The effects of benzoylecgonine, oxytocin, ritodrine and atosiban on the contractility of myometrium. An experimental study

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Summary

Purpose: To investigate the response of pregnant and non pregnant rat myometrium to benzoylecgonine, a cocaine metabolite, and oxytocin and to investigate the efficiency of ritodrine and atosiban to overcome the effects of benzoylecgonine and oxytocin. *Methods:* Isolation of rat myometrial tissue and recording of contractile activity with isotonic muscle transducer. *Results:* Benzoylecgonine and oxytocin increase myometrial contractility, while atosiban and ritodrine induce myometrial relaxation. Atosiban was able to revoke the action of oxytocin but not the action of benzoylecgonine. Ritodrine was able to induce muscle relaxation in both oxytocin and benzoylecgonine administration. *Conclusion:* Cocaine metabolites seem to act on the myometrium through different pathways compared with oxytocin. After comparing two widely used tocolytic agents: atosiban and ritodrine, it is indicated that only ritodrine, a β2 adrenergic receptor agonist, can inhibit the action of cocaine metabolites. This finding indicates that the actions of cocaine on adrenergic mechanisms are responsible to a large part for its effects on myometrium contractility. The use of β2 adrenergic receptor agonists seems to be preferable for the treatment of myometrial contractions induced by cocaine consumption.

Key words: Benzoylecgonine; Oxytocin; Ritodrine; Atosiban; Myometrial contractions.

Introduction

Myometrium is a smooth muscle tissue and its function is controlled by numerous receptors responding to hormonal, chemical or mechanical stimuli [1]. Myometrial contractions play a very important role during pregnancy. In the cases of non terminal gestations, myometrial contractions are undesirable and dangerous for the outcome of pregnancy [2]. Oxytocin and cocaine are both known as factors causing myometrium contraction [3-6]. Atosiban and ritodrine are well known tocolytic agents, causing myometrial relaxation [7, 8].

Cocaine is an alkaloid ester with molecular formula $C_{17}H_{21}NO_4$ and MW = 303.4. It is extracted from the leaves of the plant Coca *(Coca novogranatense*). For therapeutic purposes, cocaine is used as local anesthetic in ophthalmologic, ear, nose and throat surgeries [9]. Cocaine is also used as a drug of abuse with amphetamine-like effects [10]. Cocaine consumption as a drug of abuse is a continuously growing global social and economical problem and highly related to health problems of the users [11-13]. The action of cocaine on the myometrium has been shown to increase contractility both *in vivo* and *in vitro* [6, 14]. The mechanism of action involves the rise of norepinephrine levels, the rise of intracellular Ca++ levels and the increase of production and concentration of prostaglandin PGF and PGE on the myometrium. Cocaine consumption also causes decline of prostacyclin production, decrease and alternation of β-receptors as well as of dopaminergic receptors: D_1 and D_2 [15, 16]. During pregnancy cocaine consumption has been shown to be associated with high risk of spontaneous abortion during first trimester, premature labor and even uterine collapse, premature collapse of embryonic membranes, retarded endometrial development, high possibility of placenta detachments and underweight newborn [14, 17].

Oxytocin is a peptide hormone with molecular formula $C_{45}H_{66}N_{12}O_{12}S_2$ and MW = 1007.19. Oxytocin is produced by neurons of the anterior hypothalamus and is secreted in the anterior pituitary gland. Oxytocin secretion follows a circadian rhythm with the peak secretion during night hours [18-20]. Labor, breastfeeding, situations of anxiety and emotional pressure are all stimuli for the secretion of oxytocin. On its target tissue, oxytocin acts as a muscle contraction agent [19, 21-23]. Oxytocin is also used as a therapeutic agent in cases when induction of myometrial contractions is needed. The oxytocin-receptor coupling initiates a G-protein mediated response, including phospholipase C [2, 24-26]. The increase of inositol triphosphate (IP_3) induces the rise of intracellular and extracellular Ca++, which in turn activates calmodulin. Calmodulin phosphorilates the light-chain myocin kinase and results in myometrium construction [27-29].

