Identification of stage-specific changes in protein secretion by isolated seminiferous tubules from rats following exposure to short-term local testicular heating

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The objective of this study was to identify the early (after 4 and 24 h) effects of short-term local testicular heating (43°C for 30 min) on the secretion of proteins by seminiferous tubules isolated from adult rats at stages II–V, VI–VIII or IX–XII of the spermatogenic cycle, and cultured *in vitro* for 24 h with [³⁵S]methionine. Incorporation of [³⁵S]methionine into secreted and intracellular proteins was assessed and the pattern of protein secretion was evaluated using two-dimensional SDS-PAGE. Seminiferous tubules isolated from control rats exhibited the characteristic, androgen-dependent increase in protein secretion at stages VI–VIII. At 4 h after exposure to local testicular heating, seminiferous tubules at these stages showed a significant increase ($P < 0.001$) in the overall incorporation of [³⁵S]methionine into secreted proteins, whereas seminiferous tubules at stages II–V and IX–XII showed no significant change. In marked contrast, seminiferous tubules isolated from rats 24 h after local testicular heating showed a significant decrease in the incorporation of [³⁵S]methionine into secreted proteins at stages VI–VIII ($P < 0.001$) and to a lesser extent at IX–XII ($P < 0.05$), whereas seminiferous tubules at stages II–V showed no change in incorporation. Prior treatment to maintain normal intratesticular concentrations of testosterone in heat-exposed rats failed to prevent these changes. Similar results were obtained when incorporation of [³⁵S]methionine into intracellular proteins was evaluated 4 and 24 h after exposure to local testicular heating. Analysis of seminiferous tubule-secreted proteins by two-dimensional SDS-PAGE identified three androgen-regulated proteins that showed reproducible decreases in secretion following exposure to local testicular heating. These results suggest that changes in overall protein secretion by seminiferous tubules may explain some of the adverse effects of heat on the testes.

Introduction

The adverse effects of heat on the mammalian seminiferous epithelium are well documented. Early work on natural and experimental cryptorchidism in guinea-pigs and rats (Moore, 1924) and sheep (Moore and Chase, 1923) led to the conclusion that normal spermatogenesis in male mammals with a scrotum requires a temperature lower than body temperature. Quantitative analysis of changes to the seminiferous epithelium of rats exposed to short-term local testicular heating (Chowdhury and Steinberger, 1964, 1970; Bartlett and Sharpe, 1987) have found that, following exposure to temperatures of 43°C for 15 or 30 min, primary spermatocytes at stages VII–XII of the spermatogenic cycle are the germ cells most sensitive to damage by heat. The stage specificity of germ-cell degeneration after such treatment could indicate that only selected

interactions between Sertoli cells and germ cells are initially affected by local testicular heating.

It is generally believed that the Sertoli cell plays an important role in the regulation of germ-cell development through a complex series of interactions with the developing germ cells (Jégou and Sharpe, 1993; Sharpe, 1993). It is presumed that these interactions are mediated by the secretion of different proteins by the Sertoli cells and that these vary cyclically according to the stage of the spermatogenic cycle (Parvinen, 1982, 1993). Furthermore, there is increasing evidence to suggest that germ cells can directly modulate the secretory function of the Sertoli cells, according to their own specific requirements (Djakiew and Dym, 1988; Jégou and Sharpe, 1993; Sharpe, 1993). It is presumed that some of these functions are either altered selectively or are more sensitive to the adverse effects of heat. As seminiferous tubules at the various stages of the spermatogenic cycle can be isolated by transillumination-assisted microdissection (Parvinen *et al.*,

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1986), it is potentially possible to identify these alterations in function at the protein level at various time points after heat exposure. Identification of these changes would (i) identify the possible biochemical cause(s) of the adverse effects of heat, (ii) give insight into normal control mechanisms in spermatogenesis and (iii) identify proteins that could be used as early markers of the disruption of spermatogenesis, provided that they are detectable in peripheral blood and that assays can be developed for their measurement (Sharpe, 1992).

