The regulation by ovarian steroids of prostaglandin synthesis and prostaglandin-induced contractility in non-pregnant rat myometrium. Modulating effects of isoproterenol

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Abstract

The objectives of the present study were to investigate the effects of the reproductive steroids oestradiol and progesterone on myometrial levels of cyclooxygenase-2 (COX-2) mRNA and PGF_{2α} induced myometrial contractility and to study whether the effect of β₂-adrenoceptor stimulation by isoproterenol on the myometrium alters these parameters.

Oestrogen treatment of ovariectomized rats increased myometrial COX-2 mRNA whereas PGF_{2α} receptor (PGF_{2α}-R) mRNA was unchanged following this treatment and maximal contractility ($E_{\max}$) of isolated uterine strips challenged with PGF_{2α} was unaltered. Progesterone treatment alone decreased COX-2 mRNA in comparison with values obtained from oestrogen-treated animals, and in combination with oestrogen the enhancing effect of progesterone on COX-2 mRNA was curbed. EC₅₀ of uterine strips challenged with PGF_{2α} increased following oestrogen treatment whereas

this parameter was substantially decreased following progesterone treatment.

When oestrogen was combined with isoproterenol infusion mRNA values of both COX-2 and PGF_{2α}-R were reduced. Finally, when isoproterenol infusions were given in combination with both oestrogen and progesterone, PGF_{2α}-R mRNA and $E_{\max}$ were enhanced as compared with similar rats not having received isoproterenol.

We conclude that oestrogen increases COX-2 mRNA production and subsequent prostaglandin synthesis in non-pregnant rat myometrium. We further conclude that in the oestrogen-dominated rat myometrium the relaxing effect of β₂-adrenoceptor stimulation involves attenuation of both prostaglandin synthesis and PGF_{2α}-R expression. We finally conclude that in the presence of both oestrogen and progesterone this effect of β₂-adrenoceptor stimulation is restrained.

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Introduction

Prostaglandins (PGs) are believed to play pivotal roles in a number of reproductive processes such as ovulation (see Gelety & Chaudhuri 1992 for a review), luteolysis (see Poyser 1995 for a review) and parturition (Sugimoto *et al.* 1997, Uozumi *et al.* 1997). Uterine sites of prostaglandin actions include both endometrium and myometrium (Yang *et al.* 1997). The activities of cyclooxygenases represent a rate-limiting step in the formation of prostaglandins from arachidonic acid (Smith & DeWitt 1995) and the administration of inhibitors of those enzymes restrain uterine contractions and labour (MacDonald *et al.* 1974). Cyclooxygenases exist in two isozyme forms named COX-1 and COX-2. Although COX-1 is inducible in particular tissues it is constitutively expressed in most tissues (Smith & DeWitt 1996) and is considered a 'housekeeping' enzyme with small variation in synthesis

rate (DeWitt & Meade 1993). COX-2, on the other hand, is an inducible enzyme activated by a wide range of ligands (Smith *et al.* 1996).

Oxytocin releases PGF_{2α} from pregnant rat uterus *in vitro* (Chan 1977). Intraluteal PGF_{2α} in pregnant rats induces luteolysis (Stocco & Deis 1998) and thus plays a role in the initiation of parturition in this species. In addition, prostaglandins are involved more directly in the regulation of parturition, namely through modulation of uterine contractility (Novy & Liggins 1980). Thus PGF_{2α} acts as a uterine contractant in late human pregnancy and during spontaneous labour (Dray & Frydman 1976).

