Testosterone and FSH have independent, synergistic and stage-dependent effects upon spermatogenesis in the rat testis

J.B. Kerr¹, S. Maddocks², and R.M. Sharpe³

¹ Department of Anatomy, Monash University, Wellington Road, Clayton, Melbourne, Victoria 3168, Australia
² Department of Animal Sciences, Waite Agricultural Research Institute, Glen Osmond, South Australia 5064, Australia
³ MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK

Summary. Adult rats were hypophysectomized and treated with ethane dimethanesulphonate (EDS) selectively to eliminate the Leydig cells in the testis. By removing the source of endogenous gonadotrophins and androgens, the subsequent effects on the seminiferous epithelium were studied after 20 days of treatment with vehicle, or FSH (2 × 50 μg/day) or a low dose of testosterone (0.6 mg testosterone esters every 3rd day) alone or in combination. Compared to vehicle-treated hypophysectomized rats with Leydig cells, testis weight in saline-treated hypophysectomized rats treated with EDS declined by 50%, spermatogenesis was disrupted severely and only 18% of the tubules contained spermatids, these being confined to stages I–VI of the spermatogenic cycle. Treatment with either FSH or testosterone esters alone significantly (P < 0.01) increased testis weight compared to vehicle-treated hypophysectomized rats treated with EDS and 40% of the tubules contained spermatids; at other stages I–VI after FSH, or at all stages I–XIV after testosterone treatment. Treatment with FSH and testosterone esters together maintained testis weights approximately 20% above vehicle-treated hypophysectomized rats treated with EDS and 40% of the tubules contained spermatids either at stages I–VI after FSH, or at all stages I–XIV after testosterone treatment. The results suggest that, in the absence of the pituitary gland and the Leydig cells, FSH alone partially supports spermatogenesis up to the development of round spermatids whereas testosterone is capable of maintaining spermatid development at all 14 stages of the cycle. When FSH and testosterone were administered in combination, the effects upon spermatogenesis were far greater than the response expected if their individual effects were simply additive. It is therefore concluded that FSH may play a role in normal spermatogenesis and that this role is essentially that of augmenting the response of the testis to testosterone. The biochemical mechanisms via which this might occur are discussed and hypophysectomized rats treated with EDS used in the present studies should provide a useful approach for their identification.

Key words: Testis – Spermatogenesis – FSH – Testosterone – Rat (Sprague-Dawley)

Although it has long been recognized that testosterone is of prime importance in the control of the spermatogenic process (Steinberger 1971; Sharpe 1987; Rommerts 1988) there is increasing evidence that follicle-stimulating hormone (FSH) may have an important role in this regard (Matsumoto et al. 1986; Sharpe 1989). The difficulty in defining the exact roles of testosterone and FSH has been attributed to a variety of factors but none more puzzling than the apparent "synergistic" effects of these two hormones upon the development of the germ cells, first suggested in 1961 by Woods and Simpson. Despite numerous investigations since then (Steinberger 1971; Elkington and Blackshaw 1974; Hansson et al. 1975; Vernon et al. 1975; Awoniyi et al. 1989; Bartlett et al. 1989) the precise cellular targets and the biochemical mechanisms by which FSH and testosterone synergize remain unknown. The complex cooperative effects of FSH and testosterone upon the seminiferous epithelium is emphasized by the results of recent experiments exploring the relationship between testosterone availability and the qualitative or quantitative maintenance of spermatogenesis. Following the long-term removal of the Leydig cells, Sharpe et al. (1988a, b) showed that repeated subcutaneous administration of testosterone to otherwise intact adult rats could fully maintain quantitative spermatogenesis and fertility for up to 10 weeks. Although suppressed drastically by testosterone administration, FSH in serum remained just detectable by radioimmunoassay thus not excluding the possibility that low levels of FSH also may have stimulated germ cell development. Recently Awoniyi et al. (1989) showed that quantitatively normal spermatogenesis was restored by
high levels of exogenously administered testosterone in adult rats made azoospermic by active immunization against gonadotrophin-releasing hormone. Although immunoassayable FSH (and LH) were undetectable in serum, FSH bioactivity was not measured and testis weights of testosterone-treated rats were significantly (P<0.01) below accompanying control values. Quantitative restoration and maintenance of spermatogenesis with exogenous testosterone treatment (using subcutaneous implants) was also achieved in rats made previously azoospermic via selective suppression of LH but, notably, FSH levels remained in the control range (Awoniyi et al. 1990). However, when hypophysectomized rats received the same testosterone treatment, elongated spermatid numbers in the testes were only 18% of control values, again implicating the lack of FSH as a causative factor in the failure to support quantitative spermatogenesis. A direct role for FSH in spermatogenesis has been reported in FSH-treated adult hypophysectomized rats in which it was concluded that FSH partially supported the meiotic maturation of primary spermatocytes into round spermatids (Bartlett et al. 1989). In this case however, atrophied Leydig cells were still present within these testes and measurable although very low levels of testosterone were detected in both peripheral blood and within the testis, again raising the possibility of a cooperative stimulatory effect of FSH and testosterone on spermatogenesis. Other recent in vivo studies of the hormonal control of spermatogenesis have implicated FSH in potentiating the effects of testosterone upon germ cell development in the rat testis (Awoniyi et al. 1989; Sun et al. 1989, 1990; Santulli et al. 1990; Huang et al. 1991). This notion is further supported by several in vitro studies, using either isolated adult seminiferous tubules to show stage-dependent variations in FSH and androgen receptors during the spermatogenic cycle (Isomaa et al. 1985; Parvinen et al. 1986), or using immature Sertoli cell cultures to demonstrate that FSH increases both the number of androgen receptors and the expression of androgen receptor mRNA (Verhoeven and Cailléau 1988; Blok et al. 1989).