The objective of the present study was to take the first steps towards these goals by assessing whether reproducible stage-specific changes in the secretion of proteins could be identified following exposure to heat. Proteins that are secreted from the seminiferous tubules were targeted specifically because it is believed that the secretion of these proteins represents the most important of the possible routes by which Sertoli cells communicate, and thus interact, with the germ cells (Sharpe, 1993).

Materials and Methods

Animals and treatments

Young adult Wistar rats approximately 70 days of age and bred in the MRC Reproductive Biology Unit, Edinburgh, were used in studies to identify protein changes associated with heat-induced damage to the testis. They were housed under conventional conditions (12 h light:12 h dark) at a temperature of 21°C and humidity of 35–60%. Food and water were available *ad libitum*. Rats were anaesthetized with an i.p. injection of 50 mg sodium pentobarbitone kg⁻¹ (Sagatal; May and Baker Ltd, Dagenham). The lower half of their body, including the scrotum, was then immersed for 30 min in a thermostatically controlled water bath maintained at 43°C, while the animals were strapped with velcro ties to a supporting frame. Heat treatment has been shown to cause widespread early germ-cell loss (i.e. of pachytene spermatocytes and early spermatids), although this occurs in a stage-specific and time-related manner (Bartlett and Sharpe, 1987). Rats were killed 4 or 24 h after treatment by inhalation of CO₂ followed by cervical dislocation and subjected to the procedures described below. As heat treatment was found to have major effects on androgen-dependent protein secretion by seminiferous tubules, two experiments were performed to assess whether testosterone replacement could prevent these changes. Adult rats were injected s.c. with 25 mg testosterone esters (Sustanon; Organon Laboratories, Cambridge, UK) just before they were subjected to local testicular heating as described above; seminiferous tubules were isolated for culture 24 h later. The dose of testosterone esters administered has been shown to maintain quantitatively normal spermatogenesis for extended periods in rats and to maintain normal intratesticular concentrations of testosterone (Sharpe *et al.*, 1990).

Testicular morphology

At 24 h after local testicular heating, three rats were perfusion fixed and results were compared with those from two perfusion-fixed control rats. Buffered 3% glutaraldehyde was

perfused through the rat dorsal aorta using techniques described by Kerr and Sharpe (1985). After post-fixation in 2% osmium tetroxide, tissue blocks were embedded in Epon-Araldite (Bio-Rad Laboratories, Hemel Hempstead) and semi-thin (1 µm) sections were cut and stained with Toluidine blue. Cross-sections of seminiferous tubules were examined using a Zeiss photomicroscope and classified into stages as described by Leblond and Clermont (1952).

Isolation and culture of seminiferous tubules

Seminiferous tubules were isolated and cultured using slight modifications of the method of Allenby *et al.* (1991a, b). Testes were kept on ice, decapsulated within 2 h of removal from the animal and placed into a small plastic Petri dish containing ice-cold Dulbecco's PBS on a cooled (4°C) transparent Perspex stage illuminated from below. Lengths of seminiferous tubules were then isolated by gently teasing apart the central portion of the testis using fine watchmakers' forceps. Tubules were isolated in sections of > 2 cm (approximate range 2–5 cm).

