Clinical and Experimental Pharmacology and Physiology (2007) **34**, 42–47 doi: 10.1111/j.1440-1681.2007.04529.x

EFFECT OF α_1-ADRENOCEPTOR SUBTYPE-SELECTIVE INVERSE AGONISTS ON NON-PREGNANT AND LATE-PREGNANT CERVICAL RESISTANCE *IN VITRO* IN THE RAT

Zoltán Kolarovszki-Sipiczki,* Róbert Gáspár,* Eszter Ducza,* Eszter Páldy,† Sándor Benyhe,† Anna Borsodi† and George Falkay*

**Department of Pharmacodynamics and Biopharmacy, University of Szeged and †Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary*

SUMMARY

1. The aim of the present study was to compare and elucidate the effects of α_1-adrenoceptor (α_1-AR) subtype-selective inverse agonists on non-pregnant and late-pregnant rat cervical tone.

2. Cervical resistance was investigated in *in vitro* stretching tests in the absence or presence of α_1-AR subtype-selective inverse agonists (WB 4101, AH 11110A and BMY 7378; all at 10^{-6} mol/L), whereas the mRNA levels and density of the α_1-AR subtypes and the G-protein-activating effects of the inverse agonists were determined by reverse transcription–polymerase chain reaction, western blot and [^{35}S]-GTPγS binding techniques, respectively.

3. The inverse agonists did not cause any change in resistance in non-pregnant and 18-day-pregnant samples. WB 4101 increased cervical resistance from Day 20, whereas AH 11110A had no effect and BMY 7378 exhibited such an action only on Day 21. Phenylephrine (10^{-4} mol/L) had no effect on cervical resistance on Day 22. The mRNA levels and density of all α_1-AR subtypes were increased on Day 18, but no further changes were observed after that. The [^{35}S]-GTPγS binding studies revealed increased G-protein activation of α_{1A}-AR and a moderate G-protein activation of α_{1B}- and α_{1D}-AR. The effect of WB 4101 to increase [^{35}S]-GTPγS binding was blocked by pertussis toxin (50 ng/mL). Phenylephrine caused a slight and significant decrease in the amount of activated G-protein on Day 22.

4. The effects of inverse agonists on the α_{1A}-AR can enhance cervical resistance in the late-pregnant rat *in vitro*. This action is mediated, at least in part, by a pertussis toxin-sensitive G_i-protein. This effect of the α_{1A}-AR inverse agonist WB 4101 may offer a new therapeutic target in the prevention of premature labour.

Key words: α_1-adrenoceptor inverse agonists, α_1-adrenoceptor subtypes, cervical resistance, cervical ripening, G_i-protein, pertussis toxin, premature labour, [^{35}S]-GTPγS binding.

INTRODUCTION

During pregnancy, the uterus undergoes dramatic changes. Although the contractility of the corpus is steeply increased at term, the role of the cervix shifts between two opposing functions. In the early phase of pregnancy, the cervix has to resist tension and remain closed to protect the fetus. However, at term, the cervical tissue is ripened in order to accommodate stretching and delivery. The control of cervical softening is a very complex process and is only partially understood. It is known that progesterone,[1] oestrogens,[2] prostaglandins,[3] cytokines[4] and nitric oxide[5] all play essential roles in this process. It has also been clarified that oestrogens and progesterone alter the density of adrenoceptors,[6,7] but little is known about the role of these adrenoceptors in cervical ripening. Early softening of the cervical tissue could be an important step leading to preterm labour; accordingly, enhancement of cervical resistance could be beneficial in the therapy of premature birth.

The density of α_1-adrenoceptors in the rat myometrium was found to be increased by the end of gestation, which suggests that these adrenoceptors are involved in the increase of uterine contractility.[8] Cloning and pharmacological data have revealed that α_1-adrenoceptors can be classified into three subtypes: α_{1A}, α_{1B} and α_{1D}.[9] The α_1-adrenoceptors play an important role in smooth muscle contraction and hypertrophic growth.[10,11] It has also been demonstrated that α_1-adrenoceptor antagonists can reduce contractions of isolated pregnant uteri from the rat.[12] However, no study has been performed to investigate the effects of α_1-adrenoceptor subtype-selective antagonists on the resistance of pregnant cervical smooth muscle.

Characterization of the pharmacological properties of α_1-adrenoceptor ligands indicates that several antagonists appear to have inverse agonist properties at these receptors. They are thought to reduce the functional activity of the receptors below the baseline activity observed in the absence of any ligand. Conversely, neutral antagonists block receptors without affecting basal function. Many α_1-adrenoceptor subtype-selective antagonists, such as WB 4101 (α_{1A}), AH 11110A (α_{1B}) and BMY 7378 (α_{1D}), are to be known inverse agonists at concentrations of 10^{-8} to 10^{-5} mol/L.[13–16]

The aim of the present study was to investigate and elucidate the effects of the α_1-adrenoceptor subtype-selective inverse agonists WB 4101, AH 11110A and BMY 7378 and the α_1-adrenoceptor agonist phenylephrine on isolated cervices from non-pregnant and late-pregnant (Day 18–22) rats *in vitro*. Cervical resistance was characterized by tissue extensibility to mechanical stretching. The mRNA and protein levels of the α_1-adrenoceptor subtypes were

Correspondence: George Falkay, Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary. Email: falkay@pharma.szote.u-szeged.hu

Received 31 March 2006; revision 12 May 2006; accepted 13 June 2006.

determined by using reverse transcription–polymerase chain reaction (RT-PCR) and western blot analysis, respectively. The level of G-protein activation coupled to α_1-adrenoceptor subtypes was investigated via the [^{35}S]-guanosine-5′-*O*-(3-thiotriphosphate) ([^{35}S]-GTPγS) binding assay.

