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Long-term observation of the detrusor smooth muscle in rats

Its relationship to ovariectomy and estrogen treatment

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Summary. We studied the bladders of 24-month-old intact rats, rats that had been ovariectomized at the age of 6 months, and intact and ovariectomized rats treated by estrogen from the age of 16 months. The study thus comprized four groups: group I: bilaterally ovariectomized rats; group II: intact rats; group III: ovariectomized rats treated with estrogen; group IV: intact rats treated with estrogen. The weight and collagen concentration of the bladders were determined. The ovariectomized bladders weighed significantly less and had a higher collagen concentration than the intact bladders. Estrogen substitution for ovariectomized rats reversed these parameters. Detrusor strips were also used for organ bath studies. All bladders were similar in regard to the nerve-mediated frequency-response relationship. The atropine-resistant response was studied by adding scopolamine to the organ bath. Strips from ovariectomized rats had a significantly diminished atropine-resistant response, which was abolished by estrogen substitution. The present study suggests that micturition problems in menopause might have a structural as well as a pharmacological explanation.

Key words: Detrusor smooth muscle – Rats – Estrogen – Collagen concentration – Bladder

The presence of estrogen receptors has been demonstrated in the human lower urinary tract [8, 15]. This supports the widespread practice of giving estrogen treatment to postmenopausal women with lower urinary tract dysfunction.

The postmenopause period is associated with a high incidence of symptoms from the lower urinary tract [6, 9, 21]. The main urological symptoms are stress and urge incontinence and recurring urinary tract infections. Both types of symptoms show increasing incidence with age. Previous investigations have shown that estrogen can alter lower urinary tract function in postmenopausal women [4, 5, 14, 16, 22]. The decrease in maximum urethral pressure in the urodynamic parameters obtained from healthy postmenopausel women show only minor differences in comparison to these parameters in premenopausal women [20, 24].

Animal experiments on the influence of ovarian hormones on bladder function have mainly been performed in rabbits, and the results concerning the influence of estrogen on muscarinic cholinergic innervation have been contradictory. Estrogen is reported to have syncholinergic a as well as an anti-cholinergic action on the rabbit detrusor muscle [11, 12, 17]. In one study [1] ovariectomized rats were found to have a diminished response to prostaglandins of the E type. The response was restored by estrogen substitution.

Increased collagen deposits between the smooth muscle cells of the detrusor and urethra might affect the functional properties of the lower urinary tract. The collagen content of the detrusor muscle has been found to be significantly increased in females after the age of 50 when compared to younger females and to males at same age in a postmortem study [19]. The complexity of the factors resulting in coordinated bladder and urethra function may be influenced by ovarian hormons in several ways. The aim of this study was therefore to examine the effects of long-terms estrogen depletion and estrogen treatment on both structural and pharmacodynamic parameters.

Materials and methods

Forty-six female albino rats of the Wistar strain were used. At 6 months of age half the rats were ovariectomized. Ovariectomy was performed under pentobarbital anesthesia via a bilateral dorsolumbar approach. The rats were then left for 10 months without interference, allowing for estrogen deprivation manifestations to occur. From the age of 16 months and to their death at the age of 24 months, both half the intact rats and half the ovariectomized rats were treated with 17-beta-estradiol valerate [2 μg per animal, dissolved in risinus oil (Progynon Depot, Schering)] administered subcutaneously once a week. This resulted in four groups of rats: group I was ovariectomized; group II remained intact; group III was ovariectomized and received estrogen; group IV only received estrogen.

Table 1. Effects of ovariectomy and estrogen substitution in rats on body weight (g), bladder weight (mg), collagen content (mg) and collagen concentration (%). Results presented as (mean ± SD)

	Group I (ovariectomy) ($n=12$)	Group II (controls) ($n=14$)	Group III (ovariectomy + estrogen) ($n=11$)	Group IV (estrogen) ($n=13$)
Body weight prior to ovariectomy	265 ± 22	278 ± 19	274 ± 28	268 ± 22
Body weight prior to estrogen	395 ± 49*	342 ± 38	409 ± 49*	338 ± 49
Body weight at sacrifice	447 ± 71	400 ± 49	426 ± 55	372 ± 71
Dry defatted bladder weight	18.0 ± 2.4*	21.3 ± 2.8	21.3 ± 3.1	21.4 ± 3.5
Collagen content	9.9 ± 0.8*	11.1 ± 1.3	10.9 ± 1.5	11.3 ± 2.1
Collagen concentration	55.3 ± 3.7*	52.1 ± 2.9	51.7 ± 6.5	52.6 ± 3.4