Isolated tubules were transferred to a separate Petri dish containing fresh PBS and dissected into stages II–V, VI–VIII or IX–XII of the spermatogenic cycle, according to the criteria of Parvinen (1982). The selection of stage groupings was based on both the ease with which particular stages can be distinguished from one another and on the stage specificity of the early adverse effects of local testicular heating on spermatogenesis *in vivo* (Bartlett *et al.*, 1987). Seminiferous tubules were cut using a scalpel and only those seminiferous tubules > 0.5 cm in length were used. Seminiferous tubules that showed signs of damage or stretching were excluded. Staged seminiferous tubules were transferred subsequently to fresh PBS and, by reference to a transparent grid, a total length of seminiferous tubules of 10 cm at each stage grouping was transferred to a 24-well plastic culture plate containing 0.1 ml culture medium; this consisted of Eagle's minimal essential medium without methionine (Flow Laboratories, Irvine, CA) containing 4 mmol L-glutamine l⁻¹ (Sigma Chemical Co., Poole), 100 µU penicillin ml⁻¹, 100 µg streptomycin ml⁻¹ (Flow Laboratories), 25 mmol Hepes l⁻¹ (Gibco, Paisley) and 0.1% (v/v) polyvinyl alcohol (Sigma Chemical Co.) per well as a protein substitute (Sharpe *et al.*, 1992). Immediately before incubation, 60 µCi [³⁵S]methionine (specific activity, > 1000 Ci mmol⁻¹; ICN Flow, High Wycombe) was added to each culture. Seminiferous tubules were cultured for 24 h at 32°C under a humidified atmosphere of 5% CO₂ and 95% air. Each set of seminiferous tubules of total length 10 cm was incubated in a final volume of 0.4 ml. At the end of the incubation period, the medium was aspirated from the seminiferous tubules into an Eppendorf tube containing a protease inhibitor (Aprotinin at a final concentration of 5% v/v; Sigma Chemical Co.). The medium was then centrifuged at 1000 g for 5 min and the aspirate stored at -40°C. Incorporation of [³⁵S]methionine into secreted proteins (in the aspirated culture medium) and lysates was assessed after precipitation with trichloroacetic acid and scintillation counting. All experiments were repeated two to four times.

Two-dimensional SDS-PAGE

A water-cooled Protean II electrophoresis system and a model 3000xi power supply from Bio-Rad Laboratories were used in all the procedures. The method used was described by Sharpe *et al.* (1992). Tube gels for isoelectric focusing consisted of 5.5 g urea (Sigma Chemical Co.), 1.33 ml pre-mixed 30% w/v acrylamide/bis (Bio-Rad Laboratories), 2 ml 10% nonidet P-40 (BDH), 0.6 ml ampholines (0.4 ml, pH 5–7; 0.15 ml, pH 7–9; 0.05 ml, pH 3–10) (Bio-Rad Laboratories) and 2.15 ml deionized, distilled water (DD water). After degassing the monomer solution for 2 min, polymerization was initiated by adding 20 μ l fresh 10% w/v ammonium persulfate and 10 μ l TEMED (both from Sigma Chemical Co.). Isoelectric focusing gels were poured to a height of 11 cm in glass capillary tubes 160 mm high and with an internal diameter of 2.5 mm (Bio-Rad Laboratories). Gels were then overlaid with 50 μ l DD water until polymerized (2 h). At this time the overlay was replaced with 20 mmol NaOH l^{-1} and further polymerized for 1 h. The gels were then prefocused at 200 V for 15 min, 300 V for 30 min, and 400 V for 15 min, using 6 mmol phosphoric acid l^{-1} as the anolyte and freshly degassed 20 mmol NaOH l^{-1} as the catholyte.

Seminiferous-tubule-conditioned medium was mixed with an equal volume of isoelectric focusing sample buffer (9.5 mol urea l^{-1} , 2% v/v nonidet P-40, 2% w/v ampholines and 1% w/v dithiothreitol) and additional urea was added to ensure a final concentration of 9.5 mol l^{-1} . The samples were incubated at room temperature for 15 min before they were centrifuged at 1000 g for 3 min. Each tube gel was loaded with equal amounts of [35 S]methionine-labelled proteins (200–250 $\times 10^3$ c.p.m.). Blank tube gels were loaded with 50 μ l isoelectric focusing sample buffer. Gels were then prefocused at 400 V for 14 h, followed by 800 V for 2 h. Sample gels were then extruded, placed into a storage tube containing 0.5 ml sample buffer [made up of 10 ml 0.5 mol Tris-HCl l^{-1} , pH 6.8, 20 ml 10% SDS, 4 ml 0.05% w/v bromophenol blue (Bio-Rad Laboratories) and 36 ml DD water], incubated at room temperature for 3 min before being frozen rapidly in ethanol:dry ice, and stored at -40°C . Blank tube gels were then sectioned into 5 mm segments, placed into 1.4 ml DD water, and allowed to stand at room temperature for 4–6 h, before measurement of pH.

