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The effect of pregnancy and the oestrus cycle on purinergic and cholinergic responses of the rat urinary bladder

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Abstract

The urinary bladder undergoes plastic changes during physiological alterations such as pregnancy. This study has shown that bladders from pregnant rats weighed three times more than bladders from virgin rats. Each milligram of detrusor muscle from pregnant rats contracted more strongly to nerve stimulation (150% greater) and agonists (50% greater or more) compared to detrusor from virgin rats at any stage during the oestrus cycle. The purinergic component of nerve-mediated responses altered during the oestrus cycle, being greatest during oestrus (oestrogen and progesterone fall rapidly) and dioestrus (low oestrogen and progesterone), smaller during pregnancy and even smaller during pro-oestrus (high oestrogen and progesterone); in contrast the cholinergic component remained relatively unchanged. In conclusion, during pregnancy the detrusor muscle generates larger contractions compared to virgin detrusor muscle, probably due to hormonal influences on smooth muscle contraction mechanisms. As agonist responses were unchanged during the oestrus cycle, changes in the purinergic component of nerve stimulation was not due to altered P2 receptor expression but possibly to an increase in ATP release or a reduction in breakdown. The hormonal effect may have implications for the treatment of bladder disorders due to alterations in hormones, such as stress incontinence in post-menopausal women.

Keywords: Bladder; Pregnancy; Oestrus cycle; P2X receptors; Purinergic; Cholinergic

1. Introduction

The mammalian bladder receives a dual excitatory innervation; acetylcholine (ACh) and adenosine 5′-triphosphate (ATP) are co-released from parasympathetic nerves acting on muscarinic and P2 receptors, respectively (Burnstock, 2001). Stimulation of muscarinic receptors raises intracellular inositol triphosphate (IP_3) levels and releases intracellular calcium in the initiation of contraction (Iacovou et al., 1990) whereas stimulation of P2X receptors mediates rapid permeability to Ca^{2+} (Dubyak and el-Moatassim, 1993).

ATP acts through P2 receptors that have been divided into two families, P2X and P2Y, based largely on cloning studies and on signal transduction mechanisms (Burnstock and Kennedy, 1985; Abbracchio and Burnstock, 1994; Ralevic and Burnstock, 1998). P2X receptors are ionotropic while P2Y receptors are G protein-coupled. Currently seven P2X receptors and eight P2Y receptor subtypes are recognized: $P2X_{1–7}$ and $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ (see Burnstock, 2003).

Various changes can occur in the function of the urinary bladder as a consequence of diseases such as diabetes (Prosdocimi and Paro, 1990; Luheshi and Zar, 1991), multiple sclerosis (Miller et al., 1965; Blaivas et al., 1984) and interstitial cystitis (Holm-Bentzen and Lose, 1987; Palea et al., 1993). During pregnancy, urinary incontinence in women is a common problem (Turan et al., 1996), the risk increasing with multiple deliveries (Ryhammer et al., 1995). The consequences of pregnancy on mammalian bladder function have been investigated to a limited extent. Isolated whole bladders from pregnant rabbits respond to low-frequency stimulation and to ATP with a greater increase in intravesical pressure compared to virgin rabbit bladders whereas

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contractions elicited by bethanechol were reduced in the pregnant rabbits (Zderic et al., 1990; Levin et al., 1991). It was concluded that during pregnancy there was an increase in the purinergic and a decrease in the cholinergic component of nerve-mediated responses.

The aim of this present study was to examine the response of the pregnant rat bladder to electrical field stimulation and to exogenous P2X and muscarinic receptor agonists and compare them with those from virgin rat bladder at different stages of the oestrus cycle, namely pro-oestrus (during follicle maturation and the rising levels of oestrogen and progesterone), oestrus (initial high levels of oestrogen and peak in progesterone, both decreasing during oestrus) and dioestrus (the period between oestrus and the next pro-oestrus and low oestrogen and progesterone).

2. Methods

2.1. General procedures

Adult female virgin (150–200 g) and pregnant (15–18 days) Sprague–Dawley rats were used in this study. In order to assess the stage of the oestrus cycle (5 days), virgin animals were tested daily by vaginal cytology and animals were sacrificed at pro-oestrus, oestrus and dioestrus (I–III) after 2 weeks in which it was confirmed by cytology that the animals were cyclic. All groups of rats were killed by CO_2 asphyxiation and death was confirmed by cervical dislocation according to Home Office regulations covering Schedule 1 procedures. The foetuses were killed by decapitation.

