Effect of the oestrous cycle, pregnancy and uterine region on the responsiveness of the isolated mouse uterus to prostaglandin F_{2α} and the thromboxane mimetic U46619

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Abstract

Previous studies in this laboratory have suggested that the isolated uterus from non-pregnant mice has a prostaglandin F and a thromboxane receptor population similar to that found in human myometrium. The aim of this study was to investigate any regional variation in myogenic activity and responsiveness to prostaglandin F_{2α} (PGF_{2α}) and the thromboxane mimetic U46619 in the mouse uterus taken during different stages of the oestrous cycle and during pregnancy. Uterine samples from BKW mice were taken from different anatomical segments along the length of each uterine horn and set up for superfusion at 2 ml/min with Krebs solution (containing 1 μM indometacin) at 37 °C, and gassed with 95%O₂/5%CO₂. Responses (area under the curve) are expressed as a percentage of the final contraction induced by hypotonic shock. Data are expressed

as the means ± S.E.M. of $n=5-12$ and were analysed using Student's paired *t*-test or two-way ANOVA with a Bonferroni *post hoc* test. Regional variation in myogenic activity was observed in all tissues studied except those taken during labour. These tissues displayed significantly greater myogenic activity than tissues taken at late gestation and at all stages of the oestrous cycle. Tissues from pregnant animals were generally more responsive to U46619 and PGF_{2α} than tissues taken from non-pregnant animals. Tissues taken from the upper segment of the uterine horn were more responsive to both agonists during the oestrous cycle. The findings demonstrated that the hormonal milieu and site of excision are important for myogenic activity and responsiveness.

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Introduction

Prostaglandins are involved in several reproductive processes in mice including ovulation, fertilization, luteolysis and modulation of uterine contractions (Sales *et al.* 1992). The importance of prostaglandins and their receptors in reproductive function has been determined in studies using knockout mice (Tsuboi *et al.* 2002), which show that prostaglandin F (FP) receptor-deficient mice exhibited failed parturition and prostaglandin E (EP) receptor-deficient mice have a decreased litter size. Thromboxane (TP) receptor knockout mice did not show any reproductive abnormalities (Thomas *et al.* 1998). The following mouse prostaglandin receptors have been isolated and cloned: prostaglandin D (Hirata *et al.* 1994), EP₁ (Watabe *et al.* 1993), EP₂ (Honda *et al.* 1993), EP₃ (Sugimoto *et al.* 1992), EP₄ (Nishigaki *et al.* 1995), FP (Sugimoto *et al.* 1994), prostaglandin I (Namba *et al.* 1994) and TP (Namba *et al.* 1992).

Previous studies in this laboratory have suggested that the uterus from the non-pregnant mouse has a similar

TP receptor and FP receptor population (Kennedy *et al.* 1994, Hutchinson *et al.* 2003) to that found in the non-gestational human myometrium (Senior *et al.* 1992). However, the potential effect of the oestrous cycle on the prostaglandin receptor profile has not been investigated. During the oestrous cycle, oestrogen and progesterone levels vary with the stage of the cycle (Walmer *et al.* 1992). Variations in oestrogen and progesterone ratio will affect myogenic activity and may also alter agonist responses. The FP receptor has been reported to be upregulated towards term in the human (Brodt-Eppley & Myatt 1999) and mouse myometrium (Cook *et al.* 2003); however, an increase in the TP receptor population has not been reported.

To our knowledge, the topographical distribution of the FP and TP receptors in the mouse uterus has not been explored. In studies using isolated tissue the anatomical region of the uterus used should be considered. Studies using uterus from the non-pregnant rat have shown that the upper (ovarian) portion of the uterus is more sensitive to prostaglandin F_{2α} (PGF_{2α}) than the lower (cervical)

portion (Oropeza *et al.* 2000). Myogenic activity in the rat has been investigated during gestation and parturition and an increase in activity from late gestation through to labour has been shown; this study also observed regional variation in activity at late gestation (Fuchs 1969). Regional variation in responsiveness to $\text{PGF}_{2\alpha}$ has also been observed in human myometrium taken during labour, with the lower segment showing little responsiveness to $\text{PGF}_{2\alpha}$ (Wikland *et al.* 1984). However, the population of TP receptors is purported to be functionally homogenous throughout the human myometrium (Senchyna & Crankshaw 1999).