Slab gels that contained 10% w/v acrylamide were used for the second dimension of gel electrophoresis. Each of these gels consisted of 13.3 ml acrylamide in 10 ml 1.5 mol Tris-HCl l^{-1} (pH 8.8) and 16.7 ml DD water. After degassing for 15 min, polymerization was initiated by adding 600 μ l fresh 10% w/v ammonium persulfate and 18 μ l TEMED. Gels were overlaid with water-saturated iso-butanol (Aldrich Chemical Co., Gillingham) for approximately 1 h, washed with DD water and then overlaid with 0.375 mol Tris-HCl l^{-1} (pH 8.8) until required. Frozen isoelectric focusing gels were thawed at room temperature and then transferred carefully to the top of the slab gel to ensure that no air bubbles were trapped; no sealing medium was used. The electrolyte buffer used contained 0.3% w/v Tris base, 1.44% glycine and 0.1% SDS (all from Sigma Chemical Co.) in DD water. Gels were run at 38 mA per gel for approximately 2 h, fixed for 30 min in methanol:acetic acid:DD water (40:10:10; v:v:v) and then silver stained using a kit from Bio-Rad Laboratories. The gels were then soaked for 15 min in

Amplify (Amersham International, Amersham) and dried under vacuum for 2 h at 62°C before being exposed to X-ray film (Kodak X-OMAT ARS) at -80°C for 21 days.

Statistical analysis

Differences between groups were analysed by analysis of variance and Student's *t* test.

Results

Effect of local testicular heating on testicular morphology

In rats exposed to 43°C for 30 min, examination of sections from perfusion-fixed testes (Fig. 1) confirmed in general the detailed analyses of Chowdhury and Steinberger (1964, 1970). In such testes, widespread but stage-specific (stages II–XIV) damage or loss of pachytene spermatocytes was the most obvious change. Interestingly, the heads of step 19 spermatids were found to be embedded deeply in the Sertoli cell cytoplasm at stages VI–VIII rather than in their normal position of being grouped around the edge of the tubule lumen (Fig. 1a, b). Extensive vacuolation of the seminiferous epithelium, at the level of the tight junctions, of many tubules was also evident (Fig. 1b, c), and retention of sperm heads was a prominent feature of tubules at stages IX–XIV (Fig. 1d). Round spermatids at stages I–VIII were seen more frequently compared with controls. Apart from the widespread loss of pachytene spermatocytes, tubules at stages I–V were relatively unaffected by local testicular heating 24 h earlier, especially when compared with tubules at stages VII–XII.

Effect of local testicular heating on testicular mass

The mean testicular mass was reduced significantly ($P < 0.001$) 24 h after local testicular heating to 1587 ± 121 mg (mean $\pm$ SD, $n = 5$) but not 4 h after treatment (1873 ± 91 mg) when compared with controls (1878 ± 101 mg).

Stage-dependent differences in overall protein secretion

The overall incorporation of [35 S]methionine into proteins secreted into the incubation medium by seminiferous tubules at stages VI–VIII from control rats was approximately double that at stages II–V or IX–XIII, whereas incorporation into intracellular proteins showed no such difference between the stages (Figs 2 and 3). This difference was evident in the control group in each of the experiments in this study.

