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Altered Levels of mRNA Expression and Pharmacological Reactivity of α_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 α_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 α_1-ARs on days 18, 20, and 22 of pregnancy. To demonstrate the expressions of α_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 α_{1A}-AR mRNA increased from day 18 to 22, while no α_{1B}-AR mRNA was detectable. We found a small increase in the expression of α_{1D}-AR mRNA on day 20, which was not followed by a significant change in pharmacological reactivity. The α_{1D}-receptor expression and pharmacological reactivity decreased significantly up to day 22. EFS studies revealed that the α_{1A}-AR antagonist 5-methylurapidil had EC50 values (1.9×10^{-6}–6.3×10^{-6} M) about one order of magnitude lower than those of the α_{1D}-AR antagonist BMY 7378 (4×10^{-6}–3.6×10^{-5} M). However, the α_{1B}-AR antagonist cyclazosine exerted only a slight effect on the stimulated contractions. Strong correlations were found between the α_{1A}-mRNA expression and the EC50 of 5-methylurapidil ($r^2 = 0.9712$), and between the α_{1D}-AR mRNA expression and the EC50 of BMY 7378 ($r^2 = 0.9937$). Our findings suggest that both α_{1A}- and α_{1D}-ARs are involved in the regulation of the pregnant uterine contractility. The density and pharmacological reactivity indicate that the α_{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: α1-adrenergic receptors subtypes; late-pregnant rat; RT-PCR; electric field stimulation; pharmacological reactivity

INTRODUCTION

The α_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 α_1-ARs can be classified into the three subtypes: α_{1A}-, α_{1B}-, and α_{1D}-ARs (Hieble et al., 1995). The α_1-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 α-ARs in the uterine smooth muscle (Hoffman et al., 1981; Rexroad, 1981). This provides a theoretical possibility for the use of α_1-AR blockers as tocolytic agents. The β-ARs are involved in uterine relaxation (Levin et al., 1980; Tanfin-Tougui et al., 1981), which is reflected in clinical practice by the frequent application of β_2-agonists as tocolytic agents. The use of β_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 α-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 α_1/β-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 α_1-ARs during rat pregnancy (Zupkó et al., 1998). Moreover, a α_{1A}-AR knock-down transformed post-partum animal model was set up with antisense oligodeoxynucleotides in order to prove the crucial role of the α_{1A}-ARs in uterine contractility (Ducza et al., 2001).

Despite these facts, knowledge relating to the changes in α_1-AR subtype density during pregnancy is limited.

Our present aim was to determine the changes in density and pharmacological reactivity of the α_1-ARs

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subtypes in late-pregnant rats. To demonstrate the expressions of α_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

Mature 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 ice-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 $MgCl_2$ 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 α_{1A}-AR were 5′-GTA GCC AAG AGA GAA AGC CG-3′ (for the forward primer) and 5′-CAA CCC ACC ACG ATG CCC AG-3′ (for the reverse primer); these primers were anticipated to generate 212 bp PCR product. For rat α_{1B}-AR cDNA, a 300 bp PCR product resulted with forward primer 5′-GCT CTT CTA CAT CCC GCT CG-3′ and reverse primer 5′-AGGGGAGCCAACATAAGATGA-3′. The primers for the α_{1D}-AR were 5′-CGT GTG CTC CTT CTA CCT ACC-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 (α_{1B}- and α_{1D}-AR) or 50°C (α_{1A}-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. α-^{32}P-dCTP (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 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 4 NaH_2PO_4, 6 glucose, pH: 7.4). The organ bath was maintained at 37°C and carbogen (95% O_2 + 5% CO_2) 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 stimulator (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 (Gáspár 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 α_{1A}-antagonist 5-methylurapidil (5- MU; RBI, Budapest, Hungary), the selective α_{1B}-antagonist cyclazosine (RBI), and the

selective α_{1D}-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 α_{1A}-AR mRNA increased from day 18–22 (Fig. 1a), while no α_{1B}-AR mRNA expression was detected by RT-PCR analysis. The expression of α_{1D}-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 α_{1A}-antagonist 5-MU (Fig. 2a).

In the measured concentration range, the selective α_{1B}-antagonist cyclazosine had no significant action, only its highest doses displayed weak inhibitory effects on the stimulated contractions (Fig. 2b).

