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Rapid effects of estrogen and progesterone on tone and spontaneous rhythmic contractions of the rabbit bladder

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Abstract Previous studies indicate that bladder instability in man may be associated with increased spontaneous rhythmic contractile activity. Ca^{2+} influx plays a central role in smooth muscle contractions, and recent evidence suggests that steroid hormones rapidly affect Ca^{2+} influx. Therefore we tested the hypothesis that estrogen and progesterone modulates spontaneous rhythmic detrusor contractions. Tissues were secured to isometric force (F) transducers in tissue baths and length-adjusted until K^+-depolarization produced maximum contractions (F_o). Spontaneous rhythmic contractions (SRC) were sampled before and immediately after addition of estradiol or progesterone (10^{-5} M) to tissue baths. The average frequency and amplitude of SRC were, respectively, 0.156 Hz and 0.053 F/F_o ($n = 24$). Estradiol caused an immediate reduction in SRC, such that by 10 min, tone, frequency and amplitude were each reduced by, respectively, 36%, 46% and 47% ($n = 7$, $P < 0.05$). However, progesterone caused an immediate weak contraction, and at steady state (10 min), progesterone increased frequency of SRC by 152% but decreased SRC amplitude by 50% ($n = 10$, $P < 0.05$). Novel therapies using unique steroids that do not interact with genomic receptors may potentially reduce bladder smooth muscle activity, thereby reducing detrusor instability.

Key words Estradiol · Progesterone · Detrusor · Non-genomic

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Introduction

Although bladder smooth muscle strips from rabbit, pig and humans [25] are known to contract spontaneously in a rhythmic fashion, in vitro, the physiological significance of this activity remains to be determined. However, in human bladders, Kinder and Mundy [13] have shown that the incidence of spontaneous rhythmic contractions is greater in detrusor strips taken from clinically unstable bladders then in tissues taken from patients with normal bladders. This led to the hypothesis that increased myogenic detrusor activity plays a key role in bladder over activity [13].

Classically, steroid hormones are known to exert their effects by binding to intracellular receptors that modulate cell activity at the DNA transcriptional level (genomic pathway). However, there is compelling evidence that steroid hormones have additional effects via a faster "non-genomic" pathway that involves yet to be identified, non-classical, steroid receptors located in the cell membrane [15]. For example, estrogen has been shown to alter immediately the tone of vascular and other smooth muscle [20], and progesterone has been shown to rapidly increase sperm motility by increasing intracellular Ca^{2+} [1]. Urinary urgency and incontinence, which are frequently found in post-menopausal women, seem to respond clinically to the administration of estrogen [19]. However, no studies have pursued the possibility that these beneficial effects of estrogen are due, in part, to activation of non-genomic pathways. Therefore, the present study was designed to determine whether estrogen or progesterone exerts rapid effects on spontaneous rhythmic contractions of rabbit bladder smooth muscle, in vitro.

Materials and methods

Tissue preparation

Tissues were prepared as described previously [24]. Adult female New Zealand White rabbits were killed by pentobarbital overdose

and whole bladders were removed immediately. The bladders were washed several times and stored for up to 48 h in cold (4°C) physiological salt solution (PSS). The composition of PSS was in mM: NaCl, 140; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 1.6; Na_2HPO_4, 1.2; morpholinopropanesulfonic acid, 2.0 (adjusted to pH 7.4 at either 0°C or 37°C, as appropriate); Na_2 EDTA (to chelate trace heavy metals), 0.02; and dextrose, 5.6. High purity water (greater than 17 MΩ distilled and deionized) was used throughout. Longitudinal muscle strips were cut from the wall of the detrusor body. Each muscle strip was incubated at 37°C in a water-jacketed tissue bath (Radnoti Glass Technology, Monrovia, Calif.) and secured by small clips to a micrometer for length adjustments and an isometric force transducer (model 52, Harvard Apparatus, South Natick, Mass.) to measure muscle contraction.

Contractions of isolated detrusor strips

Isometric contractions were measured as described previously [24]. Voltage signals were digitized (model DIO-DAS16, Computer Boards, Mansfield, Mass.) at an acquisition rate of 200 Hz, and stored to a hard disk for later analyses. All data analyses were performed using customized computer worksheets generated using a data acquisition, process control, and analysis system (DASY-Lab+, DasyTec, Amherst, N.H.) and an electronic spreadsheet.

