Altered Levels of mRNA Expression and Pharmacological Reactivity of $\alpha_1$-Adrenergic Receptor Subtypes in the Late-Pregnant Rat Myometrium

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ABSTRACT The adrenergic system plays a major role in the regulation of the uterine contractility during pregnancy. Our previous studies have shown the significance of the $\alpha_1$-adrenergic receptors (ARs) in the control of pregnant uterine contractility both in vitro and in vivo. Our present aim was to determine the changes in mRNA expression and pharmacological reactivity of the $\alpha_1$-ARs on days 18, 20, and 22 of pregnancy. To demonstrate the expressions of $\alpha_1$-AR subtype mRNA, we used a reverse transcription-polymerase chain reaction (RT-PCR); the pharmacological reactivity was tested by electric field stimulation (EFS). The expression of $\alpha_{1A}$-AR mRNA increased from day 18 to 22, while no $\alpha_{1B}$-AR mRNA was detectable. We found a small increase in the expression of $\alpha_{1D}$-AR mRNA on day 20, which was not followed by a significant change in pharmacological reactivity. The $\alpha_{1D}$-receptor expression and pharmacological reactivity decreased significantly up to day 22. EFS studies revealed that the $\alpha_{1A}$-AR antagonist 5-methylurapidil had EC50 values ($1.9 \times 10^{-6} - 6.3 \times 10^{-5}$ M) about one order of magnitude lower than those of the $\alpha_{1D}$-AR antagonist BMY 7378 ($4 \times 10^{-6} - 3.6 \times 10^{-5}$ M). However, the $\alpha_{1B}$-AR antagonist cyclazosine exerted only a slight effect on the stimulated contractions. Strong correlations were found between the $\alpha_{1A}$-mRNA expression and the EC50 of 5-methylurapidil ($r^2 = 0.9712$), and between the $\alpha_{1D}$-mRNA expression and the EC50 of BMY 7378 ($r^2 = 0.9937$). Our findings suggest that both $\alpha_{1A}$- and $\alpha_{1D}$-ARs are involved in the regulation of the pregnant uterine contractility. The density and pharmacological reactivity indicate that the $\alpha_{1A}$-AR seems to play the major role in late-pregnant myometrial contraction. Mol. Reprod. Dev. 62: 343–347, 2002. © 2002 Wiley-Liss, Inc.

Key Words: $\alpha_1$-adrenergic receptors subtypes; late-pregnant rat; RT-PCR; electric field stimulation; pharmacological reactivity

INTRODUCTION

The $\alpha_1$-type of adrenergic receptors (ARs) plays a critical role in the regulation of the sympathetic nervous system. Cloning and pharmacological data have revealed that the $\alpha_1$-ARs can be classified into the three subtypes: $\alpha_{1A}$-, $\alpha_{1B}$-, and $\alpha_{1D}$-ARs (Hieble et al., 1995). The $\alpha_{1D}$-AR subtypes are the prime mediators of smooth muscle contraction and hypertrophic growth (Piascik and Perez, 2001).

The adrenergic system plays an important role in the regulation of the uterine motor activity (Borda et al., 1997). Contraction is mediated by the $\alpha$-ARs in the uterine smooth muscle (Hoffman et al., 1981; Rexroad, 1981). This provides a theoretical possibility for the use of $\alpha_{1A}$-AR blockers as tocolytic agents. The $\beta$-ARs are involved in uterine relaxation (Levin et al., 1980; Tanfín-Tougui et al., 1981), which is reflected in clinical practice by the frequent application of $\beta_2$-agonists as tocolytic agents. The use of $\beta_2$-mimetics, however, results in many side-effects, such as tachyphylaxis, tachycardia, pulmonary edema, hypokalemia, sodium retention, and glucose intolerance (Canadian Preterm Labour Investigators Group, 1992; Smigaj et al., 1998).

In earlier experiments, it was proved that an $\alpha$-AR antagonist induced a significant decrease in uterine activity in the rat both in vitro and in vivo (Zupkó et al., 1997; Gáspár et al., 1998). Additionally, at the end of pregnancy the $\alpha_{1}/\beta$-AR ratio of the rat uterus was found to be increased, in parallel with an increase in contractility, demonstrating a very close correlation with the density of $\alpha_{1}$-ARs during rat pregnancy (Zupkó et al., 1998). Moreover, a $\alpha_{1A}$-AR knock-down transformed post-partum animal model was set up with antisense oligodeoxynucleotides in order to prove the crucial role of the $\alpha_{1A}$-ARs in uterine contractility (Ducza et al., 2001).