METHODS

All experiments involving animals were performed with the approval of the Hungarian Ethical Committee for Animal Research (registration no. IV/1813-1/2002), which is in harmony with the control of the European Union.

Animal mating

Mature female (180–200 g) and male (240–260 g) Sprague-Dawley rats were mated in a special mating cage. A metal door, which was movable by a small electric engine, separated the rooms for the male and female animals. A timer controlled the function of the engine. Because rats are usually active at night, the separating door was opened before dawn. Within 4–5 h 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. If the search proved positive, or if smear taking was impossible because of an existing vaginal sperm plug, female rats were separated and were regarded as first-day pregnant animals.

Measurement of cervical resistance

Cervical tissues were removed from non-pregnant and late-pregnant (gestational Day 18, 20, 21 or 22) rats. The cervix was defined as the least vascular tissue with two parallel lumina between the uterine horns and the vagina. The two cervical rings were separated and mounted, with their longitudinal axis vertical, on hooks in an organ bath containing 10 mL de Jongh buffer (composition (in mmol/L): NaCl 137; KCl 3; $CaCl_2$ 1; $MgCl_2$ 1; $NaHCO_3$ 12; NaH_2PO_4 4; glucose 6, pH 7.4). The organ bath was maintained at 37°C and carbogen (95% O_2 + 5% CO_2) was bubbled through it. The lower sides of the cervices were fixed to the bottom of the tissue holders in the organ chambers, whereas the upper parts were hooked to gauge transducers (SG-02; Experimetria, Budapest, Hungary). After mounting, the rings were allowed to equilibrate for approximately 1 h before experiments were undertaken, with a buffer change every 15 min. The initial tension was set to approximately 1.00 g.

The cervical resistance was investigated by gradually increasing the tension in the tissues, as described previously.[17] The changes in cervical tension were followed with an online computer, using the SPEL Advanced Isosys Data Acquisition System (Experimetria).

When drug effects were investigated, drugs were added to the organ bath and the cervices were incubated for 5 min before stretching. The drugs investigated were as follows: (i) 10^{-6} mol/L WB 4101 (10^{-8} to 10^{-4} mol/L, with or without 10^{-6} mol/L phentolamine on Day 22), AH 11110A and BMY 7378; or (ii) 10^{-4} and 10^{-6} mol/L phenylephrine, a selective α_1-adrenoceptor agonist, in the presence of 10^{-5} and 10^{-7} mol/L propranolol, respectively, to inhibit stimulation of β_2-adrenoceptors. Data were analysed using Prism 4.00 (GraphPad Software, San Diego, CA, USA). Statistical evaluations were made using ANOVA and the Neuman–Keuls' test.

When the effects of WB 4101 on basal (2 g precontraction at the beginning of the incubation period) and precontracted (5 g precontraction at the beginning of the incubation period) cervical tension were investigated, cumulative concentration–response curves for WB 4101 were constructed over the concentration range 10^{-8} to 10^{-4} mol/L for 22-day-pregnant cervices.

Reverse transcription–polymerase chain reaction studies

Cervical tissues from non-pregnant and pregnant animals were removed on gestational Days 18, 20, 21 and 22 (n = 6 on each day), frozen in liquid nitrogen and then stored at −70°C until total RNA extraction.

Total cellular RNA was isolated by extraction with acid guanidinium thiocyanate–phenol–chloroform according to the methods of Chomczynski and Sacchi.[18] After precipitation with isopropanol, RNA was washed three times with ice-cold 75% ethanol and then dried. The pellet was resuspended in 100 μL DNase- and RNase-free distilled water. The RNA concentrations of the samples were determined from their absorbance at 260 nm.

The RNA (0.5 μg) was denatured at 70°C for 5 min in a reaction mixture containing 20 μmol/L oligo(dT) (Invitrogen, Paisley, UK), 20 U RNase inhibitor (Invitrogen), 200 μmol/L dNTP (Sigma-Aldrich, Budapest, Hungary) 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 20 μL. After the mixture had been cooled to 4°C, 20 U M-MLV reverse transcriptase (Gibco, Paisley, UK) and 40 IU/mL RNase H Minus (Invitrogen) was added and the mixture was incubated at 37°C for 60 min.