Contractile force was measured as described previously [24]. Tissues were equilibrated for 1 h at ~0.5 g passive force, then stretched to their optimum length for muscle contraction (L_o) using an abbreviated length–force determination [11, 24, 28]. The optimum force of muscle contraction (F_o) produced by 110 mM KCl at L_o was obtained for each muscle strip. To account for tissue-to-tissue variability in data analysis, subsequent contractions were reported as normalized to F_o (F/F_o). Once L_o was determined, no additional length changes were imposed on the muscle strips. Passive force values at L_o for each muscle strip were obtained at the end of each experiment by 30 min incubation of the strips in Ca^{2+}-free PSS that contained 1 mM EGTA.

Three parameters of spontaneous rhythmic contractions were recorded and analyzed during a sampling period of 10 min (Fig. 1); frequency of contractions (Hz), average amplitude of spontaneous contractions (F/F_o) and tone (F/F_o). Tone was defined as the difference between the average force generated by the muscle strips at steady state and that generated in Ca^{2+}-free PSS (Fig. 1). In our analysis of frequency, peaks that reflected force changes of greater than 0.01 F/F_o were considered true contractions. This cutoff value was derived from the analysis of recordings of muscle strips in Ca^{2+}-free PSS. Changes in force smaller than 0.01 F/F_o reflected system noise.

Fast Fourier Transform (FFT) spectral analysis was performed as an additional method to study changes in the rhythmic contractile frequency [18]. For FFT analysis, the 10-min samples of spontaneous rhythmic contractile activity were divided into four sequential 164 s segments. Frequency spectral analysis was performed for each segment, and the resulting spectra were averaged, resulting in a single average spectrum for the 10-min samples.

Experimental protocol

Once L_o and F_o were determined, each muscle strip was allowed to rest in PSS for an additional 20 min and 10 min of basal spontaneous rhythmic contractions were then sampled. Atropine (10^{-7} M) was then added to the tissue baths to eliminate any potential contributions to spontaneous rhythmic contractions of basal release of acetylcholine from cholinergic nerve terminals. An additional 10 min of basal spontaneous rhythmic contractions with atropine present were sampled. Estradiol (10^{-5} M) or progesterone (10^{-5} M) was then added to the tissue baths. Because estradiol and progesterone were dissolved in ethanol, tissues treated with these steroids were also exposed to ethanol at a final concentration of 0.1%. Therefore, to determine whether ethanol affected spontaneous rhythmic contractions or tone, some detrusor strips were exposed to 0.1% ethanol alone (vehicle control), and frequency, amplitude and tone were measured. The immediate effects of estradiol, progesterone or ethanol on spontaneous rhythmic contractions were recorded for 5–10 min. Steady-state effects of estradiol, progesterone or ethanol were then recorded for an additional 10 min. Post-treatment steady-state spontaneous rhythmic contractions were

