

Investigation of the role of the serotonergic activity of certain subtype-selective α_{1A} antagonists in the relaxant effect on the pregnant rat uterus *in vitro*

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Results from recent studies have shown that α_{1A} -adrenergic receptor (α_{1A} -AR) antagonists could offer a new alternative in the treatment of preterm delivery. However, members of this group [2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB4101), 5-methylurapidil (5-MU)] are known to influence serotonin (5-hydroxy-tryptamine) (5-HT_{1A}) receptors, too. Our objective was to clarify the role of their 5-HT_{1A} activities in the uterus relaxant effect. RT-PCR was used to determine mRNA expression of the receptor subtypes in 22 day pregnant rat uteri. Isolated uteri were stimulated by 5-HT or electrical field to investigate the contraction-inhibiting effect and the 5-HT_{1A} activity of the α_{1A} antagonists. Both receptor subtypes are present in rat myometrium. 5-HT induced contractions were inhibited by the α_{1A} antagonists. Besides shifting the dose–response curve of 5-HT to the right, 5-MU decreased its maximal effect. The α_{1A} antagonists inhibited electrical field stimulation-induced contractions. 5-HT_{1A} blockade increased the maximal effect of 5-MU but did not change that of WB4101. These results suggest that the contraction increase caused by 5-HT is mediated by α_{1A} receptors. Serotonergic activity of α_1 antagonists and especially α_{1A} antagonists should be investigated as it may alter their efficacy and could interfere with their side-effects. It is proposed that novel α_{1A} antagonists should be designed with no 5-HT_{1A} activity to achieve maximal relaxant effect.

Key words: α_1 -adrenoceptors/5-HT_{1A} receptors/rat/tocolysis/uterus

Introduction

It has been clearly established that the adrenergic system plays a major role in the regulation of myometrial contractility during pregnancy (Marshall, 1981; Legrand and Maltier, 1986). Thus, a number of attempts have been made to employ drugs that affect the adrenergic system in the treatment of myometrial contractility disorders, with special attention to premature labour. Currently, β_2 -adrenergic-receptor (β_2 -AR) agonists are among the substances most frequently used as tocolytics; however, controversy surrounds their efficacy, especially when they are administered in prolonged therapies (Lampert *et al.*, 1993; Katz and Farmer, 1999; Rosenberg, 2001). In the rat, besides the β_2 -ARs, the α_1 -adrenergic receptors (α_1 -ARs) have been found to have a great impact on myometrial contractility (Legrand and Maltier, 1986; Zupkó *et al.*, 1997). This gave the initiative for a series of new investigations exploring the possible scientific and therapeutic significance of these adrenergic receptors.

In the pregnant rat, the α_1/β_2 -AR density ratio increases towards the end of pregnancy, this increase is mainly a consequence of an elevated α_1 -AR density and not a decrease in β_2 -AR density (Gáspár *et al.*, 2001). Further studies have revealed that, of the three subtypes of α_1 -ARs, it is the α_{1A} subtype that is mostly responsible for the increase (Ducza *et al.*, 2002). It has also been reported that blockade of α_{1A} -ARs significantly inhibits contractions of post-partum rat myometrium *in vitro* (Ducza *et al.*, 2001). Subsequent investigations showed that α_{1A} -AR antagonists significantly increase the efficacy of the β_2 -AR

agonists, by raising their maximal contraction-inhibiting effect and markedly decreasing their EC₅₀ (Mihályi *et al.*, 2003).

The investigated α_1 -AR blockers, 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB 4101) and 5-methylurapidil (5-MU) are known to have serotonergic properties too. These substances bind to serotonin 1A (5-HT_{1A}) receptors and exert an agonist effect on them (Schoeffter and Hoyer, 1988; Eltze *et al.*, 1991; Moser, 1991; Chidlow *et al.*, 2001).

Data have previously been published on the interactions between 5-HT_{1A}-receptor agonists and α_1 -AR subtypes (Castillo *et al.*, 1993). The aim of the present study was therefore to clarify whether the serotonergic activities of these subtype-selective α_1 -AR antagonists have any influence on their uterus-relaxant effect.

Materials and methods

All parts of the study involving animal subjects were conducted with the approval of the Ethical Committee of Animal Experiments of the University of Szeged (registration number: 1-74-8/2002).