The PCR was performed with 5 μL cDNA, 25 μL ReadyMix REDTaq PCR reaction mix (Sigma-Aldrich) and 50 pmol of each of the forward and reverse primers. The primer sequences used to amplify the α_{1A}-adrenoceptor 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 a 212 bp PCR product. For the rat α_{1B}-adrenoceptor cDNA, a 300 bp PCR product resulted with the forward primer 5′-GCT CTT CTA CAT CCC GCT CG-3′ and the reverse primer 5′-AGG GGA GCC AAC ATA AGA TGA-3′. The primers for the α_{1D}-adrenoceptor 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).[19] A rat β-actin probe was used as an internal control in all samples.[20] The PCR was performed with a PCR Sprint thermal cycler (Hybaid, Ashford, 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 94°C and annealing at 54°C (α_{1B}- and α_{1D}-adrenoceptors) or 50°C (α_{1A}-adrenoceptor) for 1 min and at 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. The RT-PCR products were separated on 2% agarose gels, stained with ethidium bromide and photographed under an ultraviolet transilluminator. Quantitative analysis was performed by densitometric scanning of the gel using a Kodak EDAS290 (Csertex, Budapest, Hungary). For statistical evaluations, data were analysed by ANOVA with the Neuman–Keuls' post test.

Western blot analysis

In these analyses, 20 μg protein/well was subjected to electrophoresis on 10% sodium dodecyl sulphate polyacrylamide gels in Series Standard Dual Cooled Units (Bio-Rad, Budapest, Hungary). Proteins were transferred from gels to nitrocellulose membranes (Scheicher and Schuell, Darmstadt, Germany) using a semidry blotting technique (Bio-Rad). Membranes were blocked with 5% non-fat dry milk in Tris saline buffer (50 mmol/L Tris, pH 7.4, 200 mmol/L NaCl) containing 0.1% Tween overnight at 4°C. After washing, blots were incubated for 1 h on a shaker at room temperature with α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor and β-actin polyclonal antibodies (1 : 200; Santa Cruz Biotechnology, Santa Cruz, VA, California) in blocking buffer. Antibody binding was detected with a Western Breeze Chromogenic Western blot immunedetection kit (Invitrogen). Quantitative analysis was performed by densitometric scanning of the gel with a Kodak EDAS290 (Csertex). For statistical evaluations, data were analysed by ANOVA with the Neuman–Keuls' post test.

[^{35}S]-GTPγS binding assay

Rat cervix membrane fractions (approximately 10 μg protein/sample) were incubated at 30°C for 60 min in Tris-EGTA buffer (composition (in mmol/L): Tris-HCl 50; EGTA 1; $MgCl_2$ 3; NaCl 100, pH 7.4) containing 20 MBq/0.05 mL [^{35}S]-GTPγS (0.05 nmol/L) and increasing concentrations (10^{-10} to 10^{-6} mol/L) of WB 4101, AH 11110A, BMY 7378 or phenylephrine in the presence of excess GDP (30 pmol/L) in a final volume of 1 mL, as described by Sim *et al.*[21] and Traynor and Nahorski[22] with slight modifications. The G_i-protein activating effect of WB 4101 was measured in the presence of 500 ng pertussis toxin. Non-specific binding was determined with 10 μmol/L

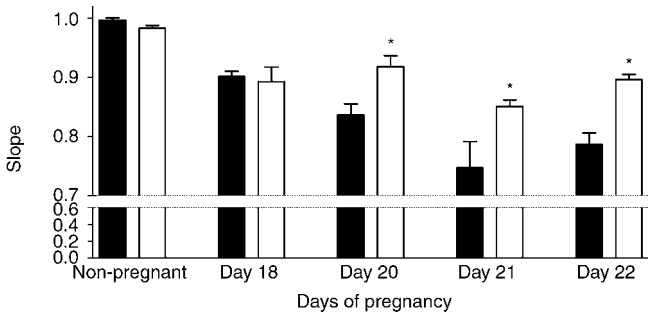

Fig. 1 Effects of the α_{1A}-adrenoceptor subtype-selective antagonist 10^{-6} mol/L WB 4101 (□) on the cervical resistance of non-pregnant and late-pregnant rat cervices *in vitro* ($n = 6$). Resistance is expressed as the slope of the regression lines fitted to the stress–strain curves. (■), slopes from non-treated (control) cervices. *$P < 0.05$ compared with resistance of the control sample for the same day.

GTPγS and subtracted. Bound and free [^{35}S]-GTPγS were separated by vacuum filtration through Whatman GF/B filters (Whatman, Dassel, Germany) with a Millipore manifold (Millipore, Budapest, Hungary). Filters were washed with three times with 5 mL ice-cold buffer and the radioactivity of the dried filters was determined in a toluene-based scintillation cocktail in a Wallac 1409 scintillation counter (Turku, Finland). The percentage stimulation caused by WB 4101 was plotted against the concentration of the drug. Concentration–response curves were fitted and the concentrations eliciting the maximum effect (E_{max}) were calculated and analysed statistically using ANOVA and the Neuman–Keuls' test.

RESULTS

In isolated organ bath studies, we found no extensibility of the non-pregnant cervices. The pregnant cervical resistance decreased continuously from Day 18 towards term, reaching minimum values on Days 21 and 22 (Fig. 1). Compared with non-treated cervices, WB 4101 had no effect on non-pregnant and 18-day-pregnant tissues, but increased cervical resistance from Day 20 to Day 22.