*$p < 0.05$

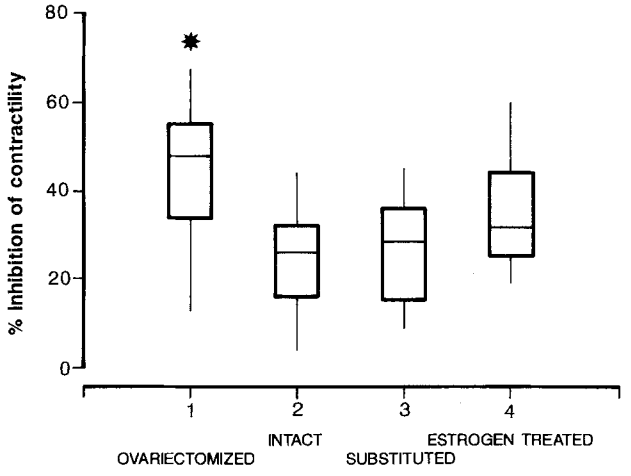

Fig. 1. The figure shows a bars and whiskers diagram of the mean percentage of inhibition on contraction exerted by scopolamine for the four groups. Total range is shown, together with framed areas with median value and first quartile on each side. * Significant difference for the ovariectomized group ($p < 0.025$) compared to the intact group

The rats were weighed and sacrified by an overdose of pentobarbital. The bladders were removed, weighed and transferred to a petri-dish containing Krebs-Ringer bicarbonate (KRB) solution with 1 mM glucose as the substrate. The tissues were kept at 37 °C and continously bubbled with 95% O_2 and 5% CO_2. The bladders were dissected free of fat and serosa, and two circular strips from the middle portion of the bladder were prepared and randomly assigned to either the pharmacological study or to serve as its own control.

The rings were attached to a glass holder and to a force transducer (Swema) coupled to an ink writer (Hokushin) and immersed in a tissue chamber filled with 10 ml KRB-glucose solution. The medium was continously bubbled with the O_2–CO_2 solution and kept at 37 °C.

A resting tension of 1 g was applied to the rings and the tissue was allowed to equlibrate for 1 h before the tissue was submitted to electrical field stimulation. Transmural stimulation was delivered by an electrical field stimulator sending pulses in 5-s trains every 4th minute at a submaximal voltage. The frequency of 20 Hz was chosen for dose-response curves to scopolamine. This frequency was chosen to ensure selective exitation of the nerves of the tissues. The scopolamine dose giving a contraction of 80% of maximum response was 1.2×10^{-7} M. At frequencies varying from 3 through 45 Hz, frequency-response curves were recorded.

The bladders were stored at −20 °C until determination of dry, defatted weight (DDW). The tissue was freeze dried and defatted by acetone. Then the collagen content of the tissue was estimated measuring hydroxyproline according to the procedure of Woessner [23]. Estradiol, estrone and estrone sulfate were determined by radioimmunoassay after ether extraction and column chromatography [3, 7]. For the statistics, Student's *t*-test was performed to analyze the group differences in sensitivity to scopolamine, DDW, and relative collagen content.

Results

Serum estradiol

The concentrations at the time of sacrifice were below 40 pmol/l, which is the lower sensitivity limit in the method applied in all four groups. Ovariectomy was followed by a significant decline in the estrogen compounds estrone sulfate and estrone. However at the time of sacrifice there were no significant differences in these compounds between the intact and ovariectomized rats, whereas estrogen-treated rats (groups III and IV) showed lower values of estrone sulfate.

The body weights

The rats body weights are shown in Table 1. Ovariectomy was followed by a significant weight increase when compared to the rats not operated upon. Estrogen substitution reduced the weight gain, although no significant difference in body weight was observed at the time of sacrifice.

Bladder weight and collagen content

The results are given in Table 1. The bladders of the ovariectomized rats weighed significantly less and had a higher collagen concentration than the control bladders. Total collagen decreased in the ovariectomized group. The bladder weights, collagen content, and collagen concentrations in the estrogen-treated group and in the substituted group did not differ from similar parameters in the control group.

Response to field stimulation

The contractile profile resulting from electrical field stimulation and expressed in terms of %s of maximum contractile response was identical in all four groups with 50% maximum response reached at 5 Hz.

The anticholinergic drug

Scoplamine resulted in a significant ($p < 0.025$) higher degree of inhibition of contraction of bladders from the ovariectomized group when compared to the intact group (Fig. 1). Thus, the atropine-resistant part of the nerve-mediated response was significantly smaller following ovariectomy. Estrogen treatment reversed the atropine-resistant response in the ovariectomized group since no difference in inhibitory action of scopolamin was observed in the estrogen-treated group when compared to the intact group.

Discussion

Lower urinary tract dysfunction is more common in women than men at all ages. The prelavence increase with age [2, 6, 21]. When looking at the etiology of dysfunction in elderly women, it is difficult to separate the influence of aging from that of menopause. The most significant effect of menopause is estrogen deprivation. Estrogen has been successfully used in the treatment of elderly women with symptoms from the lower urinary tract [16, 22]. Animal experiments concerning the influence of ovarian hormones on bladder function have mainly been performed on rabbits with contradictory results concerning the influence of estrogen on the muscarinic cholinergic innervation.

