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Operational correlates of prostanoid TP receptor expression in human non-pregnant myometrium are unaffected by excision site or menstrual cycle status of the donor

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1 Cumulative concentration-effect curves for the selective prostanoid TP receptor agonist, U46619, were constructed in strips of human non-pregnant myometrium grouped according to tissue excision site (top, lateral wall, lower uterine segment, sub-serosal or sub-endometrial), tissue orientation (strips cut either parallel or perpendicular to the serosa) and donor menstrual status (proliferative or secretory phase).

2 U46619 was excitatory in all tissues. There was no significant difference in either pEC_{50} or maximum response between groups ($P<0.05$). The range of pEC_{50} values was 6.8 ± 0.1 (lateral wall, proliferative phase, $n=5$) to 7.1 ± 0.3 (lateral wall, secretory phase, $n=5$). The range of maximum response values was 0.9 ± 0.8 N cm^{-2} (lateral wall, proliferative phase, $n=5$) to 3.1 ± 1.0 N cm^{-2} (lateral wall, secretory phase, $n=5$).

3 Saturation binding analyses were conducted using the radiolabelled TP receptor agonist, [^{125}I]-BOP. Binding parameters were estimated for membranes prepared from human non-pregnant myometrium excised from the lateral wall and grouped according to donor menstrual status.

4 There were no significant differences in the mean pK_d and $[R]_{tot}$ values for [^{125}I]-BOP binding between the two groups (proliferative phase: $pK_d=8.3\pm0.3$, $[R]_{tot}=412\pm319$ fmol mg protein^{-1}, $n=5$; secretory phase: $pK_d=8.5\pm0.4$, $[R]_{tot}=369\pm192$ fmol mg protein^{-1}, $n=6$; $P<0.05$).

5 These data indicate that U46619-mediated responses in human non-pregnant myometrium are not influenced by tissue excision site, tissue orientation or donor menstrual status and that [^{125}I]-BOP binding is not influenced by donor menstrual status. This suggests that the TP receptor population is homogeneous throughout the human non-pregnant myometrium, and not subject to hormonal regulation.

Keywords: Myometrium (human); TP receptor; [^{125}I]-BOP; U46619; response variability; menstrual cycle

Abbreviations: HMM, human myometrial membranes; MOPS, 3-[N-morpholino]propane-sulphonate; PG, prostaglandin; PSS, physiological salt solution.

Introduction

Many tissues show marked variability in their responses to the same agonist, a phenomenon that can often be explained by gradients in responsiveness which coincide with anatomical location (Kenakin, 1984). The human uterus is a classical example of such a tissue. For example, Wikland *et al.* (1984) demonstrated differences in myometrial responses to prostaglandin (PG) E_2 and $PGF_{2\alpha}$ between strips obtained from upper and lower uterine segments of pregnant donors. Whilst Melin *et al.* (1988) showed differences in the graded responses to arginine vasopressin between serosal and endometrial sections of human non-pregnant myometrium.

Additional complexity arises from evidence that variability in the response of the human myometrium can occur independently of site. Thus, Popat & Crankshaw (1997) showed that strips of human non-pregnant myometrium excised from the same site exhibited donor-dependent variability in responses to PGE_2. One possible explanation of such inter-donor variability is that some component of the signal-transduction pathway is hormonally regulated (Gannon, 1985; Europe-Finner *et al.*, 1994).

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Prostanoid TP receptors mediate contraction of human non-pregnant myometrium and methods to assess their operational expression have been well characterized (Senchyna & Crankshaw, 1996). In the present study we have used these methods to investigate two questions: Does tissue excision site or menstrual status of the donor affect the contractile response of the human non-pregnant myometrium to TP receptor stimulation? Does the menstrual status of the donor affect the binding parameters of human myometrial TP receptors? Some of these data have been communicated to the British Pharmacological Society (Senchyna & Crankshaw, 1997).