This study was designed to investigate any regional variation in myogenic activity and responsiveness to $\text{PGF}_{2\alpha}$ and the stable thromboxane mimetic U46619 (Abramovitz *et al.* 2000) in the mouse uterus taken during different reproductive stages of the oestrous cycle and during pregnancy. This work is part of a larger on-going project and is running concurrently with similar experiments using human tissue; a subsidiary aim is to determine the suitability of the mouse for future *in vivo* studies.

Materials and Methods

Tissue preparation

Non-pregnant and pregnant female, sexually mature, BKW mice (B&K Universal Ltd, Hull, Humberside, UK) housed in groups were used throughout this study. Experiments were carried out in accordance with the Animals Scientific Procedures Act 1986. The animals had free access to food and water and were exposed to a 12 h light:12 h darkness cycle. Animals were mated in a harem of three females to one male. The presence of a vaginal plug was taken as evidence of pregnancy; the day of plug detection was termed day 1 of gestation. The mice were regularly weighed to monitor the progression of pregnancy.

The mice were killed by cervical dislocation when not pregnant, at day 18 of gestation (late gestation) or during labour. Animals killed during parturition were killed after delivery had initiated and usually after at least two pups had been delivered. In non-pregnant animals, the stage of the oestrous cycle was determined by microscopic examination of the vaginal lavage (Fox & Laird 1970). The uterus was dissected from the body and uterine smooth muscle samples (8–10 cm in length and 2–3 cm in width) were taken from along the length of each uterine horn. Uterine samples from non-pregnant animals were cut transversely to leave two tube-like pieces of tissue. Samples taken from pregnant and labouring animals were cut parallel to the longitudinal fibres along the length of the uterus. These samples were taken from four anatomical regions of the horn, one immediately above the cervix (lower), two from the middle portion, one being nearer

the cervical end and the other from the ovarian end, and the last sample was taken from the uppermost section of the horn, directly below the ovary. In pregnant and labouring animals, the foetuses and placentae were completely removed from the uterus and foetuses were killed by carbon dioxide inhalation.

Uterine samples were immediately placed in oxygenated Krebs solution and set up for superfusion at a rate of 2 ml/min. The strips were attached to an isometric force transducer and a resting tension of 2 g (Chen *et al.* 2001) was applied for superfusion with oxygenated Krebs (95% O_2 /5% CO_2) (Senior *et al.* 1991) containing $1\mu\text{M}$ indometacin (Duckworth *et al.* 2002) at 37°C . Tissues were left for 30 min to equilibrate.

The prostanoid agonists were administered as $10\mu\text{l}$ bolus doses injected directly, through self-sealing silicone tubing, into the superfusate using a micropipette. Agonists were administered immediately after a spontaneous contraction to avoid superimposing responses on background activity; this did not apply if there was no background activity present. Responses were also investigated in the presence of $0.1\mu\text{M}$ SQ29548, a thromboxane receptor antagonist (Ogletree *et al.* 1985). Only one dose–response curve was performed on each tissue.

At the end of each experiment the superfusate was changed from Krebs solution to distilled water. The infusion of distilled water induced a large contraction of the tissue (hypotonic shock) (Crankshaw 2001), which was unique to that particular myometrial strip; this was used as a reference contraction. No differences in hypotonic shock were observed between the different reproductive states.

Measurement of responses

The activity of the myometrial strips was measured via isometric force transducers linked to PowerLab hardware (AD Instruments Pty Ltd). Powerlab was linked to a PC (Dell Inc.) where Chart for windows 5 (AD Instruments Pty Ltd, Chalgrove, Oxfordshire, UK) was used to display and measure the tension changes in the tissue. A 10-min period after drug administration was measured (area under the curve) and expressed as a percentage of the hypotonic shock reference contraction. Data are expressed as the means $\pm$ S.E.M.

Compounds used

Indometacin was obtained from Sigma-Aldrich Chemical Co., $\text{PGF}_{2\alpha}$, U46619 (11 α ,9 α -epoxymethano PGH_2) and SQ29548 ([1S-[1 α , 2 α (Z),3 α ,4 α]]-7-[3-[[2-[(phenylamino)-carbonyl]hydrazine]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) were acquired from Cayman Chemical (distributed by Alexis Corporation (UK) Ltd, Bingham, Notts, UK). Indometacin, $\text{PGF}_{2\alpha}$ and SQ29548 were dissolved in ethanol and U46619 in methyl acetate.

Figure 1 Typical traces (shown for 10 min) to demonstrate the spontaneous activity of isolated mouse uterine tissue taken from different anatomical locations during (a) dioestrus, (b) late gestation and (c) labour.

