Desensitization of β_2 -adrenoceptor function in non-pregnant rat myometrium is modulated by sex steroids

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Abstract

The effects of *in vivo* treatment with estrogen and progesterone on isoproterenol-induced uterine relaxation and β_2 -adrenoceptor (β_2 AR) mRNA production in non-pregnant rat myometrium were investigated. Whether homologous myometrial desensitization of β_2 AR function was dependent on or modulated by the two steroids was also examined.

Estrogen treatment alone or in combination with progesterone reduced maximal relaxation ($E_{\max}$) of isolated uterine strips subsequently challenged with isoproterenol whereas progesterone alone had no effect on this parameter. The reduction was accompanied by an enhanced β_2 AR mRNA concentration. The concentration of isoproterenol giving half-maximal relaxing response (EC_{50}) increased following estrogen treatment and this effect was curbed by progesterone.

Isoproterenol had no effect on β_2 AR transcription irrespective of the steroid regimes employed. $E_{\max}$ of

isolated uterine strips was reduced following prolonged *in vivo* treatment with isoproterenol but the effect was found only when estrogen alone was administered concomitantly. Finally, *in vivo* treatment with isoproterenol increased EC_{50} of uterine strips subsequently stimulated with isoproterenol *in vitro*. This effect was independent of steroid treatment.

We conclude that homologous desensitization of β_2 AR function in non-pregnant rat myometrium in terms of sensitivity (EC_{50}) is independent of sex steroids but in terms of maximal response ($E_{\max}$) occurs only in the presence of estrogen. We speculate whether progesterone withdrawal in connection with the well-known estrogen dominance at rat parturition may strengthen the desensitization induced by β_2 AR activation and thus contribute to the transformation of the uterus from a quiescent to a highly contractile organ.

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Introduction

β_2 -adrenoceptor (β_2 AR) stimulation inhibits myometrial contractility *in vivo* (Caritis *et al.* 1983) and *in vitro* (Fukuo *et al.* 1986, Engstrøm *et al.* 1997) and thus β_2 -agonists are widely used as tocolytic agents (Gyetvai *et al.* 1999). The effectiveness of these agents is, however, limited due to a relatively rapid development of tachyphylaxis (Lye *et al.* 1992), and consequently the incidence of preterm delivery (Hemminki & Starfield 1978) as well as perinatal morbidity and mortality (The Canadian Preterm Labor Investigators Group 1992) has remained unchanged during several years. Tachyphylaxis denotes the progressive loss of a cellular response upon continued stimulation with a given agonist. The process involves several steps. Initially, the receptor is uncoupled from the signaling cascade, thereby interrupting the chain of events necessary to elicit the cellular response (Benovic *et al.* 1988). Uncoupling is followed by endocytosis and degradation of the receptor, a process known as sequestration or

internalization (Fishman & Perkins 1988). If receptor activation persists, receptor gene expression may be impaired leading to a decreased transcription rate.

In non-pregnant estrogen dominated ovine myometrial strips β_2 AR desensitization was associated with a reduced number of β_2 AR whereas post-receptor function remained intact (Dayes & Lye 1990). On the other hand, β -adrenergic receptor density was reduced to a lesser degree than adenylate cyclase activity in ritodrine-treated pregnant ovine myometrium indicating that post-receptor events were impaired (Caritis *et al.* 1987). In rat myometrium, β_2 AR desensitisation involves up-regulation of the contractility promoting oxytocin receptor (Engstrøm *et al.* 1998, 1999). We reported that pregnancy in itself induced desensitization of rat myometrium to isoproterenol and that this process primarily occurred at the level of genetic receptor expression (Engstrøm *et al.* 1997). Yeagley *et al.* (1996) reported that the degree of rat myometrial tachyphylaxis in response to isoproterenol increased with advanced gestational age. Altogether,

β_2 AR desensitization appears to be a process dependent on both direct changes in β_2 AR function and processes that do not specifically involve this receptor. Furthermore, the process also seems to be dependent on the endocrinological environment as a whole.

