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## Localization of steroid hormone receptors in the pelvic muscles

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### Summary

After the menopause the pelvic floor becomes progressively weaker, one consequence of which is the increasing incidence of prolapse and urinary incontinence. It is plausible that the reduction in estrogen levels postmenopausally is an etiological factor in these problems. For the pelvic floor muscles to be considered as target organs for estrogen, demonstration of the presence of estrogen receptors is required. The present study aims to confirm previous findings of estrogen and progesterone receptors in the levator ani muscle, and to further localize them histologically. In seven women biopsies from the levator ani muscle were taken and analyzed immunohistologically with monoclonal antibodies for estrogen and progesterone receptors. The receptors were detected in the nuclei of connective tissue cells and striated muscle cells. These findings provide a scientific basis for a possible beneficial effect of estrogen therapy, as a complement to other therapies, in prolapse and urinary incontinence.

Estrogen receptor; Progesterone receptor; Pelvic muscles

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### Introduction

After the menopause the pelvic floor becomes progressively weaker and disorders such as prolapse and urinary incontinence increasingly common. The progressive reduction in estrogen levels postmenopausally is likely to be of importance in the etiology of these disorders. In younger women without known precipitating factors for stress incontinence, estrogen has been shown to be of significance in maintaining normal urinary tract physiology [1]. Estrogen may reduce urinary

incontinence by stimulating the growth of the urethral epithelium [2], by increasing the vascularity of the submucosal venous plexuses in the urethra [3,4] and by influencing the connective tissue within and around the urethra and bladder base [5,6].

The pelvic muscles also play an important role in maintaining urinary continence. Control of the position of the urethra, as well as of its closing, is dependent on the anterior part of the pubococcygeus muscles, the puborectal ligaments and the intimate connection between the medial portion of the levator ani muscle and the vagina [7,8].

It is generally considered that the presence of estrogen receptors is a prerequisite for a tissue to recognize estrogen and to be able to respond to

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hormonal therapy [9]. In a previous study [10] estrogen receptors and progesterone receptors were detected in female pelvic muscles and urogenital ligaments, supporting the hypothesis of a hormonal influence on these structures. In a separate study estrogen and progesterone receptors were identified in the ligamentum rotundum and shown histologically to be located in the nuclei of smooth muscle cells (Smith et al., unpublished observations).

The present study continues the histological study of the location of the steroid hormone receptors in the pelvic floor muscles, in order to determine whether the receptors are located in connective tissue cells within the levator ani muscle, in the striated muscle cells themselves, or in both.

### Material and Methods

Biopsies from the levator ani muscle were taken from seven Caucasian women during operations for benign conditions. The age range of the women was 30–85 years (mean 60 years, geometric mean 57.7). Six of the patients were postmenopausal and four of these had low dose estrogen vaginal creams or pessaries. Informed consent was obtained and the study was approved by the local ethical committee. The tissue specimens, which had wet weights between 56 and 122 mg, were immediately frozen in isopentane in the operating theatre and then stored at  $-70^{\circ}\text{C}$  until analyzed. From the biopsies frozen sections were cut at  $5\ \mu\text{m}$  and stained immunocytochemically for a qualitative evalu-

ation with the ER-ICA and PgR-ICA techniques, with monoclonal antibodies supplied by Abbott Laboratories [10]. The production of monoclonal antibodies against receptors has permitted the development of assays based on direct antigenic recognition rather than steroid binding activity. Immunocytochemical staining permits detection and localization of ER and PgR within intact tissue sections. This technique permits precise morphopathological characterization of ER- and PgR-containing cells. Assay validity is determined by staining a positive control and a negative control is also used.

### Results

Progesterone receptors were identified immunohistochemically in five of the biopsies and estrogen receptors in six (Table I). The receptors were located mainly in connective tissue cells (Fig. 1) within the muscle, but receptors were also identified in striated muscle cells. The receptors detected were all situated in the cell nuclei.

### Discussion

Estrogen and progesterone receptors were found in the female levator ani muscle, in both connective tissue cells and striated muscle cells. The location of the receptors was intranuclear, which is in accordance with the current view that estrogen receptors and progesterone receptors are nuclear proteins synthesized in the cytoplasm and then rapidly transferred to the nucleus [11,12].

TABLE I

Patient age, hormone treatment and the presence of estrogen (ER) and progesterone receptors (PgR) in biopsies from the levator ani muscle

Patient no.	Age	Hormone treatment	ER	PgR
1	30	—	++	—
2	55	Estriol vaginal cream	+	+
3	57	—	+	+
4	60	—	—	+
5	66	Dienoestrol vaginal cream	+	—
6	67	Estriol vaginal cream	+	+
7	85	Estriol vaginal cream	+	+

+, presence of receptor; —, absence of receptor.



Fig. 1. Progesterone receptors in connective tissue cells in the levator ani muscle. Nuclei stained dark.

Press [11] found an exclusively nuclear localization of estrogen receptors in epithelial cells, stromal cells of the lamina propria, and smooth muscle cells in the human vagina, uterus and fallopian tubes. Brenner [12] located estrogen and progesterone receptors exclusively in the cell nuclei when analyzing samples from the endometrium and the oviduct in macaques, with the aid of monoclonal antibodies.

The fact that we found fewer receptors in striated muscle cells than in connective tissue cells might partly be explained by the lower nucleus/cytoplasm ratio in striated muscle cells.

The presence of estrogen receptors and progesterone receptors in the levator ani muscle in females suggests that this muscle is a target organ for estrogen. The finding of steroid hormone receptors in the connective tissue cells within the muscle is also important as this tissue functions as

a 'glue' fixing different muscles to each other and to the pelvic bones [7]. Taken together this has implications concerning the etiology of postmenopausal vaginal prolapse and urinary incontinence. In addition, it constitutes a theoretical basis for the beneficial effects of estrogen treatment in these disorders, as a complement to other therapies, such as surgery.

### Acknowledgements

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