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Original Research Article

Topographic distribution of serotonin-immunoreactive urethral endocrine cells and their relationship with calcitonin gene-related peptide-immunoreactive nerves in male rats

Takuya Yokoyama[a,*], Tomoyuki Saino[a], Nobuaki Nakamuta[b], Yoshio Yamamoto[b]

[a] Department of Anatomy (Cell Biology), Iwate Medical University, Yahaba, Japan
[b] Laboratory of Veterinary Anatomy and Cell Biology, Faculty of Agriculture, Iwate University, Morioka, Japan

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ABSTRACT

We investigated the topographic distribution and morphology of serotonin (5-HT)-immunoreactive endocrine cells in the urethra of male rats, and focused on their relationship with peptidergic nerve fibers immunoreactive for calcitonin gene-related peptide (CGRP). Urethral endocrine cells immunoreactive for 5-HT were densely distributed in the epithelial layers of the prostatic part, but were sparsely distributed in the membranous and spongy parts of urethra. Distribution of urethral endocrine cells with 5-HT immunoreactivity in the prostatic part was restricted from the internal urethral orifice to the region of seminal colliculus. 5-HT-immunoreactive endocrine cells were also observed in the ductal epithelial layers of coagulating glands, prostatic glands, and seminal vesicles. 5-HT-immunoreactive endocrine cells were triangular or flask in shape and possessed an apical projection extending toward the urethral lumen, and basal or lateral protrusions intruding between other epithelial cells were also detected in some cells. Double immunolabeling for 5-HT and CGRP revealed that CGRP-immunoreactive nerve fibers attached to urethral endocrine cells with 5-HT immunoreactivity in the prostatic part. These results suggest that urethral endocrine cells may release 5-HT in response to luminal stimuli, and that these cells and CGRP-immunoreactive nerves may regulate each other by an axon reflex mechanism.

1. Introduction

In the urogenital tract, endocrine cells have been reported to be distributed in the urethral epithelium of several mammalian species such as guinea pigs (Dixon et al., 1973), dogs (Hashimoto et al., 1999), humans (Lendon et al., 1976), rabbits (Cecio and Vittoria, 1989), and rats (Ramsdale, 1974; Pinheiro et al., 2007; Aumüller et al., 2012). According to these reports, urethral endocrine cells contain serotonin (5-hydroxytryptamine; 5-HT) in both sexes, as evidenced by fluorescence microscopy and immunohistochemistry (Dixon et al., 1973; Cecio and Vittoria, 1989; Pinheiro et al., 2007; Aumüller et al., 2012). It has been reported that endocrine cells are observed most frequently in the middle portion of the urethra in female dogs and guinea pigs, but in the male animals these cells are less numerous in the spongy part than in other regions of the urethra (Håkanson et al., 1974; Hanyu et al., 1987). On the other hand, another study found numerous endocrine cells with 5-HT immunoreactivity in the spongy part of the urethra in donkeys (Arrighi et al., 2004). Moreover, immunohistochemical analysis has revealed urethral endocrine cells with 5-HT immunoreactivity to be remarkably reduced in the penis of older humans (Iwanaga et al., 1987). Thus, the distribution of endocrine cells is expected to be involved in male urogenital functions. A previous immunohistochemical investigation has revealed that peptidergic nerve fibers immunoreactive for calcitonin gene-related peptide (CGRP) are associated with urethral endocrine cells in male dogs (Tamaki et al., 1992; Iwanaga et al., 1994). However, the topographical and morphological characteristics of endocrine cells, and the interrelationship between CGRP-immunoreactive nerves and these cells in the male rat urogenital tract have not yet been elucidated in detail.

In the present study, we examined the topographic distribution and morphology of urethral endocrine cells in male rats using immunohistochemistry for the identification of 5-HT. We also performed double immunofluorescence in order to elucidate

Abbreviations: CG, coagulating gland; CGd, coagulating gland duct; CGRP, calcitonin gene-related peptide; Dd, deferent duct; Ed, ejaculatory duct; MG, mucous gland; PG, prostate gland; PGd, prostate gland duct; PS, pubic symphysis; SC, seminal colliculus; SV, seminal vesicle; SVd, seminal vesicle duct; UB, urinary bladder; 5-HT, 5-hydroxytryptamine, serotonin.

* Corresponding author at: Department of Anatomy (Cell Biology), Iwate Medical University, 2-1-1 Nishitokuta, Yahaba-cho, Shiwa gun, Iwate, 028 3694, Japan.
E-mail address: ytakuya@iwate-med.ac.jp (T. Yokoyama).

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Table 1
Antibodies used in the present study.

	Code	Host	Dilution	Source
Primary antibodies				
5-HT	ab66047	Goat	1:4000	A
CGRP	BML-CA1134	Rabbit	1:1000	B
Secondary Antibodies				
Alexa fluor 488-labeled anti-goat IgG	705–545-147	Donkey	1:600	C
Cy3-labeled anti-rabbit IgG	711–175-152	Donkey	1:600	C

A, Abcam, Cambridge, UK; B, Enzo Life Sciences, Farmingdale, NY, USA; C, Jackson ImmunoResearch, West Grove, PA, USA.

the morphological relationship between 5-HT-immunoreactive endocrine cells and CGRP-immunoreactive nerves fibers.

2. Materials and methods

2.1. Animals

Male Wistar rats (8–10 weeks old; 180–200 g) were purchased from Japan SLC. (Hamamatsu, Japan). A total of 10 rats were used in the present study. All animal experiments in the present study were approved by the Local Animal Ethics Committee of Iwate University (accession number #A201455).