Dilutions were made with 0.9% (w/v) normal saline. The vehicles (matched for solvent) were found to have had no effect on myogenicity.

Statistical analysis

Data are expressed as the means $\pm$ S.E.M. Statistical analysis of differences between two means was assessed by Student's *t*-test. Multiple means were compared by a two-way ANOVA with a Bonferroni *post hoc* test (GraphPad Prism 4.0, San Diego, CA, USA). A probability level of $P < 0.05$ was regarded as significant.

Results

Effect of region of excision and reproductive state on myogenic activity

All mouse uterine samples displayed spontaneous myogenic activity (Fig. 1). Uterine tissues taken at late gestation or from a non-pregnant animal showed variable activity that started to decline after 60 min. Large, sustainable, frequent contractions were observed in uterine tissue taken during labour. Uterine tissue taken from non-pregnant mice displayed regional variation in myogenic activity at all stages of the oestrous cycle (Fig. 1a and Table 1). The upper segment of the uterine horn displayed significantly greater activity compared with the lower segment at pro-oestrus ($P < 0.001$), metoestrus ($P < 0.05$) and dioestrus ($P < 0.001$), but not during oestrus. Myogenic activity was found to vary along the length of the uterine horn in late gestation (Fig. 1b), with the upper segment being significantly more active compared with

the lower ($P < 0.01$) and mid-lower ($P < 0.05$) segments (Table 1). The activity of tissue excised during labour was constant along the length of the horn (Fig. 1c).

Variations in activity were also observed between the different endocrine stages; tissue taken during labour displayed significantly greater myogenic activity compared with equivalent tissue taken at late gestation ($P < 0.001$, lower and mid-lower; $P < 0.01$, mid-upper and upper). Tissue taken during labour was also more active than tissue taken from non-pregnant mice ($P < 0.001$) (Table 1). Lower segment tissue taken during pro-oestrus showed significantly greater myogenic activity than uterine tissue taken during metoestrus ($P < 0.01$), dioestrus ($P < 0.001$) and late gestation ($P < 0.05$) (Table 1). Lower segment tissue taken during oestrus displayed significantly greater myogenic activity than tissue taken during dioestrus ($P < 0.05$) (Table 1).

Effect of U46619 on myogenic activity

The TP-mimetic U46619 induced dose-related excitatory responses on all uterine tissues (Figs 2 and 3a). These responses were attenuated in the presence of SQ29548 (Fig. 3b). SQ29548 caused a significant rightward shift of all dose-response curves; $P < 0.05$ to $P < 0.001$ (data not shown). As can be seen from Fig. 2(a–d), U46619 evoked a greater response in tissue from the upper segment, but this difference only became significant ($P < 0.05$) at the highest dose during dioestrus (Fig. 2d). At late gestation, regional variations in the response to U46619 were also observed (Fig. 2e). The maximum response achieved with U46619 increased progressively as segments became closer to the ovarian end of the horn, with a significantly greater

Table 1 Spontaneous myogenic activity measured for 10 min (after a 30-min equilibration period) expressed as a percentage of a reference contraction induced by the infusion of distilled water (hypotonic shock). Data are expressed as arithmetic means ± S.E.M., n=10–12

	Lower segment	Mid-lower segment	Mid-upper segment	Upper segment
Pro-oestrus	12.6 ± 2.3 ^a	N/D	N/D	43.4 ± 5.2***
Oestrus	14.4 ± 4.9 ^b	N/D	N/D	28.9 ± 6.4
Metoestrus	4.2 ± 0.9	N/D	N/D	26.2 ± 7.2*
Dioestrus	3.9 ± 0.6	N/D	N/D	32.6 ± 5.5***
Late gestation	4.7 ± 0.6	15.6 ± 3.5	35.5 ± 13.6	50.9 ± 10.2**
Labour	78.1 ± 15.4 ^{c,d}	108.5 ± 15.1 ^d	110.5 ± 18.6 ^e	108.3 ± 22.9 ^{c,e}

N/D, not determined. In non-pregnant samples the upper and lower segment values are compared using a Student's paired *t*-test. In gestational samples the lower, mid-lower, mid-upper and upper segments were all compared with each other using a two-way ANOVA with a Bonferroni *post hoc* test. Comparisons between reproductive states (equivalent anatomical regions): ^aPro-oestrus compared with metoestrus and late gestation, *P*<0.01 and dioestrus, *P*<0.001; ^boestrus compared with dioestrus, *P*<0.05; ^clabour compared with all non-pregnant groups, *P*<0.001; ^{d,e}labour compared with late gestation, ^d*P*<0.001, ^e*P*<0.01. Comparisons between anatomical regions (same endocrine stage): *upper compared with lower segment, *P*<0.05; **upper compared with lower, *P*<0.01 and mid-lower *P*<0.05; ***upper compared with lower, *P*<0.001.