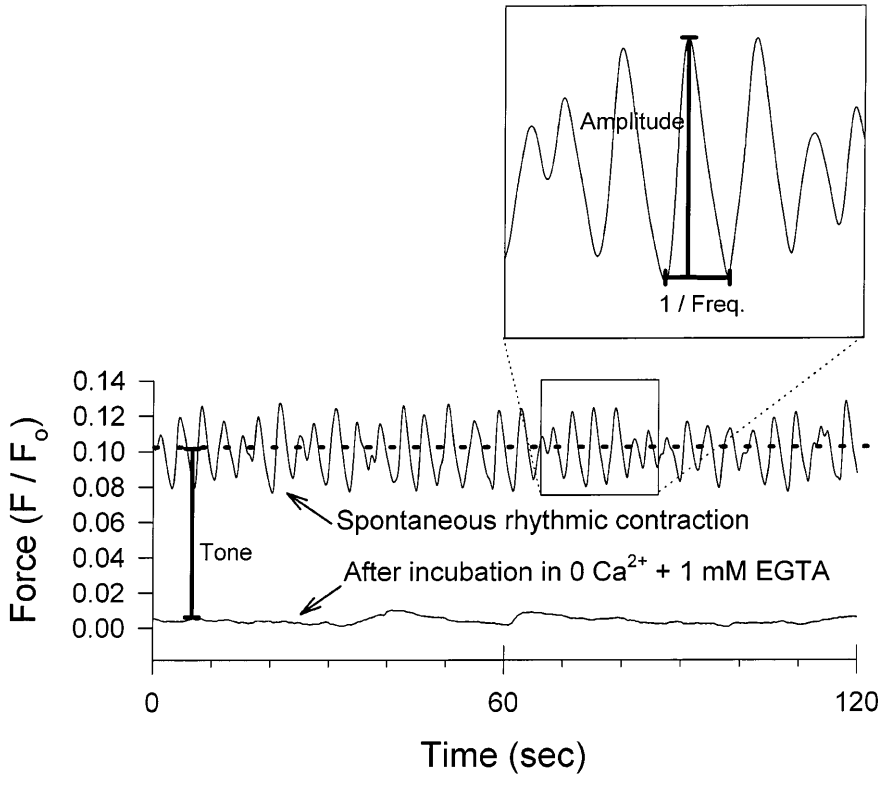

Fig. 1 A 2-minute sample of a typical recording of force generated by a detrusor smooth muscle strip in a Ca^{2+}-containing solution in which spontaneous rhythmic contractions were evident, and in a Ca^{2+}-free solution in which rhythmic contractions were eliminated. The frequency and amplitude of a single rhythm from a selected portion of the spontaneous rhythmic contraction is shown in the insert. Average values of frequency, amplitude and muscle tone were obtained by averaging signals over 10 minutes

compared to pre-treatment (basal) values. At the end of each experiment, each tissue strip was incubated in a Ca^{2+}-free solution including 1 mM EGTA to determine passive force and tone.

Statistics

Results are reported as means ± SEM. The Student's paired *t*-test was used to compare pre- with post-treatment values (frequency and amplitude of spontaneous rhythmic contractions and tone) for each muscle strip. To determine significance, the Null hypothesis was rejected at $P < 0.05$. The population sample size (n value) refers to the number of animals, not the number of tissue samples.

Results

Basal spontaneous rhythmic contractions

The mean frequency of spontaneous rhythmic contractions in detrusor muscle strips was 0.156 Hz ($n = 24$). The mean amplitude of these spontaneous contractions was 5.3% of the maximal contraction produced by 110 mM KCl (i.e., mean amplitude was 0.053 F/F_o, $n = 24$). These values agree well with that obtained by Potjer and Constantinou [18]. Average muscle tone was 0.064 F/F_o ($n = 24$). The addition of 10^{-7} M atropine had no significant effect on the frequency or amplitude of the rhythmic contractions or on basal tone.

Effect of estradiol

Estradiol (10^{-5} M) had an immediate inhibitory effect (within 20–150 s) on the spontaneous rhythmic contractions of bladder smooth muscle strips. This inhibition could, at times, be complete (for example, see Fig. 2A). Steady-state rhythmic contractions and tone were significantly decreased by estradiol (Fig. 3A and E_2 in Fig. 4) compared with the pre-treatment values. Pre- and post-estradiol values for frequency, amplitude and tone were, respectively, 0.141 ± 0.01 versus 0.065 ± 0.01, 0.057 ± 0.009 versus 0.027 ± 0.006, and 0.059 ± 0.01 versus 0.021 ± 0.004 ($n = 7$, $P < 0.05$). Spectral analysis of the rhythmic contractile activity by FFT also demonstrated a shift to a lower frequency and amplitude after estradiol treatment [Fig. 5; compare A (pre-treatment) with B (post-estradiol)].