During pregnancy, the uterus undergoes profound changes in terms of growth and contractile activity. It is transformed from a quiescent organ during the first part of gestation to a highly contractile organ at term. Sex steroids exert an overall regulation of myometrial activity and it is generally accepted that estrogen promotes uterine activity (Mercado Simmen *et al.* 1982) whereas progesterone favors uterine quiescence (Csapo 1956, Lye & Porter 1978). The regulation of uterine activity by sex steroids includes prostaglandin production (Engstrøm 2001), prostaglandin $F_{2\alpha}$ receptor function (Engstrøm *et al.* 2000) and oxytocin receptors (Engstrøm *et al.* 1999). However, reports on the effects of estrogen and progesterone on myometrial β_2 AR function are conflicting. In non-pregnant rabbit myometrium, isoproterenol-stimulated cAMP production diminished following estrogen or progesterone treatment alone but increased when both steroids were employed (Boulet & Fortier 1988). In ovariectomized guinea pigs both progesterone and estrogen increased β -adrenoceptor concentrations (Hatjis *et al.* 1988). Isoproterenol-induced adenylate cyclase activity from similar myometrial specimens was enhanced by intrauterine estrogen administration (Hatjis & Koritnik 1989). On the other hand, in estrogen-treated rats extra-uterine responses to isoproterenol were reduced possibly due to down-regulation of β -adrenoceptors (Carlberg & Fregly 1986). Nimmo *et al.* (1995) suggested that an intact myometrial β -adrenoceptor function is dependent on progesterone whereas β -adrenoceptor density is dependent on circulating estrogen (Nimmo *et al.* 1989).

The aims of the present study were: (1) to investigate the influences of estrogen and progesterone on isoproterenol-induced uterine relaxation and possible relations to the genetic expression of the β_2 AR in non-pregnant rats and (2) to examine whether desensitization of β_2 AR function is dependent on or modulated by the two steroids.

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 anesthetized with a mixture of Dormicum (1.25 mg, Roche, Basel, Switzerland) and Hypnorm (0.4 ml; Janssen, Geel, Belgium). The rats were bilaterally

ovariectomized through an incision in the lateral abdominal wall. Thereafter the incisions were sutured. Eight treatment groups were formed seven days later. The groups received either no steroid injection (groups 1–2), a daily i.m. injection of 50 μ g estradiol benzoate (LEO, Copenhagen, Denmark; groups 3–4) (Stürmer 1968), a daily i.m. injection of 100 μ g 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 estradiol 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 μ g/h; Sigma Chemical Company; 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 preliminary experiments in which a dose of 10 μ g/h was found to be the minimal effective dose to induce β_2 AR desensitization (Engstrøm *et al.* 1998). Following the treatment period, the rats were anesthetized 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.

Primers and construction of internal mRNA standard

The oligonucleotide primers (DNA Technology, Aarhus, Denmark) used for detection of β_2 AR mRNA were: sense primer: 5' TCT TCG AAA ACC TAT GGG AAC GGC 3' (nucleotides 1036–1059); antisense primer: 5' GGA TGT GCC CCT TCT GCA AAA TCT 3' (nucleotides 1355–1378).

The 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 product other than the rat β_2 AR was found. The amplified β_2 AR DNA fragments consisted of 343 bp. 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 DNA standard was 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 primer, was used for amplification of the DNA sequence by PCR. The resulting product was re-amplified using our antisense primer and a primer consisting of the initial 23 nucleotides of the T7 RNA-polymerase promoter region. Following HPLC purification, the re-amplified product was used for production of RNA by *in vitro* transcription (Riboprobe, Promega,