Mating of the animals

Mature female (180–200 g) and male (240–260 g) Sprague–Dawley (SPRD) 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 h after the possible

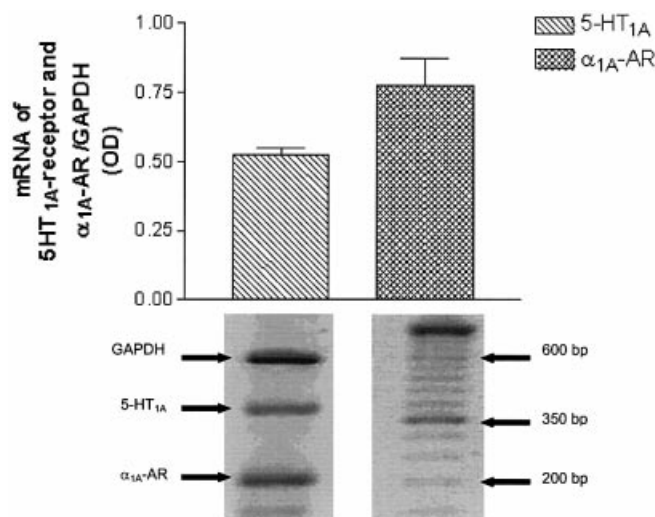


Figure 1. The expression of uterine α_{1A} -AR (212 bp) and 5-HT_{1A}-receptor (388 bp) mRNA on day 22 of pregnancy. Results of the RT-PCR study demonstrate the expressions of α_{1A} -AR mRNA and 5-HT_{1A}-receptor mRNA on day 22 of pregnancy in the rat. The receptor mRNA expression level was normalized to that of GAPDH, and is represented on the y-axis.

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.

Reverse-transcription polymerase chain reaction (RT-PCR) studies

RT-PCR was utilized to determine mRNA expression of the 5-HT_{1A} receptors and the α_{1A} -ARs.

Tissue isolation

Female SPRD rats (250–300 g) were killed by cervical dislocation on day 22 of gestation (end-term). Uterus tissue was rapidly removed. Two myometrial rings from both horns of the uterus were sliced out and prepared for isolated tissue experiments. The remainder was dissected in ice-cold saline (0.9% NaCl) containing 2 U/ml of recombinant ribonuclease inhibitor (RNasin, Promega, UK). The samples 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 (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. The RNA concentration was determined by optical density measurements at 260 nm.

RT-PCR

RNA (0.5 μg) was denatured at 70°C for 5 min in a reaction mixture containing 20 U of RNase inhibitor (Hybaid, UK), 200 $\mu\text{mol/l}$ dNTP (Sigma-Aldrich, Hungary), 20 $\mu\text{mol/l}$ oligo(dT) (Hybaid, UK) in 50 mmol/l Tris-HCl, pH 8.3, 75 mmol/l KCl and 5 mmol/l 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, UK) 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 of cDNA, 25 μl of ReadyMix REDTaq PCR reaction mix (Sigma-Aldrich, Hungary) and 50 pmol/l sense and antisense primer. The primer sequences to amplify the 5-HT_{1A}-receptor mRNA were 5'-CCA AAG AGC ACC TTC CTC TG-3' (for the forward primer) and 5'-TAC

CAC CAC CAT CAT CAT CA-3' (for the reverse primer); these primers were anticipated to generate a 388 bp PCR product (Albert *et al.*, 1989). The primers for the α_{1A} -AR were 5'-GTA GCC AAG AGA GAA AGC CG-3' and 5'-CAA CCC ACC ACG ATG CCC AG-3'; these primers generated a 212 bp PCR product (Schofield *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., UK), 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 95°C , 1 min annealing at 55°C , and 1 min at 72°C . After the last cycle, incubation was continued for 3 min at 72°C , followed by lowering of the temperature to 4°C . PCR products were used immediately or stored at -70°C . The PCR products were visualized by performing the electrophoresis on ethidium bromide (Sigma-Aldrich, Hungary) containing gel. Densitometric scanning of the gel was performed with the KODAK EDAS290 system (Cseretex Ltd, Hungary). The 5-HT_{1A}/GAPDH amplification ratio was calculated for each RNA pool. The α_{1A} -AR/GAPDH ratio was also calculated for each RNA pool.