The present study is the first to test the hypothesis that, in the absence of the pituitary and the Leydig cells, the spermatogenic cycle is regulated differentially by FSH and testosterone. We have used an in vivo approach in which the removal of endogenous gonadotrophins (via hypophysectomy) and endogenous testosterone (via selective elimination of Leydig cells) provided an opportunity to investigate the stage-specific response of spermatogenesis to administration of FSH alone or testosterone alone, or a combination of both hormones.

Materials and methods

Animals

Forty-three hypophysectomised Sprague-Dawley rats were obtained from Charles River (Kent, UK) at 10 weeks of age, and maintained under standard animal house conditions with access to 5% glucose added to drinking water. All animals were weighed upon delivery (within 48 h after surgery), the average weight being 230±2 g (mean±SD). The completeness of hypophysectomy was monitored by weighing each animal every third day thereafter until the completion of the study. Animals were eliminated from the study if their body weight remained unchanged or increased. On this basis 33 animals were finally selected for study with an average individual decline in body weight of 18 g in 20 days.

Experimental procedures

Animals were divided randomly into 5 groups. A hypophysectomized (HPX) group (n=5) received no further treatment except for twice daily subcutaneous injections of 0.5 ml vehicle (0.9% NaCl containing 2.5 mg/ml gelatin and 2.5 mg/ml bovine serum albumin, BSA; fraction V, Sigma). All remaining animals received, on the day of delivery, a single i.p. injection of ethane dimethanesulphonate (EDS; 7.5 mg/100 g body weight) in dimethylsulphoxide-water (1:3 v/v), known to selectively destroy Leydig cells within 48 h (Kerr et al. 1985). These animals were then allocated to the following groups: (1) twice daily s.c. injections of vehicle (n=7); (2) twice daily s.c. injections of 50 μg NIADDK ovine FSH S-17 in 0.5 ml vehicle (n=9); (3) subcutaneous injection of 0.6 mg long-acting testosterone esters (TE; Sustanon, Organon) in 0.1 ml arachis oil given initially on the same day as the EDS injection and repeated every three days (n=6); (4) the final group (n=6) received a combination of FSH plus TE at the same doses and injection intervals as described above for groups (2) and (3) respectively. The dose of TE administered was intentionally subnormal but was selected on the basis that it would not maintain quantitatively normal spermatogenesis on its own (Sharpe et al. 1988a, 1990) in order that any synergistic effect of the TE and FSH could be demonstrated readily. To check for the presence of antibodies developed against the oFSH, all animals receiving oFSH were lightly anaesthetized with ether on day 14, blood collected from the tail vein into tubes and plasma assayed for anti-FSH antibodies. The results were negative. Animals were killed after 20 days and the testes and epididymides were also removed and weighed as a further indicator of the completeness of hypophysectomy.

Collection of tissues

Other than those animals allocated for morphological analysis, rats were killed by CO₂ inhalation and peripheral venous blood was collected with a heparinized syringe and needle from the inferior vena cava. Plasma was separated by centrifugation and stored at -20 °C. The testes, seminal vesicles, epididymides and adrenals were excised, trimmed of fat and connective tissues and weighed.

Testosterone assay

Testosterone in plasma was measured by radioimmunoassay (Corker and Davidson 1973) as described previously (Sharpe et al. 1988a).

Testicular histology and morphometric analysis

Three animals from each group were allocated for histological study. Rats were killed with prolonged ether inhalation, blood was collected immediately from the left ventricle into heparinized tubes and the plasma separated and stored at -20 °C until assayed for testosterone concentration as described above. The thoracic aorta was cannulated and the testicular vasculature was first flushed with physiological saline containing heparin and then perfusion fixed with a mixture of 3% glutaraldehyde, 2% formaldehyde and 0.01%