Madison, WI, USA). The resulting RNA standard was quantitated by UV-detection at 260 nm (Gene-quant, Pharmacia, Stockholm, Sweden). Subsequently, the RNA standard underwent reverse transcription in order to verify that the resulting product was 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 were homogenized in 800 μ l lysis solution. An aliquot was mixed with 2 volumes dilution buffer. Tissue debris was removed by centrifugation for 10 min at 12 000 *g*, 4 °C. One milliliter 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 at 5000 *g* at room temperature. Wash buffer (500 μ l) 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 μ l 65 °C elution buffer followed by overnight precipitation in 20 μ l 5 M NH₄OAc, 1 μ l glycogen (5 mg/ml) and 500 μ l 96% ethanol. The solution was centrifuged 15 000 *g* for 20 min at 4 °C, the pellet was washed with 70% ethanol to remove remaining salts and finally the pellet was resuspended in 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, 40 U Moloney murine leukemia virus-RT (Promega), 31.2 U RNA-guard, 200 pmol antisense primer and 5 μ l internal RNA standard in Promega RT-buffer. Incubations were carried out for 60 min at 37 °C and the resulting cDNA was used immediately or stored at –80 °C.

PCR was carried out with 5 μ l cDNA, 37.5 μ M of each dNTP, 1.0 U Taq Polymerase (Pharmacia), and 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 1.5 min at 94 °C, 2 min at 60 °C and 2 min at 72 °C. 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 area of the 248 bp PCR product of the internal standard represented 0.024 amol. Hence the amount of the 343 bp PCR product of the β_2 AR could be quantitated relative to this standard and it was 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 the contractile force of myometrial strips

In vitro contractility was measured basically as described previously (Engström *et al.* 1998). 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 Krebs-Ringer buffer, at 30 °C, pH 7.4 and allowed to rest for 30 min. Every 10 min during this period the buffer was refreshed in order to wash out any isoproterenol potentially remaining in the tissue specimen. Before stimulation with isoproterenol, the specimens were further washed five times. The tissue was pre-incubated with 50 mM KCl and the relaxing effect of isoproterenol over a range of 10^{-10} to 4×10^{-6} M was examined. The response following addition of KCl was allowed to reach a plateau before the addition of the ligand. The addition of isoproterenol was carried out in a cumulative manner to obtain increasing concentrations, and phentolamine (Regitin, Ciba, Basel, Switzerland) at a concentration of 3.6 μ M was present in the incubation medium to block adrenergic α -receptors. The effect of each dose of isoproterenol was allowed to level off before addition of the next. It was ensured that the contractile response to KCl in itself did not significantly diminish during the period of the experiment. During the experiment the buffer was constantly aerated with 5% CO₂ in O₂. Responses following isoproterenol stimulation were expressed as a percentage of the potassium-induced contraction and were plotted against the logarithm to the agonist concentration.

Data analysis

A computer program (Fig.P., Biosoft, Cambridge, Cambs, UK) was used for data analysis. A four-parameter non-linear curve-fitting model was used to evaluate myometrial responsiveness to isoproterenol. Maximal relaxation ($E_{\max}$) and the agonist concentration giving half this effect (EC_{50}) were obtained from curve-fits of individual concentration–response curves using the equation: $E = E_{\min} + (E_{\max} - E_{\min}) / (1 + ([ISO]/EC_{50})^{-p})$.

A 2×4 factorial two-way analysis of variance was used to test the effects of isoproterenol (ISO) and steroid

Figure 1 Concentration–response curves of isolated uterine strips challenged with isoproterenol. Strips were obtained from rats treated with estradiol 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 three days. The rats were additionally treated with saline ($1 \mu\text{l/h}$, closed circles) or isoproterenol ($10 \mu\text{g/h}$, open circles) for three days. Values are means $\pm$ S.E.M., $n=3-4$.

treatment as well as the interaction between the two. Significant effects among individual means were subsequently separated using Student–Newman–Keul’s post-hoc test for multiple comparisons. In cases of nonhomogeneity of variances data were transformed by application of $\log_{10}$. $P<0.05$ was considered statistically significant. Results are presented as means $\pm$ S.E.M. unless otherwise stated.

