KÜLÖNLENYOMAT A

Magyar Nőorvosok Lapja

C. FOLYÓIRATBÓL

Magyar Nőorvosok Lapja 63, 377–380 (2000)

A β-adrenerg receptorok spontán deszenzitizációja terhes patkány uteruszon

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A Szegedi Tudományegyetem Gyógyszerésztudományi Kar Gyógyszerhatástani és Biofarmáciai Intézet (igazgató: Falkay György dr. tanszékvezető egyetemi tanár) közleménye

Összefoglalás: A β-mimetikumok tokolitikus terápiában való hatásossága a széles körben való alkalmazás ellenére is vitatott. Ennek egyik fő oka a β-agonistákkal szembeni receptor deszenzitizáció, mely csökkenti e szerek hatékonyságát. Kísérleteinkben azt vizsgáltuk, hogy a deszenzitizáció jelensége végbemegy-e spontán módon a terhesség során. Izolált terhes patkány uterusz modellen azt találtuk, hogy az elektromos erőtér ingerléssel kiváltott ritmikus kontrakciókat a β_2-izgató terbutalin a terhesség előrehaladtával egyre kisebb intenzitással gátolja. A radioligand kötési vizsgálatokban a β-receptorok alacsony és csökkenő tendenciát mutató mennyiségét észleltük. Eredményeink alapján terhes patkány uteruszban a β-receptorok deszenzitizációja spontán végbemegy, mely feltételezhetően a tokolitikus terápia során jelentkező deszenzitizációnak is egyik komponense lehet.

Kulcsszavak: tokolízis, deszenzitizáció, β_2-agonisták

A szülészeti gyakorlatban a tokolízis komoly kihívást jelent. A statisztikák tanúsága szerint a világon a koraszülések száma az elmúlt tíz esztendőben 10% feletti volt, ami évenként mintegy 13 millió koraszülöttet jelent [1].

A tokolitikus terápiában leggyakrabban alkalmazott szerek a β_2-adrenerg receptor izgatók. E vegyületek hatékonysága akut tokolízisben ma is vitatott. Egyes vizsgálatok arról számolnak be, hogy a β-mimetikumok csupán 24–48 óráig képesek megakadályozni a fenyegető koraszülést, más szempontok alapján (koraszülés kockázatának csökkentése, 2500 g alatti súly gyakorisága, respirációs distressz szindróma, perinatális halálozás) pedig nincs szignifikáns különbség a placebo hatáshoz képest [2, 3].

További problémát vet fel a β-agonisták alkalmazásával szemben, hogy hosszabb terápia esetén a β-adrenerg receptorok deszenzitizációjára kell számítani mind a szülészeti, mind pedig a pulmonológiai gyakorlatban [4, 5]. E jelenség lényege, hogy a β-mimetikus kezelés hatására a receptorok érzékenysége és száma csökken, így nagyobb dózisok alkalmazása szükséges [6]. Ennek következtében a β-agonisták valamennyi mellékhatása (tachikardia, aritmia, szíviszkémia, glükóz intolerancia, hipokalémia, Na+-retenció) felerősödik [7].

A deszenzitizáció kapcsán felmerül a kérdés: vajon terhességben a jelenség oka kizárólag a β-mimetikus kezelés, vagy a terhesség előrehaladtával a β_2-adrenerg receptoroknak farmakológiai beavatkozás nélkül is csökken az érzékenysége? A kérdés megválaszolására kísérleteket végeztünk elektromos erőtér ingerlés és radioligand kötési technika módszerével in vitro terhes patkány modellen.

Anyagok és módszerek

Az állatkísérleteket a Szegedi Tudományegyetem Munkahelyi Állatetikai Bizottságának engedélyével végeztük (engedélyszám: 23/1999.).

Az állatok pároztatása

Ivarérett nőstény (180–200 g) és hím (240–260 g) Sprague-Dawley patkányokat pároztattunk egy erre a célra speciálisan gyártott, saját tervezésű pároztató ketrecben. A pároztatás kezdetétől számított 4-5 órán belül a nőstény állatoktól hüvelykenetet vettünk és mikroszkóp alatt 1200-szoros nagyítással hímivarsejteket kerestünk. Amennyiben a keresés pozitív eredménnyel zárult, vagy a hüvelyből az ott lévő spermadugó miatt nem tudtunk kenetet venni, akkor az állatot elkülönítettük, mint 1. napos vemhes nőstényt.

Elektromos erőtér ingerléses vizsgálatok

Az állatok feláldozását követően a vemhesség 15., 18., 20. és 22. napjáról származó uteruszokból 0,5 cm hosszúságú gyűrűket metszettünk, melyeket két platina elektród közé függesztettünk fel és belehelyeztük egy 10 ml térfogatú, kettős falú szervfürdőbe, melybe karbogénnel (95% O_2 + 5% CO_2) folyamatosan átáramoltatott de Jongh puffert (az egyes összetevők koncentrációi mM-ban kifejezve: 137 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 4 NaH_2PO_4, 6 glükóz, pH: 7,4) helyeztünk.

Az elektromos erőtér ingerlést 1,5 g előfeszítéssel és 40 V állandó ingerlő feszültséggel végeztük. Valamennyi terhességi napon maximális ritmikus kontrakciókat váltottunk ki. Ritmikusnak akkor tekintettük a kontrakciókat, ha egy periódusidő (két inger közti időtartam) alatt a szövet egy teljes kontrakció-relaxáció ívet írt le a nyugalmi tónus emelkedése nélkül. Maximálisnak azért neveztük a kontrakciókat, mert az alkalmazható legrövidebb periódusidővel és leghosszabb jelszélességgel (egy inger időtartama) ingereltünk a ritmikusság megtartása mellett. Az egyes napokon alkalmazott jelszélesség és periódusidő értékeket az *1. táblázat* mutatja be.

1. táblázat

A maximális ritmikus kontrakciók kiváltásra használt jelszélesség és periódusidő értékek terhes patkányban

Terhesség napja	jelszélesség (ms)	periódusidő (s)
15	60	23
18	75	18
20	62	17
22	150	24

Az elektromos erőtér ingerlést ST-02 típusú stimulátorral, a kontrakciók mérését SG-02 izometriás mérőfejjel végeztük (mindettő Experimetria Ltd., U.K.). A görbék regisztrálását és feldolgozását ISOSYS Data Acquisition System (Experimetria Ltd., U.K.) segítségével végeztük.

A terbutalin (Astra, Sweden) dózis-hatás görbéit nem kumulatív módon vettük fel. A kontroll és a kezelést követő kontrakciókat egyaránt 240 s-ig vizsgáltuk. A terbutalin egyes dózisait 100 µl térfogatban adtuk a szervfürdőhöz. Az egyes dózisok hatásának mérését követően négyszeres mosást és 5 perces pihentetést alkalmaztunk. A terbutalin hatására a görbe alatti terület (AUC) változásából következtettünk a kontroll AUC-hez viszonyítva.

Az eredmények statisztikai elemzését Prism 2.01. (GraphPad Software, USA) segítségével ANOVA Newman-Keuls teszttel végeztük.

Radioligand kötési vizsgálatok

A radioligand kötési vizsgálatokat terhes patkány uterusz membrán preparátumon végeztük *Maltier* és *Legrand* szerint [8].

A reakcióelegy 100 µl membrán preparátumot (~0,5 mg/ml protein), 100 µl triciált ligandot és 100 µl jelöletlen ligandot tartalmazott a nem specifikus kötés meghatározására, vagy 100 µl inkubációs puffert (0,05 M Tris-HCL, 0,01 M $MgCl_2$, 2,5% etanol, pH=7,42) a totál kötés meghatározására. Az inkubáció a membrán preparátum hozzáadásával indult, majd az elegyet vízfürdőbe helyeztük az egyensúlyi állapot eléréséig (30 °C, 30 perc). Az inkubáció végén a szabad radioligandot Whatman GF/C filteren keresztüli gyors szűréssel távolítottuk el Brandell cell harvester segítségével, majd 3x10 ml jéghideg (4 °C) pufferes (Tris-HCl, pH=7,42) mosást végeztünk. A kötött radioaktivitást Wallac 1409 liquid szcincillációs számlálóban HighSafe szcincillációs koktélban határoztuk meg.

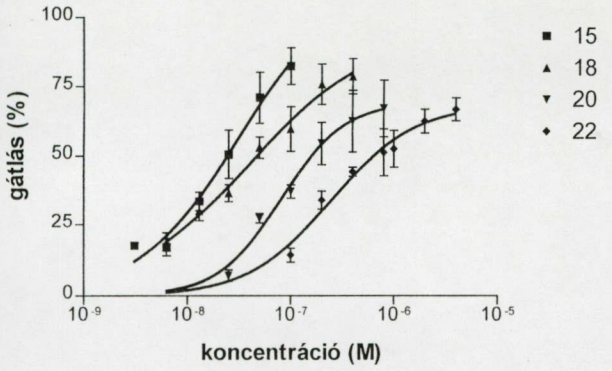
A β-adrenoceptorok telítési analízisét 0,25-10 mM ^{3}[H]-dihidroalprenolollal végeztük 1 µM jelöletlen propranolol jelenlétében vagy anélkül. A specifikus kötést a nem specifikus kötés totál kötésből való kivonásával számítottuk ki. A vizsgált szövetekben a ^{3}[H]-dihidroalprenolol Kd értéke 1,28±0,27 volt. A statisztikai analízist a fent említett módszerrel végeztük.