response being seen in the upper segment compared with the lower (*P*<0.001) and mid-lower (*P*<0.05) segment. A significantly greater response was seen in the mid-upper segment compared with the lower segment (*P*<0.01) (Fig. 2e). This pattern was not observed in tissue taken during labour (Fig. 2f) and the maximum responses attained during labour were attenuated compared with those in late gestation (Table 2); however, these trends were not statistically significant.

Variations in responses were also observed between the different reproductive states; in the upper segment tissue taken at late gestation U46619 evoked a greater response than at oestrus and metoestrus (*P*<0.01) (Table 2).

Effect of PGF_{2α} on myogenic activity

PGF_{2α} induced dose-related excitatory responses on all uterine tissues (Figs 4 and 5a). Full dose-response curves were completed in the presence of SQ29548 but no rightward shift was observed (data not shown); this lack of effect of the TP antagonist is shown in Fig. 5b. Regional variation in responsiveness to PGF_{2α} was observed in uterine tissue taken at oestrus (*P*<0.05) (Fig. 4b) and dioestrus (*P*<0.05) (Fig. 4d), with the upper uterine segments evoking a greater response than the lower segment. No regional variation in response was observed in gestational tissue (Fig. 4). In the lower segment, the response to PGF_{2α} in tissue taken during labour was significantly greater than in tissue taken during oestrus, metoestrus, dioestrus (*P*<0.001) and pro-oestrus (*P*<0.01) (Table 2). PGF_{2α} also evoked a greater response in tissue taken at late gestation compared with tissue taken during oestrus (lower segment, *P*<0.001; upper segment, *P*<0.01), metoestrus (lower segment, *P*<0.01; upper segment, *P*<0.05), dioestrus (lower segment, *P*<0.01) and pro-oestrus (lower segment, *P*<0.01). When responses to PGF_{2α} and U46619 were compared it was found that PGF_{2α} evoked a significantly greater response than

U46619 (*P*<0.05); this was observed in lower segment uterine tissue taken at late gestation (Table 2).

Discussion

The results of this study have demonstrated that mouse uterus is at its most active during labour, which is consistent with functionality during parturition. The uterus produces powerful and co-ordinated contractile activity in order to expel the foetuses at birth. This contractile activity is maintained equally along the length of the horn, which may facilitate the movement of the foetuses towards the cervix. The increased uterine activity observed during labour could be attributed to the high oestrogen concentrations observed towards term and the declining progesterone levels. Oestradiol-17β concentrations begin to rise on day 16 of gestation and continue to do so until day 20 (term), whilst peripheral progesterone concentrations begin to decline on day 17 of gestation reaching the lowest at term, day 20 (McCormack & Greenwald 1974). The change in the oestrogen to progesterone ratio from day 18 to parturition could account for the differences in activity seen at late gestation and during labour. During labour, regional differences in myogenic activity are no longer observed; this could due to the high oestrogen levels associated with parturition. The whole of the uterine horn appears to be working in synchrony to aid the expulsion of the litter.

Oestrogen is thought to modulate several factors which contribute to the contractile state of the uterus during parturition, including oxytocin production, oxytocin receptor expression (Fang *et al.* 1996), prostaglandin receptor expression (Matsumoto *et al.* 1997) and connexin-43, the gap junction protein (Petrocelli & Lye 1993). During labour, gap junctions are abundant in the mouse myometrium (Dahl & Berger 1978). Myometrium taken from non-pregnant and pregnant mice was found to

Figure 2 Dose-response curves to bolus doses of U46619 expressed as a percentage of a reference contraction induced by infusion of distilled water (hypotonic shock) in non-pregnant animals at (a) pro-oestrus, (b) oestrus, (c) metoestrus and (d) dioestrus (lower segment, open symbols and upper segment, solid symbols) and in gestational tissue at (e) gestation day 18 and (f) during parturition. Data are expressed as arithmetic means $\pm$ S.E.M., $n=5-6$ and were statistically analysed using a two-way ANOVA with Bonferroni *post hoc* test. (d) Upper segment compared with lower (* $P<0.05$). (e) Upper segment compared with lower (** $P<0.001$) and mid-lower (* $P<0.05$) and mid-upper compared with lower (** $P<0.01$).

have very few gap junctions (Dahl & Berger 1978) which may explain the inability of these tissue samples to maintain their spontaneous activity.