The urinary bladders were dissected free and placed in modified Krebs solution of the following composition (mM): NaCl, 133; KCl, 4.7; $NaHCO_3$, 16.4; $MgSO_4$, 0.6; NaH_2PO_4, 1.4; glucose, 7.7 and $CaCl_2$, 2.5; pH 7.3. The tissues were then stripped of adhering fat and connective tissue, weighed and prepared for isolated organ bath recording using a dissecting microscope. Two strips of detrusor muscle, approximately 15×2 mm, were dissected from each bladder from virgin rats and four strips were dissected from bladders from pregnant rats. Silk ligatures were applied to each end of the strip; one end was attached to a rigid support and the other end to a FT03C force–displacement transducer. Each strip was suspended in a 10 ml organ bath containing gassed (95% O_2/5% CO_2) modified Krebs solution. Experiments were carried out at 37 ± 1 °C. Separate experiments were carried out to look at electrical field stimulation (EFS) and the action of exogenously applied agonists.

Mechanical activity was recorded using the software PowerLab Chart for Windows (version 4; ADInstruments, Australia). An initial load of 1 g was applied to the detrusor strips, which were then allowed to equilibrate for not less than 45 min prior to the start of the experiment. The contraction due to a standard concentration of KCl (120 mM) was noted at the end of each experiment, as was the weight of the detrusor strip.

2.2. Frequency–response curves

Frequency–response curves to EFS were constructed for the detrusor strips (100 V, 0.3 ms, 0.5–32 Hz, 15 s stimulation every 5 min) in the absence of any antagonists, and then in the presence of either PPADS (30 μM; one detrusor strip from each bladder) or atropine (1 μM; one detrusor strip from each bladder). The frequency–response curves were then repeated in the presence of both PPADS (30 μM) and atropine (1 μM). All antagonists were incubated for 20 min. The curves were finally repeated in the presence of tetrodotoxin (TTX; 1 μM, 20 min) to identify if any part of the response was due to direct stimulation of the detrusor smooth muscle.

2.3. Concentration–response curves

Non-cumulative concentration–response curves were constructed for carbachol (CCh; 10 nM–1 mM), β,γ-methylene ATP (β,γ-meATP; 0.1–300 μM) and ATP (1 μM–1 mM). The time interval between applications of β,γ-meATP and ATP was 15–20 min to avoid desensitization.

2.4. Drugs used

Atropine, ATP, β,γ-meATP (sodium salt), CCh and TTX were obtained from Sigma Chemical Co. (Poole, UK). PPADS (tetrasodium salt) was supplied by Tocris Cookson Ltd (Bristol, UK). All drugs were dissolved in distilled water, and the volume added to the organ bath did not exceed 100 μM.

2.5. Statistical analysis

As the weight of the bladders from the pregnant rats was significantly greater than those from the virgin animals, the weight of each detrusor strip was noted and the responses to EFS and exogenously applied agonists are expressed as mean contraction in mg per mg detrusor muscle ± SE mean (n). In addition, responses to EFS were calculated as mean percent of the maximum contraction ± SE mean (n). pD_2 values ($-\log EC_{50}$ concentration) were calculated for CCh. As the concentration–response curves to β,γ-meATP and ATP did not reach maximum, it was not possible to calculate pD_2 values.

Statistical significance of frequency and concentration–response curves was tested by two-way analysis of variance (ANOVA) followed by a post hoc test

(Tukey's) using GraphPad Prism (GraphPad software, Inc., San Diego, CA). Statistical significance of percent inhibition due to either PPADS or atropine at 16 Hz was tested using a one-way ANOVA followed by a Bonferroni's post hoc test. Statistical significance between bladder weights, KCl contractions and pD_2 values for CCh was tested by one-way ANOVA followed by a Bonferroni's post hoc test. A probability of $P< 0.05$ was considered as significant for both the ANOVA tests.