Methods

Tissue collection and preparation

Human myometrial samples were obtained from premenopausal, non-pregnant women (aged 22–51), undergoing hysterectomy for benign disorders such as fibroids, menorrhagia and uterine prolapse as previously described (Fernandes & Crankshaw, 1995; Senchyna & Crankshaw, 1996). At the time of surgery none of the women had used oral contraceptives or received any hormone therapy. Only specimens from women with regular 28-day cycles were used. Tissues were grouped as proliferative phase or secretory phase, based on patient

recollection of their last menstrual period, where proliferative phase represents the time between the onset of menses to the 14th day of the cycle, and secretory phase represents the second half of the menstrual cycle, starting 15 days after the onset of menses. Specimens were taken from the anterior aspect and excised from one of three locations: the top, the lateral wall or the lower uterine segment of the corpus uteri. All specimens were trimmed of endometrial, serosal, fat and fibrous tissue prior to use. Myometrial samples used for this study were collected from a total of 48 donors. The collection protocol was approved by the Research Advisory Group, McMaster University.

Myometrial tissue intended for concentration-effect experiments, was maintained for up to 24 h post-operatively in oxygenated (95% O_2, 5% CO_2) physiological salt solution (PSS) at room temperature. Myometrial tissue strips were prepared as previously described (Fernandes & Crankshaw, 1995; Senchyna & Crankshaw, 1996). Three different types of muscle strips were obtained. In the majority of cases strips were obtained from the muscle layer directly adjacent to the serosa and were cut in a direction parallel to that of the serosa. In a second set of experiments strips were obtained from the muscle layer directly adjacent to the endometrium and were cut in a direction parallel to that of the serosa. In the third set of experiments strips were cut in a direction perpendicular to that of the serosa. In a given experiment, up to 16 strips of myometrium ($15 \times 2 \times 3$ mm) were tied at each end with silk thread and mounted longitudinally in individual 10 or 15 ml jacketed muscle baths containing oxygenated PSS at 37°C.

Recording isometric contractions

One end of each myometrial strip was anchored in the bath, the other was attached to an FT-03 force displacement transducer writing to either a 7D polygraph (Grass Instruments, Quincy, MA, U.S.A.), or a custom-made amplifier writing to the data collection software, In Vitro Collection System Ver 4.0 (J. Milton, Dundas, ON, Canada), running on a personal computer. An optimum resting force of 25 mN was applied to each strip (Crankshaw & Dyal, 1994). The mean force developed by the individual muscle strips was used as a measure of their contractility (Wainman *et al*., 1988; Cheuk *et al*., 1993; Senchyna & Crankshaw, 1996). Mean force was determined during 10 min epochs as described by Wainman *et al*. (1988), using the In Vitro Collection System software.

Effect of U46619 on spontaneous contractile activity

Tissues were challenged with KCl (90 mM) to ensure viability (Senchyna & Crankshaw, 1996). Thorough washout of KCl was followed by a 3 h equilibration period, during which time spontaneous contractile activity usually developed. At the end of the equilibration period the mean force developed during a 10 min control period was determined. Cumulative concentration-effect experiments were performed with the selective TP receptor agonist U46619 (Coleman *et al*., 1994), using approximately one-half log concentration increases. Each successive agonist concentration was in contact with the tissue for 45 s before a 10 min data collection was taken. A total of 8–10 additions were made to ensure the maximum response had been achieved.

Quantification of response to U46619

The mean force in the 10 min period following each agonist addition, minus the mean force developed during the control period was considered to be the force generated in response to that concentration of agonist. U46619 concentration-effect curves (mean force versus log molar agonist concentration) were then constructed from these data by fitting the equation:

$$E = E_{min} + (E_{max} - E_{min})/1 + e^{-1}{}_{H}{}^{*(\log C + pEC_{50})} \quad (1)$$

where E is the effect of the agonist, E_{min} is the effect in the absence of agonist, E_{max} is the maximum effect, C is the molar concentration of the agonist, n_H is the slope parameter and pEC_{50} is the negative log of the molar concentration of the agonist that produces a half-maximal response.

