

—Full Paper—

## Oxytocin Immunoreactivity in the Human Urethral (Littre's) Glands

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Denise MASSA<sup>1)</sup>, Alberto CASTI<sup>1)</sup>, Paola SOLINAS<sup>1)</sup> and M. Serenella LANTINI<sup>1)</sup>

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**Abstract.** Oxytocin is a cyclic nonapeptide whose best known effects are stimulation of uterine smooth muscle cells during labor and of milk ejection during lactation. Circulating oxytocin originates from the hypothalamus, but its production has also been documented in peripheral tissues. Furthermore, seminal plasma also contains oxytocin, but its functional role is still unknown, although its secretion is generally ascribed to the prostate. In this study, we investigated the possibility that seminal oxytocin is also secreted by other exocrine glands of the human male genital tract. Intramural (Littre's) glands isolated from bioptic specimens of normal urethrae were processed for immunogold localization of oxytocin. Immunostaining was detected in principal cells, with gold particles specifically found on secretory granules. Basal and endocrine cells were unstained. The present findings suggest that urethral glands not only produce the mucinous layer that protects and lubricates the urethral wall, but also are potential sources of other seminal components, such as oxytocin, which probably play still unclear roles in reproductive physiology.

**Key words:** Immunogold, Oxytocin, Urethral glands

(J. Reprod. Dev. 56: 94–97, 2010)

Oxytocin is a nonapeptide in which a disulfide bond between cysteine residues confers a cyclic structure. Its action is mediated by a transmembrane polypeptide receptor coupled with a G protein pathway, widely distributed throughout the human body [1, 2]. Oxytocin is mainly studied as a hypothalamic hormone as well as vasopressin, from which it differs in two amino acids only. The best known biological effects of circulating oxytocin are those on female reproductive organs, such as stimulation of uterine contractions and of milk ejection. Moreover, effects on organs of the male genital tract have also been documented, such as penile erection and ejaculation [3, 4]. In addition, a number of functions are thought to be regulated by local oxytocinergic systems, since oxytocin production has been documented in peripheral tissues [5]. In the male genital tract, paracrine secretion of oxytocin has been suggested in the testis, epididymis and prostate [1, 6, 7], where many functions such as cell growth, spermiogenesis, steroidogenesis and muscle contraction could be affected [7–10]. Furthermore, seminal plasma also contains oxytocin, whose functional role still is uncertain [11–13]. This peptide is believed to originate from the prostate [1], but it could be also supplied by other sexual glands, which to date have not been examined in this regard. In this study, we show that the intramural glands of the human urethra (Littre's glands) contain oxytocin-reactive material, suggesting the presence of the peptide in their exocrine secretions.

### Materials and Methods

Samples of the distal urethra were obtained from 4 patients aged 54–67 undergoing surgery for radical cystectomy at the Urologic

Clinic of the University of Cagliari. The procedures were approved by the Local Ethics Committee, University of Cagliari, and informed consent was obtained from all patients. The samples were immediately cut into small fragments and fixed in a mixture of 3% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h and then rinsed in the same buffer, dehydrated in ethanol and embedded in Epon resin (Glycide Ether 100, Merck, Darmstadt, Germany). Semi-thin sections were cut at 2  $\mu$ m and stained with toluidine blue. Ultrathin sections, 80 nm, were collected on nickel grids and treated with 1% bovine serum albumin (BSA) and 5% normal goat serum (NGS) in phosphate buffered saline (PBS) to block non-specific binding. Grids were incubated overnight at 4°C with a mouse monoclonal antibody directed to oxytocin (Millipore Corporation, Billerica, MA, USA) diluted 1:100 in 1% BSA-NGS-PBS solution. Control sections were incubated with a medium devoid of the primary antiserum, with non-immune mouse serum and with the primary serum absorbed with oxytocin (Sigma-Aldrich Srl). All the grids were rinsed with PBS and then incubated with goat anti-mouse IgG labeled with 10 nm gold particles (Amersham International plc, Little Chalfont, UK) diluted 1:50 in 1% BSA-PBS for 60 min at room temperature. After rinsing in PBS and distilled water, the grids were stained with uranyl acetate and bismuth subnitrate and observed and photographed using a JEOL 100S electron microscope.