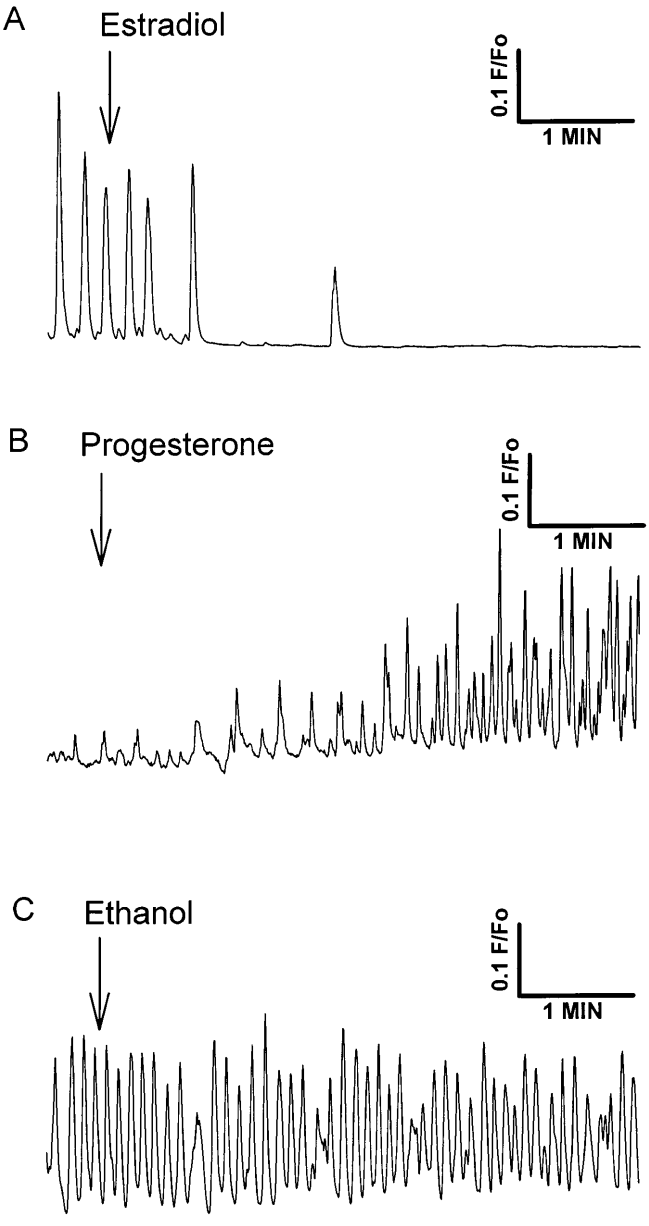

Fig. 2 Recordings showing examples of the immediate effects of addition of 10^{-5} M estradiol (**A**) 10^{-5} M progesterone (**B**) and ethanol 0.1% (**C**) on spontaneous rhythmic contractions generated by detrusor smooth muscle strips. In some cases, estradiol completely eliminated rhythmic contractile activity, and progesterone induced strong rhythmic contractions in relatively quiescent tissues. The arrows indicate when steroids or ethanol were added

Effect of progesterone

Progesterone (10^{-5} M) had an immediate excitatory effect (within 90–120 s) on the spontaneous rhythmic contractions of bladder smooth muscle strips. In tissues displaying weak basal spontaneous rhythmic activity, progesterone often produced a dramatic increase in frequency and amplitude of rhythmic contractions (Fig. 2B). In tissues displaying strong basal rhythmic activity, progesterone produced a transient increase in tone (i.e., progesterone produced a weak transient contraction; Fig. 3B). At steady state, the frequency of spontaneous rhythmic contractions was significantly increased by progesterone (Fig. 3B and P_4 in Fig. 4A). Pre- and post-progesterone frequency values were, respectively, 0.183 ± 0.04 and 0.278 ± 0.038 ($n = 10$, $P < 0.05$). Although frequency was increased, the amplitude of spontaneous rhythmic contractions at steady state was decreased by progesterone (Fig. 3B and P_4 in Fig. 4B). Pre- and post-progesterone amplitude values were, respectively, 0.05 ± 0.012 and

