The urethral glands of male mice in relation to depletion of secretory granules upon mating

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The present study describes the effects of mating on urethral gland acinar cells in male mice. Histological and morphometric analysis demonstrated that there was a depletion of secretory granules in the urethral glands during mating. However, no change occurred in the rough endoplasmic reticulum containing tubular elements. The results indicate that the urethral glands are functional during mating. The timing of their granule depletion suggests that urethral gland secretions may contribute to the formation of semen or the copulation plug.

Introduction

The male urogenital tracts of many rodents contain urethral glands in the pelvic and bulbous urethra (Hall, 1936). In mice, the pelvic urethra is visible in the pelvic cavity, whereas the bulbous urethra is surrounded and obscured from view by the bulbocavernous muscle (Hebel and Stromber, 1986). The bulbous urethra exhibits a wide, bifurcated lumen called the urogenital sinus (Hall, 1936) or the urethral diverticulum (Hebel and Stromber, 1986), into which the ducts of the bulbourethral glands (Cowper’s glands) empty. The urethral glands are compound acinar glands the acinar cells of which open into ducts that drain into the pelvic urethra or the urethral sinus.

The urethral glands have received little attention since they were described by Hall (1936), but recent evidence suggests that the glands may be sites for secretory immunity in the urogenital tract of the male rat and mouse (Parr and Parr, 1989; Parr et al., 1992). The glands contain plasma cells of the immunoglobulin A (IgA) isotype and their acinar cells express secretory component, the receptor for IgA transport. The urethral glands are targets of testosterone and contain androgen receptors (Parr et al., 1992), but their role in the physiology of the urogenital tract has not been investigated. Electron microscope studies suggest that the acinar cells may be involved in protein synthesis and secretion, since they contain stores of secretory granules and rough endoplasmic reticulum with enlarged cisternae filled with tubular material (Parr et al., 1992, 1993). These observations raise the possibility that the urethral glands, like other accessory sex glands, may contribute to the formation of seminal fluid or the copulation plug at the time of mating. The present report describes morphological changes that occur in mouse urethral gland acinar cells during mating, using histological and morphometric methods.

Materials and Methods

Animals

Fifteen male and ten ovariectomized female ICR mice (Harlan, Sprague–Dawley, Inc., Indianapolis, IN), 4–5 months old, were used. Behavioural oestrus was induced in ovariectomized female mice by injecting 10 µg oestradiol benzoate per mouse s.c., followed 48 h later by 500 µg progesterone per mouse (Mayerhofer et al., 1990). Four hours later one hormone-treated female was transferred into a cage housing one male mouse. Observations of mating behaviour continued for up to 4 h. The males were removed from the cages immediately after ejaculation and the females were checked for copulation plugs to verify that mating had occurred. Six of ten male mice that were placed with females mated and formed copulation plugs. Five males were kept isolated from females and served as nonmated controls.

Specimen preparation

Six mated mice immediately after ejaculation and five nonmated control mice were injected with 20 iu heparin. Fifteen minutes later the mice were killed by anaesthetization with tribromoethanol, and the genital tracts were fixed by vascular perfusion and processed for morphological studies (Sprando, 1990). Briefly, 0.9% (w/v) saline was perfused through the heart to clear blood vessels, followed by 3.5% (v/v) glutaraldehyde in 0.05 mol cacodylate buffer 1⁻¹, pH 7.4, or a mixture of 2.5% glutaraldehyde and 2% (w/v) paraformaldehyde in 0.1 mol cacodylate buffer 1⁻¹, pH 7.4. After perfusion for 30 min, the pelvic and bulbous urethrae were removed, fixed by immersion for 1 h, post-fixed in a mixture of 1% (w/v) osmium tetroxide and 1.5% (w/v) potassium ferrocyanide in 0.1 mol cacodylate buffer 1⁻¹ for 1 h, processed and embedded in Araldite (CY 212). Thick sections (1 µm) of the
bulbous and pelvic urethrae were stained with toluidine blue for light microscopy and thin sections of the bulbous urethra were stained with uranyl acetate and lead citrate for electron microscopy. Thin sections were examined using a Hitachi H500H electron microscope (Hitachi Scientific Instruments, San Jose, CA).