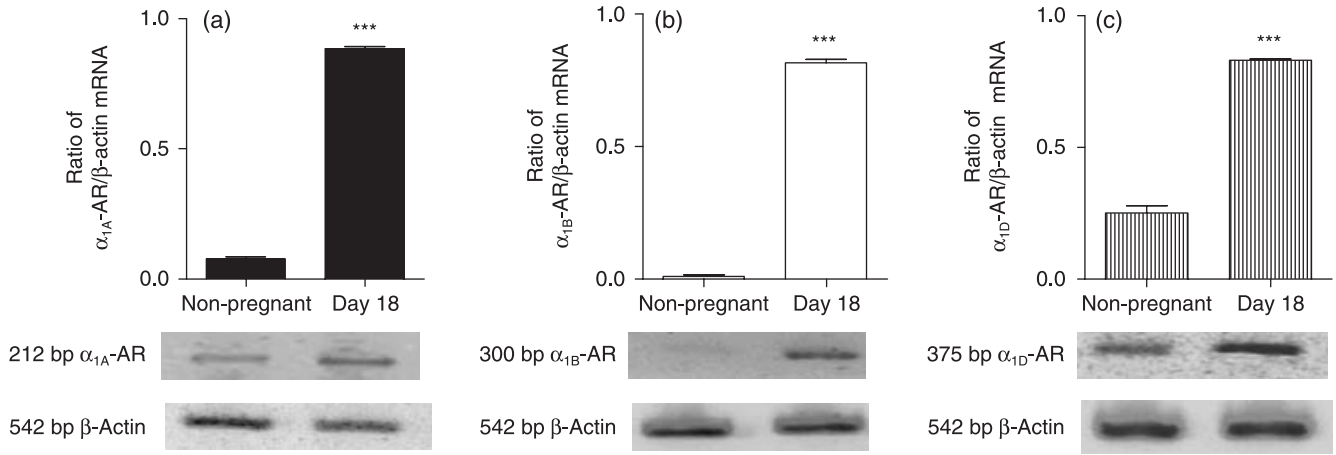

Fig. 2 Changes in mRNA levels of α_1-adrenoceptor (AR) subtypes in non-pregnant and 18-day pregnant rat cervices. (a) α_{1A}-Adrenoceptors; (b) α_{1B}-adrenoceptors; (c) α_{1D}-adrenoceptors. Each band contains RNA from the cervix of one animal. The reverse transcription–polymerase chain reaction products were stained with ethidium bromide and photographed under an ultraviolet transilluminator. Semiquantitative analysis was performed by densitometric scanning of the gel and result are expressed as the ratio of the optical densities of α_1-adrenoceptor/GAPDH. ***$P < 0.001$ compared with non-preganant samples.

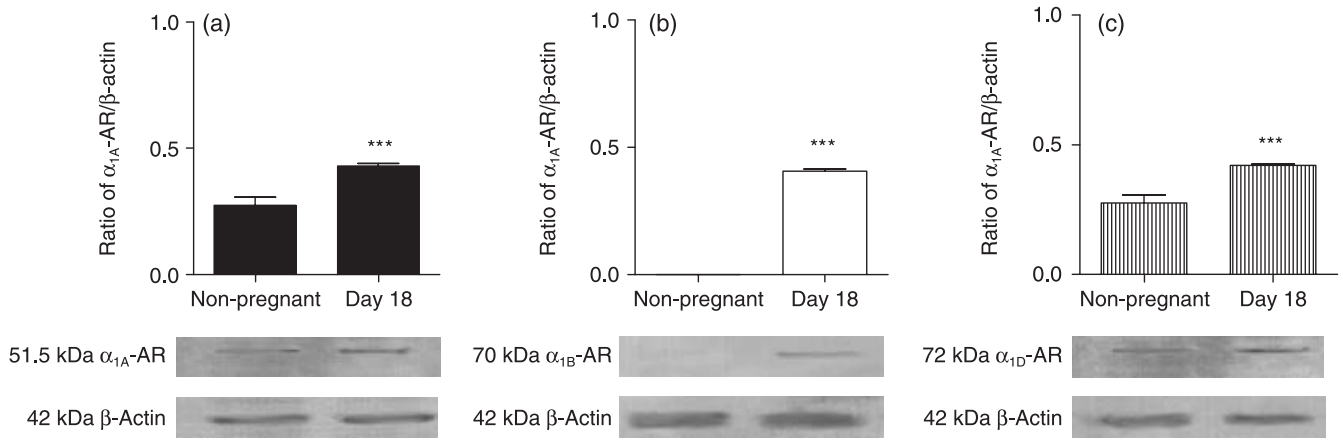

Fig. 3 Changes in α_1-adrenoceptor (AR) levels in non-pregnant and 18-day pregnant rat cervices ($n = 6$). The 51.5, 70, 72 and 42 kDa western blot products are the α_{1A}-adrenoceptor, α_{1B}-adrenoceptor, α_{1D}-adrenoceptor and β-actin, respectively. (a) α_{1A}-Adrenoceptors; (b) α_{1B}-adrenoceptors; (c) α_{1D}-adrenoceptors. Samples were subjected to gel electrophoresis on a 10% polyacrylamide gel. Antibody binding was detected with an enhanced chemiluminescence detection system and expressed as a ratio of the optical densities of α_1-adrenoceptor/β-actin. ***$P < 0.001$ compared with non-preganant samples.