2.2. Immunohistochemistry

Rats were anesthetized using pentobarbital (50 mg/kg; intraperitoneal injection) and transcardially perfused with Ringer's solution (200 ml) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4, 200 ml). The prostatic part of the urethra was exposed by cutting the pubic symphysis, and the urethra from the internal urethral orifice at the bladder neck to the external urethral orifice at the tip of glans penis was removed under a dissecting microscope. The urethra was cut into prostatic, membranous, and spongy parts, and these tissue samples were further fixed with the same fixative overnight at 4 °C. After 3 washes in phosphate-buffered saline (PBS; pH 7.4) for 10 min, tissue samples were soaked in PBS containing 30% sucrose and frozen with O.C.T. compound medium (Sakura Finetek, Tokyo, Japan). Frozen tissues were serially sectioned transversely at a thickness of 50 μm by use of a cryostat (CM1900; Leica, Wetzlar, Germany), and were then collected in PBS.

Free-floating sections were stained by immunofluorescence using an antibody against 5-HT in order to investigate the topographic distribution and morphology of urethral endocrine cells. Details of the antibodies used in the present study are summarized in Table 1. Sections were rinsed with PBS and incubated with non-immune donkey serum (1:50 dilution) for 30 min at room temperature. After blocking non-specific reactions, sections were incubated for 2 days with goat polyclonal anti-5-HT at 4 °C. Sections were then rinsed with PBS and incubated for 120 min at room temperature with Alexa Fluor 488-labeled donkey anti-goat IgG. After rinsing with PBS, sections were counterstained with DAPI for 10 min at room temperature. After rinsing with PBS, sections were mounted on gelatin-coated slides, air-dried, and coverslipped with mounting medium (Fluoromount; Diagnostic Biosystems, Pleasanton, CA, USA). Sections were observed by use of a confocal laser microscope (C2, Nikon, Tokyo, Japan), and a single confocal image or z-stacks of confocal images were obtained with ×10, ×20, or ×100 objective lens. Projection images were made from 4 to 21 series at 1 μm intervals with the computer software, NIS-Elements (Nikon). All images were analyzed with the use of Photoshop CS5 software (Adobe Systems, San Jose, CA, USA) in addition to NIS-Elements.

We also performed double immunofluorescence for 5-HT and CGRP in order to examine the morphological relationship between urethral endocrine cells and nerve fibers immunoreactive for CGRP in the prostatic part of urethra. After blocking non-specific reactions, sections were incubated for 2 days at 4 °C with a mixture of primary antibodies. Sections were then rinsed with PBS and incubated for 120 min at room temperature with a mixture of secondary antibodies. After rinsing with PBS, sections were counterstained with DAPI and coverslipped with mounting medium. Sections were observed by use of a confocal laser microscope, and z-stacks of confocal images were obtained with ×20 or ×100 objective lens. Projection images were made from 11 to 35 series at 1 μm intervals with the computer software, NIS-Elements.

PBS or non-immune serum was used for immunohistochemical controls instead of primary or secondary antisera. We confirmed the complete abolishment of specific labeling in negative controls.

3. Results

Anatomical distribution of the male rat urethra is shown in Fig. 1A. The rat urethra is divided into 3 parts: prostatic, membranous, and spongy parts, as indicated by red, blue, and green lines in Fig. 1A, respectively. The prostatic part is the portion of the urethra surrounded by the body of the prostate gland (PG), and ranged from the internal urethral orifice of the urinary bladder (UB) to the tip of the PG. The prostatic part is connected with the opening of ejaculatory ducts (Ed) formed by the union of the deferent duct (Dd) and the excretory duct of the seminal vesicle (SV) at the region of the seminal colliculus (SC), and is also connected with excretory ducts from coagulating glands (CG) and the PG. The membranous and spongy parts are situated behind the pubic symphysis (PS) and in the penis, respectively. Low magnification views of free-floating sections immunostained with 5-HT in 3 parts of urethra are shown in Fig. 1B–D. In sections of the prostatic part, 5-HT immunoreactivity was observed in numerous endocrine cells distributed in the transitional epithelium (Fig. 1B). The 5-HT-immunoreactive cells appeared to be densely distributed in the deeper part of urethral epithelial folds. Furthermore, numerous endocrine cells immunoreactive for 5-HT were also distributed in the simple columnar epithelia of coagulating gland ducts (CGd) and prostate gland ducts (PGd). However, 5-HT immunoreactivity was not detected in the lamina propria and the epithelial layers of deferent ducts (figure not shown). In sections of the membranous part, 5-HT-immunoreactive endocrine cells were sparsely scattered in the circumference of the pseudostratified columnar epithelium, but not in mucous glands (MG) of lamina propria (Fig. 1C). In sections of the spongy part, a few endocrine cells showed 5-HT immunoreactivity in the pseudostratified columnar epithelium (Fig. 1D).

Most of the 5-HT-immunoreactive endocrine cells appeared triangular or flask in shape, and were observed as solitary cells in the urethral epithelium (Fig. 2A). These cells projected a slender apical cytoplasmic process toward the urethral lumen, while short protrusions along the basement membrane were also detected. 5-HT-immunoreactive products were localized to the perinuclear cytoplasm of the endocrine cells, but strong immunolabeling was frequently observed in the apical surface of their slender cytoplasmic processes. Some endocrine cells possessed a short lateral process from the slender apical projection (Fig. 2B). The 5-HT-immunoreactive lateral processes intruded and terminated with flattened shapes between other urethral epithelial cells. A few endocrine cells were bipolar in shape and projected both apical and basal cytoplasmic processes in the urethral epithelial layers (Fig. 2C). Their apical processes reached the urethral lumen, whereas basal processes extended straight to the basement membrane. Urethral endocrine cells with 5-HT immunoreactivity