Isolated tissue studies

Preparation of the tissues

Uterus rings were taken from the above-mentioned 22 day pregnant SPRD rats. Two muscle rings were sliced from both horns of the uterus and mounted vertically between two platinum electrodes in a tissue bath containing 10 ml of de Jongh buffer solution (composition in mmol/l: NaCl, 137; KCl, 3; CaCl_2 , 1; MgCl_2 , 1; NaHCO_3 , 12; NaH_2PO_4 , 4; glucose, 6; pH 7.41). The temperature of the tissue bath was set to and maintained at 37°C , and carbogen (95% O_2 + 5% CO_2) was perfused continuously through the bath. Tissue samples were equilibrated under these conditions for 90 min before the experiments were started. The initial tension of the uterus rings was set to 1.5 g, which dropped spontaneously to 0.5 g by the end of the equilibration period.

Determination of contractility changes (5-HT stimulation).

Noncumulative concentration-response curves were constructed for 5-HT (Sigma-Aldrich, St Louis, MO, USA). The spontaneous contractions of the tissues were recorded for 4 min.

5-HT was then administered to the bath and the contractions were recorded for another 4 min. This procedure was repeated after a 5 min regeneration period during which the tissue samples were washed four times with de Jongh buffer solution. The 5-HT concentration range was 1×10^{-9} – 3×10^{-6} mol/l. Spontaneous contractions were regarded as the control. The contraction increase caused by 5-HT was expressed as a percentage of the control contractions.

In the following step noncumulative concentration-response curves were constructed for 5-HT, but this time in the presence of the subtype-selective α_{1A} -AR antagonists, WB4101 (Tocris-Cookson, Bristol, UK) (Morrow and Creese, 1986; Hieble *et al.*, 1995) and 5-MU (Sigma-Aldrich, USA) (Gross *et al.*, 1988; 1990; Valenta *et al.*, 1990) at both 1×10^{-7} and 1×10^{-6} mol/l. The procedure was similar to that described above, except recording of the control contractions was preceded by a 5 min incubation with the α_{1A} -AR antagonists. Each concentration-response curve was constructed by using newly removed uterus samples. The reason for this was that, following a 30 min regeneration period after the first series of 5-HT stimulation, the changes in the contractions caused by the same concentrations of 5-HT were different as the basal contractions were smaller than in the first series.

The tensions of the myometrial rings were measured with a strain gauge transducer (SG-02; Experimetria Ltd, London, UK) and recorded with an Isosys Data Acquisition System (Experimetria Ltd).

Electrical field stimulation (EFS)

Noncumulative concentration-response curves were constructed for the α_{1A} -AR antagonists, WB4101 and 5-MU. Contractions were elicited by a digital programmable stimulator (ST-02; Experimetria Ltd), using square pulses with a duration of 150 ms and a frequency of 23.75 s. The stimulating potential in each experiment was 40 V. After the previously described equilibration period, the tissue samples were stimulated by EFS for 4 min and the contractions were recorded and regarded as the control. The α_{1A} -AR antagonists were then added to the bath and the contractions were recorded for another 4 min. This procedure was repeated after a 5 min regeneration period, during which the

Table I. Changes in the contraction increasing effect of serotonin (5-HT) in the presence of different concentrations of the subtype-selective α_{1A} -AR antagonists WB 4101 and 5-methylurapidil (5-MU)

		EC ₅₀ (mol/l) (mean \pm SEM)	Level of significance	E _{max} (%) (mean \pm SEM)	Level of significance
1	5-HT	$8.0 \times 10^{-8} \pm 1.9 \times 10^{-8}$	—	437.9 ± 38.9	—
2	5-HT + WB4101 1×10^{-7} mol/l	$4.5 \times 10^{-7} \pm 1.1 \times 10^{-7}$	*	424.0 ± 54.8	NS
3	5-HT + WB4101 1×10^{-6} mol/l	$5.8 \times 10^{-7} \pm 1.8 \times 10^{-7}$	*	328.7 ± 66.3	NS
4	5-HT + 5-MU 1×10^{-7} mol/l	$3.3 \times 10^{-7} \pm 6.2 \times 10^{-8}$	***	384.4 ± 40.7	NS
5	5-HT + 5-MU 1×10^{-6} mol/l	$3.9 \times 10^{-7} \pm 4.8 \times 10^{-8}$	***	301.2 ± 26.2	*

NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

EC₅₀, the concentration of 5-HT producing 50% of its maximal contraction-increasing effect. E_{max}, the maximal contraction-increasing effect of 5-HT in the system.