Ritodrine is a member of β-agonists with a well established use as a tocolytic agent in preterm labor. Ritodrine has MW: 323,820 and half-life of 6-9 min and total inac-

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tivation at 1.7-2.6 hours [30-32]. The mechanism of action involves the blockage of Ca^{2+} channels on cell membranes which prevents the entrance of Ca^{2+} in the cell and the simultaneous increase of intracellular cAMP [33-35]. Ritodrine can be administered intravenously (0.3 ng/ml) or per os (10 mg or 20 mg tablets). The wide use of ritodrine is based on two decades of clinical experience, its immediate effectiveness and the low cost of the treatment [36-39].

Atosiban is a synthetic peptide with MW: 944,199. The chemical structure of atosiban is similar to that of oxytocin with differences on position 1, 2, 4 and 8. Atosiban possesses a high affinity with oxytocin receptors on the myometrium [40, 41]. Thus, atosiban acts as an oxytocin antagonist and binds to the receptors of oxytocin and vasopressin. This reduction on the number of available receptors for oxytocin and vasopressin results in myometrial relaxation [42]. The binding of atosiban on oxytocin receptors results in the inactivation of intracellular G protein system and in both the Gp/11 for V_1 receptors of vasopressin and oxytocin and the Gs for the V_2 receptors of vasopressin [43-46]. As a therapeutic agent, atosiban is used to suppress myometrial contraction in cases of preterm or endangered labor [46]. It can be administered intravenously or intramuscularly. The half life depends of the way of administration and ranges between 15 to 80 min [39, 45].

The use of ritodrine and atosiban as tocolytic agents is well established [47, 48]. However their use in cases of cocaine consumption during pregnancy has not been adequately studied.

In cases of threatened preterm labor, including cases of cocaine consumption, the therapeutic approach is to remove the causing agent and to administer tocolytic agents. As threatened labor is an emergency medical case and usually there is no time for thorough investigation, the use of the most effective and safe tocolytic agent is mandatory.

In this study we investigated the response of pregnant and non pregnant rat (*Rattus norvegicus)* myometrium to the administration of benzoylecgonine, a cocaine metabolite, and oxytocin as uterine contraction agents. Additionally, we also investigated the efficiency of ritodrine and atosiban to overcome the effects of cocaine and oxytocin on the myometrium.

Materials and Methods

Experimental Animals

We used female *Rattus norvegicus* (Wistar strain) (n = 84) with body weight 200-250 g and age 8-20 weeks. The animals were kept in stable conditions of temperature (20-22°C) and light and were fed with Mixture 510 (ELVIZ, Xanthi, Greece). Forty-two of them were non-pregnant. The rest became pregnant after placing them with male rats for 72 hours. Pregnancy was confirmed with a pregnancy test.

Tissue preparation and recording of myometrial contractions

The animals were anesthetized with ether and the uterus was extracted from each animal. The two horns were isolated and kept in Kreb's medium at 37°C.

The isolated uterine horn of each animal was suspended in the waterbath of the muscle transducer within 10 min of its extraction. The tissue was left on suspension in the waterbath for 20-30 min in order to fully resume handling and to obtain uniform mobility. The waterbath contained 100 ml of Kreb's medium and was continually supplied with O_2. The agents to be tested were suspended directly in the waterbath. We recorded the changes in the tissue contraction over the first 10 min after the administration of each agent.

To record the myometrial contractions we used an isotonic muscle transducer (Harvard Medical Apparatus, Inc Holeston, MA, USA) which transformed the mechanical movement of the muscle into electric impulse signals and then into graphic charts. The apparatus also included a transducer signal amplifier (Harvard Medical Apparatus, Inc Holeston, MA, USA), a device used to control the strength of electric potential (Attenuator, San-Ei/M-1103, Harvard Medical Apparatus, Inc Holeston, MA, USA) and a recording device (Pen Recorder, Kipp&Zonen, Delft, Netherlands). The recording of graphic charts was made on constant recording speed (5 mm/min) and sensitivity (Gain 1 mV). The rate and intensity of myometrial contractions after administration of each specific pharmaceutical agent were evaluated with graphic charts on a procedure similar to electrocardiotocography and the Montevideo recording system.