Eredmények

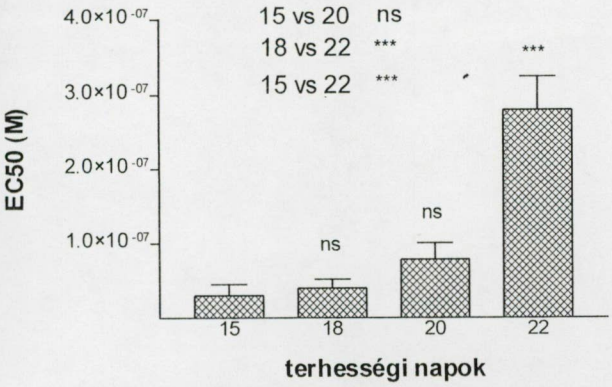
A terbutalin dózisfüggően gátolja az elektromos erőtér ingerléssel létrehozott uterusz kontrakciókat *(1. ábra)*. A terbutalin dózis-hatás görbéi a terhesség előrehaladtával fokozatosan jobbra tolódnak.

Az 50%-os hatást kifejtő terbutalin koncentrációk (EC50) a 15. naptól kezdve fokozatosan növekednek, majd a 22. napon igen jelentős emelkedés tapasztalható *(2. ábra)*.

A terbutalin maximális gátló hatása a 15. naptól egyre csökken, a 22. napra a 75%-os gátlást sem éri el *(3. ábra)*.



1. ábra A terbutalin elektromos erőtér ingerléssel kiváltott uterusz kontrakciót gátló hatása terhes patkányban in vitro



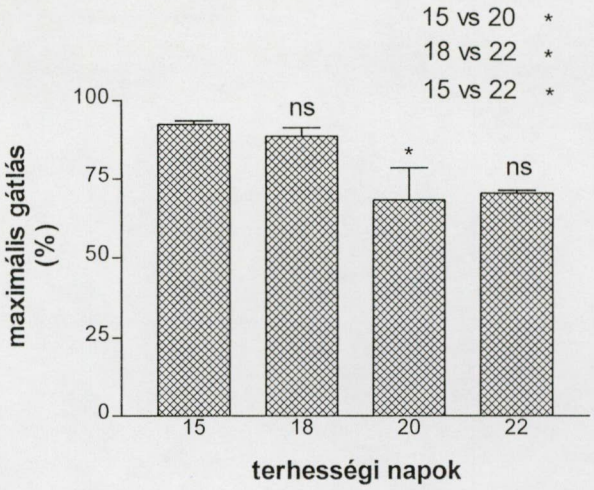
*2. ábra A terbutalin EC50 értékeinek változása a terhesség során patkányban in vitro (ns=nem szignifikáns, ***=p<0,001)*

A β-adrenerg receptorok száma a 18. napon szignifikáns csökkenést mutat a 15. naphoz képest, a terhesség végéig további változás nem tapasztalható *(4. ábra)*.

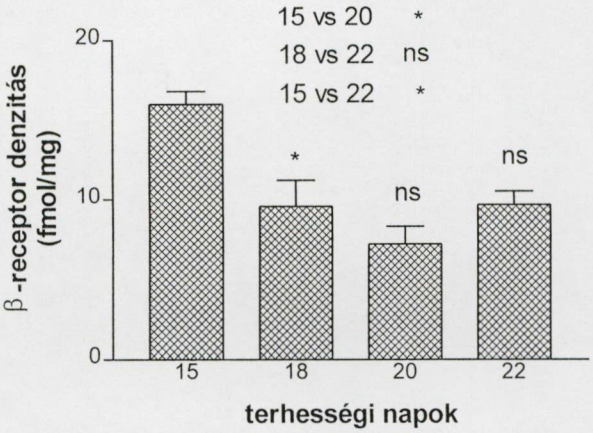
Megbeszélés

A β_2-izgató terbutalin uterusz kontrakciót gátló hatását in vitro terhes patkány modellen vizsgáltuk elektromos erőtér ingerlés módszerével. Patkányban a vemhesség 22 napos, így a terhesség utolsó harmadában (15–22. nap) végeztük a kísérleteket, mely a humán terhességben is a tokolitikus terápia indikációs időszaka. A humán terhes méh kontrakcióihoz hasonlóan ritmikus összehúzódásokat [9] váltottunk ki. Így kísérleti összeállításunk in vitro körülmények között modellezte a szülés során fellépő kontrakciókat.

Kísérleteinkben a β_2-receptor agonista terbutalin a vizsgált valamennyi terhességi napon csökkentette a stimulált kontrakciók intenzitását. A terhesség előrehaladtával azonban mind határerőssége, mind pedig hatékonysága jelentősen csökkent. A hatáserősség csökkenését a terbutalin által kiváltott maximális hatás gyengülése jelezte, különösen a terminushoz közeli időpontokban (20. és 22. nap). E jelenség azt mutatja, hogy patkányban terhesség során β_2-mimetikus előkezelés nélkül is csökken a terbutalin hatása. A terbutalin hatékonyságának csökkenésére az EC50 értékek növekedéséből következtettünk. Egy farmakon EC50 értéke számértékileg megegyezik a farmakon és a farmakológiai receptor közötti disszociációs állandó (Kd) értékével. Minél kisebb ez az érték, annál nagyobb az adott farmakon affinitása a receptorhoz és – agonisták esetében – a hatékonysága is [10]. A terbutalin EC50 értékeinek növekedése a terhesség előrehaladtával azt jelzi, hogy a β_2-adrenerg receptorok érzékenysége csökken a terbutalinnal szemben, így nagyobb dózisok szükségesek ahhoz, hogy ugyanazt a hatást váltsuk ki.



*3. ábra A terbutalin maximális gátló hatásának változása terhes patkányban in vitro (ns=nem szignifikáns, *=p<0,05)*



*4. ábra A β-adrenerg receptor denzitás változása terhes patkány uteruszban (ns=nem szignifikáns, *=p<0,05)*

A receptordenzitási vizsgálatok részben magyarázatot adnak a fent említett jelenségekre, hiszen az eredmények azt mutatják, hogy patkány

uteruszban a terhesség végén a β-receptorszám igen alacsony, amelyben még csökkenő tendencia is megfigyelhető. Ugyanakkor a receptorszám-változás a terbutalin hatásában bekövetkező változásokat nem teljesen követi, így ez csak egy tényezője lehet a deszenzitizációnak.

Eredményeink tükrében úgy gondoljuk, hogy vemhes patkányban a terhesség utolsó harmadában az uterusz β_2-adrenerg receptorainak érzékenység csökkenése spontán bekövetkezik, melyben részben szerepet játszik a receptorok alacsony száma is. Bár az állatkísérletes adatokból humán következtetéseket csak igen nagy óvatossággal lehet levonni, mégis feltételezzük, hogy eredményeink további magyarázatot adnak a β-mimetikus hatás csökkenésére a tokolitikus terápiában.

Köszönetnyilvánítás

A szerzők köszönetüket fejezik ki *Czinkotáné Nagy Judit* és *Csiszár Zoltánné* asszisztenseknek a kísérletekhez nyújtott segítségükért.

Köszönjük a Művelődési és Közoktatási Minisztérium pályázati támogatását (regisztrációs szám: MKM FKFP 0618/1999).

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Spontaneous desensitization of the β-adrenergic receptors in pregnant rat uterus

Inspite of their widespread use the efficacy of β-mimetics is debated in tocolytic therapy. One of the main reasons for this is the receptor desensitization against the β-agonists, a process which decreases their efficacy. We investigated whether this desensitization process also occurs spontaneously during pregnancy. It was found that towards the end of pregnancy contractions stimulated by electric field on isolated rat uterus were inhibited with only a reduced intensity by the β_2-agonist terbutaline. In radioliogand binding studies the small density of the β-receptors was shown to decline in their numbers. We conclude that desensitization of the β-receptors occurs even spontaneously in the pregnant rat uterus. This process is assumed to contribute to the human receptor desensitization during tocolytic therapy.

Key words: tocolysis, desensitization, β_2-agonists

Közlésre elfogadva: 2000. április 4.

Clinical and Experimental Pharmacology and Physiology (2003) 30, 164–167

SYNERGISM BETWEEN β_2-ADRENOCEPTOR AGONISTS AND SUBTYPE-SELECTIVE α_{1A}-ADRENOCEPTOR ANTAGONISTS IN THE TOCOLYTIC EFFECT ON PREGNANT RAT UTERUS *IN VITRO*

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SUMMARY

1. Despite great efforts in recent decades, premature birth is still a leading cause of perinatal morbidity and mortality. β_2-Adrenoceptor agonists are frequently used as tocolytics, although their use is rather controversial. Previous animal studies have revealed that blockade of α_{1A}-adrenoceptors results in relaxation of the pregnant rat myometrium.

2. The aim of the present study was to investigate the uterus relaxant effect of the β_2-adrenoceptor agonists (terbutaline, ritodrin) applied together with the subtype-selective α_{1A}-adrenoceptor antagonists (WB 4101, 5-methyl-urapidil) in an *in vitro* rat model. The main objective of the experiments was to clarify whether there was an additive or a potentiating synergism between the two drug classes.

3. Myometrial rings were taken from female, 22-day pregnant (end-term) Sprague-Dawley rats. Electrical field stimulation (EFS) was used to elicit rhythmical contractions. Non-cumulative concentration–response curves were constructed to the β_2-adrenoceptor agonists and the α_{1A}-adrenoceptor antagonists alone and to β_2-adrenoceptor agonists co-administered with the α_{1A}-adrenoceptor antagonists.