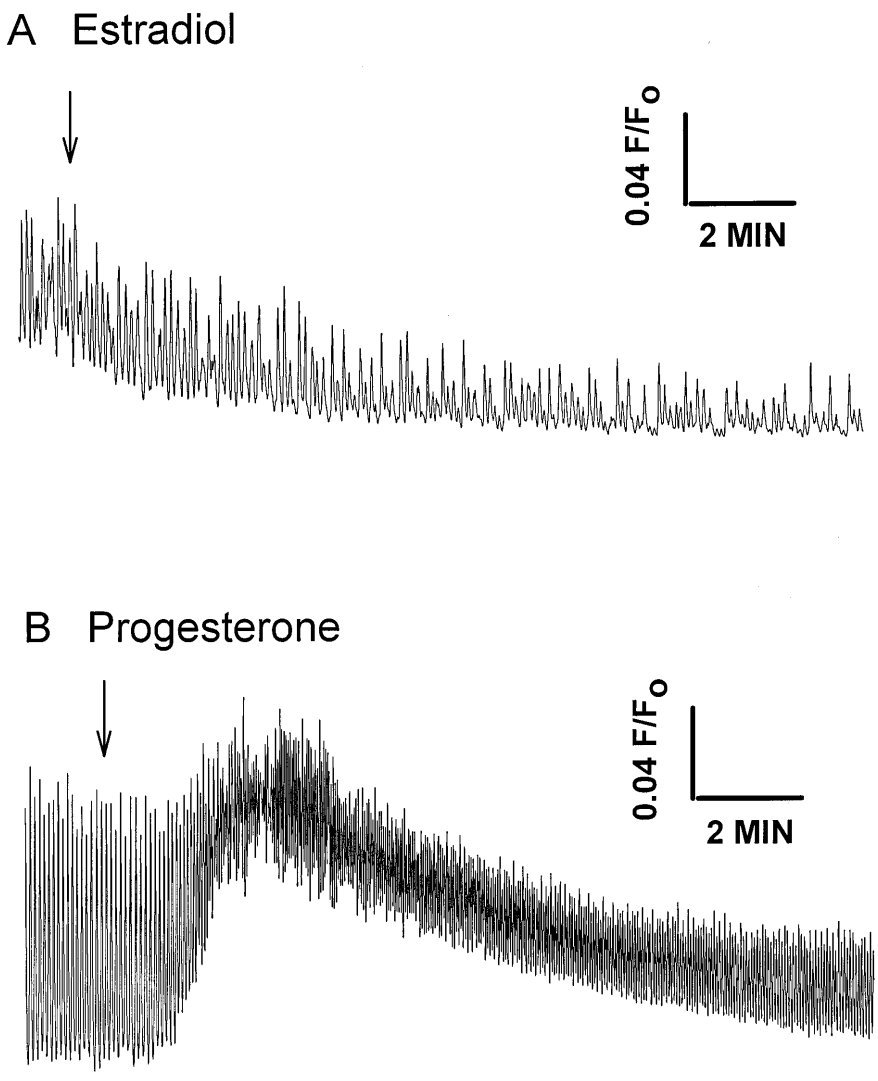

Fig. 3 Recordings showing examples of the immediate and steady-state effects of (**A**) estradiol and (**B**) progesterone (10^{-5} M) on spontaneous rhythmic contractions of detrusor muscle strips. Note that estradiol caused a rapid and maintained decrease in frequency and amplitude of rhythmic contractions and a decrease in tone, whereas progesterone caused an immediate, weak, transient contraction followed by a sustained increase in frequency but decrease in amplitude of rhythmic contractions

0.025 ± 0.003 ($n = 10$, $P < 0.05$). FFT spectral analysis confirmed that progesterone produced a shift to a higher frequency with a reduction in amplitude compared to pre-treatment control (Fig. 5C). Thus, while both estradiol and progesterone caused a decrease in the amplitude of spontaneous rhythmic contractions at steady state, estradiol decreased while progesterone increased the frequency of these contractions.

Effect of ethanol (vehicle control)

The vehicle control for estradiol and progesterone, ethanol (0.1%) had no apparent immediate effect on rhythmic contractions of bladder smooth muscle strips (Fig. 2C). Moreover, at steady state, ethanol did not significantly affect frequency, amplitude or tone of spontaneous rhythmic contractions ($n = 7$, $P < 0.05$, ETOH in Fig. 4).

Discussion

Spontaneous rhythmic contractions of detrusor muscle have been demonstrated in several mammalian species and in man [2, 25]. This contractile activity is resistant to tetrodotoxin or atropine, suggesting that the stimulus for rhythmic contractile activity originates in the smooth muscle of the detrusor (i.e., it is myogenic) [12]. In our experiments, the spontaneous rhythmic contractions were atropine resistant and similar in frequency and amplitude to those previously demonstrated for rabbit detrusor strips [18].

The physiologic role of spontaneous rhythmic contractions is not known. Interestingly, however, spontaneous rhythmic contractions have been shown to occur more commonly and to be of greater frequency and amplitude in muscle strips obtained from patients with clinically diagnosed detrusor instability and hyperreflexia compared with normal controls [13]. Therefore, increased spontaneous contractile activity may play a