3. Results

The bladders from pregnant rats were significantly heavier ($P< 0.001$) than those from the virgin animals at any stage of the oestrus cycle. The bladders from virgin animals during the different stages of the oestrus cycle did not differ significantly in weight from each other when compared using a one-way ANOVA (see Table 1). The weight of the bladder strip was used to standardize responses, and results are expressed as mean contraction in mg/mg detrusor muscle.

KCl (120 mM) induced contractions in pregnant bladders that were significantly greater ($P< 0.01$) than contractions from the virgin animals at any stage of the oestrus cycle, expressed as mg/mg tissue. Contractions to KCl (120 mM) from bladders from virgin animals during the different stages of the oestrus cycle did not differ significantly from each other when compared using a one-way ANOVA (see Table 1).

3.1. Frequency–response curves

EFS of the detrusor muscle from pregnant and virgin animals induced frequency-dependent and TTX-sensitive (1 μM) contractions and frequency–response curves were constructed (100 V, 0.3 ms, 0.5–32 Hz, 15 s stimulation every 5 min) in the absence of any blocking agents. The frequency–response curves repeated in the presence of PPADS (30 μM) alone were significantly reduced from each group of animals when compared to the corresponding control frequency–response curve. The addition of both PPADS (30 μM) and atropine (1 μM) further significantly inhibited the frequency–response curves, such that there was only a small residual response remaining (Fig. 1a–d). Similarly, the frequency–response curves when repeated in the presence of atropine (1 μM) alone were significantly reduced from each group of animals when compared to the corresponding control frequency–response curve. In the presence of both atropine (1 μM) and PPADS (30 μM), the frequency–response curves were further significantly inhibited, such that there was only a small residual response remaining (Fig. 2a–d).

The maximum contraction induced by EFS in the absence of any blocking drugs from the pregnant rats was significantly greater ($P< 0.001$) than maximum contractions from the virgin rats at any stage of the oestrus cycle (see Table 2). Maximum contractions from the virgin animals during the different stages of the oestrus cycle were not significantly different from each other.

In order to examine the effect of pregnancy and oestrus cycle on the proportions of the purinergic and cholinergic components of nerve-mediated contractions, responses to EFS from each group of animals was expressed as percent of the maximum control response. Expressing the responses thus, revealed that the PPADS-sensitive (purinergic) component of nerve-mediated responses varied with both pregnancy and oestrus cycle. The size of the PPADS-sensitive component from largest to smallest was found to be: dioestrus = oestrus > pregnant > pro-oestrus, whereas the atropine-sensitive component did not vary greatly: dioestrus = pregnant = oestrus = pro-oestrus, although the curve for dioestrus was significantly different from that of pro-oestrus.

The percent inhibition due to either PPADS (30 μM) or atropine (1 μM) at 16 Hz (approximately maximum response) from each group of animals was compared using a one-way ANOVA (see Table 2). The percent inhibition to PPADS was found to be greatest during oestrus and dioestrus, smaller in pregnancy and smaller still during pro-oestrus, although following statistical analysis, the percent inhibition at 16 Hz from oestrus and dioestrus animals was only significantly different

Table 1
Table showing bladder weights, contractions to KCl (120 mM), β,γ-meATP, ATP and CCh from bladders from pregnant rats and bladders from rats during the different stages of the oestrus cycle. Statistical significance tested by one-way ANOVA followed by a Bonferroni's post hoc test.

	Pregnant	Pro-oestrus	Oestrus	Dioestrus
Bladder weight (mg)	$101.3 \pm 8.8^{***}$ ($n = 8$)	58.8 ± 4.6 ($n = 9$)	56.4 ± 4.3 ($n = 9$)	50.3 ± 2.8 ($n = 11$)
KCl (120 mM) contraction (mg/mg tissue)	$165.2 \pm 11.2^{***}$ ($n = 8$)	99.0 ± 7.4 ($n = 9$)	90.5 ± 8.0 (n = 9)	93.0 ± 8.5 ($n = 11$)
β,γ-meATP contraction (0.3 mM) (mg/mg tissue)	$80.3 \pm 9.6^{***}$ ($n = 5$)	52.2 ± 6.2 ($n = 5$)	47.9 ± 6.8 ($n = 5$)	48.5 ± 8.0 ($n = 5$)
ATP contraction (1 mM) (mg/mg tissue)	$50.2 \pm 8.4^{***}$ ($n = 5$)	33.6 ± 4.9 ($n = 5$)	30.7 ± 3.0 ($n = 5$)	27.3 ± 3.4 ($n = 5$)
Maximum CCh contraction (mg/mg tissue)	$244.4 \pm 22.4^{***}$ ($n = 5$)	166.1 ± 12.4 ($n = 5$)	151.0 ± 15.6 ($n = 5$)	161.7 ± 15.6 ($n = 5$)
CCh pD_2 values	5.76 ± 0.18 ($n = 5$)	5.73 ± 0.06 ($n = 5$)	5.78 ± 0.09 ($n = 5$)	5.96 ± 0.07 ($n = 5$)