4. Both groups of drugs inhibited EFS-induced contractions in a dose-dependent way. Administering the β_2-adrenoceptor agonists in combination with the α_{1A}-adrenoceptor antagonists resulted in a significant decrease in the EC_{50} and an increase in the maximal contraction inhibiting effect.

5. The potentiating synergism that has been revealed between β_2-adrenoceptor agonists and α_{1A}-adrenoceptor antagonists in the uterus relaxant effect may be of great clinical importance because it could improve the efficacy of therapy of preterm delivery.

Key words: α_{1A}-adrenoceptor antagonists, β_2-adrenoceptor agonists, combination, electric field stimulation, rat, tocolysis.

INTRODUCTION

Despite great efforts in recent decades, tocolysis remains one of the greatest challenges in obstetric practice because premature birth is still a leading cause of perinatal morbidity and mortality.[1,2]

The numerous physiological mechanisms that control myometrial contractility involving the adrenergic system, oxytocin, sex steroids and prostaglandins have led to the elaboration and investigation of various therapeutic methods.

However, none of these therapeutic approaches has resulted in a serious breakthrough in the problem and none has been shown to decrease the rate of preterm delivery.[3] Of the various pharmacotherapeutic alternatives, β_2-adrenoceptor (β_2-AR) agonists (e.g. terbutaline, fenoterol, ritodrin, hexoprenaline) are applied most frequently, even though their efficacy is not fully satisfactory and their use is rather limited. The response to these drugs is reasonably good in acute situations, but the significant elongation of gestation by chronic use of β_2-AR agonists is still controversial. β_2-Adrenoceptor agonists are contraindicated in a number of situations, such as tachycardia, diabetes mellitus and arrhythmia. When not contraindicated, treatment of preterm labour with β_2-AR agonists is often accompanied by disadvantageous phenomena. These drugs often give rise to serious maternal and fetal side-effects, some of which are life threatening.[2] The cardiovascular and cardiopulmonary systems are the most severely affected (e.g. tachycardia, arrhythmia and pulmonary oedema).[4–7] It is well known that the efficacy of β_2-AR agonists decreases towards the end of pregnancy as a result of β_2-AR desensitization.[8] Accordingly, in order to prevent or inhibit preterm contractions, ever higher doses of β_2-AR agonists are required; these become less and less tolerable, increase the incidence and severity of side-effects and endanger the success of the treatment.

It has been shown in previous animal studies that, in parallel with the β_2-AR, the α_1-adrenoceptors (α_1-AR) also play a major role in the regulation of myometrial contractility.[9] The density of α_1-AR in the rat myometrium was found to be increased by the end of gestation, which suggests that these receptors are involved in the increase of uterine contractility. Further investigations revealed that, of the different α_1-AR subtypes, it is the α_{1A}-AR that are mainly responsible for the elevated α_1-AR density.[10] Blockade of α_{1A}-AR results in relaxation of rat uterine smooth muscle, while antagonism of the α_{1B}-AR subtype does not produce a significant relaxation of the rat myometrium. However, antagonism of the α_{1D}-AR subtype results in modest relaxation of the pregnant rat uterine smooth muscle.[11]

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Received 7 August 2002; accepted 23 September 2002.

The previously discussed problems relating to the use of β_2-AR mimetics show that these drugs alone do not provide a fully acceptable solution to the problem of tocolysis. The data available on the α_{1A}-AR subtype-selective antagonists also suggest that the efficiency of these compounds may not exceed that of β_2-AR mimetics.[11] Therefore, it seemed reasonable to examine the interaction between these drugs when they are coadministered in an *in vitro* study. Thus, the aim of the present study was to investigate the changes in the uterus-relaxant effect of a β_2-AR agonist when applied together with an α_{1A}-AR antagonist.

MATERIALS

Uterus rings were taken from female 22 day pregnant (end-term) Sprague-Dawley rats. Muscle rings (0.5 cm long) were sliced from both horns of the uterus and mounted vertically between two platinum electrodes in a tissue bath containing 10 mL 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 organ 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 60 min before the experiments were started. The initial tension of the uterus rings was set to 1.5 g and the tension dropped to 0.5 g by the end of the equilibration period. Following the equilibration period, rhythmic contractions were elicited by a digital programmable stimulator (ST-02; Experimetria, London, UK) using square pulses with a duration of 150 msec and a frequency of 23.75 s. The stimulating potential in each experiment was 40 V. The tension of the myometrial rings was measured with a strain gage transducer (SG-02; Experimetria) and recorded by an ISOSYS Data Acquisition System (Experimetria).

As the first step, non-cumulative concentration–response curves were constructed for the α_{1A}-AR antagonists WB 4101 (RBI, Nattick, MA, USA) and 5-methylurapidil (5-MU; Sigma-Aldrich, St Louis, MO, USA) and for the β_2-AR agonists terbutaline (Sigma-Aldrich) and ritodrin (Sigma-Aldrich). Next, concentration–response curves were constructed for both terbutaline and ritodrin in the presence of 1×10^{-6} mol/L WB 4101 and 1×10^{-6} mol/L 5-MU.

The areas under the curves (AUC) 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±SEM and in all cases $n = 10$.

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 (permission number: 1-74-8/2002).

RESULTS

Both the α_{1A}-AR antagonists (WB 4101 and 5-MU) inhibited the rhythmic contractions of the rat isolated myometrium in a dose-dependent manner, although their potencies were less than those of terbutaline and ritodrin. The EC_{50} and maximal contraction inhibiting effect of WB 4101 were found to be $2.2 \pm 0.6 \times 10^{-5}$ mol/L and 71.5 ± 9.1%, respectively. The EC_{50} and maximal contraction inhibiting effect of 5-MU were $5.3 \pm 2.7 \times 10^{-6}$ mol/L and 62.4 ± 4.4%, respectively. Similarly, the β_2-AR agonists terbutaline and ritodrin inhibited electrical field stimulation (EFS)-induced contractions in a dose-dependent manner. The EC_{50} and maximal inhibitory effect of terbutaline were 4.0 ± 0.5 10^{-7} mol/L and 73.3 ± 1.3%, respectively. The other β_2-AR agonist used in the present study, ritodrin, inhibited the EFS-elicited contractions with an EC_{50} and maximal inhibitory effect of $1.1 \pm 0.2 \times 10^{-8}$ mol/L and 60.7 ± 3.7%, respectively.

The concentration–response curve for terbutaline in the presence of 1×10^{-6} mol/L WB 4101 was shifted markedly to the left and an increase in the maximal uterus-relaxant effect was observed (Fig. 1). Similarly, when terbutaline was applied with 1×10^{-6} mol/L 5-MU, the concentration–response curve for terbutaline shifted markedly to the left and the maximal contraction inhibiting effect increased (Fig. 2). When terbutaline

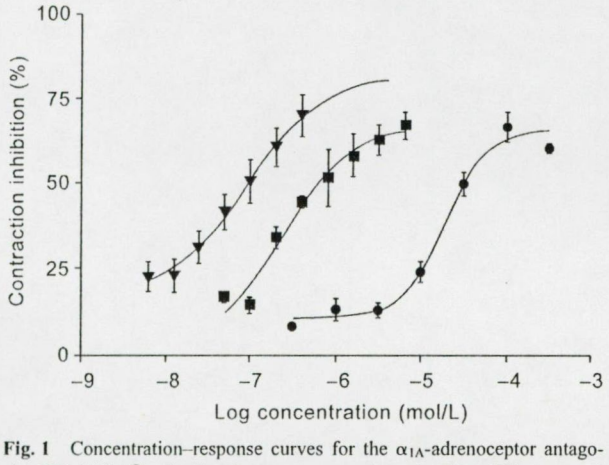

Fig. 1 Concentration–response curves for the α_{1A}-adrenoceptor antagonist WB 4101 (●), the β_2-adrenoceptor agonist terbutaline (■) alone and terbutaline in the presence of 1×10^{-6} mol/L WB 4101 (▼). Both WB 4101 and terbutaline inhibited electric field stimulation-induced contractions in a dose-dependent manner. When terbutaline was administered in the presence of 1×10^{-6} mol/L WB 4101, the dose–response curve was shifted markedly to the left and an increase in the maximal inhibitory effect was observed.

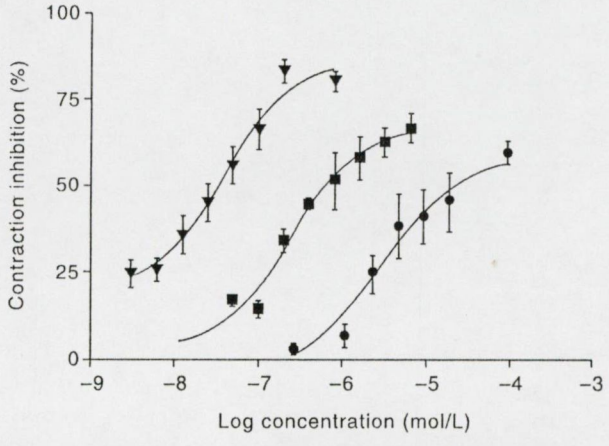

Fig. 2 Concentration–response curves for the α_{1A}-adrenoceptor antagonist 5-methylurapidil (●), the β_2-adrenoceptor agonist terbutaline (■) alone and terbutaline in the presence of 1×10^{-6} mol/L 5-methylurapidil (▼). Both 5-methylurapidil and terbutaline inhibited the electric field stimulation-induced contractions in a dose-dependent manner. When terbutaline was administered in the presence of 1×10^{-6} mol/L 5-methylurapidil, the dose–response curve was shifted markedly to the left and an increase in the maximal inhibitory effect was observed.

was co-administered with 1×10^{-6} mol/L WB 4101, the EC_{50} fell from $4.0 \pm 0.5 \times 10^{-7}$ to $4.3 \pm 1.1 \times 10^{-8}$ mol/L and the administration of terbutaline in the presence of 1×10^{-6} mol/L 5-MU caused a decrease in the EC_{50} of terbutaline to $5.1 \pm 1.9 \times 10^{-8}$ mol/L (Fig. 3).