The rate of contractions was estimated as the mean value of the number of contractions recorded over a time period of 10 min. The intensity of contractions was evaluated by estimating the mean value of the height of contractions that took place the first 10 min after administration of each agent.

Experimental groups

We divided the animals in seven groups, each containing six pregnant and six non-pregnant animals, as follows:

Group 1: to observe changes caused by benzoylecgonine, a cocaine metabolite (Abbott Laboratories, IL, USA), after administration of the first dose of 0.003 ng/ml and the second dose (0.003 ng/ml) after 10 min.

Group 2: to observe changes caused by administration of the first dose of atosiban (Tractocile, Ferring Pharmaceuticals) (0.0038 ng/ml), followed by a second dose 10 min later.

Group 3: to test after one dose of benzoylecgonine (0.003 ng/ml) followed by one dose of ritodrine (Yutopar, Solvay Pharma) at a dose of 0.001 ng/ml, after 10 min.

Group 4: to test benzoylecgonine (0.003 ng/ml) followed by the first dose of atosiban (0.0038 ng/ml) and a second dose of atosiban (0.0038 ng/ml) 10 min after the first.

Group 5: to test oxytocin (Oxytocin, G.A. Pharmaceuticals S.A., Greece) at a dose of 0.50 IU/ml, followed by one dose of ritodrine (0.001 ng/ml), 10 min later.

Group 6: to test oxytocin (0.5 IU/ml) followed by the first dose of atosiban (0.0038 ng/ml) after 10 min and the second dose of atosiban (0.0038 ng/ml) 10 min after the first dose.

Group 7: to test benzoylecgonine (one dose of 0.003 ng/ml) followed by oxytocin (one dose of 0.50 IU/ml) followed by atosiban (first dose of 0.0038 ng/ml and 10 min later a second dose of 0.0038 ng/ml) followed by ritodrine (one dose of 0.001 ng/ml).

Statistical analysis

The normality of studied parameters was evaluated with the Shapiro-Wilks test. The comparisons of the rate and strength of myometrial contractions between animals were made with the Student's t-test. Analysis of variance for repeated measurements was used to evaluate myometrial contractions after successive administration of agents. The two-tailed significant level was set at $p < 0.05$.

Table 1.

Experimental animals	Rate of contractions: Initial value	1st dose of benzoylecgonine	2nd dose of benzoylecgonine
Non-pregnant (n = 6)	0.58 ± 0.08#,a	0.75 ± 0.10#,*,b	0.77 ± 0.08#,*,c
Pregnant (n = 6)	0.78 ± 0.08#,a	1.43 ± 0.18#,*,b	1.63 ± 0.05#,*,c
Total (n = 12)	0.68 ± 0.13a	1.09 ± 0.38*, b	1.20 ± 0.46*, c

Rate of myometrial contractions (mean number of contractions/10 min ± SD) before and after administration of the 1st (0.0030 ng/ml) and 2nd (0.0030 ng/ml) dose of benzoylecgonine with 10 min time intervals between the doses, in pregnant and non-pregnant groups. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 2.

Experimental animals	Mean height of contractions in cm: Initial value	1st dose of benzoylecgonine	2nd dose of benzoylecgonine
Non-pregnant (n = 6)	1.22 ± 0.19#,a	4.28 ±0.21#,*,b	4.79 ± 0.27#,*,c
Pregnant (n = 6)	1.84 ± 0.12#,a	5.25 ± 0.87#,*,b	5.73 ± 0.74#,*,c
Total (n = 12)	1.53 ± 0.36a	4.77 ± 0.79*,b	5.26 ± 0.73*,c

Intensity of myometrial contractions (mean height of contractions in cm ± SD) before and after administration of the 1st (0.0003 ng/ml) and 2nd (0.0003 ng/ml) dose of benzoylecgonine with 10 min time intervals, in pregnant and non-pregnant groups. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Results

Benzoylecgonine

After the administration of the first dose of benzoylecgonine (0.0030 ng/ml) the rate of myometrial contractions increased significantly (compared to the initial value) in both pregnant ($p < 0.001$) and non-pregnant ($p = 0.032$) experimental groups (Table 1). After the second dose of benzoylecgonine (0.0030 ng/ml) the rate of myometrial contraction was slightly but not significantly increased in the pregnant group ($p = 0.098$). In the non-pregnant group the rate of contractions was similar before and after the administration of the second dose of benzoylegconine.