The α_{1D}-antagonist BMY 7378 inhibited the contraction in a dose-dependent manner (Fig. 2c). The EC50 was established from the dose-dependence curves. The α_{1A}-AR antagonist 5-MU had EC50 values of 1.9×10^{-6}–6.3×10^{-6} M (Fig. 3a), while the EC50 of the α_{1D}-AR antagonist BMY 7378 lay in the range 4×10^{-6}–3.6×10^{-5} M (Fig. 3b).

A strong correlation was found between the α_{1A}-AR mRNA expression and the EC50 of 5-MU ($r^2 = 0.9712$) (Fig. 4a), and between the α_{1D}-AR mRNA expression and the EC50 of BMY 7378 ($r^2 = 0.9936$) (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; Gáspár et al., 1998). The number of α_1-ARs is known to increase sharply in the last 6 hr of pregnancy, and radioligand binding assays have shown that the number of α_{1A}-ARs is increased by 88% at term. However, limited data were earlier available concerning the pharmacological reactivity of α_{1A}-ARs and the roles of the other α_1-AR subtypes (Legrand et al., 1987; Limon-Boulez et al., 1997).

The present study demonstrated the changes in the α_{1A}-, α_{1B}-, and α_{1D}-AR mRNA expressions on various days of rat pregnancy with the RT-PCR technique. We could detect no expression of α_{1B}-AR in this period. The selective α_{1B}-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 α_{1A}-AR mRNA increased from day 18 to 22, and the EFS studies revealed that the α_{1A}-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 α_{1D}-AR mRNA on day 20, the expression then decreasing to day 22. The α_{1D}-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

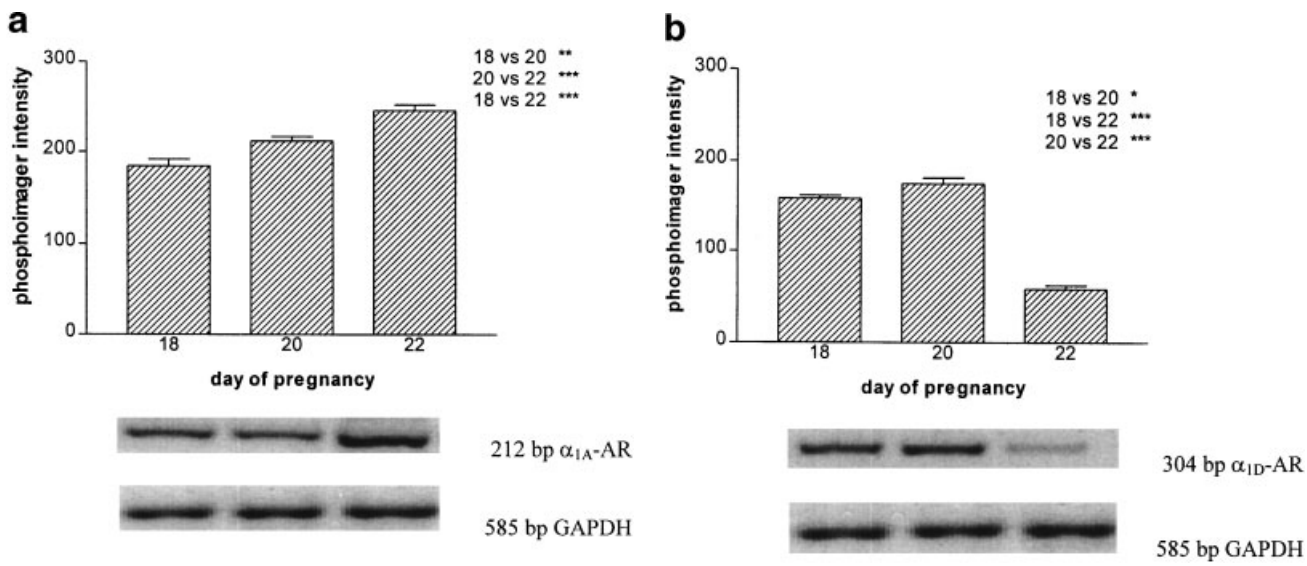

Fig. 1. Changes in expression of uterine α_{1A}- (**a**) and α_{1D}- (**b**) adrenergic receptor mRNA in the late-pregnant rat (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