The maximal inhibitory effect of terbutaline increased from 73.3 ± 1.3 to $82.5 \pm 3.0\%$ when it was administered with 1×10^{-6} mol/L WB 4101 (Fig. 4).

A similar effect was observed when terbutaline was used with 1×10^{-6} mol/L 5-MU, where the maximal inhibitory effect increased from 73.3 ± 1.3 to $86.3 \pm 4.7\%$ (Fig. 4).

The concentration–response curve for ritodrin in the presence of 1×10^{-6} mol/L WB 4101 was also shifted to the left and an increase in the maximal uterus-relaxant effect was observed, but the rate of this shift to the left was not as great as in the previous case when terbutaline was applied with the same concentration of WB 4101 (Fig. 5). When ritodrin was applied with 1×10^{-6} mol/L 5-MU, the concentration–response curve for ritodrin was shifted markedly to the left and the maximal contraction inhibiting effect increased, similar to the effects observed for the combination of terbutaline and 5-MU (Fig. 6).

When ritodrin was co-administered with 1×10^{-6} mol/L WB 4101, the EC_{50} fell from $1.1 \pm 0.2 \times 10^{-7}$ to $8.1 \pm 1.4 \times 10^{-8}$ mol/L ($n = 10$), while administration of ritodrin in the presence of 1×10^{-6} mol/L 5-MU caused the EC_{50} of ritodrin to drop to $3.4 \pm 1.2 \times 10^{-8}$ mol/L (Fig. 7).

The maximal inhibitory effect of ritodrin increased from 60.7 ± 3.7 to $87.3 \pm 7.2\%$ ($n = 10$) when it was administered with 1×10^{-6} mol/L WB 4101 (Fig. 8).

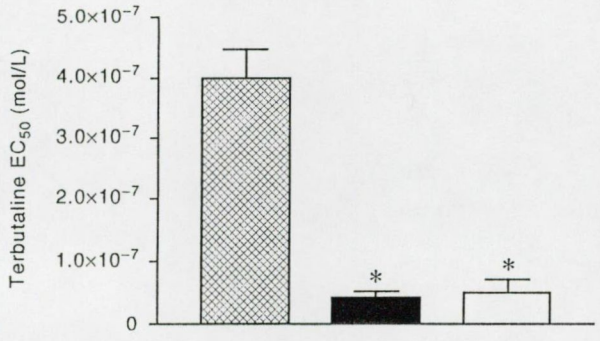

Fig. 3 Changes in the EC_{50} of terbutaline when administered in the presence of 1×10^{-6} mol/L WB 4101 (■) and 1×10^{-6} mol/L 5-methylurapidil (□). (▨), terbutaline alone. Using terbutaline in the presence of 1×10^{-6} mol/L WB 4101 resulted in a significant decrease in the concentration required to reach 50% of the maximal effect. Similarly, when terbutaline was administered in the presence of 1×10^{-6} mol/L 5-methylurapidil, the EC_{50} of terbutaline was significantly decreased. $^*P < 0.001$.

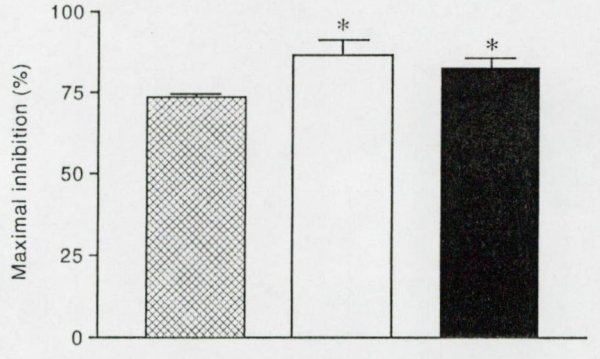

Fig. 4 Changes in the maximal contraction inhibiting effect of terbutaline when administered in the presence of 1×10^{-6} mol/L WB 4101 (■) and 1×10^{-6} mol/L 5-methylurapidil (□). (▨), terbutaline alone. Using terbutaline in the presence of 1×10^{-6} mol/L WB 4101 resulted in a significant increase in the maximal inhibitory effect of terbutaline. The maximal inhibitory effect was also increased significantly when terbutaline was administered in the presence of 1×10^{-6} mol/L 5-methylurapidil. $^*P < 0.01$.

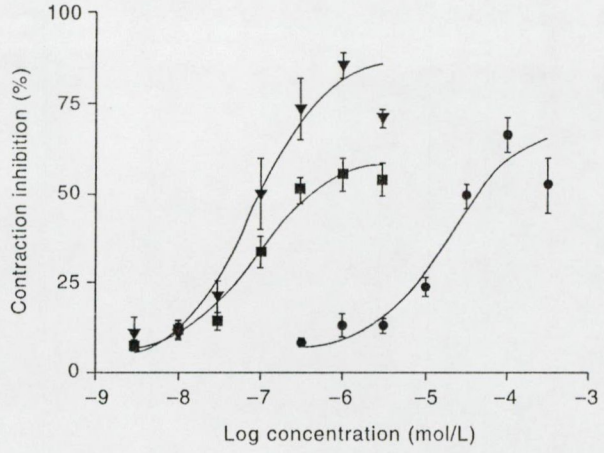

Fig. 5 Concentration–response curves for the α_{1A}-adrenoceptor antagonist WB 4101 (●), the β_2-adrenoceptor agonist ritodrin (■) alone and ritodrin in the presence of 1×10^{-6} mol/L WB 4101 (▼). Both WB 4101 and ritodrin inhibited electric field stimulation-indeced contractions in a dose-dependent manner. When ritodrin was administered in the presence of 1×10^{-6} mol/L WB 4101, the dose–response curve was shifted to the left and an increase in the maximal inhibitory effect was observed.

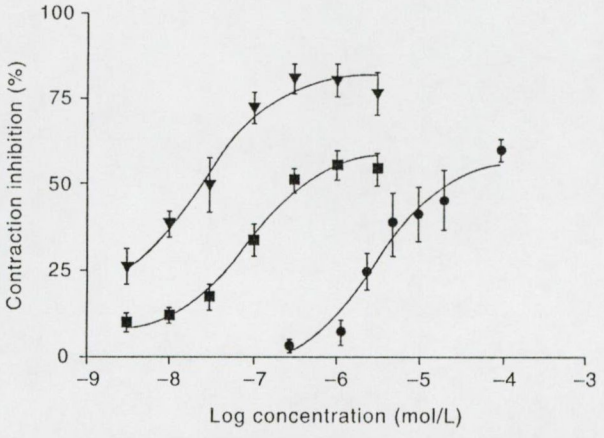

Fig. 6 Concentration–response curves of the α_{1A}-adrenoceptor antagonist 5-methylurapidil (●), the β_2-adrenoceptor agonist ritodrin (■) alone and ritodrin in the presence of 1×10^{-6} mol/L 5-methylurapidil (▼). Both 5-methylurapidil and ritodrin inhibited the electric field stimulation-induced contractions in a dose-dependent manner. When ritodrin was administered in the presence of 1×10^{-6} mol/L 5-methylurapidil, the dose–response curve was shifted markedly to the left and an increase in the maximal inhibitory effect was observed.

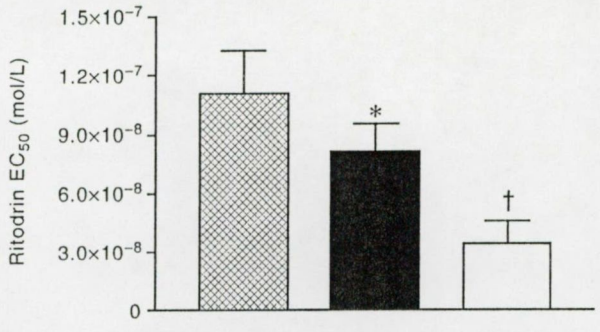

Fig. 7 Changes in the EC_{50} of ritodrin when administered in the presence of 1×10^{-6} mol/L WB 4101 (■) and 1×10^{-6} mol/L 5-methylurapidil (□). (▨), ritodrin alone. Using ritodrin in the presence of 1×10^{-6} mol/L WB 4101 resulted in a significant decrease in the concentration required to reach 50% of the maximal effect; however, this change was not as great as in case of terbutaline. Similarly, when ritodrin was administered in the presence of 1×10^{-6} mol/L 5-methylurapidil, the EC_{50} of ritodrin was significantly decreased. $^{\dagger}P < 0.01$, $^{*}P < 0.05$.

