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Localization of manserin, a secretogranin II-derived neuropeptide, in the oviduct of female rats

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ABSTRACT

Gynecological disorders related to menstrual cycle may be affected by stress and can cause infertility. Manserin is a stress-related neuropeptide that is present in the neuroendocrine system. In the present study, we determined the localization of manserin in the oviduct of adult Wistar rats using immunohistochemical techniques. Manserin was detected on the surface of the epithelium of the oviduct, but not in the ovary and uterus. Localization of manserin was specific to a large portion of the isthmus and to a small portion of the ampulla. These results suggest that manserin localizes to secretory cells in the oviduct and may be involved in stress-induced gynecological disorders.

Introduction

Gynecological disorders, such as amenorrhea, polycystic ovary syndrome, premenstrual syndrome, endometriosis, and uterine fibroids, related to menstrual cycle are regulated by hormones (Petraglia et al., 2008). The hypothalamus–pituitary–gonad (HPG) axis plays a critical role in the functioning of the reproductive system. However, activation of the hypothalamic–pituitary–adrenal (HPA) axis exerts an inhibitory effect on the female reproductive system (Ferin, 1999). Corticotropin-releasing hormone (CRH) inhibits gonadotropin-releasing hormone (GnRH), and glucocorticoids inhibit luteinizing hormone (LH), estrogen, and progesterone secretion and cause menstrual cycle-related diseases, such as menstrual disorders and amenorrhea (Kalantaridou et al., 2004, 2010). Moreover, an epidemiological study found that stress is correlated to menstrual cycle irregularities and menstrual pain among Japanese female workers (Nohara et al., 2011). However, the mechanisms behind these associations are not well understood.

The vertebrate oviduct is composed of the infundibulum, ampulla, and isthmus. When an egg is released from the ovary, it is received in the fimbria of the infundibulum and transported to the uterus through the oviduct. The oviduct provides the environment required for egg maturation, fertilization, and implantation (Croxatto, 2002; Coy et al., 2012). The environment of the oviduct is regulated by the sex hormones estrogen and progesterone. The production of these hormones is regulated by the HPG axis, which controls the female estrus cycle (Fink, 2000; Schwartz, 2000).

Manserin is a 40-amino acid peptide derived from its precursor secretogranin II (Yajima et al., 2004) and is distributed in the brain and organs, such as duodenum (Yajima et al., 2008), pancreas (Tano et al., 2010), cerebellum (Ohkawara et al., 2011), and inner ear (Ida-Eto et al., 2012). Secretogranin II is the precursor of other peptides, such as secretoneurin and EM66 (Marksteiner et al., 1993; Anouar et al., 1998). Because these peptides are present in the hypothalamic nuclei, pituitary and adrenal glands, we reasoned that they may function as neurotransmitters in the endocrine and neuronal systems, although their receptors have not yet been identified. We recently reported that physical stress upregulates manserin levels in the adrenal gland (Kamada et al., 2010), indicating that manserin may be involved in the stress response.

As part of our efforts to identify the function of neuropeptides in the female reproductive tract, in the present study we report, for the first time to the best of our knowledge, that manserin is localized in the oviduct.

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Materials and methods

Animals and tissue preparation

Wistar rats were purchased from CLEA Japan, Inc. (Tokyo, Japan). All animal experiments were approved by the Committee of Laboratory Animal Research Center of Mie University. Female Wistar rats (12–15-weeks-old) were anesthetized with pentobarbital and transcardially perfused with 4% paraformaldehyde in 10 mM phosphate buffered saline (PBS), pH 7.4, and the reproductive organs ovary, oviduct, and uterus were dissected and excised for analysis. The samples were immersed in the same fixative overnight at 4 °C. After washing with PBS, the tissues were cryoprotected in sucrose overnight at 4 °C, embedded in Tissue-Tek O.T.C. compound (Sakura Finetek, Torrance, CA, USA), and stored at −80 °C. The tissues were cryosectioned (10 μm thick sections), mounted on glass slides, dried for 30 min, and stored at −80 °C until use.

Antibodies

An affinity-purified rabbit anti-manserin antibody was prepared as previously described (Yajima et al., 2004; Kamada et al., 2010; Tano et al., 2010). The specificity of anti-manserin antibody has been previously confirmed by immunoblotting (Yajima et al., 2004). The anti-β-tubulin IV antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA) and anti-oviductin antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The secondary antibodies used were as follows: biotinylated goat anti-rabbit IgG (Chemicon, Temecula, CA, USA), Alexa Fluor® 488-conjugated donkey anti-goat IgG, Alexa Fluor® 568-conjugated donkey anti-rabbit IgG, Alexa Fluor® 568-conjugated goat anti-rabbit IgG, and Alexa Fluor® 488-conjugated goat anti-mouse IgG (Invitrogen, Carlsbad, CA, USA).

Immunohistochemistry

Sections were immunostained as previously described (Ida-Eto et al., 2011, 2012). In brief, frozen sections were washed with PBS and were treated with 3% hydrogen peroxide to quench endogenous peroxidase activity. After washing with PBS, sections were blocked with 3% skim milk in PBS and incubated with the anti-manserin antibody. Then the sections were sequentially treated with biotinylated goat anti-rabbit IgG. Finally, sections were immunostained using the ABC method (Vectastain® ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA) and were visualized using 3,3′-diaminobenzidine hydrochloride (DAB) and were counterstained with hematoxylin.