$^{***}P< 0.001$

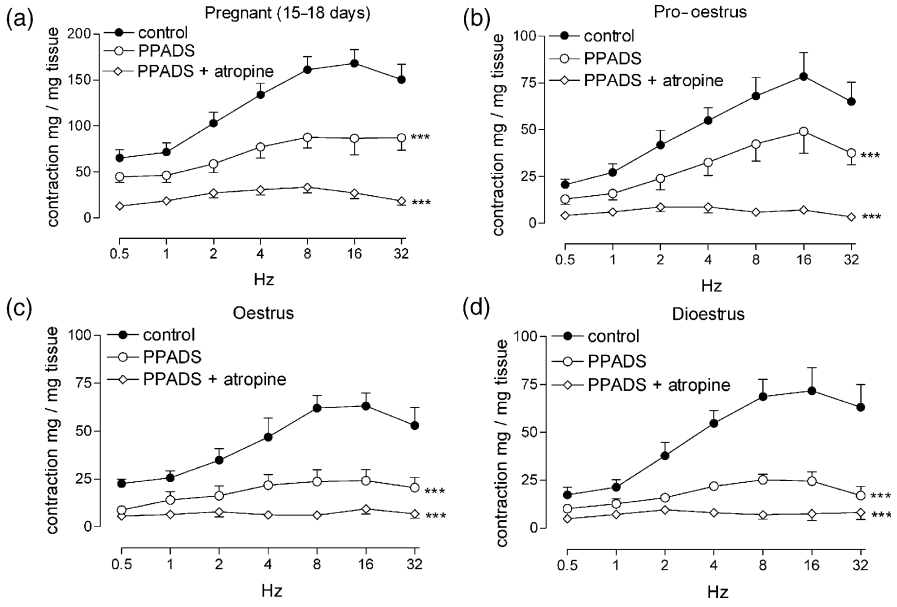

Fig. 1. Effect of P2 receptor and cholinoceptor antagonists on electrical field stimulation of the rat urinary bladder (100 V, 0.3 ms, 0.5–32 Hz, 15 s stimulation every 5 min) during pregnancy (15–18 days) and the oestrus cycle. (a) Frequency–response curves in the absence (control; $n = 6$) and presence of either PPADS (30 μM; $n = 6$) alone or PPADS (30 μM) plus atropine (1 μM; $n = 6$) on bladders from pregnant animals. (b) Frequency–response curves in the absence (control; $n = 5$) and presence of either PPADS (30 μM; $n = 5$) alone or PPADS (30 μM) plus atropine (1 μM; $n = 5$) on bladders from animals during pro-oestrus. (c) Frequency–response curves in the absence (control; $n = 5$) and presence of either PPADS (30 μM; $n = 5$) alone or PPADS (30 μM) plus atropine (1 μM; $n = 5$) on bladders from animals during oestrus. (d) Frequency–response curves in the absence (control; $n = 5$) and presence of either PPADS (30 μM; $n = 5$) alone or PPADS (30 μM) plus atropine (1 μM; $n = 5$) on bladders from animals during dioestrus. All symbols are means ± SE (unless error bars masked by the symbol), expressed as contraction mg/mg tissue. Statistical significance was tested by two-way ANOVA. $^{***}P < 0.0001$ (applicable to the whole curve).