Results

Contractile activity of isolated uterine strips

Concentration–response curves from isoproterenol-stimulated uterine strips are shown in Fig. 1. Maximal relaxation (E_{max}) was affected by steroid treatment itself when evaluated by two-way ANOVA ($P<0.001$). In the rats which did not receive isoproterenol infusion post hoc

analysis revealed that estrogen treatment and combined steroid treatment decreased E_{max} whereas progesterone had no effect on this parameter when compared with control rats ($P<0.05$, Student–Neuman–Keul’s test, Fig. 2). In addition, progesterone was not able to curb the effect of estrogen as indicated by the non-significant difference between the estrogen treatment group and the combined steroid treatment group. Overall, isoproterenol treatment decreased E_{max} as compared with saline ($P<0.001$, two-way ANOVA) but since the interaction between isoproterenol and steroid treatment was also significant ($P<0.001$, two-way ANOVA) the differences within each steroid treatment group had to be evaluated. Post hoc analysis showed that isoproterenol decreased E_{max} in the estrogen treatment group only ($P<0.05$, Fig. 2). In general, curves from saline-treated animals showed that the first concentration of isoproterenol induced

Figure 2 Maximal isoproterenol-induced relaxation of isolated uterine strips following treatment for three days with estradiol 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 three days whereas grey bars indicate additional treatment with saline ($1 \mu\text{l}/\text{h}$) for three days. The difference in the mean values between the steroid-treatment groups was significant when evaluated by two-way ANOVA ($P < 0.001$) after allowing for the effects of differences between isoproterenol and saline treatments. Different letters (a-c) within the steroid-treatment groups which received saline (grey bars) indicate statistically different values between those four groups ($P < 0.05$) when evaluated post hoc by Student-Newman-Keul's test. Different letters (x-z) within the steroid-treatment groups which received isoproterenol (hatched bars) indicate statistically different values between the other four groups ($P < 0.05$) when evaluated post hoc by Student-Newman-Keul's test. A significant interaction between the two treatment regimes was also found (isoproterenol $\times$ steroid, $P < 0.001$). * $P < 0.05$ when isoproterenol was substituted with saline as evaluated post hoc by Student-Newman-Keul's test. Values are means $\pm$ S.E.M., $n = 3-4$.

a substantial relaxation. Thus, in comparison with isoproterenol-treated specimens, saline-treated strips were very sensitive to isoproterenol stimulation. Long-term isoproterenol treatment significantly increased EC_{50} values ($P < 0.001$, two-way ANOVA, Table 1). EC_{50} values were significantly different among the different steroid treatments ($P = 0.004$) whereas the interaction between isoproterenol and steroid treatment was not different ($P = 0.389$, Table 1). Estrogen treatment increased EC_{50} and this effect was blocked by concomitant administration of progesterone ($P < 0.05$, Student-Newman-Keul's test).

$\beta_2\text{AR}$ mRNA

Myometrial $\beta_2\text{AR}$ mRNA results are shown in Fig. 3. Neither isoproterenol treatment ($P = 0.646$) nor the interaction between isoproterenol and steroid treatment ($P = 0.163$) were significantly different. Thus, in Fig. 3, data from rats which had or had not received isoproterenol are combined within each steroid treatment group. Steroid treatment significantly changed mRNA values ($P = 0.005$, two-way ANOVA). Post hoc analysis revealed differences between the estrogen-treatment group or the

Table 1 EC₅₀ values of isolated uterine strips challenged with isoproterenol. Values are means $\pm$ S.E.M., $n=3-4$

Treatment	EC ₅₀ (nM)	
Saline	0.99 $\pm$ 0.76	
Saline+isoproterenol	4.60 $\pm$ 2.31	a
Estrogen	2.68 $\pm$ 1.05	
Estrogen+isoproterenol	30.9 $\pm$ 9.89	b
Progesterone	0.29 $\pm$ 0.17	
Progesterone+isoproterenol	6.66 $\pm$ 1.77	a
Estrogen+progesterone	0.56 $\pm$ 0.28	
Estrogen+progesterone+isoproterenol	19.2 $\pm$ 4.35	a,b