During pregnancy, the uterus is associated with quiescence maintained by several inhibitory factors. Although at day 18 of gestation oestrogen levels have begun to rise, they have not yet peaked and quiescence appears to be maintained. Differences in activity with respect to reproductive state were also observed by Mackler *et al.* (1999); however, samples were not taken during parturition but after 6 h. Mackler *et al.* (1999) found that the postpartum uterus was most active which indicates that the uterus can maintain its contractile state for a least a day after

parturition. They also found that tissue taken at oestrus was more active than tissue taken at late pregnancy. This is not consistent with the results reported here, but could be accounted for by the regional variations in activity observed in both non-pregnant and late gestational samples.

Hormone levels are also continuously changing during the oestrous cycle. Pro-oestrus is characterized by an increase in oestrogen levels that peak just before oestrus (Walmer *et al.* 1992). Progesterone concentrations are highest during metoestrus and dioestrus (Walmer *et al.* 1992). The change in the oestrogen and progesterone ratio would account for the increased myogenic activity observed at pro-oestrus and oestrus compared

Figure 3 Sample traces showing activity of isolated uterine samples taken from non-pregnant animals at dioestrus to bolus doses of U46619 (a) alone and (b) in the presence of 0.1 μ M SQ29548.

with metoestrus and dioestrus in the lower uterine segment.

Ovarian steroid production may account for the regional variation in myogenic activity observed in the uterine horn in non-pregnant mice and at late gestation. The activity was greatest in the upper segment of the uterine horn, which is nearest the site of production. This was particularly marked during dioestrus and metoestrus when myogenicity in the upper segment is six and eight times respectively greater than that in the lower segment. No regional variation in myogenicity was observed during oestrus; the high levels of oestrogen observed during

pro-oestrus might have lasting contractile effects on the uterus, which may facilitate the transport of sperm along the uterine horn during oestrus. The site of sex steroid production may also account for regional differences observed during late gestation, with the uterine sample taken from the closest proximity to the ovaries being most active.

The contractile effect of U46619 was attenuated significantly in the presence of the selective TP antagonist SQ29548, confirming that the responses were mediated via the TP receptor. Our data have shown that there are no differences in the response to U46619 between the different stages of the oestrous cycle. This was comparable with data obtained from non-pregnant human myometrium that suggested that the response to U46619 is not influenced by menstrual status (Senchyna & Crankshaw 1999).

Regional variations in response to U46619 observed during dioestrus and at late gestation may be due to a greater number of TP receptors in the ovarian region of the uterus at this stage of the oestrous cycle; however, to our knowledge, the quantitative distribution of TP receptors along the length of the mouse uterine horn has not been measured. The response to U46619 in non-pregnant human myometrium is not believed to be dependent on the excision site (Senchyna & Crankshaw 1999); unfortunately this information is not available for myometrium from pregnant and labouring women at present.

In general, the response to U46619 was less influenced by hormonal milieu than the response to $\text{PGF}_{2\alpha}$. The role of thromboxane and the TP receptor in pregnancy and parturition is currently under further investigation in this laboratory.

Responses to $\text{PGF}_{2\alpha}$ were not altered in the presence of SQ29548, indicating that the excitation observed with $\text{PGF}_{2\alpha}$ is not due to any off-target TP activity. $\text{PGF}_{2\alpha}$ is believed to play a crucial role in the induction of parturition in the mouse (Tsuboi *et al.* 2002). FP knockout mice fail to undergo parturition (Sugimoto *et al.* 1999);

Table 2 Comparison of the uterine response to bolus doses of 100 nmol U46619 and $\text{PGF}_{2\alpha}$ expressed as a percentage of the hypotonic shock in different reproductive states. Data are expressed as arithmetic means $\pm$ S.E.M., $n=5-6$