Despite these facts, knowledge relating to the changes in $\alpha_1$-AR subtype density during pregnancy is limited. Our present aim was to determine the changes in density and pharmacological reactivity of the $\alpha_1$-ARs.

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subtypes in late-pregnant rats. To demonstrate the expressions of $\alpha_1$-AR subtype mRNA, we used a reverse transcription-polymerase chain reaction (RT-PCR) on days 18, 20, and 22. Electric field stimulation (EFS) was applied to test the pharmacological reactivity of the rat uterus in late pregnancy.

**MATERIALS AND METHODS**

Animal investigations were carried out with the approval of the Ethical Committee for Animal Research, University of Szeged (registration number: 23/1999).

**Mating of the Animals**

Female (180–200 g) and male (240–260 g) Sprague–Dawley rats were mated in a special mating cage. A metal door, movable by a small electric engine, separated the rooms for the male and female animals. A timer controlled the function of the engine. Since rats are usually active at night, the separating door was opened before dawn. Within 4–5 hr after the possibility of mating, vaginal smears were taken from the female rats, and a sperm search was performed under a microscope at a magnification of 1200 times. If the search proved positive, or when smear taking was impossible because of an existing vaginal sperm plug, the female rats were separated and were regarded as first-day pregnant animals.

**RT-PCR Studies**

**Tissue isolation.** Female Sprague–Dawley rats (250–300 g) were anesthetized with sodium pentobarbital (1 g/kg i.p.). Uterus tissues from nonpregnant animals and on gestational days 18, 20, and 22 were rapidly removed and dissected in iced-cold saline (0.9% NaCl) containing 2 U/ml of recombinant ribonuclease inhibitor (RNasin, Promega, Southampton, UK). The tissues were frozen in liquid nitrogen and then stored at −70°C until total RNA extraction.

**Total RNA preparation.** Total cellular RNA was isolated by extraction with guanidinium thiocyanate-acid-phenol-chloroform according to the procedure of Chomczynski and Sacchi (1987). After precipitation with isopropanol, the RNA was treated with RNase-free DNase I for 30 min at 37°C, re-extracted with phenol, precipitated with ethanol, washed with 75% ethanol, and then resuspended in diethyl pyrocarbonate-treated water, and the RNA concentration was determined by optical density measurements at 260 nm.

**RT-PCR.** The RNA (0.5 µg) was denatured at 70°C for 5 min in a reaction mixture containing 20 U of RNase inhibitor (Hybaid Corp., Middlesex, UK), 200 µM dNTP (Sigma-Aldrich, Budapest, Hungary), 20 µM oligo(dT) (Hybaid Corp.) in 50 mM Tris-HCl, pH 8.3, 75 mM KCl, and 5 mM MgCl2 in a final reaction volume of 19 µl. After the mixture had been cooled to 4°C, 20 U of M-MLV reverse transcriptase, RNase H Minus (Promega) was added, and the mixture was incubated at 37°C for 60 min and then at 72°C for 10 min.