Two short-term studies [11, 12] treating immature rabbits with estradiol for 4 days showed an increase in response to the parasympatomimetic bethanechol and evidence of increased muscaninic cholinergic receptor (MChR) density. A later long-term study [17] in which estrogen was administered for 3 weeks to mature rabbits found estrogen administration associated with a decrease in MChR density, whereas ovariectomy was not associated with differences in MChR density. The different design and the difference in age of the animals used could explain the discrepancy between the studies. In our experiment we used rats instead of rabbits. Also, we studied the effect of ovariectomy and estrogen treatment on a long-term basis. The amount of estradiol used was much smaller than that used in other animal experiments. We used approximately 5 μg/kg per week. In other studies 150 μg/kg per day [11] and 250 μg/kg per day [17] were used. Unfortunately, the sensitivity of the kit used for estradiol determination had a sensitivity (≥ 40 pmol/l) that did not allow us to distinguish between the various groups. The measurements of the other estrogen compounds showed great variation within the groups.

Our study showed that ovariectomy was followed by a significant incrase in body weight. Although the body weight at the time of sacrifice showed no significant difference between groups there, was a tendency for estrogen treatment to counteract the weight-gain effect of an ovariectomy. A possible explanation for the changes in body weight observed could be that ovariectomy is followed by a reduction in physical activity and that estrogen treatment abolishes this reduction.

The bladders from the ovariectomized rats were smaller and had a higher collagen concentration than the control bladders. Substitution with estrogen reversed these parameters. Estrogen administration to intact animals did not cause changes in bladder size or collagen concentration. A reduction in bladder capacity is associated with symptoms such as urge incontinence and increased frequency symptoms that are of typical postmenopausal voiding problems [16, 22]. In an earlier short-term high-dose study [17], estrogen treatment to mature rabbits were followed by a threefold increase in bladder weight when compared to intact animals, whereas no differences were observed in ovariectomized rabbits. It may be that changes in bladder weight in relation to ovariectomy need time to develop. The reason we did not observe any changes in mean bladder weight in the estrogen-treated intact group might be that we did not reach a serum estradiol level that was sufficient to create such changes.

The collagen concentration in ovariectomized bladders increased when compared to the intact bladders. Estrogen substitution to ovariectomized rats seemed to counteract the increase in collagen concentration. The information regading collagen content in lower urinary tract in relation to menopause is very limited. An age-related increase in collagen content has been reported in human postmortem studies [19]. In animal experiments using mice, an age-related increase in collagen and a concomitant loss of smooth muscle was observed in the urethra but not in the bladder [13]. Changes in collagen concentration might change the functional properties of the detrusor muscle [10]. It has been suggested that an inversely proportional relationship exists between the detrusor contraction and collagen content [18]. We found the contractile profiles identical in all four groups. However, the response was expressed as a percentage of maximal contraction. More refined studies are needed to deduce knowledge about the contractile quality in relation to estrogen status. Incrases in collagen concentration might also influence the collecting phase of micturition by decreasing the wall compliance of the detrusor, thereby creating the symptoms often associated with postmenopausal micturition disorders: urgency and frequency. In the ovariectomized rats we found a significantly ($p < 0.025$) greater inhibition of contractility exerted by scopolamine than in the intact group. Estrogen substitution to ovariectomized rats abolished this extra inhibition. It has been known for a long time that the detrusor muscle of several animals exerts a certain atropine-resistant contractile activity that is nonadrenergic. Its existence in the human detrusor muscle is more controversial. It has been demonstrated that prostaglandins of the E and F series play a role in the noncholinergic, nonadrenergic response. The importance of prostaglandins in relationship to estrogens was investigated in vitro

in rats by Borda and associates [1]. They found that an ovariectomy performed 3 weeks prior to the study resulted in reduced sensitivity to prostaglandins of the E type and that the detrusor strips regained their sensitivity of the ovariectomized rats were treated with 17-beta-estradiol. Thus, the micturition problems found in menopause could partly be explained by reduced atropine resistance and diminished sensitivity to prostaglandins associated with low estrogen levels.

In conclusion, we found that estrogen depletion resulted in increased body weight, diminished bladder size, increased collagen concentration and decreased atropine resistance. All these changes were partly or completely reversed by estrogen substitution in ovariectomized rats. Estrogen treatment of intact rats did not influence any of the parameters studied.

Since clinical as well as experimental studies show a discrepancy, there is reason to believe that changes associated with lack of estrogen must be very subtle. Also, we think it is likely that symptoms related to menopause depend on several factors rather than one single factor.

There is a need for systematic studies that combine functional and structural parameters and relate them to the symptomatology of postmenopausal voiding problems.

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