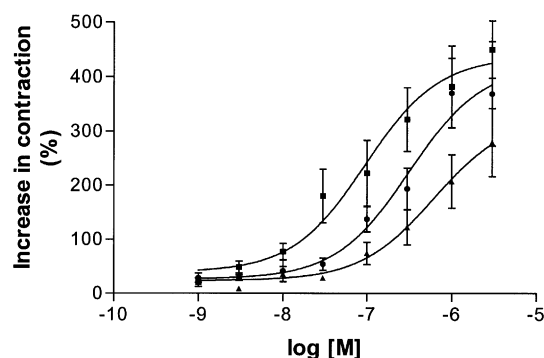


Figure 2. Concentration–response curve of 5-HT alone and in the presence of different concentrations of the subtype-selective α_{1A} -AR antagonist WB 4101. 5-HT (squares) increased the contractility of the pregnant rat myometrium in a concentration-dependent manner. 1×10^{-7} mol/l WB 4101 shifted the concentration–response curve of 5-HT to the right (circles). When WB 4101 was present at 1×10^{-6} mol/l the right-shift of the curve was more explicit (triangles). The y-axis represents the contractions expressed as percentages of the basal contractions.

tissues were washed four times. Following this, noncumulative concentration–response curves were constructed for the α_{1A} -AR antagonists in the presence of 1×10^{-7} and 5×10^{-7} M (*S*)-*N*-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide (WAY100135) (Fletcher *et al.*, 1993a; 1993b), a subtype-selective 5-HT_{1A}-receptor antagonist. The experimental design was similar to the previous one, but the control phase was followed by a 5 min incubation period with WAY100135. Here, and also in the previous case when 5-HT was used to elicit contractions, newly removed uterus rings were used for each concentration–response curve.

Data analysis

The areas under the curves (AUCs) were analysed statistically with Prism 2.01 software (GraphPad Software, San Diego, CA, USA), using ANOVA with the Neuman–Keuls' *post hoc* test. Data are given as the mean \pm SEM and in all experiments $n = 10$.

Results

Receptor mRNA expression

We demonstrated the expression of α_{1A} -AR mRNA and 5-HT_{1A}-receptor mRNA on day 22 of pregnancy in the rat by RT–PCR. Both receptor subtypes are present in pregnant rat myometrium (Figure 1).

Effects of α_{1A} -AR antagonists on 5-HT-induced contractions

5-HT increased the contractions of pregnant rat myometrium in a concentration-dependent manner (Figure 2). The EC₅₀ and the maximal effect of 5-HT are presented in the first row of Table I.

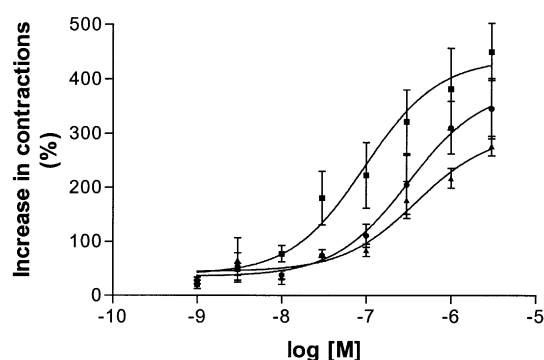


Figure 3. Concentration–response curve of 5-HT alone and in the presence of different concentrations of the subtype-selective α_{1A} -AR antagonist 5-MU. 5-HT (squares) increased the contractility of the pregnant rat myometrium in a concentration-dependent manner. When the concentration–response curve of 5-HT was constructed in the presence of 1×10^{-7} mol/l 5-MU (circles), the α_{1A} -AR antagonist shifted the curve to the right. On increasing the concentration of 5-MU to 1×10^{-6} mol/l, the dose–response curve of 5-HT was further shifted to the right (triangles). The y-axis represents the contractions expressed as percentages of the basal contractions.