The PCR was carried out with 5 µl cDNA, 25 µl ReadyMix REDTaq PCR reaction mix (Sigma-Aldrich) and 50 pm sense and antisense primer. The primer sequences used to amplify the $\alpha_1_A$-AR were 5′-GTA GCC AAG AGA GAA GC-3′ (for the forward primer) and 5′-CAAGG ACC ACC ACG ATG CCC AG-3′ (for the reverse primer); these primers were anticipated to generate 212 bp PCR product. For rat $\alpha_1_D$-AR cDNA, a 300 bp PCR product resulted with forward primer 5′-GCC CTT CAT CAT CCC GCT CG-3′ and reverse primer 5′-AGGGAGCCTACATAGATGA-3′. The primers for the $\alpha_1_B$-AR were 5′-CTG CTC CTT CCA ACC CG-3′ (for the forward primer) and 5′-GCA CAG GAC GAA GAC ACC CAC-3′ (for the reverse primer) (Scofield et al., 1995). A rat GAPDH probe was used as internal control in all samples (Tso et al., 1985). The PCR was performed with a PCR Sprint thermal cycler (Hybaid Corp.), with the following cycle parameters: after initial denaturation at 95°C for 3 min, the reactions were taken through 35 cycles of 1 min at 94°C, 1 min annealing at 54°C ($\alpha_1^B$- and $\alpha_1^D$-AR) or 50°C ($\alpha_1^A$-AR), and 72°C for 2 min. After the last cycle, incubation was continued for 10 min at 72°C, followed by lowering of the temperature to 4°C. $\alpha_1^B$- and $\alpha_1^D$-AR (1 µCi) was added to the above reaction mixture to quantify the amplified product. PCR products were used immediately or stored at −70°C. The PCR products were electrophoresed in 1.8% agarose gels, dried under vacuum, and placed into a PhosphorImager (Molecular Dynamics, Amersham Biosciences, Buckinghamshire, UK) exposure cassette. Quantification was carried out by ImageQuant software (Molecular Dynamics).

**Uterus Preparation and EFS**

Uteri were removed from rats (250–350 g) on day 18, 20, or 22 of pregnancy. Muscle rings 0.5 cm long were sliced from the uterine horns and mounted vertically between two platinum electrodes in an organ bath containing 10 ml de Jongh solution (in mM: 137 NaCl, 3 KCl, 1 CaCl2, 1 MgCl2, 12 NaHCO3, 4 NaH2PO4, 6 glucose, pH: 7.4). The organ bath was maintained at 37°C and carbogen (95% O2 + 5% CO2) was bubbled through it. After mounting, the rings were equilibrated for about 1 hr before experiments were begun, with a solution change every 15 min. The initial tension was set to about 1.25 g, which had relaxed to about 0.5 g by the end of equilibration. Maximum rhythmic contractions were elicited with a digital, programmable stimulus (ST-02, Experimetria Ltd. Budapest, Hungary), using different values of pulse width (PW, the duration of the electric field as a single stimulus) and period time (PER, the time interval between two stimuli) at 40 V. The applied PWs and PERs were published earlier (Gaspár et al., 2001). The tension of the myometrial rings was measured with a gauge transducer (SG-02, Experimetria U.K. Ltd.) and recorded with an ISOSYS Data Acquisition System (Experimetria U.K. Ltd.). Noncumulative concentration-response curves for the selective $\alpha_1^A$-antagonist 5-methylurapidil (5-MU; RBI, Budapest, Hungary), the selective $\alpha_1^B$-antagonist cyclazosine (RBI), and the
selective α₁D-antagonist BMY 7378 (RBI) were constructed in each experiment. The drug effects were detected during another 240 sec. After this period, the electric field was switched off and the tissues were washed out three times and left to rest for 5 min. Concentration-response curves were fitted and areas under curves (AUCs) were evaluated and analyzed statistically with the Prism 2.01 (GraphPad Software, San Diego, CA) computer program. For statistical evaluations, data were analyzed by ANOVA with the Neuman–Keuls test.

RESULTS

The expression of α₁A-AR mRNA increased from day 18–22 (Fig. 1a), while no α₁B-AR mRNA expression was detected by RT-PCR analysis. The expression of α₁D-AR mRNA was highest on day 20 and then decreased to day 22 (Fig. 1b).

The electric field-stimulated contraction on days 18, 20, and 22 of pregnancy was inhibited concentration dependently by the selective α₁A-antagonist 5-MU (Fig. 2a). In the measured concentration range, the selective α₁B-antagonist cyclazosine had no significant action, only its highest doses displayed weak inhibitory effects on the stimulated contractions (Fig. 2b).

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The α₁D-antagonist BMY 7378 inhibited the contraction in a dose-dependent manner (Fig. 2c). The EC50 was established from the dose-dependence curves. The α₁A-antagonist 5-MU had EC50 values of 1.9 × 10⁻⁶–6.3 × 10⁻⁶ M (Fig. 3a), while the EC50 of the α₁D-AR antagonist BMY 7378 lay in the range 4 × 10⁻⁶–3.6 × 10⁻⁶ M (Fig. 3b).