Effect of local testicular heating on overall protein secretion

After short-term local testicular heating, there was a significant increase ($P < 0.001$) in the incorporation of [35 S]methionine into newly synthesized proteins secreted into the incubation medium by seminiferous tubules at stages VI–VIII when isolated 4 h after treatment; seminiferous tubules at stages II–V or IX–XII showed no significant change in incorporation when compared with controls (Fig. 2a). In

Fig. 2. Incorporation of [³⁵S]methionine over 24 h into newly synthesized proteins secreted *in vitro* by seminiferous tubules isolated at different stages of the spermatogenic cycle from control rats (■) or rats that had been exposed to short-term local testicular heating (43°C for 30 min) (▨) either (a) 4 or (b) 24 h earlier, and cultured for 24 h. Values are means ± SD; *n* = 4. **P* < 0.05; ****P* < 0.001 compared with the respective control group.

contrast, seminiferous tubules isolated from rats 24 h after heat treatment showed a marked decrease in incorporation of radioactivity into secreted proteins at stages VI–VIII (*P* < 0.001) and a small decrease at stages IX–XII (*P* < 0.05; Fig. 2b), whereas seminiferous tubules at stages II–V showed no change in incorporation at this time. This heat-induced decrease in the concentration of secreted protein by seminiferous tubules at stages VI–VIII 24 h after treatment effectively abolished the normal, androgen-dependent increase in protein secretion observed at these stages.

Fig. 3. Incorporation of [³⁵S]methionine into newly synthesized intracellular proteins by seminiferous tubules isolated at different stages of the spermatogenic cycle from control rats (■) or rats that had been exposed to short-term local testicular heating (43°C for 30 min) (▨) either (a) 4 or (b) 24 h earlier, and cultured for 24 h. Values are means ± SD; *n* = 4. **P* < 0.05; ****P* < 0.001 compared with the respective control group.

Effect of local testicular heating on synthesis of intracellular proteins

After short-term local testicular heating, the pattern of change in the synthesis of intracellular radiolabelled proteins was comparable to that for secreted proteins. Thus, 4 h after heat treatment there was a significant increase (*P* < 0.001) in the incorporation of [³⁵S]methionine into intracellular proteins in seminiferous tubules at stages VI–VIII, whereas seminiferous tubules at stages II–V or IX–XII showed no significant change

Fig. 1. Effect of local testicular heating 24 h earlier on the cross-sectional morphology of rat seminiferous tubules at different stages of the spermatogenic cycle. (a) Stage VII in a control rat, exhibiting a full complement of germ cells. (b) Stage VII (based on the acrosomal development of round spermatids) in a rat exposed to local testicular heating. Note the complete absence of pachytene spermatocytes but the near-normal complement of other germ types. Note, however, the markedly abnormal position of the step 19 spermatids (curved arrows), which are embedded deep within the epithelium instead of grouped around the edge of the lumen as in a normal tubule at stage VII (see (a)). Note also the abnormal vacuolation (arrowheads) towards the base of the seminiferous epithelium. (c) Stage III in a rat exposed to local testicular heating. Note the complete absence of pachytene spermatocytes but the near-normal complement of other germ cell types. Note also the abnormal vacuolation (arrowheads) towards the base of the seminiferous epithelium. (d) Stage X in a rat exposed to local testicular heating. Note that pachytene spermatocytes are absent or degenerative (short arrows), whereas zygotene spermatocytes (long arrows) appear normal. Other abnormalities include retained sperm heads and reduced numbers of condensing spermatids. E: elongate spermatids; P: pachytene spermatocytes; R: round spermatids. Scale bar represents 100 μm.