Preparation of myometrial membranes

Enriched human myometrial membranes (HMM) were prepared from proliferative and secretory phase myometrial tissues excised from the uterine lateral wall within 2 h of the operation as previously described (Senchyna & Crankshaw, 1996). Myometrial tissue was lightly blotted, weighed, minced with a razor blade and suspended in six volumes of sucrose-3-[N-morpholino]propane-sulphonate (MOPS) buffer (pH 6.5) and homogenized with a Polytron PT20 (Brinkmann; Westbury, NY, U.S.A.). The Polytron probe was washed with six volumes of sucrose-MOPS buffer. Homogenates and washings were combined and centrifuged at 3000 r.p.m. ($1400 \times g$) for 15 min in a Sorvall RC5 centrifuge with a SS-34 rotor (Fisher Scientific; Nepean, ON, Canada). Supernatants were filtered through three layers of cheese cloth and the collected filtrate was centrifuged at 35,000 r.p.m. ($87{,}000 \times g$) for 30 min in a Beckman L7 ultracentrifuge using a Beckman 60Ti rotor (Beckman; Rexdale, ON, Canada). The final pellet was resuspended in sucrose-MOPS buffer and was stored at −20°C for no more than 2 months. Final protein concentrations were measured according to the method of Bradford (1976), using bovine serum albumin as standard.

Saturation radioligand binding studies

Saturation experiments using HMM were performed in triplicate with the iodinated TP receptor agonist [^{125}I]-BOP (Morinelli *et al*., 1989), as previously described (Senchyna & Crankshaw, 1996). Reactions contained 100 μg of protein; ~15,000 c.p.m. (30 pM) of [^{125}I]-BOP; 4.6 mM $MgCl_2$; 0.5 mM MOPS; 6 μM indomethacin; 2.3 μM phenylmethylsulfonylfluoride and increasing concentrations of [^{127}I]-BOP in a total volume of 250 μl. The final pH was 6.5. Reactions were incubated at 30°C for 60 min. Reactions were terminated by the addition of 4 ml of ice cold sucrose-MOPS buffer, pH 7.4, followed by filtration under vacuum through Whatman GF/C glass fibre filters (Whatman INC; Clifton, New Jersey, U.S.A.). The filters were washed with three additional 4 ml volumes of ice cold buffer and then counted for bound radioactivity in a LKB 1261 Gamma Counter (Pharmacia; Baie D'Urfe, PQ, Canada). The filtration procedure was complete within 10 s. Non-specific binding was defined as the amount of radioactivity bound in the presence of the TP receptor antagonist L670596 (Ford-Hutchinson *et al*., 1989) at 10 μM. Specific binding was determined by subtracting non-specific binding from total binding. Binding data were fitted to the following equation:

$$[RL] = ([R]_{tot}{}^{*}[L])/K_d + [L] \quad (2)$$

where [RL] is the molar concentration of specifically bound ligand, $[R]_{tot}$, represents the total concentration of binding sites, K_d is the concentration of ligand that produces half-

maximal saturation, and [L] is the molar concentration of free ligand.

Compounds

U46619 ([1R-[1a,4a,5b(Z),6a(1E,3S*)]]-7-[6-(3-hydroxy-1-octenyl)-2-oxabicyclo[2.2.1]hept-5-yl]-5-heptenoic acid), [^{125}I]-BOP and IBOP ([1S[1α,2α(Z),3β(1E,3S*),4α)]]-7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo-[2.2.1]hept-2-yl]5-heptanoic acid) were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.). L670596 ((1)6,8-di-fluoro-9-*p*-methylsulphonyl benzyl-1,2,3,4-tetra-hydrocarbazol-1-yl-acetic acid) was from Merck Frosst (Point Claire-Dorval, PQ, Canada). Indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid) was purchased from Sigma (St. Louis MO, U.S.A.). All other chemicals were of the finest quality available and purchased from either Sigma or BDH (Toronto, ON, Canada).

Solutions

The PSS was composed as follows (mmol): KCl 4.6, $MgSO_4$ 1.16, NaH_2PO_4 1.16, $CaCl_2$ 2.5, NaCl 115.5, $NaHCO_3$ 21.9 and *d*-glucose 11.1 with indomethacin at 10 μM. The sucrose MOPS buffer contained (mmol): sucrose 250, MOPS 20, EDTA 25, the pH was adjusted with NaOH.

Statistics

All data are expressed as means$\pm$s.d.mean. Statistical comparisons of U46619 potency and tissue maximum response were performed using one-way analysis of variance followed by the Newman-Keuls multiple comparisons *post-hoc* test. Statistical comparisons of [^{125}I]-BOP saturation binding data were performed using one-way analysis of variance. In all cases differences were considered significant if $P<0.05$.