WB4101 (1×10^{-7} mol/l) shifted the concentration–response curve of 5-HT to the right (Figure 2), significantly increasing its EC₅₀, but not markedly altering its maximal effect (Table I, row 2). When WB4101 was present at 1×10^{-6} mol/l the right-shift of the concentration–response curve of 5-HT was more explicit (Figure 2) and its EC₅₀ increased further, but the maximal effect was not altered (Table I, row 3).

Similar phenomena were observed when the concentration–response curve of 5-HT was constructed in the presence of 5-MU (1×10^{-7} mol/l). The α_{1A} -AR antagonist shifted the curve to the right (Figure 3) and, as in the previous case, the EC₅₀ of 5-HT was significantly elevated whilst there was no significant change on the maximal effect (Table I, row 4).

Interestingly, when 5-MU was applied at 1×10^{-6} mol/l concentration the dose–response curve of 5-HT was again shifted to the right (Figure 3), but the increase in the EC₅₀ was accompanied by a significant decrease in the maximal effect of 5-HT (Table I, row 5).

Changes in contractility caused by α_{1A} -AR blockade and simultaneous 5-HT_{1A}-receptor blockade

WB4101 inhibited EFS-elicited contractions in a concentration-dependent manner (Figure 4). The EC₅₀ and the maximal contraction-inhibiting effect are presented in the first row of Table II.

5-HT_{1A}-receptor blockade with WAY100135 (1×10^{-7} mol/l) did not change the contraction-inhibiting features of WB 4101. The 5-

Table II. Changes in the contraction inhibiting effects of the subtype-selective α_{1A} -AR antagonists WB 4101 and 5-methylurapidil (5-MU) in the presence of different concentrations of the 5-HT $_{1A}$ -receptor antagonist WAY100135

		EC $_{50}$ (mol/l) (mean \pm SEM)	Level of significance	E $_{max}$ (%) (mean \pm SEM)	Level of significance
1	WB4101	$2.5 \times 10^{-5} \pm 5.2 \times 10^{-6}$	—	76.5 ± 6.4	—
2	WB4101 + WAY100135 1×10^{-7} mol/l	$2.5 \times 10^{-5} \pm 6.9 \times 10^{-6}$	NS	76.2 ± 18.4	NS
3	WB4101 + WAY100135 5×10^{-7} mol/l	$2.0 \times 10^{-5} \pm 2.8 \times 10^{-6}$	NS	73.6 ± 5.6	NS
4	5-MU	$2.9 \times 10^{-6} \pm 9.3 \times 10^{-7}$	—	53.7 ± 9.7	—
5	5-MU + WAY100135 1×10^{-7} mol/l	$3.7 \times 10^{-6} \pm 8.9 \times 10^{-7}$	NS	78.23 ± 11.51	NS
6	5-MU + WAY100135 5×10^{-7} mol/l	$3.8 \times 10^{-6} \pm 8.6 \times 10^{-7}$	NS	89.6 ± 5.4	*

NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
EC $_{50}$, the concentration of the α_{1A} -AR antagonists producing 50% of their maximal contraction inhibiting effect; E $_{max}$: the maximal contraction-inhibiting effect of the α_{1A} -AR antagonists in the system.

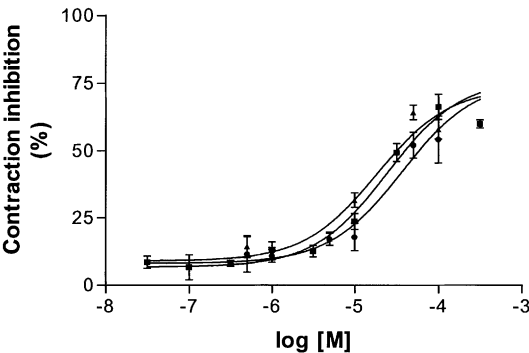


Figure 4. Contraction-inhibiting effect of WB 4101 alone and in the presence of different concentrations of the 5-HT $_{1A}$ -receptor antagonist WAY100135. WB 4101 alone (squares) inhibited the EFS-elicited contractions in a concentration-dependent manner. 5-HT $_{1A}$ -receptor blockade with 1×10^{-7} mol/l WAY100135 changed neither the shape nor the position of the concentration–response curve of WB 4101 (circles). Elevation of the concentration of the 5-HT $_{1A}$ -receptor antagonist to 5×10^{-7} mol/l did not significantly alter the dose–response curve of WB 4101 either (triangles). The y-axis represents the contractions expressed as percentages of the control contractions elicited by EFS.