### Results

The urethral glands, defined as ramified tubulo-alveolar glands, showed a normal morphology, with principal exocrine, basal and endocrine-like cells (Fig. 1). Oxytocin reactivity appeared restricted to the principal cells, where gold particles were found on some Golgi cisternae and secretory granules with different substructures (Figs. 1–4). The mucous-like granules, the most

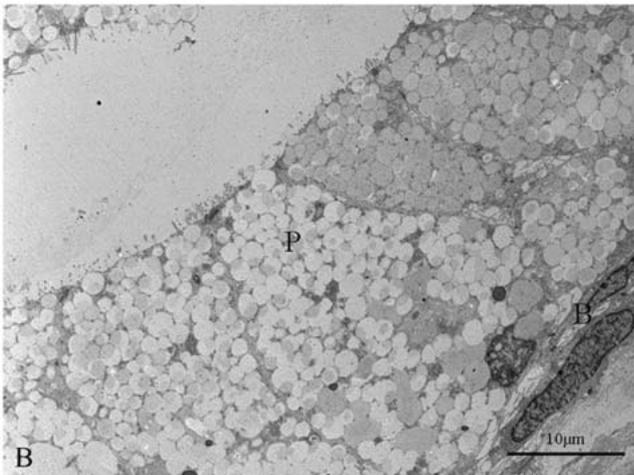
Received: April 9, 2009

Accepted: October 5, 2009

Published online in J-STAGE: November 5, 2009

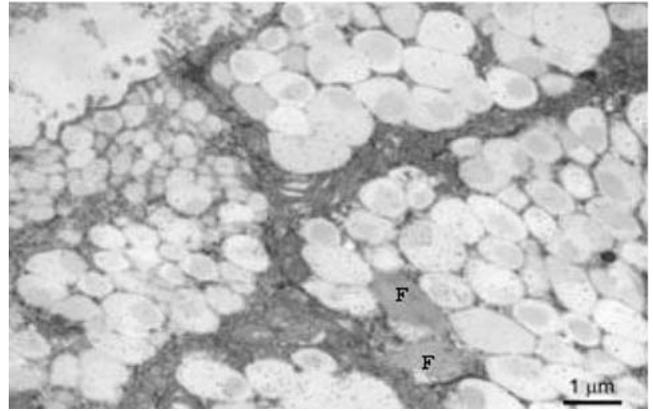
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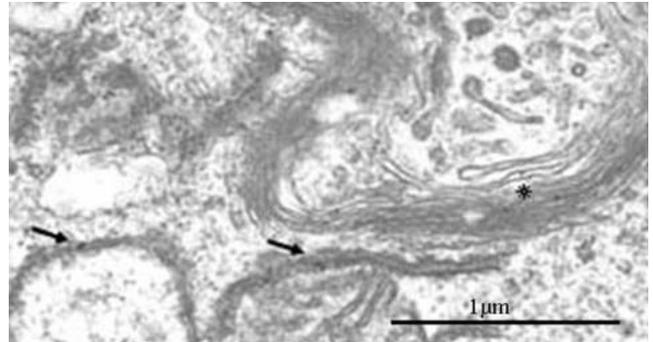


**Fig. 1.** Human urethral gland morphology. A: Light microscopic image of urethral tubulo-alveolar glands. B: Transmission electron microscopic (TEM) image showing a general view of the urethral gland epithelium, where principal (P) and basal (B) cells are clearly distinguishable. The cytoplasm of the principal cells is filled with secretory granules of variable density, that are immunoreactive for oxytocin, but gold particles are not discernible at this magnification.

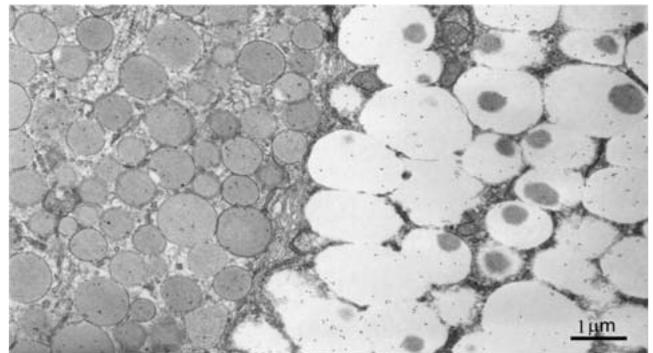
common type, were specifically labeled in their pale matrix, while the dark intragranular masses were less stained or completely unstained (Fig. 2). Typical filamentous bodies, which represent another secretory product, were unreactive (Figs. 2, 3). Oxytocin reactivity was also revealed in a few cells housing only dense granules (Fig. 4). The nuclei and all other cytoplasmic membranous organelles were always unreactive, as were the cytosol and cell surfaces. Endocrine-like elements, characterized by the abundance of small secretory vesicles of heterogeneous morphology, were always negative, as were basal cells (Fig. 5). The sections incubated with the control media were completely unstained (Fig. 6).