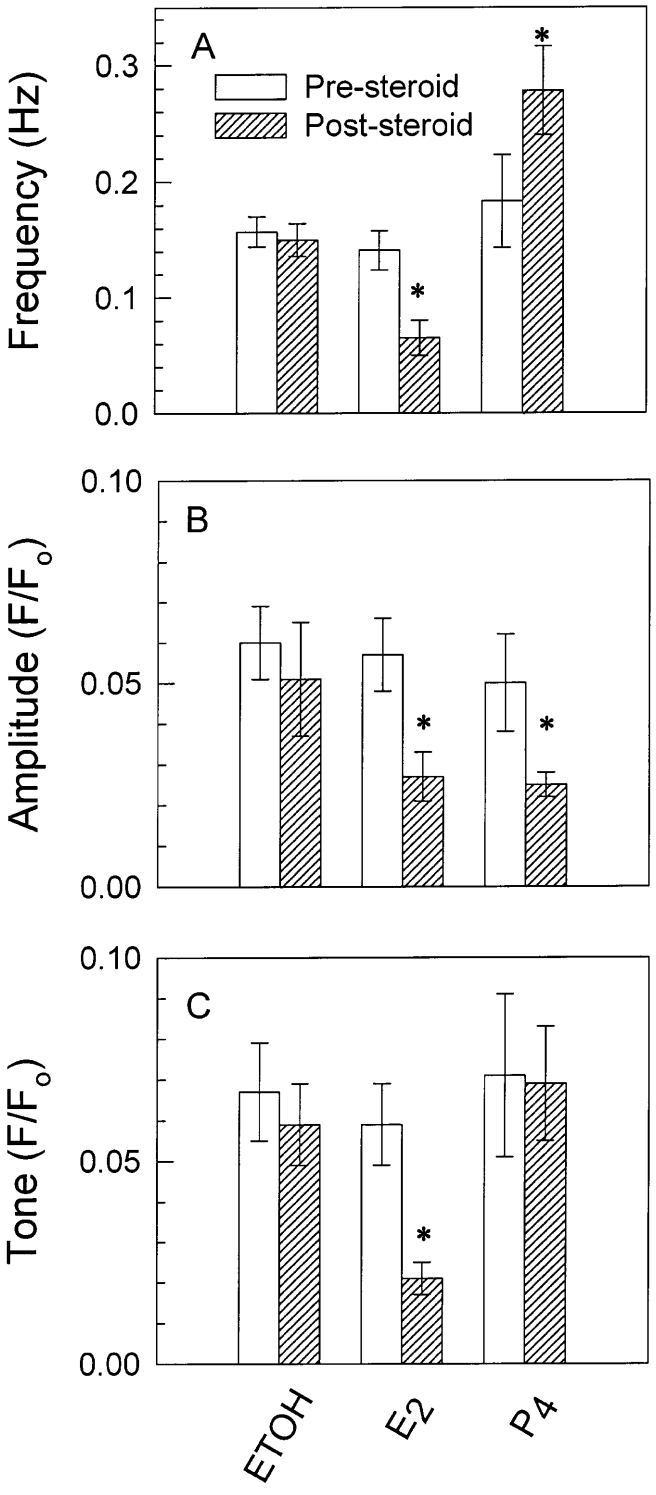

Fig. 4 Steady-state values of (**A**) frequency, (**B**) amplitude and (**C**) tone, before (pre-steroid) and 5 minutes after (post-steroid) the addition of estradiol (E_2), progesterone (P_4), or the vehicle control for the steroids, ethanol (ETOH), to detrusor smooth muscle strips. * $P < 0.05$ post-treatment compared with pre-treatment values