The hormonal control of uterine PGF_{2α} secretion has, in view of its luteolytic capability, been extensively investigated. Thus both oestradiol (Pakrasi *et al.* 1983, Nakayama *et al.* 1991) and progesterone (Franchi *et al.* 1982) administration to ovariectomized rats increased uterine PGF_{2α} output. However, when administered together in rats

progesterone was able to prevent the increase in $\text{PGF}_{2\alpha}$ secretion induced by oestradiol (Kuehl *et al.* 1975, Pakrasi *et al.* 1983). In these studies it was, however, difficult to distinguish between endometrial and myometrial prostanoid production. Roberts *et al.* (1976) suggested that the primary source of uterine $\text{PGF}_{2\alpha}$ was endometrial and that the enhancing effect of oestradiol was a result of an increased oxytocin receptor formation. On the other hand, Fuchs *et al.* (1999) demonstrated that cow myometrium expresses both COX-1 and COX-2 mRNA, indicating that this tissue is competent of prostanoid production. Similarly we recently found that COX-2 and $\text{PGF}_{2\alpha}$ receptor ($\text{PGF}_{2\alpha}\text{-R}$) mRNA increase in rat myometrium during pregnancy with maximal values obtained during labour (Engstrøm *et al.* 2000).

Stimulation of β_2 -adrenoceptors inhibits myometrial contractions (Anderson *et al.* 1975, Berg *et al.* 1982, Caritis *et al.* 1983) and consequently β_2 -adrenomimetics are used clinically in the treatment of pre-term labour. Continuous occupancy of β_2 -adrenoceptors leads to the loss of ovine myometrial responsiveness to subsequent agonist stimulation (Lye *et al.* 1992). However, as mentioned by Lye *et al.* (1998) β_2 -adrenoceptor down-regulation and uncoupling *per se* does not necessarily induce tachyphylaxis.

The present study had two aims: (1) to investigate the effects of the reproductive steroids oestrogen and progesterone on myometrial levels of COX-2 mRNA and $\text{PGF}_{2\alpha}$ induced myometrial contractility, and (2) to investigate whether the well-known attenuation of prolonged β_2 -adrenoceptor stimulation on the myometrium involves an altered response to $\text{PGF}_{2\alpha}$. We measured mRNA for COX-2 and $\text{PGF}_{2\alpha}\text{-R}$ in ovariectomized rats pre-treated with oestrogen, progesterone or a combination of both. In additional studies the effects of prolonged isoproterenol infusion in identical treated rats were examined.

Materials and Methods

Animals

Female Wistar rats (250–350 g) were maintained under controlled conditions in the Panum Institute Animal House. Food and water were freely available. All experiments conformed to the *Guidelines on the Handling and Training of Laboratory Animals* by UFAW.

Rats were anaesthetized with a mixture of Dormicum (1.25 mg, Roche, Basel, Switzerland) and Hypnorm (0.4 ml, Janssen, Geel, Belgium). Through lateral abdominal incisions the rats were bilaterally ovariectomized. Thereafter the incisions were sutured. Eight treatment groups were formed 7 days later. The groups received either no steroid injection (groups 1–2), a daily i.m. injection of 50 μg oestradiol-benzoate (LEO, Copenhagen, Denmark, groups 3–4) (Stürmer 1968), a daily i.m. injection of 100 μg progesterone (Sigma Chemical Company, St Louis, MO, USA, groups 5–6) or both steroids

(groups 7–8) for three consecutive days. The higher dosage of progesterone compared with oestradiol-benzoate was chosen due to the high metabolic degradation rate of progesterone (Ganjam *et al.* 1975). During the steroid treatment period the rats were additionally given continuous infusions of isoproterenol (10 $\mu\text{g}/\text{h}$, Sigma, groups 1, 3, 5, 7) or saline (groups 2, 4, 6, 8) by means of osmotic mini-pumps (Engstrøm *et al.* 1998). The dosage regime of isoproterenol was selected from our previous experiment where the dosage was found to affect oxytocin receptor binding, oxytocin receptor mRNA and oxytocin-induced myometrial contractility (Engstrøm *et al.* 1998). After the treatment period the rats were anaesthetized with CO_2 and decapitated. The abdomen was opened longitudinally and the uterine horns were removed. Some uterine horns were used for measurements of *in vitro* contractility. Others were freed from endometrium and parametrium and were subsequently used for isolation of mRNA. Both inner and outer layers of the myometrium were used for mRNA isolation. Histological examination secured that the specimens were free from endometrium.