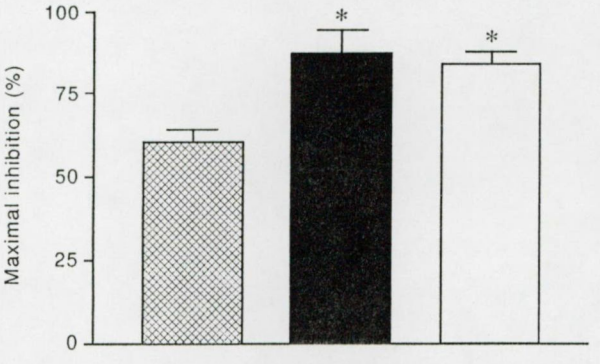

Fig. 8 Changes in the maximal contraction inhibiting effect of ritodrin when administered in the presence of 1×10^{-6} mol/L WB 4101 (■) and 1×10^{-6} mol/L 5-methylurapidil (□). (▨), ritodrin alone. Using ritodrin in the presence of 1×10^{-6} mol/L WB 4101 resulted in a significant increase in the maximal inhibitory effect of terbutaline. The maximal inhibitory effect also increased significantly when ritodrin was administered in the presence of 1×10^{-6} mol/L 5-methylurapidil. $^{*}P < 0.01$.

When ritodrin was used with 1×10^{-6} mol/L 5-MU, the maximal inhibitory effect increased from 60.7 ± 3.7 to $84.2 \pm 3.3\%$ ($n = 10$; Fig. 8).

DISCUSSION

The results of the present *in vitro* animal study indicate that the combination of β_2-AR agonists with α_{1A}-AR subtype-selective antagonists appreciably increases the efficacy of the β_2-AR mimetics in terms of uterus relaxation as a result of the potentiating synergism revealed. The significant decrease in the EC_{50} and the increase in the maximum inhibitory effect of the β_2-AR agonists in the presence of α_{1A}-AR subtype-selective antagonists may be of great importance, because it could offer a possibility to use a considerably lower dose of β_2-AR agonists in order to achieve the desired uterus-relaxant effect. Furthermore, these results suggest that there could possibly be a markedly lower risk of the severe, sometimes life-threatening, side-effects of tocolytic therapy with β_2-AR mimetics that is usually induced by the very large doses of β_2-AR agonists required. Supplementation of β_2-AR agonist therapy with α_{1A}-AR antagonists could result in beneficial effects on pregnancy related disorders such as pre-eclampsia, which is associated with increased sympathetic activity.[12] Furthermore, the interaction revealed could be even more important in cases of pregnancies where the mother suffers from hypertension, because these mothers have a greater risk of preterm delivery.[13] Supplementation with α_{1A}-AR antagonists could not only improve the tocolytic effect, but could also exert a beneficial antihypertensive effect.

These findings suggest that the combination of β_2-AR mimetics and α_{1A}-AR antagonists may provide a better solution to the clinical problem of premature labour.

Because α_{1A}-AR subtype-selective antagonists are not used in obstetric practice, further *in vivo* experiments and clinical trials should be planned in order to explore the possible benefits of this drug interaction.

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Molecular Human Reproduction Vol.9, No.8 pp. 475–480, 2003 DOI: 10.1093/molehr/gag061

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: alpha1A-adrenoceptors/5-HT1A 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 EC_{50} (Mihalyi *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 serotonin1A (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

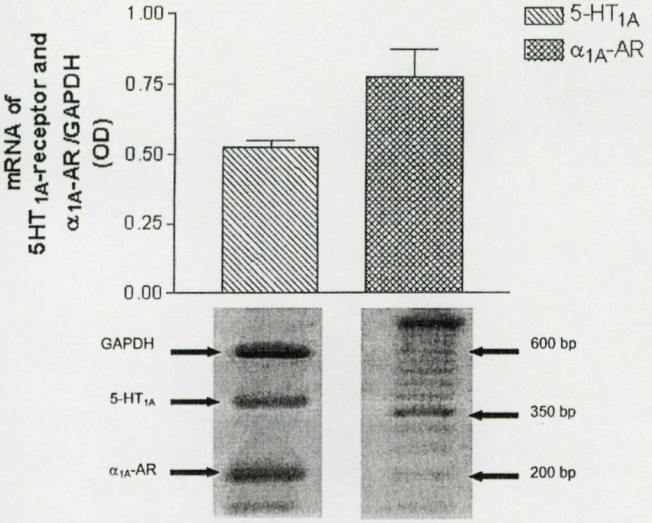

Figure 1. The expression of uterine α_{1A}-AR (212 bp) and 5-HT_{1A}-receptor (388 bp) mRNA on day 22 of pregnancy. Results of the RT–PCR study demonstrate the expressions of α_{1A}-AR mRNA and 5-HT_{1A}-receptor mRNA on day 22 of pregnancy in the rat. The receptor mRNA expression level was normalized 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 denaturated at 70°C for 5 min in a reaction mixture containing 20 U of RNase inhibitor (Hybaid, UK), 200 µmol/l dNTP (Sigma-Aldrich, Hungary), 20 µmol/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 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 (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., 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 (Csertex 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 ± SEM)	Level of significance	E_{max} (%) (mean ± SEM)	Level of significance
1	5-HT	$8.0\times10^{-8} \pm 1.9\times10^{-8}$	–	437.9 ± 38.9	–
2	5-HT + WB4101 1×10^{-7} mol/l	$4.5\times10^{-7} \pm 1.1\times10^{-7}$	*	424.0 ± 54.8	NS
3	5-HT + WB4101 1×10^{-6} mol/l	$5.8\times10^{-7} \pm 1.8\times10^{-7}$	*	328.7 ± 66.3	NS
4	5-HT + 5-MU 1×10^{-7} mol/l	$3.3\times10^{-7} \pm 6.2\times10^{-8}$	***	384.4 ± 40.7	NS
5	5-HT + 5-MU 1×10^{-6} mol/l	$3.9\times10^{-7} \pm 4.8\times10^{-8}$	***	301.2 ± 26.2	*

NS, $P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
EC_{50}, 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.

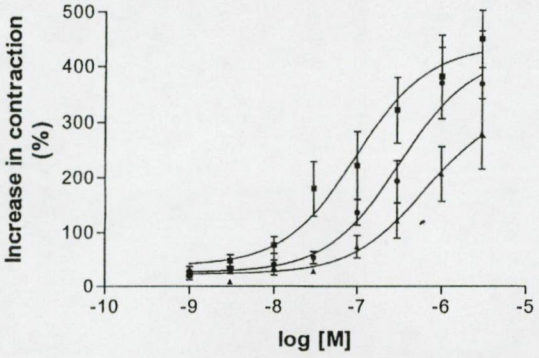

Figure 2. Concentration–response curve of 5-HT alone and in the presence of different concentrations of the subtype-selective α_{1A}-AR antagonist WB 4101. 5-HT (squares) increased the contractility of the pregnant rat myometrium in a concentration-dependent manner. 1×10^{-7} mol/l WB 4101 shifted the 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.

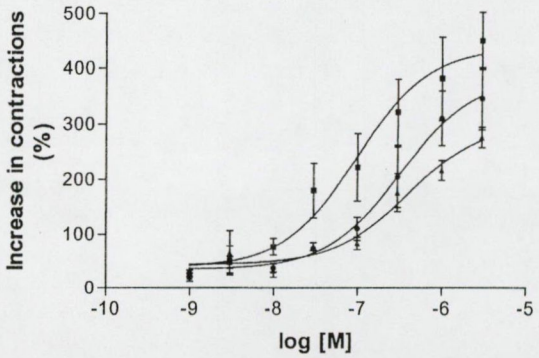

Figure 3. Concentration–response curve of 5-HT alone and in the presence of different concentrations of the subtype-selective α_{1A}-AR antagonist 5-MU. 5-HT (squares) increased the contractility of the pregnant rat myometrium in a concentration-dependent manner. When the concentration–response curve 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.

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 ± 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 EC_{50} and the maximal effect of 5-HT are presented in the first row of Table I.

WB4101 (1×10^{-7} mol/l) shifted the concentration–response curve of 5-HT to the right (Figure 2), significantly increasing its EC_{50}, 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 EC_{50} 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 EC_{50} 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 EC_{50} 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 EC_{50} 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 ± SEM)	Level of significance	E_{max} (%) (mean ± SEM)	Level of significance
1	WB4101	$2.5\times10^{-5} \pm 5.2\times10^{-6}$	–	76.5 ± 6.4	–
2	WB4101 + WAY100135 1×10^{-7} mol/l	$2.5\times10^{-5} \pm 6.9\times10^{-6}$	NS	76.2 ± 18.4	NS
3	WB4101 + WAY100135 5×10^{-7} mol/l	$2.0\times10^{-5} \pm 2.8\times10^{-6}$	NS	73.6 ± 5.6	NS
4	5-MU	$2.9\times10^{-6} \pm 9.3\times10^{-7}$	–	53.7 ± 9.7	–
5	5-MU + WAY100135 1×10^{-7} mol/l	$3.7\times10^{-6} \pm 8.9\times10^{-7}$	NS	78.23 ± 11.51	NS
6	5-MU + WAY100135 5×10^{-7} mol/l	$3.8\times10^{-6} \pm 8.6\times10^{-7}$	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.