The intensity of myometrial contractions was increased in both pregnant ($p < 0.001$) and non-pregnant ($p < 0.001$) experimental groups after the administration of the first dose of benzoylecgonine (0.0030 ng/ml) (Table 2). A further increase in the intensity of contractions was observed after the administration of the second dose of benzoylecgonine (0.003 ng/ml) in both pregnant ($p = 0.007$) and non-pregnant ($p < 0.001$) experimental groups.

Atosiban

The administration of the first dose of atosiban induced a decrease in the rate of myometrial contractions in both the pregnant ($p = 0.001$) and non-pregnant ($p = 0.002$) group. The second dose of atosiban induced a further decrease in the rate of contractions ($p = 0.009$ for pregnant, $p = 0.018$ for non-pregnant) (Table 3).

Table 3.

Experimental animals	Mean number of contractions / 10 min: Initial value	1st dose of atosiban	2nd dose of adosiban
Non-pregnant (n = 6)	0.60 ± 0.11#,a	0.23 ± 0.05#,*,b	0.05 ± 0.05#,*,c
Pregnant (n = 6)	1.00 ± 0.27#,a	0.38 ± 0.12#,*,b	0.17 ± 0.05#,*,c
Total (n = 12)	0.80 ± 0.29a	0.31 ± 0.12*,b	0.11 ± 0.008*,c

Rate of myometrial contractions (mean number of contractions/10 min ± SD) before and after administration of the 1st (0.0038 ng/ml) and 2nd (0.0038 ng/ml) dose of atosiban, with 10 min time interval between the doses. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 4.

Experimental animals	Height of myometrial contractions: Initial value	1st dose of atosiban	2nd dose of adosiban
Non-pregnant n = 6	3.56 ± 0.84#,a	1.39 ± 0.31#,*,b	0.25 ± 0.27#,*,c
Pregnant n = 6	8.08 ± 2.27#,a	3.25 ± 1.51#,*,b	1.88 ± 0.83#,*,c
Total n = 12	5.82 ± 3.03a	2.32 ± 1.42*,b	1.06 ± 1.03*,c

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) before and after administration of the 1st (0.0038 ng/ml) and 2nd (0.0038 ng/ml) dose of atosiban, with 10 min time interval between the doses. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 5.

Experimental animals	Mean height of contractions per min: Initial value	Benzoylecgonine	Ritodrine
Non-pregnant n = 6	0.63 ± 0.08#,a	0.75 ± 0.08#,*,b	0.15 ± 0.05#,*,c
Pregnant n = 6	0.98 ± 0.08#,a	1.48 ± 0.12#,*,b	0.27 ± 0.08#,*,c
Total n = 12	0.81 ± 0.19a	1.12 ± 0.39*,b	0.21 ± 0.09*,c

Rate of myometrial contractions (mean number of contractions/10 min ± SD), after administration of one dose of benzoylecgonine (0.003 ng/ml) followed by one dose of ritodrine (0.001 ng.ml). *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

The intensity of contractions was significantly decreased after the first dose of atosiban in both pregnant ($p = 0.001$) and non-pregnant groups ($p = 0.014$), and further decreased by the administration of the second dose of atosiban ($p = 0.039$ for pregnant and $p = 0.039$ for non-pregnant). The intensity of contraction after the second dose of atosiban was lower compared to the initial values ($p = 0.002$ for pregnant, $p < 0.001$ for non-pregnant). The decrease of the intensity of contractions was greater in the pregnant compared to the non-pregnant group (Table 4).

Benzoylecgonine and ritodrine

The response of myometrium in terms of rate and intensity of contractions, to the administration of benzoylecgonine first and then to ritodrine is shown in Table 5. The rate of contractions significantly increased after benzoylecgonine administration in the pregnant ($p < 0.001$) and non-pregnant ($p = 0.038$) group. Administration of ritodrine significantly reduced the number of myometrial contractions by 80% in pregnant ($p < 0.001$) and by 81,8% in non-pregnant ($p < 0.001$) rats.