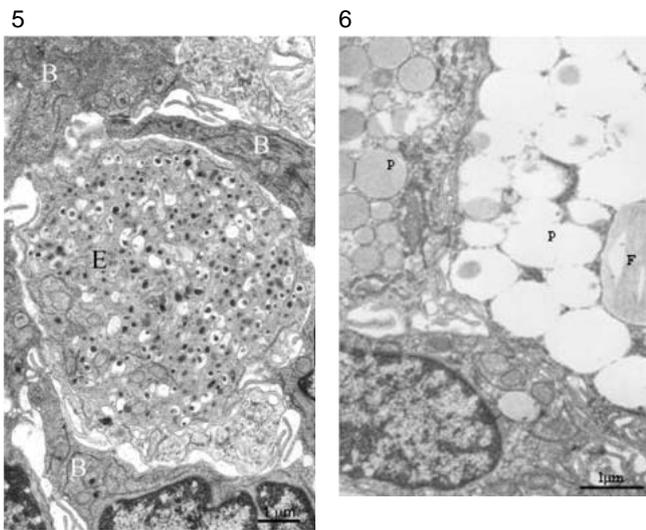
**Fig. 2.** Principal cells of the human urethral gland. Oxytocin reactivity is specific for bipartite granules, i.e., granules with an electron dense core, whereas filamentous bodies (F) are unstained.



**Fig. 3.** Principal cell of the human urethral gland. Cisternae of the Golgi apparatus (\*) and RER (arrows) decorated by gold particles labeling oxytocin.



**Fig. 4.** Adjacent principal cells of the human urethral gland showing different types of secretory granules, both labeled for oxytocin.



**Fig. 5.** Endocrine-like (E) and basal cells (B) located deeply in the epithelium of the human urethral glands appear unstained.

**Fig. 6.** Human urethral gland control section. Incubation of the sections without the primary antiserum does not produce any labeling. Principal cell (P). Basal cell (B). Filamentous body (F).

## Discussion

Oxytocin reactivity in the secretory granules of human urethral glands suggests that these glands secrete this peptide. Immunohistochemical demonstration of oxytocin is usually coupled with that of its carrier molecule neurophysin I in order to prove local production of the peptide [6, 7, 13]. In our experiments, specific oxytocin labeling of granules and Golgi apparatus could indicate that the peptide follows the common intracellular route of secretion. Thus, demonstration of neurophysin I appeared superfluous. Actually, we expected oxytocin reactivity in endocrine-like cells, which are morphologically similar to those described in the prostatic and urethral epithelia. These cells are believed to release a variety of peptides and amines, such as serotonin, chromogranin A, calcitonin, CGRP and somatostatin [14], not only basally but also, as cells of the open type, into the glandular lumen. Given the lack of immunoreactivity, it appears that oxytocin peptide does not belong to the pool of substances produced by endocrine-like cells. Urethral glands are known to secrete the mucus layer, which protects and lubricates the urethral wall. Their principal cells share many morphological features with typical mucin secreting elements, except for some peculiar aspects of their secretory granules [15]. What we know about their secretion dates back to a few histochemical studies documenting the presence of acidic glycoproteins [16] bearing AB0 and Lewis oligosaccharides [17]. Demonstration of oxytocin reactivity suggests that urethral glands do not provide only mucus components. Oxytocin production has previously been noted in organs of the male reproductive tract, such as the testis, epididymis and prostate gland, in several mammals including man [9, 10, 12, 18]. However, in most studies, attention was chiefly devoted to the oxytocinergic paracrine mechanisms, which locally

regulate many processes, such as steroid metabolism, muscle contraction and cell growth, rather than oxytocin release into the semen. It is generally believed that the prostate gland provides the seminal oxytocin [1, 19], but our results suggests that prostatic secretion is not the only source of the seminal hormone, since at least a small additional fraction could be supplied by the urethral glands. Oxytocin secretion by human urethral glands could be confirmed by the presence of oxytocin in human pre-ejaculatory fluid, which consists of urethral and bulbourethral secretions. To date, oxytocin in pre-ejaculatory fluid has only been demonstrated in the stallion [13]. The functional significance of oxytocin in semen still is obscure. It might influence some sperm activities as well as interact with female tissues. The direct effects on sperm number and motility have been investigated, but the resulting data were not statistically significant [12]. Although we ignored whether spermatozoa have oxytocin receptors, the presence of oxytocinase on their surfaces [20] suggests that the hormone might be active in spermatozoa. Moreover, since it has been demonstrated that oxytocin stimulates at least 5- $\alpha$ -reductase activity [21], it can be hypothesized that seminal oxytocin might influence the metabolism of androgen hormones occurring in semen [22]. Finally, oxytocin produced by exocrine cells might even have intracellular effects, since epithelial cells express the enzyme oxytocinase, also called leucyl aminopeptidase [23]. It is conceivable that this intracellular function might be related with the antioxidant properties ascribed to oxytocin and other peptidic hormones [24], which regulate the homeostasis of cytoplasmic membranes.

## Acknowledgments

The authors thank Mrs S Bernardini and Mr A Cadau for their excellent technical assistance. This investigation was supported by Fondazione Banco di Sardegna.

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