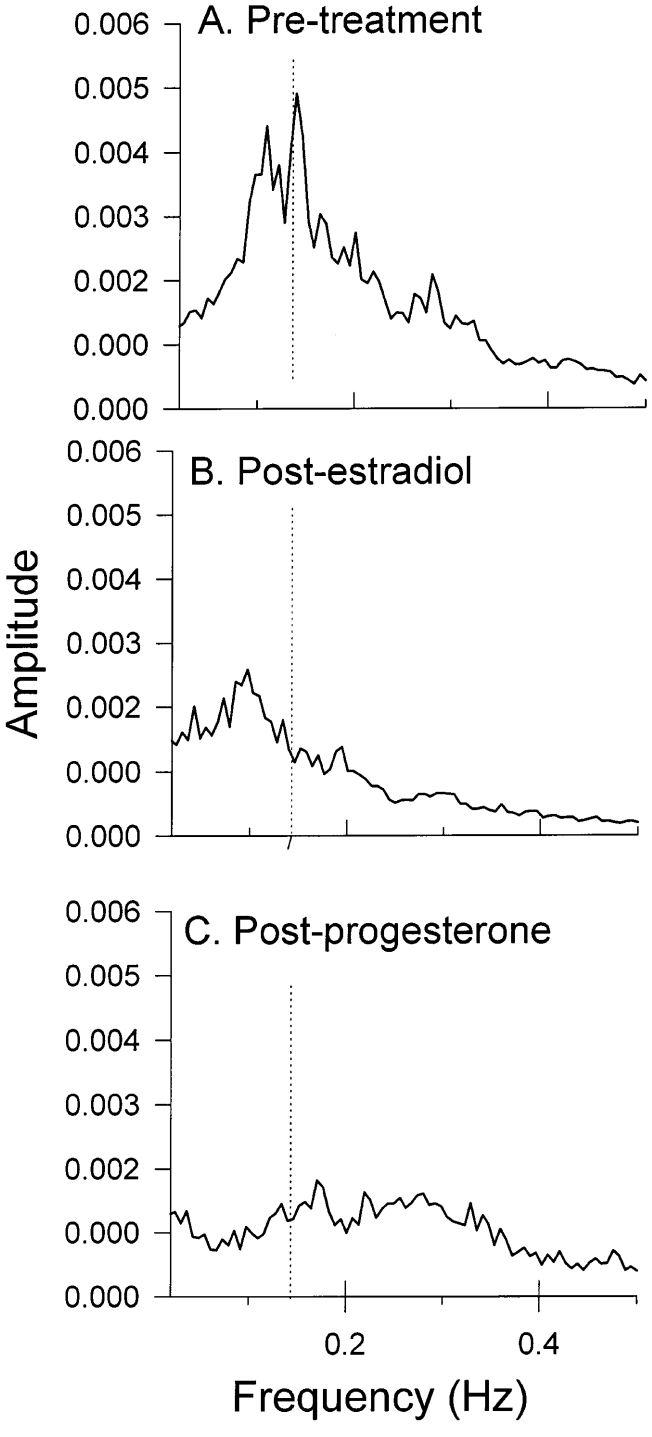

Fig. 5 Averaged Fast Fourier Transform (FFT) spectra. Dotted line indicates location of dominant peak in control (pre-treatment) tissues. (**A**) Control, basal spontaneous rhythmic contractions. (**B**) After the addition of estradiol, the dominant peak was diminished and shifted to the left, compared with **A**, signifying a lower frequency and amplitude of spontaneous rhythmic contraction. (**C**) After the addition of progesterone the dominant peak was again diminished but shifted to the right, compared with **A**, signifying decreased amplitude but increased frequency of rhythmic contractions

role in the pathogenesis of detrusor instability, and modulation of this activity may reduce or prevent the symptoms of a clinically unstable bladder.

The cellular mechanisms that initiate and sustain spontaneous rhythmic contractions in bladder smooth

muscle are not known. Nifedipine blocks rhythmic contractions in guinea pig detrusor strips [21]. Thus, L-type Ca^{2+} channels probably participate in the initiation of spontaneous rhythmic contractions. Spontaneous transient increases in intracellular Ca^{2+} were found to occur in cultured human single detrusor muscle cells. The Ca^{2+} transients were rhythmic and occurred at the same frequency reported for spontaneous rhythmic contractions [8]. Chambers et al. [8] therefore suggested that such rhythmic changes in intracellular Ca^{2+} concentrations may be associated with the spontaneous rhythmic contractions observed in detrusor strips. Moreover, the percentage of cells showing these spontaneous Ca^{2+} transients was higher in cells cultured from patients with unstable bladders compared with normal bladders [8], which further supports the contention that spontaneous activity plays a role in clinically relevant bladder instability. These spontaneous Ca^{2+} transients were inhibited when the cultured detrusor cells were placed in Ca^{2+}-free conditions or in the presence of 10 μM verapamil, an L-type Ca^{2+} channel blocker. Our data showing that rhythmic contractions in detrusor strips were promptly abolished in a Ca^{2+}-free solution containing 1 mM EGTA supports the contention that spontaneous rhythmic contractions are dependent on Ca^{2+} influx.

Estrogen receptors have been identified in the tissues obtained from the lower urinary tract in women [30] and in the posterior urethra of male rabbits [6] and men [7]. Estrogen has profound effects on the lower urinary tract and is used clinically in the treatment of incontinence in post-menopausal women [19]. The positive effects of estrogen on continence in woman may be derived from its ability to enhance urethral coaptation and function [27, 22]. In the rabbit, long-term estrogen treatment has been shown to increase urethral contractility [9]. This may be caused by an increase in adrenergic innervation and alpha-adrenergic receptor density [16]. All these changes are probably mediated by intracellular estrogen receptors, the activation of which initiates DNA transcription and protein synthesis.