Table 6.

	Mean height of contractions in cm		
Experimental animals	Initial value	Benzoylecgonine	Ritodrine
Non-pregnant n = 6	1.33 ± 0.06$^{\#,a}$	4.14 ± 0.04$^{\#,*,b}$	1.09 ± 0.11$^{\#,*,c}$
Pregnant n = 6	2.05 ± 0.16$^{\#,a}$	5.28 ± 0.49$^{\#,*,b}$	1.38 ± 0.38$^{\#,*,c}$
Total n = 12	1.69 ± 0.40^{a}	4.71 ± 0.68^{*,b}	1.24 ± 0.31^{*,c}

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) after the administration of benzoylecgonine (0.003 ng/ml) followed by ritodrine (0.001 ng/ml), with 10 min time interval between the doses. In pregnant and non-pregnant groups. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 7.

	Mean number of contractions per min			
Experimental animals	Initial value	Benzoylecgonine	1st dose of atosiban	2nd dose of adosiban
Non-pregnant n = 6	0.63 ± 0.08$^{\#}$	0.78 ± 0.12$^{\#,*,a}$	0.75 ± 0.11$^{\#}$	0.73 ± 0.08$^{\#}$
Pregnant n = 6	0.80 ± 0.14$^{\#}$	1.38 ± 0.32$^{\#,*,a}$	1.32 ± 0.33$^{\#}$	1.35 ± 0.37$^{\#}$
Total n = 12	0.72 ± 0.14	1.08 ± 0.39^{*,a}	1.03 ± 0.38	1.04 ± 0.41

Rate of myometrial contractions after the administration of one dose of benzoylecgonine (0.003 ng/ml), the 1st dose of atosiban (0.0038 ng/ml) and the 2nd dose of atosiban (0.0038 ng/ml). The time intervals between the administrations of each agent were 10 min. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 8.

	Mean height of contractions (cm / 10 min)			
Experimental animals	Initial value	Benzoylecgonine	1st dose of atosiban	2nd dose of adosiban
Non-pregnant n = 6	1.34 ± 0.06$^{\#}$	4.40 ± 0.28$^{\#,*,a}$	4.38 ± 0.38$^{\#,*}$	4.42 ± 0.25$^{\#,*}$
Pregnant n = 6	1.99 ± 0.20$^{\#}$	5.08 ± 0.35$^{\#,*,a}$	4.95 ± 0.24$^{\#,*}$	4.90 ± 0.38$^{\#,*}$
Total n = 12	1.67 ± 0.37	4.74 ± 0.46^{*,a}	4.66 ± 0.43*	4.66 ± 0.40*

Changes of the intensity of myometrial contractions (mean height of contraction in cm) after the administration of one dose of benzoylecgonine (0.0030 ng/ml) followed by the 1st dose of atosiban (0.0038 ng/ml) and the 2nd dose of atosiban (0.0038 ng/ml), in pregnant and non-pregnant groups. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 9.

	Mean number of contractions per min		
Experimental animals	Initial value	Oxytocin	Ritodrine
Non-pregnant n = 6	0.63 ± 0.10$^{\#}$	1.27 ± 0.14$^{\#,*,a}$	0.23 ± 0.08$^{\#,*,b}$
Pregnant n = 6	1.13 ± 0.36$^{\#}$	1.65 ± 0.39$^{\#,*,a}$	0.28 ± 0.13$^{\#,*,b}$
Total n = 12	0.88 ± 0.36	1.46 ± 0.34$^{\#,*,a}$	0.26 ± 0.11$^{\#,*,b}$

Rate of myometrial contractions (mean number of contractions/10 min ± SD) after the administration of one dose of oxytocin (0.50 IU/ml) followed by one dose of ritodrine (0.0005 ng/ml). The time intervals between the administration of each agent were 10 min. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

The effects of benzoylecgonine and ritodrine on the intensity of myometrial contractions are shown in Table 6. Similarly to the pattern of changes in the number of contractions, the intensity of myometrial contractions was significantly increased after administration of benzoylecgonine and reduced after the subsequent administration of ritodrine. The effects of benzoylecgonine and ritodrine was significantly higher in the pregnant compared to non-pregnant group ($p = 0.028$ for benzoylecgonine and $p < 0.001$ for ritodrine).