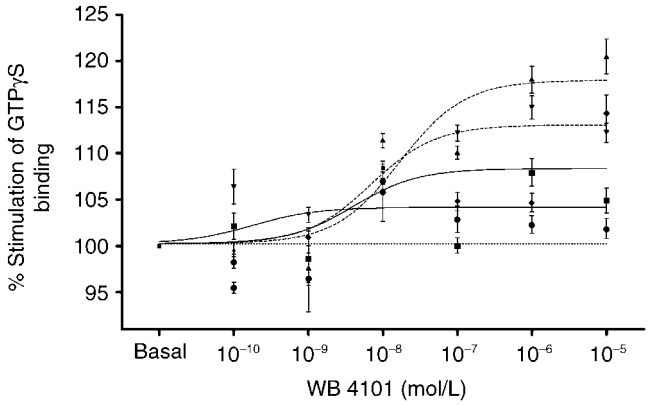

Fig. 4 Changes in [^{35}S]-GTPγS binding following treatment with the α_{1A}-adrenoceptor subtype-selective inverse agonist WB 4101 in non-pregnant and late-pregnant rat cervical membranes. The percentage stimulation caused by the compound was plotted against the concentration of the drug. Basal refers to the value of [^{35}S]-GTPγS binding in the absence of WB 4101. Data are given as the percentage stimulation over basal (non-stimulated taken as 100%). The rising curves indicate increased G-protein activation following the addition of WB 4101 to the cervical membrane preparations. (▲), Day 22; (▼), Day 21; (◆), Day 20; (■), Day 18; (●), non-pregnant.

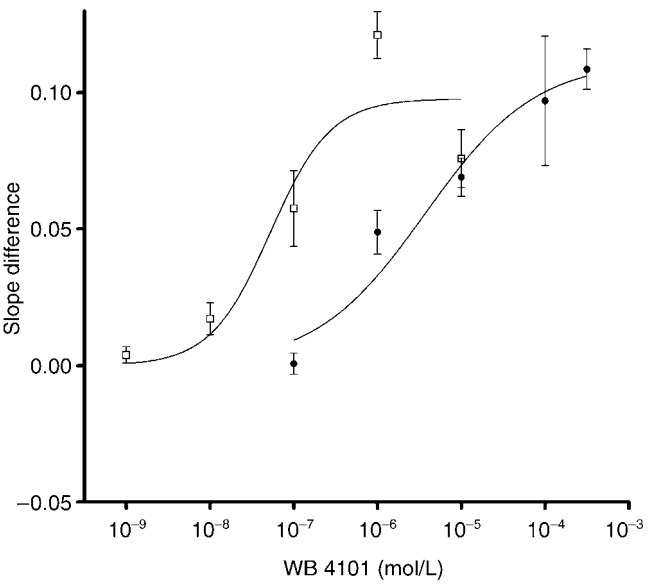

Fig. 5 Effect of phentolamine on the effect of WB 4101 in increasing cervical resistance in 22-day-pregnant cervices. The effect of WB 4101 on cervical resistance was expressed as the slope difference, calculated by subtraction of the average slope (0.7867) for non-treated samples from that for treated samples. The drug elicited a concentration-dependent increase in slope difference (□). True antagonists, such as phentolamine, which is an α_1-adrenoceptor antagonist, can antagonize the effects of inverse agonistic drugs. In the presence of 10^{-6} mol/L phentolamine (●), the slope-difference curve was shifted to the right, without a significant change in its maximum value, suggesting an α-adrenoceptor-mediated action of WB 4101 on the cervical resistance.

This effect was most marked on Days 21 and 22 (Fig. 1). AH 11110A and BMY 7378 had no effect on the cervical resistance *in vitro*, except on Day 21, when BMY 7378 treatment resulted in a weak (but significant) increase in cervical resistance (data not shown).

We demonstrated the expression of α_1-adrenoceptor subtype mRNA in non-pregnant and late-pregnant rat cervices using RT-PCR. α_{1B}-Adrenoceptors were not present in non-pregnant samples, whereas low levels of α_{1A}- and α_{1D}-adrenoceptor mRNA were detected. On Day 18, there were increases in the mRNA levels of all cervical α_1-adrenoceptor subtypes and no further changes were detected up until the end of pregnancy (Fig. 2). The results of western blot analysis at the level of receptor protein expression were in agreement with the PCR results (Fig. 3).

In the GTPγS binding studies, WB 4101 caused a stimulation of [^{35}S]-GTPγS binding compared with basal values up to Day 22 of pregnancy (Fig. 4). AH 11110A did not activate the G-proteins, whereas BMY 7378 significantly increased [^{35}S]-GTPγS binding only on Day 21 by 93.5% (data not shown).

The effect of WB 4101 in increasing cervical resistance concentration dependent over the range 10^{-8} to 10^{-4} mol/L on Day 22. This concentration–response curve was shifted to the right in the presence of 10^{-6} mol/L phentolamine (Fig. 5). WB 4101 over the concentration range 10^{-8} to 10^{-4} mol/L had no effect on basal and precontracted cervical muscle tone on Day 22. Phenylephrine, a non-selective α_1-adrenoceptor agonist, had no effect on the cervical resistance on Day 22 at concentrations of 10^{-6} and 10^{-4} mol/L.

