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Identification of Androgen Receptors in the Motoneurons of the External Urethral Sphincter in the Spinal Cord of Female Rats

Pascal Blanchet[a,b], El-Djouher Yaici[b], Laure Cayzergues[b], François Giuliano[a,b], Alain Jardin[a,b], Gérard Benoit[a,b], Stéphane Droupy[a,b,*]

[a]*Department of Urology, Bicêtre Hospital AP-HP, Paris-Sud University School of Medicine, Kremlin-Bicêtre, France*
[b]*Experimental Surgery Laboratory–UPRES 1602, Bicêtre Hospital AP-HP, Paris-Sud University School of Medicine, Kremlin-Bicêtre, France*

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Abstract

Objectives: In women, aging is associated with profound hormonal changes. Menopause has been implicated in the etiology of urinary tract complaints including incontinence, urgency and recurrent urinary tract infections. However, the use of hormone replacement therapy for these conditions has given conflicting and disappointing results. The role of androgen changes on urinary continence in perimenopausal women has not been studied. We studied the presence of androgen receptors in pudendal motoneurons controlling the external urethral sphincter of female rats.
Materials and methods: A combination of retrograde labeling of pudendal motoneurons from the external urethral sphincter and immunohistochemistry for the N-terminal portion of androgen receptors on spinal cord sections was performed in adult female rats.
Results: Androgen receptors were identified in the nuclei of the ventral horn of the L5–L6 spinal cord. Pudendal motoneurons, retrogradely labeled from the external urethral sphincter, were identified in the dorsolateral nucleus of the ventral horn at the same levels. Confocal microscopy demonstrated the presence of nuclear and cytoplasmic androgen receptors in the cell bodies of these retrogradely labeled pudendal motoneurons.
Conclusion: Our study demonstrated the presence of androgen receptors in the cell bodies of retrogradely labeled pudendal motoneurons controlling the urethral sphincter of female rats suggesting a role of androgens in the neuromodulation of urethral sphincter function at the spinal level.

Keywords: Androgen receptors; Urethral sphincter; Pudendal motoneurons; Continence

1. Introduction

In women, aging is associated with profound hormonal changes. Menopause has been implicated in the etiology of urinary tract complaints including incontinence, urgency and recurrent urinary tract infections. The marked decline in sex steroid production by both the ovaries and the adrenals may have very deleterious effects on several organ systems in postmenopausal women. Androgen, estrogen and progesterone receptor expression has been found in the muscles and stroma cells of the pelvic floor musculature including the levator ani muscle and external urethral sphincter. Changes in the expression of sex steroid receptors have also been identified in the levator ani and underlying fascia in postmenopausal women [1]. However, the use of hormone replacement therapy using estrogens and progesterone in postmenopausal women with urinary stress incontinence has given conflicting and disappointing results [2–4].

* Corresponding author. Present address: Service d'Urologie, CHU de Bicêtre, 78 rue du Général Leclerc. 94270 Le Kremlin Bicêtre, France. Tel. +33 1 45 21 36 98; Fax: +33 1 45 21 21 70.
E-mail address: sdroupy@aol.com (S. Droupy).

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In animal experiments, estrogen and androgen receptors have been identified in neurons controlling pelvic organs in the sacral spinal cord of rats. Estrogen receptors are present in the nuclei of neurons predominantly located in the dorsal horn, lamina V and sacral parasympathetic nucleus, while androgen receptors are present in the nuclei and cytoplasm of neurons located in the dorsal and ventral horns and dorsal root ganglia [5–7].

The role of androgen changes on urinary continence in perimenopausal women has not been studied.

We aimed to study the presence of androgen receptors (AR) in pudendal motoneurons controlling the external urethral sphincter of adult female rats.