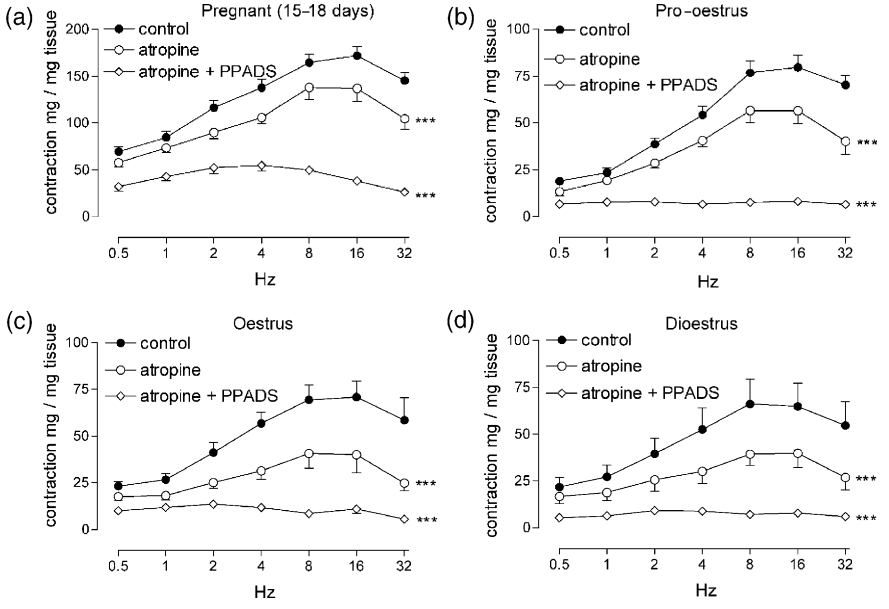

Fig. 2. Effect of cholinoceptor and P2 receptor antagonists on electrical field stimulation of the rat urinary bladder (100 V, 0.3 ms, 0.5–32 Hz, 15 s stimulation every 5 min) during pregnancy (15–18 days) and the oestrus cycle. (a) Frequency–response curves in the absence (control; $n = 5$) and presence of either atropine (1 μM; $n = 5$) alone or atropine (1 μM) plus PPADS (30 μM; $n = 5$) on bladders from pregnant animals. (b) Frequency–response curves in the absence (control; $n = 5$) and presence of either atropine (1 μM; $n = 5$) alone or atropine (1 μM) plus PPADS (30 μM; $n = 5$) on bladders from animals during pro-oestrus. (c) Frequency–response curves in the absence (control; $n = 5$) and presence of either atropine (1 μM; $n = 5$) alone or atropine (1 μM) plus PPADS (30 μM; $n = 5$) on bladders from animals during oestrus. (d) Frequency–response curves in the absence (control; $n = 5$) and presence of either atropine (1 μM; $n = 5$) alone or atropine (1 μM) plus PPADS (30 μM; $n = 5$) on bladders from animals during dioestrus. All symbols are means ± SE (unless error bars masked by the symbol), expressed as contraction mg/mg tissue. Statistical significance was tested by two-way ANOVA. $^{***}P < 0.0001$ (applicable to the whole curve).

Table 2
Table showing maximum contractions to EFS and the percent of the response to EFS at 16 Hz that could be attributed to be either purinergic or cholinergic. Statistical significance tested by one-way ANOVA followed by a Bonferroni's post hoc test.

	Pregnant	Pro-oestrus	Oestrus	Dioestrus
Maximum EFS contraction (mg/mg tissue)	$173.5 \pm 7.4^{***}$ ($n = 6$)	77.2 ± 6.7 ($n = 6$)	69.6 ± 5.9 ($n = 5$)	71.5 ± 7.8 ($n = 6$)
Percent purinergic component of EFS at 16 Hz (% max response)	45.8 ± 8.4 ($n = 6$)	39.0 ± 6.9 ($n = 5$)	$69.4 \pm 4.7^{*}$ ($n = 5$)	$69.6 \pm 4.64^{*}$ ($n = 5$)
Percent cholinergic component of EFS at 16 Hz (% max response)	34.2 ± 9.3 ($n = 5$)	30.6 ± 5.5 ($n = 6$)	25.8 ± 9.4 ($n = 5$)	38.9 ± 5.1 ($n = 5$)

$^{*}P < 0.05$, $^{***}P < 0.001$.

from those during pro-oestrus. In contrast the percent inhibition due to atropine did not vary with either pregnancy or the oestrus cycle.