Results

U46619-induced myometrial response

Figure 1 is a representative tracing of the effect of cumulative addition of U46619 on a strip of human myometrium obtained from the top of the corpus uteri from a non-pregnant donor. Similar graded-response data were obtained for all strips tested in each of the experimental groups under study. The concentration-effect curves resulting from such experiments were sometimes bell-shaped in appearance. For purposes of analysis, data points falling below and to the right of the concentration-effect asymptote were omitted (Senchyna & Crankshaw, 1996). No significant differences were found in either pEC_{50} or maximum response values between any of the groups under investigation (Table 1).

Saturation binding of [^{125}I]-BOP

Binding of [^{125}I]-BOP to HMM was saturable over a concentration range of 300–4000 pM (Figure 2. Mean pK_b and $[R]_{tot}$ values did not differ between the proliferative phase ($pK_d=8.3\pm0.3$, $[R]_{tot}=412\pm319$ fmol mg protein^{-1}, $n=5$) and the secretory phase ($pK_d=8.5\pm0.4$, $[R]_{tot}=369\pm192$ fmol mg protein^{-1}, $n=6$).

Discussion

We investigated whether tissue excision site or menstrual status of the donor affected the cumulative concentration-effect curves to U46619 in strips of human myometrium derived from different sites and from different non-pregnant donors. U46619 is generally recognized as a selective TP receptor agonist (Coleman *et al.*, 1994) and we have confirmed that it behaves in this manner in the human non-pregnant myome-

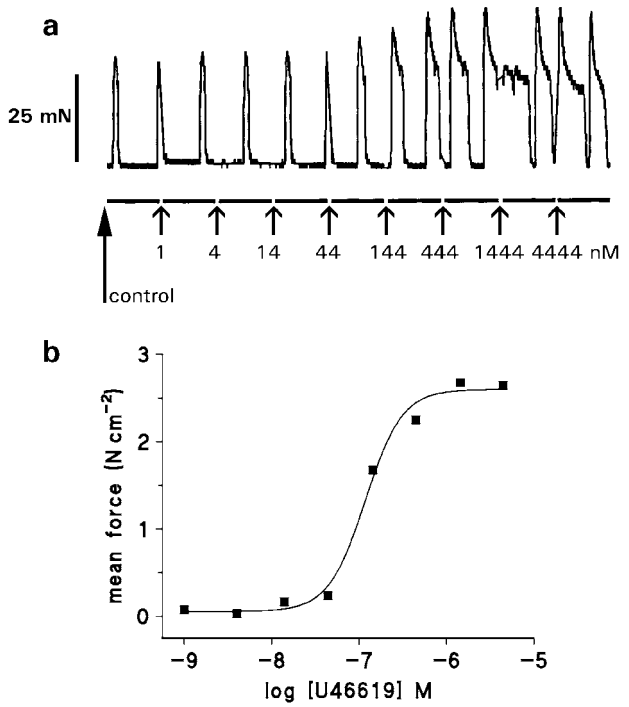

Figure 1 (a) Sample trace showing the effect of cumulative addition of U46619 on the contractile activity of a strip of myometrium excised from the top of the corpus uteri from a non-pregnant donor in the proliferative phase of the menstrual cycle. Horizontal bars indicate 10 min collection periods, the first of which is the control. Thereafter, U46619 was added to the baths at the points indicated by the arrows to give the cumulative concentrations shown. (b) Concentration-effect curve, constructed from the data in (a).

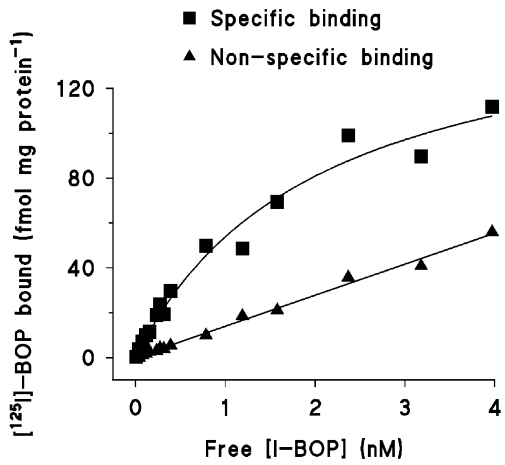

Figure 2 Effect of the free concentration of [^{127}I]-BOP on the amount of [^{125}I]-BOP bound to HMM prepared from human myometrium excised from the lateral wall of the corpus uteri from a non-pregnant donor in the secretory phase of the menstrual cycle. Binding parameters calculated for this experiment were: $pK_d=8.7$, $[R]_{tot}=163$ fmol mg protein^{-1}. Representative of 11 experiments.