Primers and construction of internal mRNA standard

The primers used for COX-2 mRNA detection were:

Sense primer: 5' ACG CCA CCC CAA ACA CAG TA 3' (nucleotide 327–346)

Antisense primer: 5' CCC AGG TCC TCG CTT CTG A 3' (nucleotide 703–721)

The primers used for $\text{PGF}_{2\alpha}\text{-R}$ detection were:

Sense primer: 5' ACG GCG TTT ATC TCC ACA AC 3' (nucleotide 113–122)

Antisense primer: 5' CCG ATG CAC CTC TCA ATG 3' (nucleotide 506–523)

Basic Local Alignment Search Tool (Blast) (Altschul *et al.* 1990) was used to search all non-redundant databases (GenBank+EMBL+DDBJ+PDB) for sequence homology. No homology with any known products other than the actual receptors was found. The amplified DNA fragments consisted of 413 bp (COX-2) and 428 bp ($\text{PGF}_{2\alpha}\text{-R}$). The exact identity of the PCR product was confirmed by sequencing (Engstrøm *et al.* 1998). Using a polymerase chain reaction MIMIC construction kit (Clontech, Palo Alto, CA, USA) an internal 238 bp ($\text{PGF}_{2\alpha}\text{-R}$) and 239 bp (COX-2) DNA standard were constructed (Engstrøm *et al.* 1997). The internal standard RNA was constructed mainly as described by Faure *et al.* (1995). A composite primer, comprising 37 nucleotides of bacteriophage T7 RNA polymerase promoter followed by the sequences of our usual sense primers, was used for amplification of the 238 bp and 239 bp DNA sequences by PCR. The resulting 275 bp and 276 bp products were re-amplified using our antisense primers and a primer consisting of the initial 23 nucleotides of the T7 RNA polymerase promoter region. Following HPLC purification the re-amplified products were used for production

of RNA by *in vitro* transcription (Riboprobe, Promega, Madison, WI, USA). The resulting RNA standards were quantitated by UV detection at 260 nm (Gene-quant, Pharmacia, Stockholm, Sweden). Subsequently the RNA standards underwent reverse transcription in order to verify that the resulting products were indistinguishable from the internal DNA standard.

Isolation of myometrial mRNA

The isolation of poly(A⁺) mRNA was performed using a MicroPoly(A)Pure kit (Ambion Inc., Austin, TX, USA). Approximately 30–300 mg myometrium free from para- and endometrium was homogenized in 800 µl lysis solution. An aliquot was mixed with 2 volumes of dilution buffer. Tissue debris was removed by centrifugation for 10 min at 12 000 g, 4 °C. One millilitre of the supernatant was combined with 20 mg oligo dT resin and agitated for 60 min at room temperature. The oligo dT resin was washed thrice with 1 ml binding buffer followed by three washings with 1 ml wash buffer. It was subsequently transferred to a spin column and centrifuged 5000 g at room temperature. A 500 µl volume of wash buffer was added to the column and the oligo dT resin re-centrifuged. To ensure complete removal of ribosomal RNA this step was repeated until the absorbance of the flow-through wash at 260 nm was below 0.05. Poly(A⁺) mRNA was thereafter eluted with 200 µl 65 °C elution buffer followed by overnight precipitation in 20 µl 5 M NH₄Oac, 1 µl glycogen (5 mg/ml) and 500 µl 96% ethanol. The solution was centrifuged 15 000 g for 20 min at 4 °C, the pellet washed with 70% ethanol to remove remaining salts and finally the pellet was re-suspended in DEPC-H₂O and stored frozen at –80 °C.

RT-PCR

Reverse transcription (RT) was performed on mRNA from 0.1–1.0 mg tissue in a mixture consisting of 1 mM dNTP, 25–35 U MMLV-RT (Promega), 40 U RNA-guard, 200 pmol antisense primer and either 2.2×10^{-15} M (COX-2) or 26×10^{-15} M (PGF_{2α}) internal RNA standard in Promega RT buffer. Incubations were carried out for 60 min at 37 °C and the resulting cDNA used immediately or stored at –80 °C.