	Maximum response achieved with 100 nmol U46619		Maximum response achieved with 100 nmol $\text{PGF}_{2\alpha}$	
	Lower segment	Upper segment	Lower segment	Upper segment
Pro-oestrus	140.5 $\pm$ 13.4	178.0 $\pm$ 14.3	145.9 $\pm$ 12.4	179.8 $\pm$ 18.5
Oestrus	102.5 $\pm$ 24.2	128.9 $\pm$ 24.3	89.3 $\pm$ 6.7	129.3 $\pm$ 8.0
Metoestrus	131.7 $\pm$ 33.0	145.6 $\pm$ 17.8	112.5 $\pm$ 33.7	152.0 $\pm$ 20.6
Dioestrus	149.4 $\pm$ 8.6	209.7 $\pm$ 25.1 ^b	132.0 $\pm$ 16.6	187.8 $\pm$ 25.7
Late gestation	143.6 $\pm$ 49.1 ^a	280.6 $\pm$ 36.6 ^c	262.2 $\pm$ 58.5 ^d	272.9 $\pm$ 36.4 ^f
Labour	192.3 $\pm$ 23.5	185.7 $\pm$ 37.3	277.9 $\pm$ 25.8 ^e	196.0 $\pm$ 46.7

Data were statistically analysed using a two-way ANOVA with Bonferroni *post hoc* test. For the statistical analysis of anatomical differences see Figs 2 and 4. Differences between reproductive states (equivalent anatomical region): ^aresponse to U46619 in lower segment at late gestation compared with the responses to $\text{PGF}_{2\alpha}$ in both lower and upper segments ($P<0.01$); ^bresponse to U46619 in upper segment during dioestrus compared with oestrus ($P<0.05$); ^cresponse to U46619 in upper segment at late gestation compared with oestrus and metoestrus ($P<0.01$); ^dresponse to $\text{PGF}_{2\alpha}$ in lower segment at late gestation compared with oestrus ($P<0.001$); metoestrus, dioestrus and pro-oestrus ($P<0.01$); ^eresponse to $\text{PGF}_{2\alpha}$ in lower segment during labour compared with oestrus, metoestrus, dioestrus ($P<0.001$) and pro-oestrus ($P<0.01$); ^fresponse to $\text{PGF}_{2\alpha}$ in upper segment at late gestation compared with oestrus ($P<0.01$) and metoestrus ($P<0.05$).

Figure 4 The dose-response of bolus doses of PGF_{2α} expressed as a percentage of a reference contraction induced by infusion of distilled water (hypotonic shock) in non-pregnant animals at (a) pro-oestrus, (b) oestrus, (c) metoestrus and (d) dioestrus (lower segment, open symbols and upper segment, solid symbols) and in gestational tissue at (e) gestation day 18 and (f) during parturition. Data are expressed as arithmetic mean $\pm$ S.E.M., $n=5-6$ and were statistically analysed using a two-way ANOVA with Bonferroni *post hoc* test. Upper compared with lower (* $P<0.05$).

this is due, however, to the role of PGF_{2α} in luteolysis (Horton & Poyser 1976) rather than simply its contractile effects on the uterus. The luteolytic function of PGF_{2α} appears to be less crucial during the oestrous cycle (Sugimoto *et al.* 1999). The importance of uterine FP receptor activation in the initiation and progression of parturition is not yet fully understood, but it is known that uterine FP receptor mRNA and uterine tissue concentrations of PGF_{2α} increase in the mouse at late gestation (Cook *et al.* 2003). The similar responses to PGF_{2α} observed at late gestation and during labour are consistent with upregulation of the FP receptor by

day 18 of gestation and expression remaining high during parturition. This is reaffirmed by the significant increase in responsiveness seen in tissue taken during late gestation and labour compared with tissue taken from non-pregnant animals.

The results have demonstrated that the uterine contractile response to PGF_{2α} varied anatomically in uterine tissue taken during oestrous and dioestrous stages, but no differences were observed in gestational tissue. These findings are similar to those observed in the non-pregnant rat where the uterus demonstrates regional variation in responsiveness to PGF_{2α} (Oropeza *et al.* 2002). These

Figure 5 Sample traces showing activity of isolated uterine samples taken from non-pregnant animals at dioestrus during bolus doses of PGF_{2α} (a) alone and (b) in the presence of 0.1 μM SQ29548.

studies suggest that the FP receptor population is greater in the upper region of the uterus; topographical distribution of prostanoid receptors are currently being investigated on human myometrium.

It is possible that differences in responsiveness to both agonists may not be solely attributable to changes in receptor density but could be influenced by other factors, for example, changes in gap junction numbers. The expression of connexin 43 is regulated by prostaglandins and steroids (Garfield *et al.* 1988, Kilarski *et al.* 2000). Increased responsiveness could also be affected by changes in signal transduction pathways.

These data have demonstrated that the hormonal milieu and the anatomical region influence uterine activity and responsiveness. Such factors must be considered when making inter-species comparisons and extrapolating results to predict *in vivo* responses.

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