A strong correlation was found between the α₁A-AR mRNA expression and the EC50 of 5-MU (r² = 0.9712) (Fig. 4a), and between the α₁D-AR mRNA expression and the EC50 of BMY 7378 (r² = 0.9936) (Fig. 4b).

DISCUSSION

The α-adrenergic part of the autonomic nervous system exerts a great influence in the control of the contractions of the pregnant myometrium.

In previous experiments, an α-AR dominance was proved at the end of pregnancy (Zupkó et al., 1997; Gáspar et al., 1998). The number of α₁A-ARs is known to increase sharply in the last 6 hr of pregnancy, and radioligand binding assays have shown that the number of α₁A-ARs is increased by 88% at term. However, limited data were earlier available concerning the pharmacological reactivity of α₁A-ARs and the roles of the other α₁-AR subtypes (Legrand et al., 1987; Limon-Boulez et al., 1997).

The present study demonstrated the changes in the α₁A-, α₁B-, and α₁D-AR mRNA expressions on various days of rat pregnancy with the RT-PCR technique. We could detect no expression of α₁B-AR in this period. The selective α₁B-antagonist cyclazosine exerted only weak action in the measured concentration range, which could be explained by some nonspecific activity at high concentration.

The expression of the α₁A-AR mRNA increased from day 18 to 22, and the EFS studies revealed that the α₁A-antagonist 5-MU exhibited a well-balanced inhibitory effect, without a significant decrease in efficacy or effectivity at term.

We detected a significant increase in the expression of α₁D-AR mRNA on day 20, the expression then decreasing to day 22. The α₁D-antagonist BMY 7378 elicited a quite strong inhibitory effect, but we did not observe significant changes in efficacy or effectivity on days 18 and 20. At term, the inhibitory effect of BMY
7378 was significantly decreased. Our findings suggest that both \( \alpha_{1A} \)- and \( \alpha_{1D} \)-ARs are involved in the regulation of the pregnant uterine contractility.

The increases in \( \alpha_{1A} \)-AR mRNA and the pharmacological reactivity demonstrate the important role of the \( \alpha_{1A} \)-ARs near term. The \( \alpha_{1A} \)-ARs seem to play the major role as regards the \( \alpha_{1} \)-subtypes in the late-pregnant myometrium.

The \( \alpha_{1A} \)-AR antagonist 5-MU had EC50 values (1.9 \( \times \) 10\(^{-6}\)–6.3 \( \times \) 10\(^{-6}\) M) about one magnitude lower than those of the \( \alpha_{1D} \)-AR antagonist BMY 7378 (4 \( \times \) 10\(^{-6}\)–3.6 \( \times \) 10\(^{-5}\) M). The RT-PCR and EFS findings reveal a strong correlation between the mRNA expression and the pharmacological reactivity for the \( \alpha_{1A} \)- and \( \alpha_{1D} \)-ARs. These correlations show that the syntheses of \( \alpha_{1A} \)- and \( \alpha_{1D} \)-ARs are in harmony with the change in pharmacological reactivity.

We consider that the strong correlation between the receptor synthesis and pharmacological reactivity could be beneficial compared to the \( \beta_2 \)-mimetics where the process of receptor desensitization may decrease the effectivity of these compounds (Yeagly et al., 1996; Engelhardt et al., 1997). A similar effect was not experienced in the case of \( \alpha_1 \)-AR blockers.

Moreover, the side-effects of the \( \alpha_1 \)-AR antagonists during pregnancy might possibly be moderated or even advantageous (e.g., pregnancy-induced hypertension).

**CONCLUSIONS**

Our findings suggest that both \( \alpha_{1A} \)- and \( \alpha_{1D} \)-ARs are involved in the regulation of the pregnant uterine contractility. We found a strong correlation between the mRNA expression and the pharmacological reactivity. The \( \alpha_{1A} \)-ARs seem to play the major role as regards the \( \alpha_1 \)-subtypes in the late-pregnant myometrium.
In light of these facts, $\alpha_1$-blockers might offer new perspectives in tocolysis. However, further investigations are required, including thorough density mapping of the $\alpha_1$-AR subtypes and clinical trials are planned for the human myometrium.

REFERENCES