PCR was carried out with 5 µl cDNA, 37.5 µM of each dNTP, 1.0 U Taq polymerase (Pharmacia, Sweden), 40 pmol of both sense primer and antisense primer in PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, pH 9.0). Amplification took place in a Perkin Elmer Model 460 thermocycler. Cycling parameters were: 95 °C for 2 min followed by 27 cycles consisting of 90 s at 94 °C, 56 °C for 45 s and 70 °C for 2 min. After the last cycle the incubations continued for 5 min at 72 °C followed by lowering of the temperature to 4 °C. PCR products were used immediately or stored at –80 °C.

Quantitation of PCR products was carried out by means of HPLC using a TSK DEAE-NPR column (Engström *et al.* 1997). Following chromatography, PCR products were UV-detected at 254 nm. The areas of the PCR products of the internal standards represented 0.011 (COX-2) and 0.130 (PGF_{2α}-R) amol respectively. Hence the amounts of the 413 and 428 bp PCR products could be quantitated relative to those standards and they were finally related to tissue wet weight. The average value of the uterine horns of each animal was calculated to represent the amount of specific mRNA.

In vitro examination of contractile force of myometrial strips

One uterine horn was opened longitudinally and a middle segment measuring 5 mm was mounted in an isometric myograph connected to a Grass force transducer, the resting tension being 1.5 g. The strip was placed in an organ bath containing 7 ml of Krebs–Ringer buffer (NaCl 119 mM, KCl 4.6 mM, NaHCO₃ 20 mM, CaCl₂ 1.5 mM, NaH₂PO₄ 1.2 mM, glucose 11 mM, pH 7.4) and allowed to rest for 30 min. Every 10 min during this period the buffer was refreshed. Before stimulation with PGF_{2α} the specimen was further washed five times. The temperature was kept at 30 °C and the solution constantly aerated with 5% CO₂ in O₂. Contractile responsiveness to PGF_{2α} (Sigma) was measured using doses over the range of 1.1×10^{-8} – 4.7×10^{-5} M. The addition of PGF_{2α} was done in a cumulative manner in order to obtain increasing concentrations. The effect of each dose was allowed to level off before the addition of the next. When the dose–response curve had been obtained each strip was thoroughly washed with Krebs–Ringer buffer and the contractile response induced by modified Krebs–Ringer buffer containing 50 mM KCl (and 73 mM NaCl) was recorded. All PGF_{2α}-induced responses were finally expressed as a percentage of the KCl-induced contraction and were plotted against the logarithm to the agonist concentration.

Data analysis

A computer program (Fig.P., Biosoft, Cambridge, UK) was used for data analysis. A four parameter non-linear curve-fitting model was used to evaluate myometrial responsiveness to PGF_{2α}. Maximal contraction ($E_{\max}$) and the agonist concentration giving half this effect (EC_{50}) were obtained from curve-fits of individual dose–response curves using the equation:

$$E = E_{\min} + (E_{\max} - E_{\min}) / (1 + ([\text{PGF}_{2\alpha}] / EC_{50})^{-p}).$$

A 2×4 factorial two-way analysis of variance was used to test the effects of isoproterenol and steroid treatment as well as the interaction between the two. Significant effects among individual means were subsequently separated using Student–Newman–Keuls *post hoc* test for multiple

Figure 1 $\text{PGF}_{2\alpha}$ receptor mRNA following treatment for 3 days with oestradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens). Hatched bars indicate additional treatment with isoproterenol ($10 \mu\text{g}/\text{h}$) for 3 days whereas grey bars indicate additional treatment with saline ($1 \mu\text{l}/\text{h}$) for 3 days. The difference in the mean values was not significant concerning either isoproterenol or steroid treatment. The interaction between isoproterenol and steroid treatment was, however, significant (two-way ANOVA, $P=0.040$). *Post hoc* analysis showed a significant decrease in mRNA when saline was substituted with isoproterenol in the oestrogen treatment group only (* $P<0.05$). Values are means $\pm$ S.E.M., $n=3-4$.

comparisons. In cases of inhomogeneity of variances data were transformed by application of $\log_{10}$ or the square root. One-way analysis of variance was used to compare the slopes of the linear parts of the dose-response curves. $P<0.05$ was considered statistically significant. Results are presented as means $\pm$ S.E.M.s unless otherwise stated.