2. Materials and methods

Five adult male and fifteen adult female Sprague-Dawley rats weighing 250–280 g and provided by Elevage René Depré (Saint-Doulchard, France) were used in the present experiments. Rats were kept in the animal facilities under a 12:12 light-dark cycle, lights on at 8:00 am, and food and water were delivered *ad libitum*. Care and use of animals complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.1. Retrograde labelling using fast blue tracer

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg). The pelvis was dissected through an abdominal midline vertical incision. The urethral sphincter was exposed using a cue-tip inserted into the vagina. In all rats, a solution of fast blue (FB) (Sigma, 3% in sterile water, 3 μl per rat in 3 separate points) was injected into the left external urethral sphincter, using a Hamilton microsyringe, in order to retrogradely label spinal motoneurons. The needle was kept in the injection site for 1 minute post-injection to prevent dye leakage. The external urethral sphincter and the opened cavity were then abundantly rinsed with saline. Surgical wounds were closed in separate muscle and skin layers. After a survival period of 72 h, rats were reanesthetized with sodium pentobarbital and intracardially perfused with 300 ml of PBS followed by cold 4% PAF in PBS (pH 7.4). Spinal cords were removed and postfixed in the same fixative for 2 h at 4 °C. The spinal cords were then placed in a Petri dish and the dorsal roots of each spinal segment were identified. The L5 and L6 spinal segments were identified by counting the dorsal roots and were cut into 50 μm thick transverse sections using a vibratome. One series of sections were examined with a Nikon microscope equipped for epifluorescence to visualize the distribution of FB-labelled neurons. Retrogradely labeled motoneurons were counted in ten randomly chosen sections from each rat. The total number of motoneurons was averaged in the same rat, then the mean of the mean was calculated for all rats. Results are presented as mean ± S.E. per section.

The remaining sections were kept for further immunofluorescence analysis.

2.2. Immunohistochemistry for androgen receptors

The second series of sections were processed for immunohistochemical localization of androgen receptors. We used a commercial polyclonal antibody PG21 generated in rabbit and directed against the NH2-terminal domain of human AR (Euromedex, France). The specificity of the PG21 antiserum and the reliability of the AR immunohistochemistry (AR ICC) method for detecting AR in pudendal motoneurons are well established [8–10]. Floating sections were rinsed several times in PBS and incubated in solution of PBS containing 0,5% H2O2 (30 min) followed by a solution of PBS containing 1% normal goat antiserum for a further 30 min. Sections from the males were then incubated with a rabbit polyclonal antibody to AR (PG21) diluted 1:2000 in a solution of PBS containing 1% of normal goat antiserum and 0,1% Triton X-100 during 24 hours at 4 °C. Different dilutions were tested for sections from the females: 1/2000, 1/1000, 1/500 and 1/200.

After several rinses, sections were incubated in biotinylated goat anti-rabbit Immunoglobulin G (IgG) (dilution 1:200, Vectastain, Vector Laboratories) for 2 h at room temperature. Sections were then rinsed in PBS and incubated in the avidin-biotin complex (dilution 1:100, Elite Kit, Vectastain) for 1 h at room temperature. Sections were washed and the peroxidase activity was visualized using a nickel chloride enhanced solution of 0.05% 3,3′-diaminobenzidine and 0.01% H_2O_2. Sections were thoroughly washed in distilled water and mounted on gelatin coated slides, dried overnight and covered with Entellan before coverslipping. The sections were then examined using light microscope and photographed.

3. Results

3.1. Fast blue retrograde labelling from the external urethral sphincter

Following injection of FB into the external urethral sphincter none of the rats displayed any signs of illness or distress during the post injection period. Retrogradely labeled motoneurons were found in the dorsolateral part of the Onuf's nucleus of the ventral horn of the L5–L6 spinal cord (Fig. 2). All labeled cells were found ipsilateral to the injection side. They were located in the lateral part of the nucleus. In both L5 and L6 segments, we observed the same number of retrogradely labeled motoneurons per section (5 ± 2; mean ± S.E.; $n = 10$).

3.2. Distribution of androgen receptor immunoperoxidase labelling in the L5–S1 levels of the spinal cord

In male rats, androgen receptor immunoreactivity, revealed by the ABC method using DAB as chromogen, was homogeneously distributed within the grey matter in the ventral horn of the L5–S1 spinal cord segment (Fig. 1A). The immunoreactivity was present in three motoneuron pools: dorsomedial, dorsolateral and retrodorsolateral nuclei (Fig. 1B). These motoneurons presented a homogeneous immunoreactivity, cells presenting immunostaining of the nucleus and a discrete cytoplasmic immunostaining (Fig. 1C).