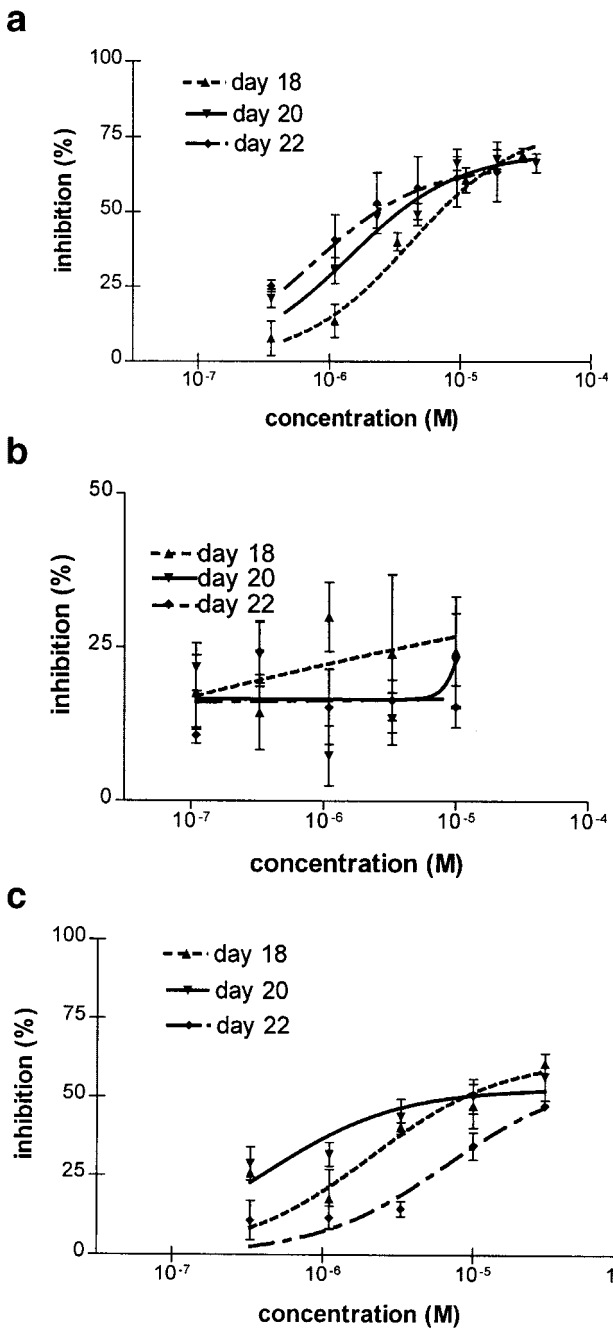

Fig. 2. Inhibitory effects of the α_{1A}-antagonist 5-MU (**a**), the α_{1B}-antagonist cyclazosine (**b**), and the α_{1D}-antagonist BMY 7378 (**c**) on electric field-stimulated contractions on different days of pregnancy in the isolated rat myometrium.

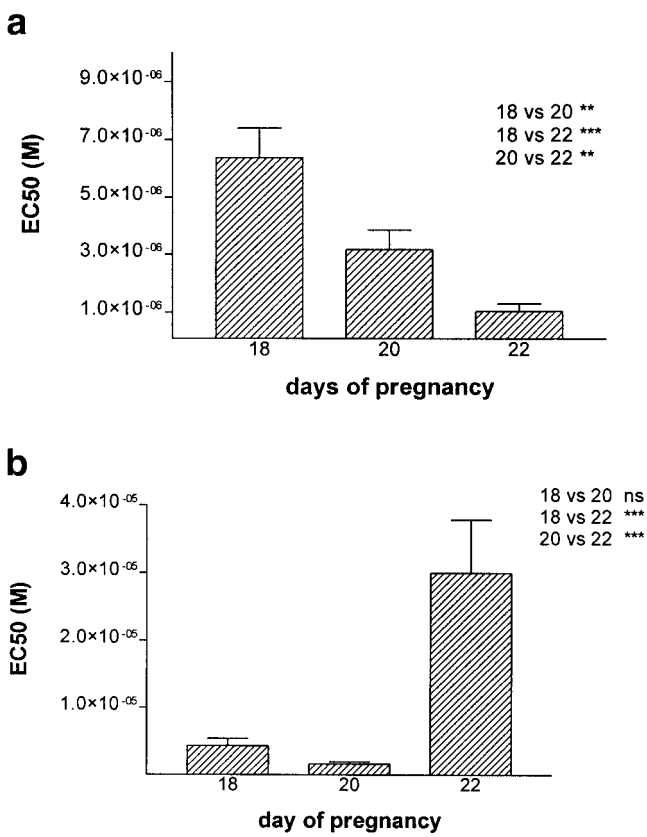

Fig. 3. Changes in EC50 of the α_{1A}-antagonist 5-MU (**a**) and the α_{1D}-antagonist BMY 7378 (**b**) on different day of pregnancy in vitro (ns, not significant; $^{**}P < 0.01$, $^{***}P < 0.001$).