Results

Messenger RNA

Myometrial $\text{PGF}_{2\alpha}$ -R mRNA results are shown in Fig. 1. Analysis of variance revealed no overall statistical difference between the steroid treatment groups ($P=0.575$). Furthermore, isoproterenol had no effect on $\text{PGF}_{2\alpha}$ -R mRNA ($P=0.801$). A significant interaction between steroid treatment and isoproterenol was found ($P=0.040$). Isoproterenol infusion decreased $\text{PGF}_{2\alpha}$ -R mRNA more than threefold in the oestrogen treatment group ($P<0.05$, Student-Newman-Keul). $\text{PGF}_{2\alpha}$ -R mRNA was enhanced threefold in the combined steroid treatment group but this effect was not significant when evaluated by the *post hoc* test.

Figure 2 COX-2 receptor mRNA following treatment for 3 days with oestradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens). Hatched bars indicate additional treatment with isoproterenol ($10 \mu\text{g}/\text{h}$) for 3 days whereas grey bars indicate additional treatment with saline ($1 \mu\text{l}/\text{h}$) for 3 days. The differences in the mean values concerning isoproterenol treatment ($P=0.046$) and steroid treatment ($P<0.001$) were significant when evaluated by two-way ANOVA. A significant interaction between the two treatment regimes was also found (isoproterenol $\times$ steroid, $P<0.001$). Groups with different letters within the steroid treatment groups having not received isoproterenol are statistically different when evaluated by *post hoc* analysis ($P<0.05$). * $P<0.05$ when isoproterenol was substituted with saline. Values are means $\pm$ S.E.M., $n=3-4$.

COX-2 mRNA differed between the steroid treatment groups ($P<0.001$, two-way ANOVA, Fig. 2) and between groups having or having not received isoproterenol ($P=0.046$). In addition a significant interaction between the two factors was found ($P<0.001$). Within the rats having received saline, *post hoc* analysis revealed statistically differences between the oestrogen treatment group and all other groups and between the progesterone treatment group and the combined treatment group ($P<0.05$, Student-Newman-Keul). Isoproterenol infusion decreased COX-2 mRNA in the oestrogen treatment group ($P<0.05$, Student-Newman-Keul).

Contractile activity of isolated uterine strips

In Figs 3 and 4 dose-response curves from $\text{PGF}_{2\alpha}$ -stimulated uterine strips are shown. The slopes of the curves were not statistically different ($P=0.295$, one-way ANOVA). Maximal contractile effect (E_{max}) was affected by steroid treatment when evaluated by two-way ANOVA ($P=0.024$). *Post hoc* analysis revealed differences

Figure 3 Dose–response curves of isolated uterine strips challenged with $\text{PGF}_{2\alpha}$. Strips were obtained from rats treated with oestradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens) for 3 days. The rats were additionally treated with saline ($1 \mu\text{l/h}$) for 3 days. Values are means $\pm$ S.E.M., $n=4$.

within the rats having received isoproterenol between the oestrogen treatment and the progesterone treatment and the combined treatment group respectively ($P<0.05$, Student–Newman–Keul, Fig. 5). The interaction between steroid treatment and isoproterenol treatment was significant ($P=0.045$, two-way ANOVA). Among the rats having both oestrogen and progesterone administered isoproterenol enhanced E_{max} by 53% ($P<0.05$, Student–Newman–Keul). EC_{50} values were significantly different among the different steroid treatments ($P<0.001$, two-way ANOVA, Fig. 6). Oestrogen treatment increased EC_{50} whereas this parameter was reduced by progesterone ($P<0.05$, Student–Newman–Keul). When rats were treated with the combined steroid regimen the increasing effect of oestrogen was partly blunted ($P<0.05$, Student–Newman–Keul). No interaction was found between

steroid treatment and isoproterenol treatment ($P=0.087$, two-way ANOVA).