Uteri were isolated from rats treated for three days with estradiol benzoate (50 $\mu\text{g} \times 1$), progesterone (100 $\mu\text{g} \times 1$) and isoproterenol (10 $\mu\text{g/h}$) in the combinations indicated. The difference in the mean values between groups having received or not received isoproterenol was significant when evaluated by two-way ANOVA ($P<0.001$). Steroid treatment ($P=0.004$) significantly affected EC₅₀ values whereas the interaction between the two treatment regimes (isoproterenol $\times$ steroid, $P=0.389$) was statistically insignificant. Different letters indicate statistically different values among steroid treatment regimes ($P<0.05$, Student-Newman-Keul).

combined-treatment group and either the control or the progesterone-treatment group ($P<0.05$, Student-Newman-Keul's test).

Discussion

The present paper describes the regulation by estrogen and progesterone of isoproterenol-induced uterine relaxation as well as the effect of these steroids on the development of myometrial desensitization to β_2 -adrenergic stimulation. Five significant observations can be outlined. First, estrogen either alone or in combination with progesterone reduces E_{max} of isolated uterine strips, whereas progesterone alone has no effect on this parameter. Secondly, estrogen by itself increases EC₅₀ and thus decreases uterine sensitivity to isoproterenol, but this effect is curbed by simultaneous administration of progesterone. Thirdly, isoproterenol treatment attenuates E_{max} but only when rats are treated with estrogen alone. Fourthly, isoproterenol increases EC₅₀ independently of the steroid regimes employed. Fifthly, β_2 AR mRNA increases following estrogen treatment alone or in combination with progesterone. No effect on this parameter is found following isoproterenol treatment *per se* and no interaction between isoproterenol treatment and steroid treatment is seen.

The fact that estrogen affects both E_{max} and EC₅₀ indicates that both β_2 AR concentrations and the coupling between receptor occupancy and relaxation are under the influence of this steroid. Our results disagree with studies in guinea pig myometrium in which estrogen up-regulated β -adrenergic receptors (Hatjis *et al.* 1988) and in rabbit uterine tissue in which estrogen was not able

Figure 3 β_2 AR mRNA following treatment for three days with estradiol 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). No effect of isoproterenol infusion and no interaction between steroid treatment and isoproterenol were found when evaluated by two-way ANOVA. Thus data from rats with saline treatment or with isoproterenol treatment were combined within each steroid-treatment group. Steroid treatment affected mRNA values ($P=0.005$, two-way ANOVA). Different letters indicate significant differences when evaluated post hoc by Student-Newman-Keul's test ($P<0.05$). Values are means $\pm$ S.E.M., $n=7-8$.

to alter the number of β -adrenoceptors (Roberts *et al.* 1977). Therefore, species differences may exist or alternatively the reduced E_{max} might reflect a decreased adenylate cyclase activity or an increased phosphodiesterase activity.

Nevertheless, the regulation by sex steroids of E_{max} appears to be controlled primarily by estrogen since progesterone was not able to overcome the influence of estrogen. The effect on E_{max} was actually slightly more pronounced when both steroids were administered.

It appears unlikely that the reduced maximal relaxation to isoproterenol challenge following estrogen treatment is caused by a decreased genetic expression of β_2 AR. On the contrary, mRNA encoding this receptor increased more than twofold following estrogen or combined steroid treatment. In view of the reduced E_{max} , this may seem to

be an apparent contradiction. Estrogen does, however, in some tissues decrease the half-life of mRNA molecules (El Meskini *et al.* 1997, Saceda *et al.* 1998) and therefore the net result of estrogen domination may be an attenuated translation of β_2 AR transcripts. In addition, mRNA reduction as a mechanism of receptor down-regulation has primarily been observed in cell culture lines, where rapid cell division necessitates continuous receptor synthesis (Hadcock & Malbon 1988, Bouvier *et al.* 1989). *In vivo*, however, β -adrenergic receptors have half-lives of several days to weeks (Mahan *et al.* 1987). Therefore, changes in β_2 AR mRNA may not be able to alter the receptors themselves within the treatment period employed in the present study. In agreement hereof, Engelhardt *et al.* (1997) observed no change in β_2 AR mRNA in human myometrium despite the development of tachyphylaxis to β_2 -stimulation.