Recent studies have suggested that steroid hormones also have additional, more rapid, effects through accessory ''non-genomic'' pathways. These non-genomic responses to estrogen have been demonstrated in neurons where estradiol probably binds to membrane receptors and activates cellular messenger systems [15]. Recent studies have shown estrogen to have a rapid relaxant effect on vascular [20] and bladder [31] smooth muscle. Progesterone has a rapid dose-dependent inhibitory effect on colon smooth muscle [10], but causes a rapid increase in sperm motility [4] by its ability to bind to cell surface receptors [1, 3] and activate a non-genomic pathway [5].

Because spontaneous rhythmic contractions of bladder smooth muscle appear to depend on rhythmic changes in cellular Ca^{2+} influx, and estrogen and progesterone have been shown to rapidly affect Ca^{2+} influx in various tissues, we tested the hypothesis that estradiol and progesterone may rapidly modulate detrusor spontaneous rhythmic contractions and tone. We found that estrogen significantly decreased rhythmic contractile frequency and amplitude by approximately 50% and detrusor muscle tone by approximately 60% compared with pre-treatment values.

Though the exact mechanism by which estrogen inhibits bladder contractions is not known, it is known that intracellular Ca^{2+} mobilization is not affected by estradiol, and RNA and protein synthesis inhibitors do not affect the ability of estradiol to inhibit contractions in vascular smooth muscle [14]. Estradiol was shown to reduce the contractile response to KCl and Ca^{2+} in isolated strips of detrusor smooth muscle [31]. Moreover, electrophysiological studies of the immediate effect of estradiol on single, guinea pig smooth muscle cells demonstrate dose-dependent inhibition of Ca^{2+} influx [23]. These data, taken together, support the hypothesis that estrogen may affect bladder smooth muscle contractility by reducing Ca^{2+} influx through a rapid non-genomic mechanism.

In our experiments, unlike estrogen, progesterone caused an immediate excitatory effect on rabbit detrusor muscle strips, and at steady state, after the immediate effect, progesterone caused a 30% increase in the frequency of spontaneous rhythmic contractions. However, at steady state, progesterone, like estrogen, caused a 50% decrease in the amplitude of spontaneous contractions. The opposite immediate effects of progesterone and estradiol, and opposite effects on the frequency of rhythmic contractions at steady state, suggest that progesterone and estradiol may exert their actions on bladder smooth muscle by different mechanisms. For example, whereas estradiol appears to decrease Ca^{2+} influx, progesterone may rapidly increase Ca^{2+} influx in detrusor smooth muscle, as is the case in human sperm [5].

To measure the frequency of spontaneous rhythmic contractions, contraction peaks were counted using a computer algorithm. A more sophisticated approach to frequency analysis, the use of FFT, has previously been shown to be an effective means for the description and analysis of rhythmic contractions of bladder strips [18]. We found that FFT spectral analysis confirmed that estradiol decreased and progesterone increased the frequency of spontaneous rhythmic contractions.

In our experiments, the onset of noticeable changes in rhythmic contractions caused by estradiol and progesterone in rabbit detrusor strips were quite rapid, occurring within 20–150 s of steroid application. We found these effects to be easily reversible with buffer change and tissue wash-out, as has been previously demonstrated [23]. These data support the hypothesis that changes in rhythmic contractions and basal tone produced by estradiol and progesterone were caused by activation of a rapid non-genomic pathway.

In conclusion, our data show that estrogen and progesterone rapidly modulate detrusor smooth muscle spontaneous rhythmic contractions and tone. It is

interesting to speculate, therefore, that a decrease in the levels of these steroids plays a role in the pathogenesis of voiding dysfunction in post-menopausal woman [17, 19]. One hypothesis is that the decrease in estradiol and progesterone levels associated with menopause may cause an increase in the amplitude of spontaneous rhythmic contractions inherent to the detrusor causing clinically significant detrusor instability. Moreover, the rapid inhibitory effects of estrogen on bladder smooth muscle strips may be responsible for some of the therapeutic effects of estrogen in the treatment of urinary incontinence in post-menopausal woman [17, 19]. The modulatory effects of estradiol and progesterone on detrusor spontaneous rhythmic contractions may also explain the association of voiding dysfunction with the phases of the menstrual cycle and pregnancy in some women [29, 26]. Future studies, directed towards identifying the subcellular mechanisms by which sex steroids rapidly modulate spontaneous rhythmic contractions in the detrusor could lead to the development of selective medications for the treatment of overactive bladders while avoiding the adverse effects associated with the activation of the genomic sex hormone receptors.

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