**Morphometry**

A morphometric analysis of the volumes of acinar cells and their nuclei from the urethral glands in the bulbous urethra was performed by standard stereological methods. Nuclear volumes were obtained by serially sectioning tissue blocks, tracing randomly selected nuclei, and measuring the surface area of the nuclear profiles (Sinha Hikim et al., 1988, 1989). Twenty consecutive sections from each block per animal were taken at a thickness of approximately 0.92 μm using a calibrated ultramicrotome (Sinha Hikim et al., 1988). The number of sections in each case was sufficient to traverse the entire nucleus. The outlines of each nuclear profile were copied on to tracing paper using a camera lucida attached to a light microscope (Nikon Microphot, Garden City, NJ) at ×1000 magnification. Five nuclei of each animal were randomly selected (lottery method) and traced throughout the serial section series. The surface area of each nuclear profile was digitized three times using a calibrated automatic digitizer (Model 1224; Numonics, Lansdale, PA). The nuclear volume for the acinar cells of each animal was calculated as the sum of all of the individual areas of each nucleus multiplied by the section thickness. A mean for each group was obtained. The acinar cell volume was determined by point counting according to the following formula:

\[ V_{\text{cell}} = V_{\text{nucleus}} \times \text{points over cell/points over nucleus}. \]

The sections in which cell volumes were determined were randomly selected by a lottery method and examined under a Microphot-FX microscope equipped with a bright-field condenser, a ×40 objective and a ×10 eyepiece fitted with a square lattice containing 441 intersections. The number of pertinent structures, that is, nuclei and gland cells, over the entire section was counted by moving the sections across the grid without overlapping them. A total of 1000 points was counted per section. The cytoplasmic volume of the acinar cells was determined by subtracting the nuclear volume from the cell volume.
Urethral gland secretory acini were selected from histological sections 1 μm thick by lottery (Sinha Hikim et al., 1989) to determine the area to be trimmed for electron microscopy. In ultrathin sections, acini were divided arbitrarily into four equal parts, and the quadrant to be photographed was selected by a lottery method. If acini were small, the entire acinus was photographed. Electron micrographs thus selected were used to measure the volume densities of secretory granules and rough endoplasmic reticulum containing tubular material (Sinha Hikim et al., 1989). All negatives (×3000) were printed at a magnification of 2.6 times the negative magnification (final magnification ×7800). Each acinus was considered to be a sphere with the lumen as its centre and each acinus was divided into a number of pie-shaped wedges that were used for morphometric determinations. The volume density (Vv) of acinar cell organelles was determined using the point-counting method (Weibel, 1979). A transparent overlay bearing a double-lattice grid was superimposed on each micrograph. The Vv of each organelle was obtained by dividing the number of points falling on each structure (Pv) by the number of points counted over the entire cell (P). The results were expressed as percentages of the acinar cell volume (Vv, %) by multiplying the volume density by 100. The absolute volume of each organelle (Vv) was then calculated by multiplying its volume density (Vv, %) by the average volume of the acinar cells (Vv). Six to nine micrographs of acini from each of 11 mice were used for point counting (83 micrographs in total).

Results

Urethral glands in nonmated mice

The urethral glands in the pelvic and bulbous urethrae were histologically similar. Both consisted of acini composed of pyramidal cells the apices of which bordered on a narrow lumen (Fig. 1). The apical region of acinar cells contained prominent Golgi complexes and numerous granules up to 2 μm in diameter. The granules were either uniformly dense (Fig. 2) or showed regions of lesser density (Fig. 3a). The basal regions of acinar cells contained elongated mitochondria and abundant rough endoplasmic reticulum of unusual appearance (Fig. 3). The cisternae of the rough endoplasmic reticulum were often dilated and contained small, closely packed, tubular structures that were randomly arranged, oriented parallel to one another, or that radiated outward from aggregates of dense material. The tubular structures were straight and unbranched and were never seen to be continuous with the rough endoplasmic
Mating caused a striking depletion of granules from the acinar cells of pelvic urethral glands (Fig. 4). Although degranulation was less obvious in acinar cells in the bulbous urethral gland (Fig. 5), morphometric analysis (Table 1) revealed a statistically significant ($P < 0.05$) reduction by 38% in the volume of granules in these cells. In contrast, mating caused small increases in the mean volume of the rough endoplasmic reticulum, the whole cell, the cytoplasm, and the nucleus, of which only the change in nuclear volume was statistically significant ($P < 0.05$).

Discussion

The results of these experiments suggest that the urethral glands discharge stored secretory granules into the acinar lumen and presumably into the urethral lumen during exposure to females and during mating. Morphological evidence of secretion, such as fusion of the secretory granule membrane with the apical cell membrane, was not observed. However, such a fusion event may be too rapid to be seen routinely, or it may be completed before the mice were killed in this study. In a similar study, Geuze and Slot (1976) found that synthesis and secretion of glycoproteins in the rat bulbourethral gland (Cowper’s gland) was strongly influenced by copulation. Cells in Cowper’s glands from control rats were packed with secretory granules, but secretion began immediately after the start of sexual intercourse. Granule depletion was completed...
after 4 h, concurrent with the end of mating. The glandular epithelial cells became low and cuboidal, containing few if any secretory granules, and showed a ruffled apical membrane. The nuclei of these cells appeared swollen and less dense than in control cells. Geuze and Slot (1976) also reported that the cellular changes in Cowper’s glands appeared to depend on the time that elapsed between the first intromission and death rather than on the number of intromissions or copulation plugs.