Fig. 4. Analysis by two-dimensional SDS-PAGE of radiolabelled proteins secreted *in vitro* by seminiferous tubules isolated at stages VI–VIII from (a) control rats or (b) rats that had been exposed to short-term local testicular heating (43°C for 30 min) 24 h earlier and then cultured for 24 h. Previously identified androgen-regulated proteins (ARPs) which decreased in relative abundance following local testicular heating are labelled ARP-1, ARP-3 and ARP-4. The positions of the three major Sertoli-cell-secreted proteins, sulfated glycoprotein-1 (SGP-1), sulfated glycoprotein-2 (SGP-2) and cyclic protein-2 (CP-2), are indicated for reference. Both gels were loaded with equal amounts of [³⁵S]methionine-labelled protein.

in incorporation when compared with controls (Fig. 3a). By 24 h after treatment the incorporation of radiolabel into intracellular proteins was decreased in seminiferous tubules at stages II–V, VI–VIII and IX–XII (Fig. 3), although the decrease at stages VI–VIII was not as marked as for secreted proteins (Figs 2 and 3).

Effect of testosterone administration to heat-treated rats

The maintenance of normal intratesticular concentrations of testosterone by injecting 25 mg testosterone esters failed to alter the decrease in the overall secretion of radiolabelled protein by seminiferous tubules at stages VI–VIII, induced by heat treatment 24 h earlier [mean ($\pm$ SD) control value: $28\,562 \pm 895 \times 10^5$ c.p.m. per 10 cm length of seminiferous tubule ($n = 3$); mean ($\pm$ SD) heat-treated value: $12\,911 \pm 1426$ ($n = 3$); mean ($\pm$ SD) heat- plus testosterone-ester-treated value: $12\,297 \pm 1455$ ($n = 3$)].

Two-dimensional SDS-PAGE

As the effect of local testicular heating had the most marked effect on overall protein secretion at stages VI–VIII, newly synthesized proteins secreted at these same stages, from control and heat-treated rats, were analysed by two-dimensional SDS-PAGE (Fig. 4). After heat treatment 4 h earlier, no major changes in the secretion of specific proteins were observed (data not shown). However, by 24 h after treatment, three previously identified androgen-regulated proteins (ARP-1, ARP-3 and ARP-4) (Sharpe *et al.*, 1992) showed a pronounced and repeatable decrease in relative abundance.

Discussion

The objective of the present study was to identify early changes in protein secretion by seminiferous tubules following disruption of spermatogenesis induced by short-term local testicular heating. Unexpectedly, the results have shown that this treatment has major effects on the androgen-dependent increase in the overall secretion of protein by seminiferous tubules at stages VI–VIII. Changes in the secretion of three specific proteins that have been shown to be androgen-dependent were also identified. These findings also shed light on the mechanism of testicular damage induced by heat.

The present study has confirmed that local testicular heating causes damage to spermatogenesis that is both stage- and cell-specific (Chowdhury and Steinberger, 1964, 1970; Collins and Lacy, 1969), and that spermatocytes are most affected. The present study has also shown for the first time that exposure to short-term local testicular heating (43°C) for 30 min (Bartlett and Sharpe, 1987) results in stage-specific changes in the overall incorporation of [³⁵S]methionine into both secreted and intracellular proteins by seminiferous tubules isolated 4 and 24 h after treatment. The most striking of these changes is the effect of local testicular heating on the overall amount of secretion of radiolabelled proteins by seminiferous tubules at stages VI–VIII. Previous studies have shown that protein secretion at these stages is approximately double that of seminiferous tubules at other stages, an increase that is totally androgen dependent (Sharpe *et al.*, 1992, 1993; McKinnell and Sharpe, 1992). The present findings show that, within 4 h of local testicular heating, this stage-dependent increase in protein secretion is exacerbated, whereas by 24 h it is abolished.

completely; at the same time, changes in protein secretion by tubules at stages II–V and IX–XII were either not evident or were minor in comparison with those observed at stages VI–VIII. This heat-induced perturbation of protein secretion by tubules at stages VI–VIII is unlikely to be related to alterations in testosterone concentrations for the following reasons. First, previous studies have shown that intratesticular testosterone concentrations are not reduced by local testicular heating (Bartlett and Sharpe, 1987) and that after complete withdrawal of testosterone it takes 72 h to reduce protein secretion by tubules at stages VI–VIII (Sharpe *et al.*, 1992). Second, in the present study it was shown that administering a dose of testosterone esters known to maintain quantitatively normal spermatogenesis and fertility in rats (Sharpe *et al.*, 1990) completely failed to alter the deleterious effects of heat treatment 24 h earlier on protein secretion by seminiferous tubules at stages VI–VIII.