Table 1 Effect of excision site, orientation and menstrual cycle status of the donor on U46619-induced contractile activity of human non-pregnant myometrium

	Proliferative phase		*Secretory phase*	
Excission site	pEC_{50}	*max* (N cm^{-2})	pEC_{50}	*max* (N cm^{-2})
Top	6.9±0.2	2.6±1.0	6.9±0.3	1.8±1.9
Lower uterine segment	6.8±0.3	2.4±2.4	7.0±0.2	2.2±0.9
Lateral wall	6.9±0.3	1.4±0.9	7.1±0.3	3.1±1.0
Lateral wall (sub-endometrial)	6.8±0.1	0.9±0.8	7.0±0.4	1.1±0.4
Lateral wall (perpendicular to serosa)	6.8±0.2	1.3±0.7	6.7±0.2	1.1±0.6

All strips were taken from sub-serosal layer and cut parallel to the serosa except where indicated. Values are means±s.d.mean, $n=5$ in all cases).

trium (Senchyna & Crankshaw, 1996). Thus the pEC_{50} and maximum response data obtained in the present study can be taken as operational correlates of TP receptor expression.

The lack of effect of any of the variables that we studied on the functional response of the tissue to U46619 strongly suggests that operational expression of TP receptors is homogeneous throughout the human non-pregnant myometrium, and unaffected by the phase of the menstrual cycle. The mean maximum responses induced by U46619 were highly variable within all groups studied (Table 1). Such variability was not attributable simply to inter-donor differences, as substantially different maximum responses were recorded in parallel strips prepared from the same donor. It is conceivable that the 'noise' in our estimates of maximum responses hides some real differences. If functional TP receptor expression increases in a system where there is no receptor reserve – a likely situation in human non-pregnant myometrium considering the relatively low pEC_{50} for U46619 – the consequence will be an increased maximum response. It was therefore necessary to estimate TP receptor binding parameters.

The radioligand [^{125}I]-BOP has been shown to selectively label TP receptors in a number of tissues (Morinelli *et al*., 1989, 1990) including human myometrium (Senchyna & Crankshaw, 1996). Therefore our present results with [^{125}I]-BOP binding suggest that TP receptor affinity and density are not altered, at least by the hormonal status of the donor. Unfortunately we were unable to collect sufficient quantities of myometrial tissue from different excision sites, so our investigation of this variable *via* radioligand binding was precluded. Nevertheless, these data are consistent with our conclusions from the U46619 experiments.

The similarity in responses of a wide variety of myometrial samples to U46619 suggests a number of conclusions. Firstly, although the response to arginine vasopressin varies between endometrial and serosal sections of human non-pregnant myometrium (Melin *et al*., 1988), and the response to PGE_2 varies between top and bottom of the uterus (Wikland *et al*., 1984) as well as between different donors (Popat & Crankshaw, 1997), none of these types of variability were seen with U46619 in the present study. This implies that endometrial to serosal, top to bottom and inter-donor differences are not universal phenomena of agonist-induced responses of human non-pregnant myometrium. Furthermore, they are unlikely to result from pathways that are shared by different agonists, and consequently are more likely to be receptor-specific. Secondly, the lack of effect of menstrual status suggests that operational expression of the TP receptor is not influenced by differences in steroid hormones or related transcription factors between the proliferative and secretory phases of the cycle. A complete lack of regulation of the TP receptor pathway is unlikely since human myometrium from term-pregnant donors is a log unit more sensitive to U46619 (Dyal & Crankshaw, 1988). Thirdly, the lack of effect of orientation of the muscle strips (parallel to serosa *versus* perpendicular to serosa) indicates that TP receptor stimulation is equi-effective in provoking longitudinal and transverse contractions of human non-pregnant myometrium.

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