In the present study, mating also caused a significant increase in the size of acinar cell nuclei in bulbous urethral glands. This finding is interesting given the short time that males were exposed to females. There is, however, some basis for short-term nuclear changes relating to testosterone stimulation. Within 30–60 min after males are placed with strange females there is a threefold rise in the serum concentration of testosterone in males (Macrides et al., 1975). It is well known that androgen receptors are present within nuclei of androgen-responsive tissues and that androgen binding initiates transcriptional events (Sar et al., 1990). Androgen-responsive tissues that have been deprived of androgen respond by increasing their nuclear size when androgen production is resumed, even in relatively short-term experiments (Sinha Hikim et al., 1988, 1989). We assume that androgen-stimulated nuclear transcriptional events are responsible for replenishing the secretory granules in acinar cells after mating, since it has been shown that acinar cells, their nuclei, and their secretory granule population in castrated males are substantially reduced in volume and that the volumes of these parameters in castrated males can be maintained by androgen (Parr et al., 1992).

The degranulation that occurs in the urethral glands at the time of mating suggests that these glands, like other accessory sex glands, may play a role in the formation of semen or the copulation plug. It is known that secretions of the seminal vesicles, coagulating glands, and Cowper’s glands undergo complex biochemical interactions that result in semen coagulation at the time of mating (Price and Williams-Ashman, 1961; Hart and Greenstein, 1968). The coagulating glands secrete a heat-labile enzyme called vesiculase, which coagulates a basic protein secreted by the seminal vesicles in vitro (Gottler et al., 1955; Notides and Williams-Ashman, 1967). Cowper’s glands secrete a heat-stable, sialic acid-rich complex that coagulates a seminal vesicle protein in vitro that is electrophoretically distinct from the substrate of vesiculase (Hart and Greenstein, 1968). In addition, clotting of the seminal vesicle substrate by Cowper’s gland secretions is enhanced when the substrate is incubated with coagulation gland secretions for a period before adding Cowper’s gland secretions (Beil and Hart, 1973). The function, if any, of urethral gland secretions in these complex biochemical interactions awaits further study.

We have previously shown that the urethral glands of the male mouse are dependent on testosterone and contain androgen receptors (Parr et al., 1992). The results of the present study indicate that the acinar cells of urethral glands degranulate during mating, suggesting that they contribute to the formation of semen or the copulation plug. Thus, the urethral glands show similarities to other male reproductive accessory

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**Table 1.** Morphometric analysis of acinar cells of the bulbous urethral gland in male mice.a

<table>
<thead>
<tr>
<th>Mice</th>
<th>Nucleus</th>
<th>Cytoplasm</th>
<th>Cell</th>
<th>Granules</th>
<th>Rough endoplasmic reticulum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmated</td>
<td>190.1 ± 6.1</td>
<td>1832.3 ± 110.6</td>
<td>2022.4 ± 116.3</td>
<td>732.3 ± 44.2</td>
<td>243.5 ± 69.2</td>
</tr>
<tr>
<td>Mated</td>
<td>222.3 ± 11.6</td>
<td>2019.6 ± 146.2</td>
<td>2241.9 ± 151.2</td>
<td>452.7 ± 84.4</td>
<td>316.6 ± 67.4</td>
</tr>
</tbody>
</table>

aValues are means ± SEM (µm² per cell).
bSignificantly different from nonmated mice (P < 0.05; Student’s t test).
glands in being dependent on androgen (Price and Williams-Ashman, 1961; Spring-Mills and Hafez, 1979; Cooke et al., 1987), containing epithelial androgen receptors (Sar et al., 1990; Takeda et al., 1990; Cooke et al., 1991; Husmann et al., 1991) and participating in the formation of semen or the copulation plug. In addition, previous investigations of local immunity in the male mouse and rat urogenital tracts have indicated that secretory component and IgA plasma cells are localized mainly in the urethral glands (Parr and Parr, 1989). A study of the role of these glands in the secretion of secretory IgA into the semen would therefore be of interest.

This work was supported by an NIH grant HD-17737 to M. B. Parr.

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