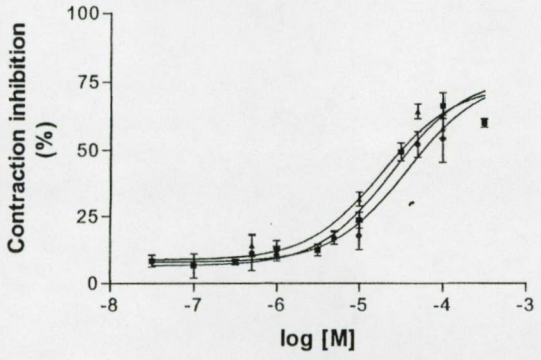

Figure 4. Contraction-inhibiting effect of WB 4101 alone and in the presence of different concentrations of the 5-HT_{1A}-receptor antagonist WAY100135. WB 4101 alone (squares) inhibited the EFS-elicited contractions in a concentration-dependent manner. 5-HT_{1A}-receptor blockade with 1×10^{-7} mol/l 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.

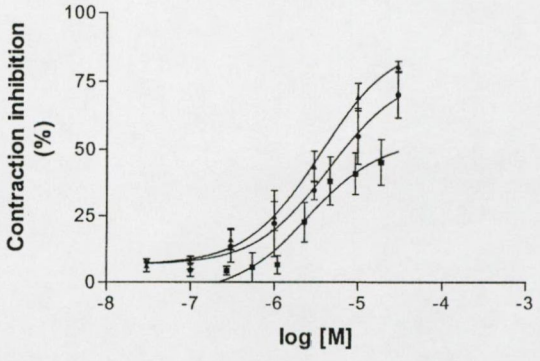

Figure 5. Contraction-inhibiting effect of 5-MU alone and in the presence of 1×10^{-7} and 5×10^{-7} mol/l WAY100135. The subtype-selective α_{1A}-AR antagonist 5-MU (squares) inhibited the EFS-induced contractions of the isolated rat myometrium in a dose-dependent way. In the presence of 1×10^{-7} mol/l 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 (Mihalyi *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

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

In contrast, the contraction-inhibiting properties of 5-MU changed hen it was applied together with the 5-HT_{1A}-receptor antagonist. ven in the presence of 1×10^{-7} mol/l WAY100135, 5-MU exhibited increased maximal contraction-inhibiting effect. Elevation of the ncentration of the 5-HT_{1A}-receptor antagonist to 5×10^{-7} mol/l sulted in an even greater increase in the maximal effect of 5-MU, is increase proving to be statistically significant.

Impeding the serotonin agonist effect of 5-MU by 5-HT_{1A}-receptor ockade raised the maximal contraction-inhibiting effect of 5-MU to .6 ± 5.4%, which is even higher than the maximal effect of B4101, 76.5 ± 6.4%. These results suggest that the serotonergic tivity of 5-MU is more pronounced than that of WB4101. The fferent chemical structures may offer an explanation for the teworthy difference between the serotonergic properties of the o α_{1A}-AR antagonists. It seems probable that 5-MU has a dual fluence on the pregnant rat myometrium: a dominant α_{1A}-AR tagonist effect that inhibits contractions, and a less expressed onist effect on the 5-HT_{1A} receptors that increases the contractility the pregnant rat uterus.

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

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

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Submitted on March 13, 2003; accepted on April 23, 2003

Volume 15
Supplement 1
July 2001

Fundamental & Clinical Pharmacology

Abstracts of the 3rd Meeting of the Federation of the European Pharmacological Societies (EPHAR)

6–9 July 2001
Lyon, France

Blackwell Science

0767-3981(2001)15+1;1-J

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ynergism in the uterus-relaxant effects of terbutaline nd 5-methylurapidil on the pregnant rat uterus *in vitro*

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bjectives: Despite many efforts in recent decades, preterm delivery remains the main eason for perinatal morbidity and mortality. Although β_2-agonists are widely used as ocolytics in the gynaecological practice, their efficacy decreases towards the term of regnancy in consequence of β-desensitization and the increasing incidence of side-effects ypertension, pulmonary oedema). The aim of the present study was to investigate the nteraction between the β_2-agonist terbutaline (T) and 5-methylurapidil (5MU)- an $_{1A}$-antagonist which in our earlier studies was found to be suitable to inhibit ontractions of the uterus *in vitro*.

esign and methods: Uterus rings taken from 22 days pregnant rats were mounted etween two platinum electrodes, through which the samples were stimulated with a eriod time of 24 s and pulse width of 150 ms. In the first step, T was used in increasing oncentrations to inhibit the contractions in the presence of a constant 5MU concentra- on. The following step was to record the dose–response curve of 5MU at a constant oncentration of T.

esults: It was found that in the presence of 5MU the dose–response curve of T was ifted to the left and its maximum value increased. In the presence of 5MU, the EC50 alue and the maximal inhibiting value were about 5 times lower and 13% higher, espectively. Similar phenomena were experienced for the dose–response curve of 5MU.

onclusions: Analysis of the study data revealed a strong synergism between the two harmacons. The results suggest that the disadvantages of β-desensitization and the erious side-effects may be reduced by the synergic relation between the two substances in ossible clinical application.

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lteration of α-adrenergic receptor subtypes and pharmacological reactivity f the late-pregnant myometrium in the rat

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bjective: The adrenergic system plays a major role in the contractility of the uterus uring pregnancy. Our previous studies have shown the significance of α_1-adrenergic eceptors (ARs) in the control of pregnant uterine contractility. Our present aim was to etermine the changes in the expression, density and pharmacological reactivity of -ARs in the late-pregnant rats.

esign and methods: RT-PCR method was used to demonstrate the expressions of $_1$- and β_2-AR mRNA. The densities of receptor proteins were determined by adioligand binding assay (RBA); the pharmacological reactivity was tested by electric eld stimulation (EFS).

esults: The α_1/β-ratios determined by RBA were 21.6, 43.9, 33.9, and 51.1 on days 5, 18, 20 and 22 of pregnancy, respectively; this was confirmed by RT-PCR analysis. he expression of the α_{1A}-ARs increased from day 15 to day 22, while α_{1B}-AR xpression was not detectable. EFS studies revealed that the α_{1A}-AR antagonist -methylurapidil had EC50 values (1×10^{-6}–3×10^{-6} M) about one magnitude lower han those of the α_{1D}-AR antagonist BMY 7378 (5×10^{-6}–1×10^{-5} M). However, the $_{1B}$-AR antagonist cyclazosine exerted no action on stimulated contractions. A strong orrelation was found between the α_{1A}/α_{1D}-AR expression ratio and the ratio of the C50 values of BMY 7378 and 5-methylurapidil ($r^2 = 0.9993$).

onclusions: There is an α-AR dominance at the end of pregnancy in the rat. Our ndings suggest that both α_{1A}- and α_{1D}-ARs are involved in the regulation of pregnant terine contractility. Nevertheless, the α_{1A}-AR seems to play the major role among the $_1$-AR subtypes in late-pregnant myometrium contractions.

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timulatory effect of β-carbolines on locus coeruleus neurons n anaesthetized rats: an electrophysiological study

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armane, harmaline and norharmane, all β-carboline related compounds have been roposed as endogenous ligands for imidazoline receptors [1]. We have studied the ffect of these substances on locus coeruleus (LC) neuronal activity by extracellular ecordings in anaesthetized rats. Harmane (100 μg i.c.v.) caused an increase of LC euron firing rate in control and vagotomized rats (E_{max}: 79% ± 32.2, $n = 5$, < 0.05 and 73.6% ± 17.8, $n = 5$, $P < 0.01$ respectively) that lasted for 10–5 min. Efaroxan (100 and 500 μg/kg i.v.), an I_1 imidazoline receptor antagonist, ailed to prevent or reverse the harmane effect. Local application of harmane (0.55–7.6 pmoles) also caused an increase in the firing rate of LC cells (E_{max}: 65.1 ± 12.4, $= 5$, $P < 0.001$) which was reversible and dose-dependent. Similarly, intracerebro-entricular administration (20, 40 and 80 μg) and local application (1.1–17.6 moles) of harmaline increased the firing rate of LC neurons (E_{max}: 52.9% ± 9.8, $= 6$, $P < 0.01$ and 75.3 ± 14.4, $n = 8$, $P < 0.05$ respectively). Finally, norhar-ane (2.5–10 mg/kg i.v.) also stimulated LC neuronal activity in a dose-dependent anner ($n = 5$, $P < 0.001$). We conclude that β-carboline compounds, increase LC euronal activity. This effect is not due to changes in blood pressure and is not ediated by I_1 imidazoline receptors, but is induced by a mechanism located in the LC.

Supported by the CICYT (SAF 99/0046) and UPV-EHU 026.327-EC238/97. . Ruiz-Durántez was supported by a fellowship from the Gobierno Vasco.

eference

Hudson. Br J Pharmacol (1999) **126**: 2.

8P118

Phenylephrine-induced contractions of perforating branch of the human internal mammary artery: role of adrenergic α receptors

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The role of the adrenergic receptors in the phenylephrine (Phe)-induced contractions of the perforating branch of the human internal mammary artery (HIMA) was investigated. Arterial segments were obtained from 28 women undergoing mammectomy. Standard procedure for *in vitro* blood vessels investigation was used. Phe (10^{-9}–10^{-5} mol/L) induced concentration-dependent contractions of perforating branch of HIMA with intact and denuded endothelium with similar pEC_{50} values (intact: $pEC_{50} = 6.72 \pm 0.04$, denuded: $pEC_{50} = 6.87 \pm 0.01$). In both type of preparations, the α-adrenergic receptor antagonists, prazosin, a selective α_1 antagonist (4×10^{-10}–4×10^{-9} mol/L) and rauwolscin, a selective α_2 antagonist (10^{-6}–10^{-5} mol/L) antagonized the Phe-induced contractions. Thus, the following pA_2 values were obtained (intact vs. denuded): 10.05 ± 0.13 vs. 10.10 ± 0.23 for prazosin and 6.03 ± 0.12 vs. 7.22 ± 0.13 for rauwolscin. In conclusion, this study showed, following the affinities of antagonists and affinity of Phe itself, that identical subtype of α-adrenergic receptors, probably α_1 subtype, is involved in the Phe-induced contractions of the perforating branch of the HIMA with intact or denuded endothelium.