Phenylephrine caused a slight and significant decrease in the amount of activated G-protein on Day 22. The effect of WB 4101 in increasing [^{35}S]-GTPγS binding was blocked by pertussis toxin (Fig. 6).

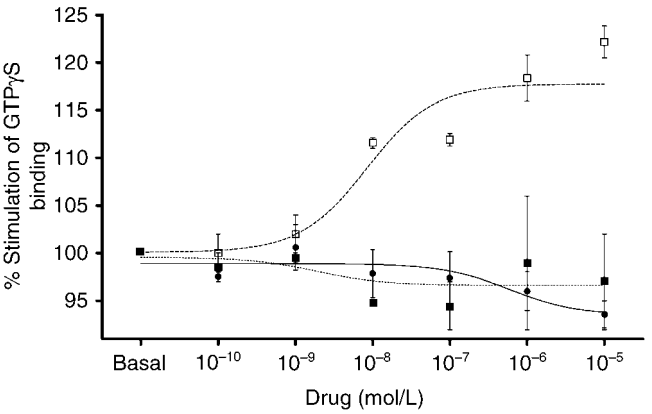

Fig. 6 Changes in [^{35}S]-GTPγS binding following treatment with phenylephrine (●) and WB 4101, with (■) and without (□) pertussis toxin, in non-pregnant and late-pregnant rat cervical membranes. The percentage stimulation caused by the compounds was plotted against the concentration of the drug. Basal refers to the value of [^{35}S]-GTPγS binding without the substance. Data are given as the percentage stimulation over basal (non-stimulated taken as 100%). The α_1-adrenoceptor agonist phenylephrine, at a high concentration, slightly and significantly decreased the activated G-protein level on Day 22. The effect of WB 4101 in increasing [^{35}S]-GTPγS binding was blocked by 50 ng/mL pertussis toxin.

DISCUSSION

The harmonized co-operation between the cervix and the myometrium results in safe delivery. Because premature cervical dilation can contribute to preterm birth of the fetus,[23] compounds that increase cervical resistance could be beneficial in the therapy of premature complications. Although α_1-adrenoceptor inverse agonists are known to decrease uterine contractility,[28,29] no reliable information is available concerning their action on the cervix.

In the present study, of the three inverse agonists examined, only WB 4101, an α_{1A}-adrenoceptor subtype-selective agonist, elicited a considerable increase in cervical resistance to mechanical stretching in the late-pregnant rat cervix. The α_{1B}-adrenoceptor subtype-selective agonist AH 11110A had no effect on cervical resistance, whereas the effects of BMY 7378, an α_{1D}-adrenoceptor-selective agonist, to increase cervical resistance were manifested on Day 21 only.

To clarify the differences in the effects of the different α_1-adrenoceptor inverse agonists on cervical tone, we first determined the presence and changes in α_1-adrenoceptor mRNA and protein expression using RT-PCR and western blot analysis, respectively. Receptor synthesis and expression were found to be elevated in late-pregnant rat cervices compared with non-pregnant samples, but no differences were detected between levels on the investigated days of pregnancy. This elevation itself partially explains the inefficiency of WB 4101 in the non-pregnant cervix, but gives no explanation for the inefficiency of AH 11110A and BMY 7378 on both non-pregnant and pregnant cervices.

For further clarification, we performed [^{35}S]-GTPγS binding studies. α_1-Adrenoceptors are mainly coupled to $G_{q/11}$-protein and mainly activate phospholipase C, thereby increasing the levels of inositol 1,4,5-triphosphate and 1,2-diacylglycerol.[24,25] Conversely, it has been proven that these receptors can be coupled to G_0- or G_i-proteins in some cases.[26,27] The [^{35}S]-GTPγS binding assay measures the level of G-protein activation following antagonist occupation of the G-protein-coupled receptor. This method detects the functional consequence of receptor occupancy in one of the earliest receptor-mediated events. In the assay, [^{35}S]-GTPγS replaces endogenous GTP and binds to the α-subunit of the G-protein (G_α). The γ-thiophosphate bond is resistant to the hydrolysis of G_α by GTPase. Accumulation of the labelled G_α subunits can be measured by counting the amount of [^{35}S] incorporated.[30] This method is most useful for investigating activation of G_i-coupled receptors.

In the present studies of α_{1A}-adrenoceptor occupancy, WB 4101 resulted in a moderate increase in [^{35}S]-GTPγS binding compared with basal values in pregnant samples, whereas no effect was detected in non-pregnant cervices. Stimulation of α_{1B}- and α_{1D}-adrenoceptors was found to have no and a time-dependent effect in the [^{35}S]-GTPγS binding assay, respectively. In subsequent experiments, the effects of WB 4101 were investigated further, whereas no further investigations were performed for the other two inverse agonists.