3.2. Concentration–response curves

Both β,γ-meATP (0.1–300 μM) and ATP (1 μM–1 mM) induced concentration-dependent transient contractions of the detrusor muscle in both pregnant and virgin rats. Contractions to β,γ-meATP from the pregnant rats were significantly greater ($P < 0.001$) than the virgin rats at any stage in the oestrus cycle. Contractions to β,γ-meATP from the virgin animals during the different stages of the oestrus cycle were not significantly different from each other (Fig. 3a). Similarly, contractions to ATP from the pregnant rats were significantly greater ($P < 0.01$) than the virgin rats at any stage in the oestrus cycle. Contractions to ATP in virgin bladders during the different stages of the oestrus cycle did not differ significantly from each other (Fig. 3b). Concentration–response curves to β,γ-meATP and ATP did not reach a maximum response and as such pD_2 values could not be calculated.

CCh (10 nM–1 mM) induced concentration-dependent, sustained contractions in both pregnant and virgin rats. Contractions to CCh from the pregnant rats were significantly greater ($P < 0.001$) than the virgin rats at any stage in the oestrus cycle. Contractions to CCh from virgin rats during the different stages of the oestrus cycle did not differ significantly from each other (Fig. 3c). The pD_2 values were calculated (see Table 1) and found not to differ significantly from each other.

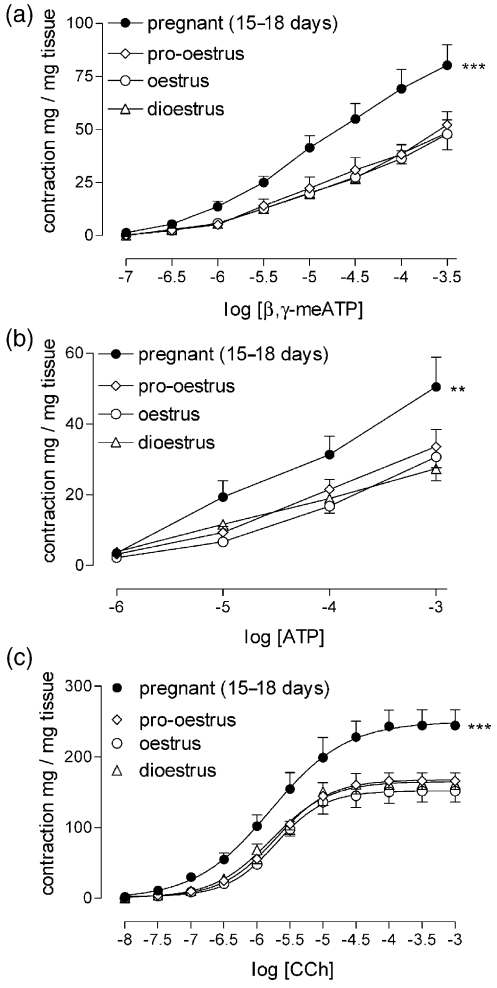

Fig. 3. Effects of exogenously applied P2 receptor and cholinergic agonists on the rat urinary bladder. (a) Non-cumulative concentration–response curves for β,γ-meATP (0.1 μM–0.3 mM; all $n = 5$) on urinary bladders from pregnant animals and at different stages of the oestrus cycle. (b) Non-cumulative concentration–response curves for ATP (1 μM–1 mM; all $n = 5$) on urinary bladders from pregnant animals and at different stages of the oestrus cycle. (c) Cumulative concentration–response curves for CCh (10 nM–1 mM; all $n = 5$) on urinary bladders from pregnant animals and at different stages of the oestrus cycle. All symbols are mean ± SE (unless error bars masked by the symbol) expressed as contraction mg/mg tissue. Statistical significance was tested by two-way ANOVA. $^{**}P < 0.001$, $^{***}P < 0.0001$ (applicable to the whole curve).

4. Discussion

This study has shown that bladders from pregnant rats are larger and contract more strongly in response to EFS and P2 receptor and muscarinic receptor stimulation. An interesting finding of this study is that the size of the purinergic component of the parasympathetic innervation alters during the oestrus cycle, whereas the cholinergic component remains relatively unchanged.