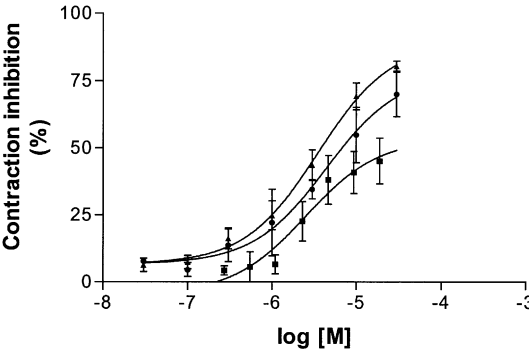


Figure 5. Contraction-inhibiting effect of 5-MU alone and in the presence of 1×10^{-7} and 5×10^{-7} mol/l WAY100135. The subtype-selective α_{1A} -AR antagonist 5-MU (squares) inhibited the EFS-induced contractions of the isolated rat myometrium in a dose-dependent way. In the presence of 1×10^{-7} mol/l WAY100135 (circles) the concentration–response curve of 5-MU was slightly shifted to the right and the top of the curve was higher, though these changes were not significant. When the 5-HT $_{1A}$ -receptor antagonist was applied at 5×10^{-7} mol/l (triangles) the concentration–response curve of 5-MU was further (but still not significantly) shifted to the right; however, the top of the curve was significantly elevated. The y-axis represents the contractions expressed as percentages of the control contractions elicited by EFS.

HT $_{1A}$ -receptor antagonist had no effect on the shape or the position of the concentration–response curve of WB4101 (Figure 4). No significant change was observed regarding the EC $_{50}$ or the maximal contraction-inhibiting effect of WB4101 (Table II, row 2). Raising the concentration of the 5-HT $_{1A}$ -receptor antagonist to 5×10^{-7} mol/l did not significantly alter the dose–response curve of WB4101 either (Figure 4). The EC $_{50}$ and the maximal contraction-inhibiting effect of the α_{1A} -AR antagonist remained unchanged (Table II, row 3).

The other subtype-selective α_{1A} -AR antagonist, 5-MU, also proved to inhibit the EFS- induced contractions of the isolated rat myometrium in a dose-dependent way (Figure 5). The EC $_{50}$ and maximal inhibitory effect are given in the fourth row of Table II. In the presence of WAY100135 (1×10^{-7} mol/l) the concentration–response curve of 5-MU was shifted slightly to the right (Figure 5), but no essential change was observed in the EC $_{50}$. The maximal contraction-inhibiting effect of 5-MU showed an increase in the presence of WAY100135 (1×10^{-7} mol/l), but statistically this change did not prove to be significant (Table II, row 5). When the 5-HT $_{1A}$ -receptor antagonist was applied at 5×10^{-7} mol/l concentration the concentration–response curve of 5-MU was shifted further to the right (Figure 5). The EC $_{50}$ of 5-MU did not display a considerable deviation in this case either, but the maximal contraction-inhibiting effect of the α_{1A} -AR antagonist increased to a greater degree, and this increase was statistically significant (Table II, row 6).

Discussion

Recent studies have shown that the subtype-selective α_{1A} -AR antagonists have a pronounced relaxant effect on the pregnant rat uterus (Ducza *et al.*, 2002) and they appreciably potentiate the effects of β_2 -AR agonists (Mihályi *et al.*, 2003). These promising results may afford an adequate basis for a new approach in the treatment of preterm delivery, which is still a leading cause of perinatal morbidity and mortality, costing approximately 13 million lives annually (Althabe *et al.*, 1999).