7378 was significantly decreased. Our findings suggest that both α_{1A}- and α_{1D}-ARs are involved in the regulation of the pregnant uterine contractility.

The increases in α_{1A}-AR mRNA and the pharmacological reactivity demonstrate the important role of the α_{1A}-ARs near term. The α_{1A}-ARs seem to play the major role as regards the α_1-subtypes in the late-pregnant myometrium.

The α_{1A}-AR antagonist 5-MU had EC50 values (1.9×10^{-6}–6.3×10^{-6} M) about one magnitude lower than those of the α_{1D}-AR antagonist, BMY 7378 (4×10^{-6}–3.6×10^{-5} M). The RT-PCR and EFS findings reveal a strong correlation between the mRNA expression and the pharmacological reactivity for the α_{1A}- and α_{1D}-ARs. These correlations show that the syntheses of α_{1A}- and α_{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 β_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 α_1-AR blockers.

Moreover, the side-effects of the α_1-AR antagonists during pregnancy might possibly be moderated or even advantageous (e.g., pregnancy-induced hypertension).

CONCLUSIONS

Our findings suggest that both α_{1A}- and α_{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 α_{1A}-ARs seem to play the major role as regards the α_1-subtypes in the late-pregnant myometrium.

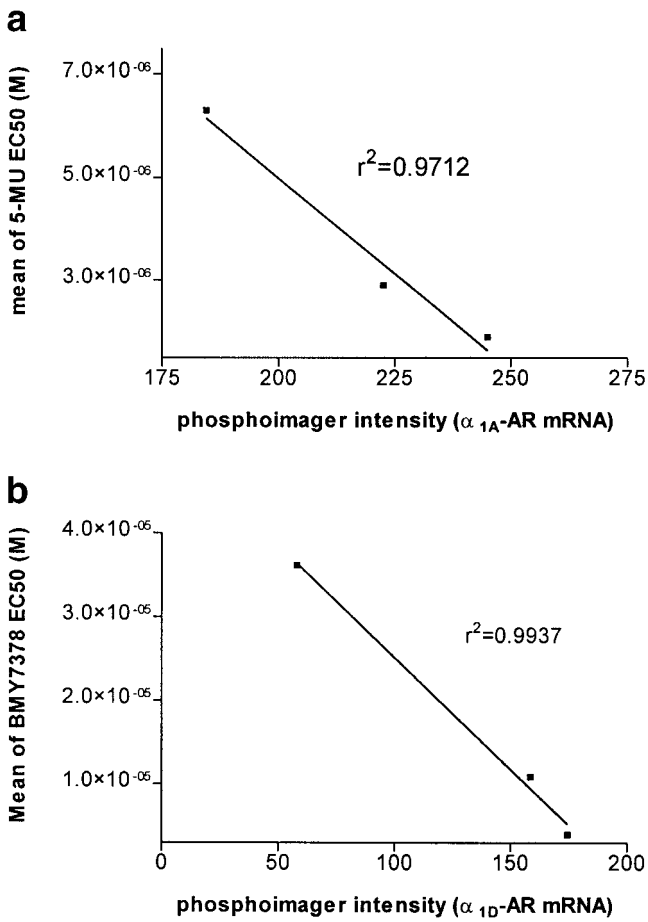

Fig. 4. Correlations between α1A-adrenergic receptor mRNA expression and EC50 of the α_{1A}-antagonist 5-methylurapidil (**a**), and between α_{1D}-adrenergic receptor mRNA expression and EC50 of α_{1D}-antagonist BMY 7378 (**b**).

In light of these facts, α_{1A}-blockers might offer new perspectives in tocolysis. However, further investigations are required, including thorough density mapping of the α_1-AR subtypes and clinical trials are planned for the human myometrium.

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