Discussion

The results of the present study show that oestrogen treatment of ovariectomized rats increases myometrial genetic expression of the prostaglandin-promoting enzyme COX-2. On the other hand, $\text{PGF}_{2\alpha}$ receptor mRNA was unchanged following treatment with this steroid and maximal contractility of isolated uterine strips challenged with $\text{PGF}_{2\alpha}$ remained unaltered. Progesterone treatment alone decreased the production of COX-2 mRNA when compared with oestrogen treatment and when the two compounds were administered together the enhancing

Figure 4 Dose-response curves of isolated uterine strips challenged with $\text{PGF}_{2\alpha}$. Strips were obtained from rats treated with oestradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens) for 3 days. The rats were additionally treated with isoproterenol ($10 \mu\text{g/h}$) for 3 days. Values are means $\pm$ S.E.M., $n=4-6$.

effect of the latter substance was curbed. When oestrogen treatment was combined with isoproterenol infusion mRNA values of both $\text{PGF}_{2\alpha}$ -R and COX-2 were lowered. When isoproterenol infusions were given in combination with both oestrogen and progesterone E_{max} was enhanced as compared with similar rats having not received isoproterenol and as compared with rats having received isoproterenol and oestrogen alone. Finally, EC_{50} of isolated uterine strips was increased by oestrogen and decreased by progesterone without any influence of isoproterenol treatment.

Rat (Dong *et al.* 1996, Engström *et al.* 2000) and human (Slater *et al.* 1999) myometrial COX-2 protein and mRNA accumulate at parturition suggesting a role for COX-2 in the initiation of labour. In bovine parturition oestrogen levels rose rapidly during labour (Fuchs *et al.*

1992) and from day 19 till day 22 of rat gestation plasma oestradiol and uterine oestrogen receptors rose (Fang *et al.* 1996). In accordance with the present data we suggest that the enhanced influence of oestrogens on the myometrium during delivery may be responsible for the increased COX-2 mRNA production. In view of the tenfold increased EC_{50} and the unaltered levels of $\text{PGF}_{2\alpha}$ -R mRNA and E_{max} it appears unlikely that an increased myometrial prostaglandin production primarily serves a contracting role in the oestrogen dominated uterus. However, contractions may be elicited through prostaglandin-mediated ovarian release of oxytocin (McCracken *et al.* 1999) or the targets for myometrial prostaglandin production may alternatively be the ovary for the induction of luteolysis (Fiedler *et al.* 1999) or cervix uteri for the induction of ripening (Trofatter *et al.* 1985, Bernstein *et al.* 1987).

Figure 5 Maximal contractile responsiveness of isolated uterine strips following treatment for 3 days with oestradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens). Hatched bars indicate additional treatment with isoproterenol ($10 \mu\text{g/h}$) for 3 days whereas grey bars indicate additional treatment with saline ($1 \mu\text{l/h}$) for 3 days. The difference in the mean values between the steroid treatment groups was significant when evaluated by two-way ANOVA ($P=0.024$). *Post hoc* analysis revealed that the differences were found within the groups having received isoproterenol. A significant interaction between the two treatment regimes was also found (isoproterenol $\times$ steroid, $P<0.045$). Groups with different letters within the steroid treatment groups having received isoproterenol are statistically different ($P<0.05$). * $P<0.05$ when isoproterenol was substituted with saline. Values are means $\pm$ S.E.M., $n=4-6$.

A decline in serum progesterone prior to delivery has been observed in sheep (Fuchs *et al.* 1992), rat (Sanyal 1978) and mouse (Soares & Talamantes 1984). Indeed Csapo (1956) suggested that the withdrawal of progesterone was a prerequisite for the initiation of labour. The ability of progesterone to suppress uterine activity includes a decreased gap junction formation (Chwalisz *et al.* 1991), an enhanced β -adrenergic receptor expression (Krall *et al.* 1978), a reduced oxytocin receptor formation (Larcher *et al.* 1995) and an up-regulation of iNOS (Dong *et al.* 1998). Our present data suggest that inhibition of oestrogen-stimulated COX-2 expression by progesterone may additionally contribute to those effects. In this context it is, however, enigmatic that EC_{50} was substantially decreased by progesterone indicating that the coupling between the $\text{PGF}_{2\alpha}$ -R and the contractile response was altered towards a more sensitized state. It is, however, well-known that in the absence of a ligand homologous sensitization may occur (Liang and Hirsch 1993) and thus the reduced EC_{50} may reflect a lack of $\text{PGF}_{2\alpha}$ -R stimulation due to the reduced