An increase in bladder size and weight and alterations in responses to agonists and electrical stimulation has been reported previously. Pregnant rats were shown to have an increased bladder capacity and weight, thought to be due to an increase in compliance (Hsia and Shortliffe, 1995). Increased contractions due to ATP and CCh (Tong et al., 1995; Grandadam et al., 1999) were thought to be due to an increase in receptor sensitivity. During pregnancy, adrenergic nerves in the rat bladder degenerate and re-innervate within 10 days of parturition (Qayyum et al., 1989). In contrast, in the rabbit bladder during pregnancy, responses to cholinergic agonists were reduced compared to the non-pregnant state (Zderic et al., 1990; Brandes and Ruggieri, 1995) that corresponded to a decrease in muscarinic receptor density (Baselli et al., 1999), whereas responses to ATP were increased (Levin et al., 1991).

The increase in responses to EFS and the contractile agents used in this study suggest that pregnancy affects the contractile properties of the detrusor smooth muscle, since in addition to receptor-mediated contractions, contractions to KCl were augmented in pregnancy. Histological examination of bladders from pregnant rats revealed that the bladder walls were significantly thicker with interstitial oedema and urothelium changes associated with proliferation to a papillary configuration (Tong et al., 1995). A separate study found a significant decrease in sarcolemmal caveolae and the membrane protein caveolin-1 in the smooth muscle of bladders from pregnant rats. It was thought that changes in the caveolae and caveolin might play a role in the functional changes associated with pregnancy (Bakircioglu et al., 2000). Such changes in the urinary bladder during pregnancy are now thought not to be due to obstruction, but due to hormonal influences (Hvidman et al., 2002). Oestrogen and progesterone inhibit myometrium activity and progesterone blocks gap junction synthesis in myometrial cell membranes. Since the uterus and bladder have a common embryological origin, an endocrine etiology may explain the morphological changes associated with pregnancy (Hsia and Shortliffe, 1995). Oestrogen and progesterone receptors are present in the urinary tract of women and female rabbits, rats and mice (Iosif et al., 1981; Batra and Iosif, 1987; Uotinen et al., 1999; Bennett et al., 2003) and micturition is known to be affected by oestrogen in cats (VanderHorst et al., 2001) and oestrogen has been implicated in decreased urethral tone associated with pregnancy in rabbits (Callahan and Creed, 1985).

A change in the distribution of P2X receptor clusters on smooth muscle cells of the rat bladder during pregnancy has been reported; clusters at junctional varicosities of $P2X_1$, $P2X_2$, $P2X_3$ and $P2X_5$ receptors decreased by approximately 80%, while the extent of $P2X_4$ and $P2X_6$ junctional clusters increased by more than 80% (Yunaev et al., 2000). This may account for the smaller PPADS-sensitive component of nerve-mediated responses seen in this study during pregnancy (a period of increasing oestrogen and progesterone) compared to the purinergic component when circulating hormones are reducing or low (oestrus and dioestrus).

Levin and co-workers (1991) reported an increase in the purinergic component of nerve-mediated contractions during pregnancy in the rat; this was accompanied with an increase in the response to ATP. These findings differ from the present study and may reflect a difference in methodology. In the study by Levin and co-workers (1991), the stage in the oestrus cycle of the virgin animals was not ascertained, which this investigation has shown can influence responses to nerve stimulation. For comparison, when the results from the three virgin groups in this study are combined, the purinergic component from the pregnant rat appears to be greater than the purinergic component of the combined virgin animals, although individually, the purinergic component was only greater in the pregnant rats compared to those in pro-oestrus.

In addition to an increase in responses to EFS during pregnancy, the proportion of the response that was either PPADS- or atropine-sensitive altered with hormonal status. The purinergic component of nerve-mediated responses was greatest in the rats during oestrus and dioestrus, smaller in the pregnant and smaller still in rats in pro-oestrus. In contrast, the cholinergic component did not differ greatly with pregnancy or hormonal status; the only significant difference in the curves was seen in those for dioestrus and pro-oestrus. The alteration in the purinergic component of nerve-mediated responses within the oestrus cycle may be due to an alteration in transmitter release (pre-junctional effect) or an alteration in the post-junctional receptor ($P2X_1$ receptors) or to ectoenzymatic breakdown of ATP. The concentration–response curves to the purinergic and cholinergic agonists were not significantly different during the oestrus cycle suggesting that there is no change in the post-junctional receptors.