8P119

Pharmacologicological effects of novel imidazoline-based derivatives

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Objective: Over the last two decades significant work has been done on the field of imidazoline-derived drugs and imidazoline receptors. Moxonidine and rilmenidine, representatives of the second generation of centrally acting antihypertensive drugs, have been recently introduced into therapeutic use but the work for identifying new compounds continue.

Design and methods: In this study we investigated effects of 9 novel clonidine-like compounds on isolated rabbit aortic rings. Clonidine, well known representative of the first generation of centrally acting antihipertensive drugs, exhibit affinity not only to central α2, I1 receptors but peripheral α1 and α2 receptors as well.

Results: Inquired compounds could be devided into 3 groups, regarding to their constricting activity. The first group (laboratory codes SK-P-1 to 4) characterised by 2 methyl or chloride moieties by the benzene ring, produce irreversible contraction that is close to phenylephrine. Compounds SK-P-5 and 6, that form the second group deprived of methyl and chloride moieties, displayed only minimal but reversible contracting activity. The last group of compounds SK-Fw-I,-II, and III do not have any contractile effect on isolated rabbit aortic rings but after incubation reinforce phenylephrine contractions. One compound-SK-P-2 (strong irreversible contractile activity)-has been subdued more detailed analysis in the presence of specyfic receptor/channel blockers such as prazosin, yohimbine, diltiazem, clotrimazole and rimalkalim. Rimalkalim-K/ATP channel opener produced dose-dependent relaxation of the vascular smooth muscle preconstricted with SK-P-2.

Conclusions: On the basis of these experiments we think that investigated imidazolines exhibit affinity not only to peripheral α1 receptor but K/ATP channel as well.

8P120

Characterization of α_1-adrenoceptor subtypes in rat cerebral cortex: evidence that α_{1L} represents a conformational state of the α_1-adrenoceptor

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The α_1-adrenoceptor subtypes of rat cerebral cortex were characterized in binding studies. Saturation binding experiments performed with [^{3}H]-prazosin (0.025–6 nM) in rat cerebral cortex evidenced the presence of two distinct affinity sites for prazosin ($pK_{D,high} = 10.35 \pm 0.09$, $pK_{D,low} = 8.84 \pm 0.15$, $R_{high} = 70.2 \pm 10.5$ fmol/mg, $R_{low} = 40.0 \pm 2.5$ fmol/mg, $n_H = 0.44 \pm 0.08$, $n = 6$). The prazosin low-affinity sites disappeared when the experiments were performed in presence of 100 μM GppNHp ($pK_D = 10.16 \pm 0.06$, $R_{high} = 118.0 \pm 6.8$ fmol/mg, $n_H = 0.87 \pm 0.02$, $n = 3$), thus suggesting that the α_{1L} subtype is a conformational state modulated by G proteins. Competition binding experiments were performed with two [^{3}H]-prazosin concentrations and the results shown in Table 1 confirm the existence of two prazosin binding sites modulated by G proteins and demonstrate the presence of two α_1-adrenoceptor subtypes: α_{1A} and α_{1B} in rat cerebral cortex.

Table 1

	[^{3}H]-prazosin 0.2 nM			[^{3}H]-prazosin 3 nM		
	$pK_{i\ high}$	$pK_{i\ low}$	n_H	$pIC_{50\ high}$	$pIC_{50\ low}$	n_H
Prazosin (P)	9.75 ± 0.08		0.88	9.31 ± 0.40	7.13 ± 0.46	0.58
P + GppNHp				8.02 ± 0.14		0.94
5-methylurapidil	8.72 ± 0.13	6.92 ± 0.08	0.60	6.74 ± 0.29	4.95 ± 0.30	0.51
BMY 7378	7.07 ± 0.06		0.86	5.68 ± 0.18		0.75

Mean ± SEM $pK_i = -\log K_i$, n_H = Hill coefficient, n = 3–6 experiments.

Supported by CICYT SAF98 0123.

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The findings support the notion that capsaicin-sensitive afferent nerves, by the release of sensory neuropeptides, significantly contribute to the modulation of uterine contractility. It is suggested that uterine sensory nerve activation may be part of a trigger mechanism leading to preterm contractions evoked by, e.g. inflammatory processes.

University of Szeged, Department of Pharmacodynamics and Biopharmacy - Hungary

P19-06

ROLE OF SEROTONERGIC ACTIVITY IN THE ALPHA1A-BLOCKADE IN THE PREGNANT RAT MYOMETRIUM IN VITRO

Mihalyi A., Ducza E., Gáspár R., Falkay G.

The adrenergic system plays an important role in the regulation of myometrial contractility. It had been revealed that blockade of the alpha1A-adrenoceptors inhibits contractions of the pregnant rat uterus elicited either by electrical field stimulation (EFS) or norepinephrine. Some alpha1A-adrenoceptor antagonists exert stimulatory effect on 5-HT1A receptors. The aim of the study was to clarify whether this feature has any influence on the uterus relaxant effect.
Uterus rings were taken from 22-day pregnant SPRD rats and mounted in a tissue bath. Concentration-response curve of 5-HT was constructed alone and in the presence of subtype-selective alpha1A-adrenoceptor antagonists, WB4101 and 5-methylurapidil. Next, the concentration-response curves of the alpha1A-antagonists were constructed in the presence of 5-HT1A – antagonist WAY100135, using EFS. RT-PCR was used to determine the mRNA expression of the two receptor types.
The mRNA expression of the alpha1A-adrenoceptors is significantly greater than that of the 5-HT1A receptors. Serotonin increased the contractility of the myometrium dose-dependently. In the presence of the alpha1A-antagonists the concentration-response curve of serotonin was shifted to the right in each case. The uterus-relaxant effect of WB4101 did not change in the presence of 5-HT1A –antagonist WAY100135. The maximal inhibition of 5-methylurapidil increased in the presence of the 5-HT1A –antagonist.
These results suggest that the contractions induced by serotonin are mediated by alpha1A-receptors. Serotonergic activity of WB4101 does not influence its uterus-relaxant effect. Concerning 5-methylurapidil, its serotonin activity depresses its efficacy in terms of uterus relaxation. These findings provide further proofs for the interaction between the adrenergig and serotonergic systems.

University of Szeged, Department of Pharmacodynamics and Biopharmacy - Hungary

P19-07

STUDY ON MYOMETRIAL ACTIVITY AS A FUNCTION OF INTRAUTERINE PRESSURE IN PERFUSED RAT UTERUS IN VITRO

Zupkó I., Bokor D., Falkay G.

In spite of the increasing knowledge concerning the regulation of the motor activity of the pregnant uterus the mechanism which is responsible for the initiation of delivery remains unknown. The intrauterine volume and pressure is proved to be one of the crucial factors determining the contractility of the myometrium [1]. Up to now the effect of intrauterine pressure on the activity of the uterine smooth muscle could be investigated only in vivo, arising a methodological limitation to most investigation on drugs with myometrial site of action.
We therefore elaborated a unique system in which the isolated uterine horn is continuously perfused by a peristaltic pump. The longitudinal contraction of the horn and the inner pressure are registrated in the same time. Due to perfusion, non pregnant uterine horns, which had no spotaneous activity in a traditional in vitro chamber, showed a pressure dependent motor activity.
The spontaneous activity of the myometrium was recorded as a function of the perfusing pressure during the time course of the pregnancy of the rat.
We believe that the involvement of intrauterine pressure as an experimental parameter into this in vitro system gives a substantial contribution to the understanding of the initiation of labor as well as to the development of new tocolytic agents and uterotonics.
1. Csapo AI: Model experiments and clinical trials in the control of pregnancy and parturition. Am. J. Obstet. Gynecol. 85: 359-376 (1963)

University of Szeged, Department of Pharmacodynamics and Biopharmacy - Hungary

P19-08

ALTERATION OF ESTROGEN RECEPTOR SUBTYPES OF THE PREGNANT MYOMETRIUM IN THE RAT

Minorics R., Ducza E., Márki Á., Falkay G.

Estrogens exert numerous biologic effect in large number of targets, including the uterus. Two subtypes of estrogen receptors (ERs) have been described to date, ERalpha and ERbeta. The timecourse density of these subtypes in the pregnant rat uterus is not completely examined. Our present aim was to determine the changes in the expression of ER subtypes proteins and mRNA on days 4, 5, 6, 7, 8, 10, 15, 18, 20 and 22 of pregnancy.
To demonstrate the expression of ER subtypes mRNA we used reverse transcription-polymerase chain reaction (RT-PCR), and the densities of receptor proteins were determined by radioligand binding assay (RBA).
This was the first characterisation of ERalpha and ERbeta during pregnancy in the rat myometrium. The first maximum of ERalpha mRNA expression was found on day 5-6, then the receptor expression increased again from day 8 to 22. ERbeta1 and beta2 were detectable from day 7 to 15 only. The maximum levels of ERbeta1 and beta2 mRNA were on day 7 and slowly decreased to day 15. These results were supported by the measurment of RBA.
In light of these facts it can be concluded, that the presence of ERalpha is dominant on the days of pregnancy. The continuous increase in the expression of ERalpha mRNA until the end of pregnancy correlates with the expression pattern of alpha1A-adrenergic receptor mRNA investigated by earlier studies. Therefore the interaction between estrogens and the adrenergic system, a previous hypothesis, might be realized through the ERalpha receptor.