This study also showed that local testicular heating altered the incorporation of [35 S]methionine into intracellular proteins at stages VI–VIII in a comparable manner to that of secreted proteins. This may indicate an alteration in one or more basic aspects of metabolic activity in tubules at these stages. These changes may explain some of the adverse effects of heat on spermatogenesis. It is also noteworthy that within 24 h of treatment of rats with testicular toxicants at doses known to cause subsequent disruption of spermatogenesis, precisely the same changes are observed to those shown to occur at 24 h after local testicular heating (McLaren *et al.*, 1993); that is, the normal stage-dependent increase in protein secretion at stages VI–VIII fails to occur, despite the maintenance of normal concentrations of testosterone.

Despite a significant increase in the overall incorporation of [35 S]methionine into secreted proteins by seminiferous tubules isolated at stages VI–VIII 4 h after local testicular heating, analysis by two-dimensional SDS-PAGE revealed that no major changes had occurred in the secretion of specific proteins. However, by 24 h after local testicular heating, the secretion of three androgen-regulated proteins was found to have changed. Furthermore, the secretion of these three proteins has been shown to be affected adversely following exposure to testicular toxicants (McLaren *et al.*, 1993). These earlier findings and those from the present study thus suggest that disruption of spermatogenesis by various agents may involve common pathways. Moreover, these pathways may involve downstream events of androgen action on the seminiferous tubule.

In this study, the morphological effects of local heating on the rat testis were identified histologically within 24 h of treatment. These initial effects were manifest as the loss or degeneration of pachytene spermatocytes at all stages of the spermatogenic cycle, while the nuclei of elongate spermatids at stages VI–VIII were found to be embedded deep within the cytoplasm of Sertoli cells. Round spermatids in stages I–VII appeared normal, compared with controls. The reason for the initial susceptibility of pachytene spermatocytes to the adverse effects of local testicular heating is unknown. However, treatment of rats with chemicals such as *m*-dinitrobenzene (Blackburn *et al.*, 1988), nitrobenzene (Bond *et al.*, 1981), ethylene glycol monomethyl ether/methoxyacetic acid (Chapin *et al.*, 1984; Creasy *et al.*, 1985) and an imidazole compound

(De Martino *et al.*, 1975) have all been shown to cause rapid degeneration of pachytene spermatocytes.

As specific depletion of pachytene spermatocytes from seminiferous tubules at stages VI–VIII also considerably reduces the amount of protein secreted at stages VI–VIII (McKinnell and Sharpe, 1992; Sharpe *et al.*, 1993), it is possible that it is the loss of these cells induced by heat treatment that is responsible for the alterations in seminiferous tubule protein secretion. However, the pattern of change in androgen-regulated proteins induced by heat treatment is substantially different from that induced by depletion of pachytene spermatocytes (McKinnell and Sharpe, 1992). In addition, depletion of pachytene spermatocytes has no effect on the incorporation of [35 S]methionine into intracellular proteins at stages VI–VIII (C. McKinnell and R. Sharpe, unpublished), whereas heat treatment 24 h earlier caused a reduction.

In conclusion, the present study has shown that exposure to short-term local testicular heating causes stage-specific changes in the incorporation of [35 S]methionine into both secreted and intracellular proteins, changes that particularly affect androgen-dependent aspects of seminiferous tubule function and which may be an important cause of the disruption of spermatogenesis following heat treatment.

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