Oestrogen and progesterone are known to alter vascular, airway, bladder and gastrointestinal smooth muscle contractility (Gill et al., 1985; Rodriguez et al., 1996; Freay et al., 1997; Perusquia et al., 1997; Shenfeld et al., 1999) and oestrogen replacement therapy in ovariectomized rabbits resulted in an increase in bladder wet weight and smooth muscle cell density (Hashimoto et al., 1999) and decreased muscarinic receptor density (Shapiro, 1986) but an increased response to cholinergic and purinergic agonists (Levin et al., 1980, 1981). In similarly treated rats, there was also an increase in bladder body mass following oestrogen supplementation, together with an increased contractility to cholinergic and purinergic stimulation (Longhurst et al., 1992; Diep and Constantinou, 1999). A

study of the oestrus cycle of the guinea pig, which is particularly long (between 13 and 20 days), revealed that inhibition of nerve-mediated responses by α,β-meATP in the bladder was greatest on day 6 of the cycle which corresponded to high circulating levels of oestrogen (Liu et al., 1998). In contrast, in the rat bladder, high circulating oestrogen during pregnancy and pro-oestrus corresponded to when the purinergic component of nerve-mediated contractions was the smallest.

Although one must be cautious in comparing bladder function between laboratory animals and humans, immunohistochemical investigations of the expression of P2X receptor in the adult rat and humans reveal that the distribution of receptors is very similar (Dutton et al., 1999; Yunaev et al., 2000), even though the function of normal bladders of rat and humans vary in respect to the purinergic component of parasympathetic innervation. In the human condition of idiopathic detrusor instability (IDI) the expression of P2X receptors are altered. In a study using RT-PCR, $P2X_2$ receptor mRNA was found to be increased and $P2X_1$, $P2X_4$ and $P2X_7$ receptor subtype mRNA decreased compared to normal bladder (O'Reilly et al., 2002); in the same investigation, functional studies revealed that bladder samples from IDI patients exhibited a significant purinergic component to nerve-mediated responses, whereas in control samples a purinergic component was hardly detectable. In an immunohistochemical study it was shown that $P2X_3$ and $P2X_5$ immunoreactivity was absent from the bladders from IDI patients (Moore et al., 2001) suggesting that the lack of these receptors may contribute to urge incontinence. It is known that pregnancy places a great deal of stress on the urinary bladder and many women report urinary incontinence, particularly as pregnancy progresses. It is thought to arise as a result of detrusor instability resulting from changes in hormone levels (Miodrag et al., 1988) although anatomical changes due to a growing uterus may also contribute (Wijma et al., 2001). Previous studies indicate that detrusor instability may be associated with increased spontaneous rhythmic contractile activity (Kinder and Mundy, 1987). Exogenous oestradiol inhibited spontaneous rhythmic contractions of the rabbit bladder, whereas progesterone increased spontaneous rhythmic contractions (Shenfeld et al., 1999). It has been postulated that a decrease in the circulating levels of oestrogen and progesterone may be important in the pathogenesis of voiding dysfunction in post-menopausal women (Miodrag et al., 1988) by increasing the amplitude of spontaneous rhythmic contractions causing clinically significant detrusor instability, and as such, the rapid inhibitory effects of oestrogen on detrusor smooth muscle may be responsible for some of the therapeutic effects of oestrogen in the treatment of incontinence in post-menopausal women (Ravn et al., 1994). Whether changes in expression of purinoceptors in bladder play a role in human pregnancy remains to be investigated.

In conclusion, this study has confirmed that in pregnancy the detrusor smooth muscle of the rat generates larger contractions in response to both nerve stimulation and exogenous agonists compared to virgin animals. This augmentation of contractility is probably due to hormonal influences on the smooth muscle as a result of pregnancy. In addition, this study has interestingly shown a difference in the size of the purinergic component of nerve-mediated responses corresponding to the stage of the oestrus cycle, being largest when circulating levels of oestrogen are low or falling (i.e. during dioestrus and oestrus). The effect of sex hormones on the activity of the urinary bladder may have implications for the treatment of bladder disorders, such as stress incontinence.

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