Figure 6 EC_{50} values of isolated uterine strips following treatment for 3 days with oestradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens). Hatched bars indicate additional treatment with isoproterenol ($10 \mu\text{g/h}$) for 3 days whereas grey bars indicate additional treatment with saline ($1 \mu\text{l/h}$) for 3 days. The difference in the mean values between the steroid treatment groups was significant when evaluated by two-way ANOVA ($P<0.001$). No significant interaction between isoproterenol and steroid treatment was found. Groups with different letters within all steroid treatment groups are statistically different ($P<0.05$). Values are means $\pm$ S.E.M., $n=4-6$.

expression of COX-2. Nevertheless, the observation is in accordance with that of Basu *et al.* (Basu & Chatterjee 1978), who found that progesterone sensitized ovariectomized pregnant rat uterus to $\text{PGF}_{2\alpha}$. Blockade of oxytocin receptors considerably reduces EC_{50} and increases E_{max} of $\text{PGF}_{2\alpha}$ stimulated uterine strips from rats in labour (Engström *et al.* 2000), suggesting that $\text{PGF}_{2\alpha}$ may compensate the action of oxytocin on term myometrium. Suppression of rat myometrial oxytocin receptors by progesterone (Larcher *et al.* 1995) may therefore possibly be responsible for the decreased EC_{50} in this study.

β_2 -adrenomimetics are potent inhibitors of myometrial contractions (Anderson *et al.* 1975, Berg *et al.* 1982, Caritis *et al.* 1983) and are as such used clinically in the treatment of pre-term labour. Crankshaw & Gaspar (1995) showed that β_2 -adrenoceptor stimulation *in vitro* was able to inhibit ovine myometrial contractions induced by the $\text{PGF}_{2\alpha}$ analogue cloprostenol. The present data indicate that β_2 -adrenoceptor stimulation of oestrogen dominated rat myometrium *in vivo* modulates both prostaglandin synthesis and $\text{PGF}_{2\alpha}$ -R expression although neither E_{max} nor EC_{50} were affected by the combined treatment with oestrogen and isoproterenol. However, uterine strips

contained endometrium from which other myometrial stimulants, e.g. oxytocin (Lefebvre *et al.* 1994), may be released in response to PGF_{2α} and may thus subsequently blunt the reduced myometrial PGF_{2α}-R expression. Indeed, Lefebvre *et al.* (1994) found oestrogen to increase endometrial oxytocin mRNA production in ovariectomized rats, and in guinea pig Coleman & Parkinson (1988) found the endometrium to play a substantial role in the establishment of uterine contractions.

When both oestrogen and progesterone were administered in isoproterenol-infused rats PGF_{2α}-R mRNA returned to a level comparable to that seen following treatment with oestrogen alone and the effect on COX-2 mRNA was reversed. As indicated by the increased $E_{\max}$ of PGF_{2α} challenged strips following treatment with both steroids and isoproterenol we speculate whether progesterone withdrawal in pregnant rats may be redundant provided β_2 -adrenoceptor activation is present. Given high levels of circulating catecholamines as shown in human (Suzuki *et al.* 1989) we therefore suggest that PGF_{2α}-R-induced myometrial contractions may secure proper delivery if progesterone withdrawal fails.

Altogether the inhibitory effect of β_2 -adrenoceptor stimulation on the uterus may involve modification of myometrial PGF_{2α}-R though this is seen only after progesterone is withdrawn. In accordance, β_2 -induced rat uterine relaxation was more pronounced during labour than at day 21 of pregnancy (Engstrøm *et al.* 1999).

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