The existence of a close relationship between the serotonergic and adrenergic systems has been supported by a number of studies (Castillo *et al.*, 1993). However, the data available concerning this relationship regarding the myometrium are limited, and the information relating to the different receptor subtypes involved in the relationship between the two systems is incomplete. With regard to the severity of the problem of preterm labour and the possible benefits of the use of subtype-selective α_1 -AR antagonists, it seemed to be particularly important to elucidate the role of the serotonergic features of these substances.

5-HT itself increased the contractility of the pregnant rat myometrium. Both α_{1A} -AR antagonists inhibited the contraction-increasing effect of 5-HT. When 5-HT was administered in the presence of 1×10^{-7} mol/l WB4101, its concentration–response curve

was shifted to the right, and when the concentration of WB4101 was increased by one magnitude the shift was even greater. In parallel with this, the maximal effect of 5-HT did not change significantly. These results suggest that there is a competitive antagonism between 5-HT and WB4101. Furthermore, since WB4101 binds to α_{1A} -ARs with greater affinity than to 5-HT $_1$ A receptors, these results suggest that the contractility-increasing effect of 5-HT in the rat myometrium is (at least partially) mediated by α_{1A} -ARs. Similarly, when the concentration–response curve of 5-HT was constructed in the presence of 1×10^{-7} mol/l 5-MU, the curve was moved to the right, and elevation of the concentration of 5-MU to 1×10^{-6} mol/l further increased this right-shift. However, when 5-HT was applied together with 5-MU, the coadministration led not only to a right-shift of the curve but also to a decrease in the maximal effect of 5-HT. These observations suggest that the serotonergic activities of the two α_{1A} -AR antagonists differ substantially. To determine the difference between the substances in this respect, the dose–response curves of the α_{1A} -AR antagonists were constructed alone and in the presence of the subtype-selective 5-HT $_1$ A-receptor antagonist WAY100135, EFS being used to elicit contractions. The contraction-inhibiting characteristics of WB4101 were not changed when the 5-HT $_1$ A-receptor antagonist at 1×10^{-7} mol/l was added. When a five times larger concentration of WAY100135, 5×10^{-7} mol/l, was applied, it did not alter the uterus-relaxant effect either. This again indicates that WB4101 inhibits contractions of the pregnant rat myometrium through α_{1A} -ARs. Since WB4101 inhibited the 5-HT-induced contractions, these results strengthen the hypothesis that 5-HT-elicited contractions may also be mediated by α_{1A} -ARs in the rat myometrium.

In contrast, the contraction-inhibiting properties of 5-MU changed when it was applied together with the 5-HT $_1$ A-receptor antagonist. Even in the presence of 1×10^{-7} mol/l WAY100135, 5-MU exhibited an increased maximal contraction-inhibiting effect. Elevation of the concentration of the 5-HT $_1$ A-receptor antagonist to 5×10^{-7} mol/l resulted in an even greater increase in the maximal effect of 5-MU, this increase proving to be statistically significant.

Impeding the serotonin agonist effect of 5-MU by 5-HT $_1$ A-receptor blockade raised the maximal contraction-inhibiting effect of 5-MU to $89.6 \pm 5.4\%$, which is even higher than the maximal effect of WB4101, $76.5 \pm 6.4\%$. These results suggest that the serotonergic activity of 5-MU is more pronounced than that of WB4101. The different chemical structures may offer an explanation for the noteworthy difference between the serotonergic properties of the two α_{1A} -AR antagonists. It seems probable that 5-MU has a dual influence on the pregnant rat myometrium: a dominant α_{1A} -AR antagonist effect that inhibits contractions, and a less expressed agonist effect on the 5-HT $_1$ A receptors that increases the contractility of the pregnant rat uterus.

Although subtype-selective α_{1A} -AR antagonists are not yet used in pharmacotherapeutic practice, the data obtained on these substances so far suggests that they could improve the armamentarium of tocolytic agents. The results of the present study allow the assumption that in the future, subtype-selective α_{1A} -AR antagonists should be designed with no or only negligible 5-HT $_1$ A-agonist activity so as to provide a maximal contraction-inhibiting effect.

Since the demand for highly specific, subtype-selective substances is great not only in the field of obstetrics but in other areas as well, the serotonergic activities of α_1 antagonists and especially the α_{1A} antagonists should be investigated, as this may alter the efficacy of such substances and could interfere with their side-effects.

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