The β_2 AR are coupled to adenylate cyclase via a stimulatory guanine nucleotide-dependent protein (G_s) (Rodbell 1980). Uncoupling of β_2 AR from G_s shifts the concentration–response curve to the right thereby increasing EC_{50} . As reported by Riemer *et al.* (1988) estrogen treatment *in vivo* reduces G_s labeling in rabbit myometrium and this reduction is curbed by concomitant employment of progesterone. Both findings are compatible with the present results.

The decrease in tissue responsiveness following continuous exposure to catecholamines has been extensively described (Lefkowitz 1979, Levin *et al.* 1980, Engstrøm *et al.* 1998). The increasing effect of isoproterenol on EC_{50} seen in the present study is independent of the steroid regimes employed and therefore appears to be exclusively related to β_2 AR stimulation. As to the mechanism behind this effect, mRNA levels were unchanged following isoproterenol infusions indicating that EC_{50} is not regulated via an altered genetic transcription rate. This is in agreement with our previous studies (Engstrøm *et al.* 1998) and with observations in women (Engelhardt *et al.* 1997).

Isoproterenol infusions reduced $E_{\max}$ to a greater extent than estrogen alone and consequently the two compounds together effectively induced sequestration and degradation of β_2 AR. In addition, isoproterenol did not alter $E_{\max}$ following progesterone treatment even if estrogen was administered simultaneously. In rat uterus (Kano 1982) and guinea pig myometrium (Hatjis *et al.* 1988) progesterone up-regulates β -adrenergic receptors. Taken together, this suggests that progesterone favors uterine quiescence both directly via regulation of β -adrenoceptors and indirectly through the inhibition of the influence of estrogen on the receptors.

It cannot be excluded that heterologous up-regulation of contractile receptors in response to isoproterenol and estrogen treatment *in vivo* accounts for the reduced $E_{\max}$ *in vitro*. Thus isoproterenol up-regulates oxytocin receptors in estrogen-dominated rat myometrium (Engstrøm *et al.* 1998) and estrogen itself is a strong promoter of oxytocin

receptor formation (Bale & Dorsa 1997). α_2 -receptors are up-regulated by estrogen (Roberts *et al.* 1981, Riemer *et al.* 1987) but since we routinely blocked these receptors with phentolamine in the *in vitro* studies it is unlikely that any isoproterenol effect on these receptors contributes to the weakened relaxing response.

Yeagley *et al.* (1996) observed that tachyphylaxis to β -agonist stimulation increases with advanced gestational age in rat myometrium. They speculated that this effect might be related to an increased myometrial expression of inactivating β -adrenoreceptor kinase (β ARK) as previously shown by Ruzicky & DeLoia (1997). A high estrogen level (Fuchs *et al.* 1992) and a fall in circulating progesterone (Thorburn & Challis 1979) are characteristics of parturition in mammals. However, since estrogen induces a modest decrease in β ARK and progesterone reverses this effect (Ruzicky & DeLoia 1997), the present data indicate that other mechanisms must contribute to the observed tachyphylaxis. Alternatively, we suggest that progesterone withdrawal (Csapo 1956) in connection with estrogen dominance at rat term may strengthen the tachyphylaxis induced by β_2 AR activation and thus contribute to the transformation of the uterus from a quiescent to a highly contractile organ. Nevertheless, the precise mechanism behind this differentiated steroid action still needs to be elucidated.

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