Immunofluorescence assays were performed as previously described (Ida et al., 2006; Ida-Eto et al., 2012). For double immunostaining, sections were incubated with the anti-manserin antibody and either anti-β-tubulin IV or anti-oviductin antibodies. To identify the nucleus, the nuclear-DNA staining dye TO-PRO®-3 (Invitrogen) was used. Sections were then incubated with secondary antibodies conjugated to the fluorescent molecules as described above. After mounting with Prolong® Gold Antifade reagent (Invitrogen), the sections were examined using a laser-scanning confocal microscope (FV1000, Olympus, Japan). Confocal images were saved as TIFF files and imported into Adobe Photoshop software (Adobe Systems, San Jose, CA, USA) for figure preparation.

Results

Localization of manserin in female reproductive organs

Immunohistochemical analysis of female reproductive organs revealed positive signals for manserin in the oviduct (Fig. 1A, arrows) but not in the ovary or uterus. Signals were not detected without the anti-manserin antibody (Fig. 1B). Manserin signals were strongly detected at the luminal surface (open arrowhead), epithelial cells (arrow) of the isthmus (Fig. 1C) and in a part of the luminal surface of the ampulla (Fig. 1D and E, arrows). No signals were detected at the fimbriae of the infundibulum (Fig. 1E, arrowhead) and the uterus (Fig. 1F). These results indicate that manserin is localized in the oviduct, and in particular in the isthmus.

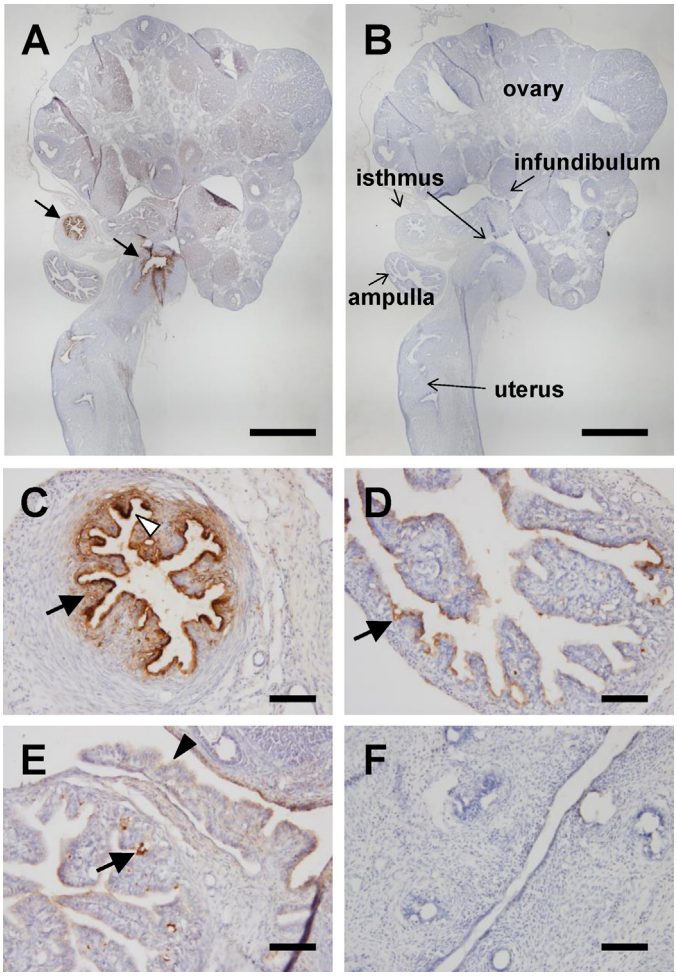

Fig. 1. Manserin immunoreactivity in female rat reproductive organs. Sections were incubated with an anti-manserin antibody and immune complexes were detected using DAB staining with a hematoxylin counterstain. (A) Panoramic view of manserin immunoreactivity. Strong immunoreactivity was observed in the isthmus (arrows). (B) Signals were not detected without the anti-manserin antibody. (C–F) Higher magnifications of the corresponding areas shown in A. Manserin immunoreactivity was detected on the luminal surface (open arrowhead) and the epithelium (arrow) of the isthmus (C) and in a part of the luminal surface in the ampulla (D and E, arrow). Manserin immunoreactivity was absent in the fimbriated infundibulum (E, arrowhead) and uterus (F). Scale bars, 1 mm (A and B) and 100 μm (C–F).

Immunolocalization of manserin in the oviduct

Next, the localization of manserin was examined in detail by immunofluorescence analysis. Manserin-immunopositive signals were observed along the apical surface of the epithelium in the ampulla and isthmus (Fig. 2A–D) but not in the nucleus because manserin did not colocalize with the DNA marker TO-PRO-3. The merged image of manserin/TO-PRO-3 and differential interference contrast (DIC) image demonstrated that manserin was localized on the epithelial microvilli (Fig. 2E–H). Manserin was also detected in