University of Szeged, Department of Pharmacodynamics and Biopharmacy - Hungary

P19-09

ONTOGENESIS OF MUSCARINIC ACETYLCHOLINE RECEPTORS IN SERTOLI CELLS.

Caviglia D., Angelini C., Scarabelli L., Voci A., Palmero S.

Cholinergic-like molecules have been previously localized in rat testis during postnatal development by immunological assays.
In a recent study all the M1-M5 mAChR mRNA subtypes were detected in Sertoli cell primary cultures from 30-d-old rats.
The aim of the present study was to evaluate by RT-PCR the ontogenesis of mAChR mRNA isoforms during postnatal development in Sertoli cell isolated from prepubertal 8-15-21-d-old rats. Moreover, the localization of the mAChRs at Sertoli cell level was investigated on piglet Sertoli cell primary cultures as well as in a pure mouse clonal Sertoli cell line (42GPA9).The presence of molecules immunologically-related to cholinergic system has been then validated by specific monoclonal antibodies.
Our results suggest an age-dependent expression of the different muscarinic receptor isoforms.Actually, M1 and M2 mAChRs mRNAs appear to be the earliest (8-d-old rats) subtypes expressed in rat Sertoli cells, while all the five isoforms were identified in Sertoli cells from older rats.
The presence of muscarinic receptors in Sertoli cells from piglet testes as well as in a pure mouse Sertoli cell line was demontrated both at mRNA (RT-PCR) and protein (Immuno-cytochemistry) level.
Cholinergic molecules in Sertoli cell might play a role in cell-to-cell communication affecting cell differentiation and co-ordinating cell functions.

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P19-11

ACTIVITY OF BGT: QUALITATIVE AND QUANTITATIVE ANALYSIS OF BILIRUBIN COMPOUNDS THROUGHOUT DEVELOPMENT

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Hepatic bilirubin excretion requires bilirubin UDP-glucuronosyltransferase (BGT)-mediated glucuronidation. Patients with type I Crigler-Najjar syndrome and Gunn rats inherit deficiency of BGT activity towards bilirubin as an autosomal recessive trait and, as a result, exhibit marked hyperbilirubinemia.In fetal life placental clearance of bilirubin of fetal origin is efficient via conjugation in maternal liver. At birth, once the neonatal pup

Magyar Szülészeti és Nőgyógyászati Endokrinológiai Társaság II. Nemzeti Kongresszusa

„Kecskemét látképe"
Bozsó János festménye
(1968)

Program és összefoglalók

Kecskemét, 2002. április 11-13.

uterusz szövetmintákban, mint a nem terhes és a fájásgyengeség miatt végzett császármetszésből nyert mintákban. Következtetés – megbeszélés: Eredményeink tükrében megállapíthatjuk, hogy az α_1–adrenerg receptorok a humán uteruszban jelentős mennyiségben kimutathatók, és szerepük a fájástevékenység kialakulásában bizonyítható. Ebből adódóan a jövőben az α_1-adrenerg receptor blokkolók a tokolitikus terápia fontos részévé válhatnak.

Interakció a terbutalin és altípus szelektív α_{1A}-antagonisták között az uterus relaxáló hatásban

Dr. Mihályi Attila, Dr. Gáspár Róbert, Csonka Dénes, Prof. Dr. Falkay György
Szegedi Tudományegyetem Gyógyszerhatástani és Biofarmáciai Intézet, Szeged

Bevezetés - a munka célkitűzései: A tokolitikus céllal széles körben alkalmazott β_2-agonista vegyületek terápiás értéke erősen vitatott. Korábbi állatkísérletes vizsgálatok kimutatták, hogy az altípus szelektív α_{1A}-antagonista vegyületek jelentős mértékben képesek relaxálni a terhes patkány uterus kontrakcióit. Vizsgálatunk célja a két hatóanyag típus közötti interakció felderítése volt együttes alkalmazás esetén in vitro patkány modellen.

Anyagok és módszerek: Az izolált 22 napos terhes patkány uterus mintákon ritmikus kontrakciókat hoztunk létre elektromos erőtér ingerléssel. Nonkumulatív módon megszerkesztettük a terbutalin dózis-hatás görbéjét önmagában illetve WB 4101 valamint 5-metilurapidil konstans koncentrációi mellett. Az interakció típusának meghatározására izobola analízist végeztünk.

Eredmények: A terbutalin dózis-hatás görbéje mind WB 4101, mind 5-metilurapidil jelenlétében balra tolódott, mellyel párhuzamosan a maximális kontrakció gátló hatás is szignifikánsan emelkedést mutatott. Az izobola analízis igazolta, hogy a fennálló interakció potenciáló szinergizmus.

Következtetések - megbeszélés: A potenciáló szinergizmus révén egy ilyen gyógyszerkombináció lehetőséget nyújthat a mellékhatások kockázatának csökkentésére, a terápia eredményességének növelésére. Ennek igazolása további in vivo vizsgálatokat igényel.

Az erythropoetin közvetlen hatásának vizsgálata humán méhlepény ereken in vitro

Dr. Resch Béla Endre[1], Dr. Sonkodi Sándor[2], Prof. Dr. Falkay György[1]
[1]Szegedi Tudományegyetem Gyógyszerhatástani és Biofarmáciai Intézet, Szeged
[2]Szegedi Tudományegyetem Általános Orvostudományi Kar,
I. sz. Belgyógyászati Klinika, Szeged

Bevezetés - a munka célkitűzései: Az erythropoietin (EPO) direkt vazokonstriktor hatása állatkísérletekben igazolt. Vizsgálatink célja a rekombináns

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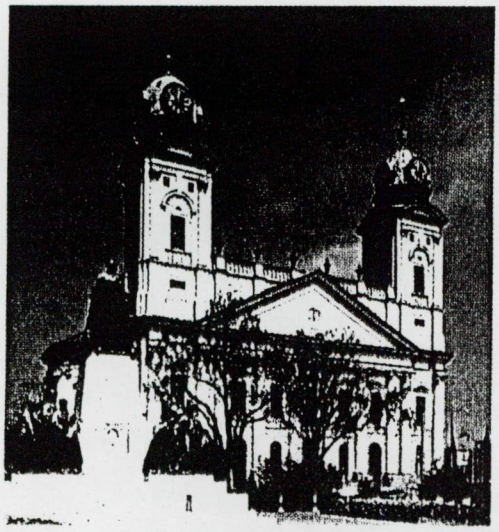

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2002. DECEMBER 12-14.

15:30 – 16:00
Szünet

16:00 – 17:15
IVARSZERVEK MŰKÖDÉSÉNEK FARMAKOLÓGIAI MODULÁCIÓJA
Előadások
Üléselnökök: Dr. Szolcsányi János, Dr. Pórszász Róbert

Klukovits A.[1], Gáspár R.[1], Sántha P.[2], Jancsó G.[2], Falkay Gy.[1] *(SZTE ÁOK Gyógyszerhatástani és Biofarmáciai Int.[1], Élettani Int.[2], Szeged):* Capsaicin-szenzitív érző rostok funkcionális jelentősége patkány uterusban
Zupkó I., Márki Á., Falkay Gy. *(SZTE ÁOK Gyógyszerhatástani és Biofarmáciai Int., Szeged):* Alfa-adrenerg blokád: a benzodiazepinek tokolitikus hatásának feltételezett mechanizmusa
Ducza E. Gáspár R., Falkay Gy. *(SZTE ÁOK Gyógyszerhatástani és Biofarmáciai Int., Szeged):* Az alpha 1-Adrenerg receptor altípusok szerepe az uterusz kontraktilitás szabályozásában patkány uteruszban és human miometriumban
Mihályi A., Ducza E., Gáspár R., Falkay Gy. *(SZTE ÁOK Gyógyszerhatástani és Biofarmáciai Int., Szeged):* A WB 4101 toxolytikus hatásának vizsgálata terhes patkány uteruson in vitro
Resch B., Ducza E., Gáspár R., Falkay Gy. *(SZTE ÁOK Gyógyszerhatástani és Biofarmáciai Int., Szeged):* Adrenerg receptor altípusok vizsgálata humán méhlepény erekben RT-PCR és elektromos téringerlés módszerekkel

17:15 – 17:45
Szünet

17:45 – 19:00
CHEMOPROTEKTÍV GYÓGYSZERKUTATÁSI IRÁNYOK (N-GENE SZIMPÓZIUM)
Szimpózium
Üléselnökök: Dr. Rablóczky György, Dr. Szilvássy Zoltán

Rablóczky Gy[1]., Tory K.[1], Literati Nagy P.[1], Szilvássy Z.[2] *(N-Gene Kut. Kft., Budapest[1], DE OEC ÁOK Farmakol. Farmakoter. Int., Debrecen[2]):* Hatástani stratégiák az N-GENE gyógyszerfejlesztéseiben
Ábrahám Cs.[1], Tory K.[1], Rablóczky Gy.[1], Literati Nagy P.[1], Szilvássy Z.[2] *(N-Gene Kut. Kft., Budapest[1], DE OEC ÁOK Farmakol. Farmakoter. Int., Debrecen[2]):* Kemoprotektív hatású N-GENE molekulák keresése