The smooth muscle content of the cervix is known to be quite low and is further reduced by pregnancy induced apoptosis.[31] Therefore, the possibility that the effect of WB 4101 is not mediated by α_{1A}-adrenoceptors was also considered. In order to create a concentration–response curve, 22-day-pregnant cervical samples were used because the difference in cervical resistance between non-treated and 10^{-6} mol/L WB 4101-treated cervices was highest on this day. In organ bath studies, we proved that the effect of WB 4101 in increasing cervical resistance is concentration dependent and that this effect can be antagonized by the non-selective α-adrenoceptor antagonist phentolamine. This is a further evidence for the inverse agonist property of WB 4101, because true antagonists (e.g. phentolamine) can antagonize the effects of inverse agonists.[32]

WB 4101 is highly selective for α_{1A}-adrenoceptors; accordingly, it can be concluded that the effect of WB 4101 in increasing cervical resistance is predominantly related to these receptors. However, WB 4101 proved ineffective on basal cervical muscle tone when gradual stretching was omitted. These results suggest that WB 4101 increases the resistance of the pregnant cervix against incremental stretching, but apparently does not affect basal smooth muscle tone.

Drugs that behave as inverse agonists commonly decrease both [^{35}S]-GTPγS binding and G-protein activation, whereas real agonists elicit the opposite effects.[30] Conversely, we have demonstrated recently in [^{35}S]-GTPγS binding studies that the β_2-adrenoceptor agonist terbutaline behaves as an inverse agonist in the pregnant rat cervix.[17] Accordingly, it may be concluded that the G-protein-activating properties of agonists and inverse agonists may be reversed in the pregnant rat cervix.

In order to acquire more evidence in this respect, the effect of a real agonist was also tested in our system. Phenylephrine had no effect on cervical resistance *in vitro*, but at a high concentration, it decreased the activated G-protein level. These results support our theory that the activation of G-proteins in the cervical adrenergic system by an agonist or an inverse agonist is controlled in an opposite way compared with that in the myometrium. The ineffectiveness of phenylephrine on cervical resistance can be explained by its very weak G-protein-moderating action.

It is known that the catalytic A subunit (S1) of pertussis-toxin (PTX) transfers the ADP ribosyl moiety of nicotinamide adenine dinucleotide phosphate (NAD) to the membrane-bound regulatory protein G_i, which normally inhibits adenylate cyclase. This inhibitory action of PTX is special for the G_i-protein and enables distinction of the different G-protein-mediated signal transduction pathways. In the present study, PTX inhibited the effect of WB 4101 in increasing [^{35}S]-GTPγS binding (and, thus, G-protein activation); consequently, we assume that the effects of WB 4101 are connected, at least partially, with PTX-sensitive G-proteins, supposedly G_i-proteins, although an interaction with the $G_{q/11}$-protein ($G_{q/11}$) cannot be excluded. We therefore presume that these changes in the level of activated G_i and/or $G_{q/11}$ mediate an intracellular process that is not sufficient to cause a change in basal smooth muscle tone, but can provide a stronger resistance against stretching forces. This effect may offer new perspectives in the therapy of preterm labour. Although the α_{1A}-adrenoceptor inverse agonist WB 4101 is not yet used in obstetric practice, its ability to increase cervical resistance may be applicable in the prevention or treatment of early dilation of the pregnant cervix.

ACKNOWLEDGEMENTS

This study was sponsored by the Szeged Neurobiological Knowledge (DNT) Center, Hungary (project no. RET-08/04). Special thanks are due to Zsuzsanna Magyar and Zsuzsanna Canjavec for technical assistance.

REFERENCES

1. Bienkiewicz A. Influence of progesterone and its antagonist mifepristone (RU 486) on collagen content in uterine cervix in pregnant rats. *Endocr. Res.* 1995; **21**: 615–22.
2. Wang H, Stjernholm Y, Ekman G, Eriksson H, Sahlin L. Different regulation of oestrogen receptors alpha and beta in the human cervix at term pregnancy. *Mol. Hum. Reprod.* 2001; **7**: 293–300.
3. Hertelendy F, Zakar T. Prostaglandins and the myometrium and cervix. *Prostaglandins Leukot. Essent. Fatty Acids* 2004; **70**: 207–22.
4. Sennstrom MB, Ekman G, Westergren-Thorsson G *et al.* Human cervical ripening, an inflammatory process mediated by cytokines. *Mol. Hum. Reprod.* 2000; **6**: 375–81.
5. Vaisanen-Tommiska M, Nuutila M, Aittomaki K, Hiilesmaa V,

