituitary response to LPS, however, it has already been demonstrated that the suppression of testosterone following LPS treatment is due to a direct inhibition of the Leydig cell, rather than an effect on serum LH (O'Bryan *et al.* 2000). The failure of DEX to inhibit testosterone production in the current model, even though it suppressed both liver IL-1 β expression and endogenous corticosteroid levels, suggests that glucocorticoids are not responsible for the initial phase of testosterone suppression after LPS treatment (i.e. at 6 h) and that this inhibition was more likely due to local inflammatory events that were not affected by DEX treatment. On the other hand, treatment with DEX did bring about a more rapid recovery in both serum testosterone and LH concentrations evident by 18 h after LPS administration. In contrast to the early phase, the later inhibition of testos-

terone might be attributable to the systemic inflammatory response, including up-regulation of circulating inflammatory mediators or adrenal corticosteroids exerting a secondary effect at the testicular level. The serum LH data also indicate that a similar pattern of inhibition by local inflammatory events, followed by longer-term systemic inflammatory effects, may have occurred at the hypothalamo-pituitary level as well.

The dose of DEX employed in this study was chosen on the basis of inhibition of the inflammatory and stress responses in previously published studies (Nakano *et al.* 1987, Bradbury *et al.* 1994, Kapcala *et al.* 1995, Gomez *et al.* 1998). That this dose was effective was confirmed by the inhibition of basal and LPS-stimulated IL-1 β production in the liver. A significant inhibition of serum corticosterone was also observed, even though the control levels of corticosterone appeared to be artificially elevated, most likely as a consequence of ether-induced stress at the time of death. Normal serum corticosterone concentrations in rats are generally in the order of 100–150 ng/ml, rising to above 500 ng/ml upon application of stress (Bradbury *et al.* 1991). In spite of the profound inhibition of inflammation and adrenal responses, however, DEX alone appeared to have little or no direct effect

Figure 5 The effect of LPS and DEX on IL-1 β mRNA in the testis and liver 18 h after treatment. Upper panels: representative duplicate examples of a Northern blot of IL-1 β and GAPDH mRNAs in the testis (left panel) and liver (right panel) 18 h after treatment with saline or LPS (0.1 mg/kg i.p.) in combination with saline or DEX (50 μ g/kg i.p.). Lower panels: relative expression of IL-1 β mRNA in the testis (left panel) and liver (right panel) of rats (means $\pm$ S.E.M., $n=5-8$ /group) 18 h after treatment with saline or LPS (0.1 mg/kg i.p.) in combination with saline (open bars) or DEX (solid bars, 50 μ g/kg i.p.). ** $P<0.01$ LPS vs saline treatment.

on the hypothalamo–pituitary–Leydig cell axis. Glucocorticoids, including DEX, have been shown to inhibit testicular steroidogenesis in rats *in vivo* when administered at high doses or for extended periods (Saez *et al.* 1977, Lerman *et al.* 1997); however, the single dose used in the present study appears to have been below the threshold necessary to exert a detectable effect at the Leydig cell level.

Since the animals used in this study were not held under pathogen-free conditions, it is not surprising that constitutive expression of endogenous IL-1 β production should have been detected in these animals – this is the normal state in any population, including humans (Eastgate *et al.* 1988, Tovey *et al.* 1988). This low level of expression was only detected after very long exposure times in the present study. Previous studies showing no IL-1 β in the normal testis of rats raised under standard laboratory conditions (Khan *et al.* 1987, Jonsson *et al.* 1999) may reflect differences in ongoing health status from population to population, as much as the relative sensitivity of the assay methods used.

In the present study, it was found that DEX was able to inhibit liver production of IL-1 β in response to LPS as

expected, but not in the testis. This observation suggests that the regulation of IL-1 β production in the testis may be significantly different from that seen in other tissues. Glucocorticoids normally block inflammation by inhibiting the production of a number of pro-inflammatory mediators by monocyte/macrophages, lymphocytes and endothelial cells (Kapcala *et al.* 1995). IL-1 β , in particular, is regulated both transcriptionally and post-transcriptionally by glucocorticoids (Zuckerman *et al.* 1989, Scheinman *et al.* 1995). At this stage, the site of production of IL-1 β in the rat testis is unknown. Evidence indicates that resident testicular macrophages in the rat do not produce significant amounts of either isoform of IL-1 in response to LPS stimulation (Kern *et al.* 1995, Hayes *et al.* 1996), although circulating leukocytes which may express IL-1 β progressively increase in number within the testicular vasculature and interstitium following LPS treatment (O'Bryan *et al.* 2000). Sertoli cells, which produce IL-1 α constitutively, do not appear to produce IL-1 β , but rat and human Leydig cells express IL-1 β in response to inflammatory stimulation *in vitro* (Lin *et al.* 1993, Cudicini *et al.* 1997, Jonsson *et al.* 1999). It is possible that, even though the Leydig cells possess glucocorticoid receptors

(Stalker *et al.* 1989), the production of IL-1 β by these cells may not be responsive to glucocorticoid control due to differences in the regulatory regions of the IL-1 β gene, for example. Alternatively, the different effects of DEX on IL-1 β mRNA in the testis and liver might be explained by differential access of DEX to the two organs. DEX is a substrate of P-glycoprotein, the product of the multi-drug resistance (Schinkel *et al.* 1995), which limits access of DEX to the brain and also appears to be expressed in the microvessels of the testis (Holash *et al.* 1993). Metabolism of DEX by this enzyme could account for the differences in the effect of DEX on IL-1 β expression between the liver and testis. In either case, this tissue-specific difference in the response to DEX is deserving of further investigation.

In conclusion, these data clearly support a direct effect of LPS at the testis level, possibly even a direct effect of LPS on the Leydig cell itself, leading to acute inhibition of steroidogenesis. The secondary inhibition of testosterone production after LPS treatment seen at longer time-points, however, may occur in response to systemic inflammatory events, including the secretion of endogenous glucocorticoids. These data need to be taken into consideration when designing studies on the effects of inflammation on testicular function. These data also indicate a number of fundamental differences in the process of inflammation and its control in the rat testis, compared with other tissues. Finally, they have important implications for treatment of systemic or testicular inflammatory disease in subfertile or infertile patients, which may guide the appropriate use of glucocorticoids in such patients.

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