Ylikorkala O. Nitric oxide metabolites in cervical fluid during pregnancy: Further evidence for the role of cervical nitric oxide in cervical ripening. *Am. J. Obstet. Gynecol.* 2003; **188**: 779–85.
6. Hurd WW, Riemer RK, Goldfien A, Roberts JM. Prostaglandins modulate hormonal effects on rabbit myometrial alpha 1-adrenergic responses. *Endocrinology* 1991; **123**: 1436–42.
7. Falkay G. Effects of antiprogestogen (RU-486) treatment on myometrial and cervical alpha and beta-adrenoreceptors in pregnant rabbits. *Hum. Reprod.* 1990; **5**: 924–7.
8. Legrand C, Maltier JP. Evidence for a noradrenergic transmission in the control of parturition in the rat. *J. Reprod. Fertil.* 1986; **76**: 415–24.
9. Hieble JP, Bylund DB, Clarke DE *et al.* International Union of Pharmacology X. Recommendation for a nomenclature of α_1-adrenoreceptors consensus update. *Pharmacol. Rev.* 1995; **47**: 267–70.
10. Piascik MT, Perez DM. α_1-Adrenergic receptors: New insight and directions. *J. Pharm. Exp. Ther.* 2001; **298**: 403–10.
11. Ducza E, Gáspár R, Márki Á, Gyula P, Bottka S, Falkay G. Use of antisense oligonucleotides to verify the role of the α_{1A}-adrenergic receptor in the contractility of the rat uterus post partum. *Mol. Pharmacol.* 2001; **59**: 1235–42.
12. Ducza E, Gáspár R, Falkay G. Altered levels of mRNA expression and pharmacological reactivity of α_1-adrenergic receptor subtypes in the late-pregnant rat myometrium. *Mol. Reprod. Dev.* 2002; **62**: 343–7.
13. Noguera MA, Ivorra MD, D'Ocon P. Functional evidence of inverse agonism in vascular smooth muscle. *Br. J. Pharmacol.* 1996; **119**: 158–64.
14. Rossier O, Abuin L, Fanelli F, Leonardi A, Cotecchia S. Inverse agonism and neutral antagonism at α_{1A}- and α_{1B}-adrenergic receptor subtypes. *Mol. Pharmacol.* 1999; **56**: 858–66.
15. McCune DF, Edelmann SE, Olges JR *et al.* Regulation of the cellular localization and signaling properties of the α_{1B}- and α_{1D}-adrenoreceptors by agonists and inverse antagonists. *Mol. Pharmacol.* 2000; **57**: 659–66.
16. Ibarra M, Pardo JP, López-Guerrero JJ, Villalobos-Molina R. Differential response of chloroethylclonidine in blood vessels on normotensive and spontaneously hypertensive rats: Role of α_{1D}- and α_{1A}-adrenoreceptors in contraction. *Br. J. Pharmacol.* 2000; **129**: 653–60.
17. Gáspár R, Kolarovszki-Sipiczki Z, Ducza E *et al.* Terbutaline increases the cervical resistance of the pregnant rat *in vitro*. *Naunyn Schmiedebergs Arch. Pharmacol.* 2005; **371**: 61–71.
18. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* 1987; **162**: 156–9.
19. Scofield MA, Liu F, Abel PW, Jeffries WB. Quantification of steady state expression of mRNA for alpha-1 adrenergic receptor subtypes using reverse transcription and competitive polymerase chain reaction. *J. Pharmacol. Exp. Ther.* 1995; **275**: 1035–42.
20. Murata T, Higuchi T. Progesterone receptor mRNA levels during pregnancy, labor, lactation and the estrous cycle in rat uterus. *J. Reprod. Dev.* 2003; **49**: 425–32.
21. Sim LJ, Selley DE, Childers SR. *In vitro* autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5′-[gamma-[^{35}S]thio-triphosphate binding. *Proc. Natl Acad. Sci. USA* 1995; **92**: 7242–6.
22. Traynor JR, Nahorski SR. Modulation by mu-opioid agonists of guanosine-5′-*O*-(3-[^{35}S]thio) triphosphate binding to membranes from human neuroblastoma SH-SY5Y cells. *Mol. Pharmacol.* 1995; **47**: 848–54.
23. Bouyer J, Papiernik E, Dreyfus J, Collin D, Winisdoerffer B, Gueguen S. Maturation signs of the cervix and prediction of preterm birth. *Obstet. Gynecol.* 1986; **68**: 209–14.
24. Minneman KP. Alpha 1-adrenergic receptor subtypes, inositol phosphates, and sources of cell Ca^{2+}. *Pharmacol. Rev.* 1988; **40**: 87–119.
25. Berridge MJ. Inositol trisphophate and calcium signaling. *Nature* 1993; **361**: 315–25.
26. Gurdal H, Seasholtz TM, Wang H-Y *et al.* Role of $G_{\alpha q}$ or $G_{\alpha 0}$ proteins in α_1-adrenoceptor subtype-mediated responses in Fischer 344 rat aorta. *Mol. Pharmacol.* 1997; **52**: 1064–70.
27. Otani H, Oshiro A, Yagi M, Inagaki C. Pertussis toxin-sensitive and -insensitive mechanisms of alpha$_1$-adrenoceptor-mediated inotropic responses in rat heart. *Eur. J. Pharmacol.* 2001; **419**: 249–52.
28. Zupkó I, Gáspár R, Kovács L, Falkay G. Are α-adrenergic antagonists potent tocolytics? *In vivo* experiments on postpartum rats. *Life Sci.* 1997; **61**: 159–63.
29. Gáspár R, Márki A, Zupkó I, Falkay G. Evidence of non-synaptic regulation of post-partum uterine contractility in the rat. *Life Sci.* 1998; **62**: 1119–24.
30. Zhu J, Taniguchi T, Takauji R, Suzuki F, Tanaka T, Muramatsu I. Inverse agonism and neutral antagonism at a constitutively active alpha-1a adrenoreceptor. *Br. J. Pharmacol.* 2000; **131**: 546–52.
31. Harrison C, Traynor JR. The [^{35}S]GTPγS binding assay: Approaches and applications in pharmacology. *Life Sci.* 2003; **74**: 489–508.
32. Leppert PC, Yu SY. Apoptosis in the cervix of pregnant rats in association with cervical softening. *Gynecol. Obstet. Invest.* 1994; **37**: 150–4.