Benzoylecgonine and atosiban

The effects of benzoylecgonine and atosiban administration on the pregnant and non-pregnant animals are shown in Table 7. Benzoylecgonine administration resulted in a significant increase on the rate of myometrial contractions in both pregnant ($p = 0.03$) and non-pregnant ($p = 0.007$) animals. The administration of the first and second dose of atosiban did not affect the rate of contractions at a significant level. The effect of benzoylecgonine was significantly higher in the pregnant compared with non-pregnant group (0.58 ± 0.30 vs 0.15 ± 0.05, $p = 0.016$). On the contrary, the effect of atosiban did not differ in the two groups.

The intensity of myometrial contractions after the administration of benzoylecgonine and atosiban are shown in Table 8. The administration of benzoylecgonine caused an increase on the intensity of myometrial contractions in the pregnant ($p < 0.001$) and non-pregnant ($p < 0.001$) group while the administration of the first and second dose of atosiban did not cause significant changes on the intensity of contractions. The changes on the intensity of myometrial contractions caused by benzoylecgonine and atosiban did not differ between the pregnant and non-pregnant group.

Oxytocin and ritodrine

The response of myometrium of pregnant and non-pregnant groups to the administration of oxytocin and subsequently to ritodrine is presented in Table 9.

After oxytocin administration the rate of myometrial contractions increased in both the pregnant ($p = 0.004$) and non-pregnant ($p = 0.001$) group. After the administration of ritodrine, the number of contractions was reduced to levels lower than the initial (those before administration of oxytocin) in both pregnant ($p < 0.001$) and non-pregnant ($p < 0.001$) groups. Furthermore, the effect of oxytocin and ritodrine on the rate of myometrial contractions was greater in the pregnant compared to non-pregnant group (-1.37 ± 0.30 vs -1.03 ± 0.14, $p = 0.003$).

The intensity of myometrial contractions increased after administration of oxytocin in the pregnant ($p = 0.008$) and non-pregnant ($p = 0.001$) group (Table 10). After administration of ritodrine the intensity of myometrial contractions was decreased in both pregnant ($p = 0.002$) and non-pregnant ($p < 0.001$) groups. There were no significant differences in the strength of the effects of oxytocin and ritodrine between the pregnant and non-pregnant group.

Oxytocin and atosiban

The administration of oxytocin caused a significant increase in the rate of myometrial contractions in pregnant ($p < 0.001$) and non-pregnant ($p < 0.001$) groups, as shown in Table 11. The rate of myometrial contractions decreased after the administration of the first dose of atosiban in both pregnant ($p < 0.001$) and non-pregnant ($p < 0.001$) experimental groups. The administration of

Table 10.

Experimental animals	Height of contractions (cm)		
	Initial value	Oxytocin	Ritodrine
Non-pregnant n = 6	2.33 ± 0.54	7.06 ± 0.90^{*,a}	1.30 ± 0.36^{*,b}
Pregnant n = 6	2.85 ± 0.40	7.94 ± 1.98^{*,a}	1.53 ± 0.26^{*,b}
Total n = 12	2.59 ± 0.53	7.50 ± 1.54^{*,a}	1.42 ± 0.32^{*,b}

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) after the administration of one dose of oxytocin (0.50 IU/ml) followed by one dose of ritodrine (0.0005 ng/ml), in pregnant and non-pregnant groups. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 11.

Experimental animals	Mean number of contractions per min			
	Initial value	Oxytocin	1st dose of atosiban	2nd dose of adosiban
Non-pregnant n = 6	0.62 ± 0.08$^{\#}$	1.22 ± 0.16$^{\#,*,a}$	0.22 ± 0.10$^{\#,*,b}$	0.05 ± 0,05$^{\#,*,c}$
Pregnant n = 6	0.88 ± 0.19$^{\#}$	2.74 ± 0.19$^{\#,*,a}$	0.33 ± 0.10$^{\#,*,b}$	0.22 ± 0.04$^{\#,*,c}$
Total n = 12	0.75 ± 0.20$^{\#}$	1.98 ± 0.17$^{\#,*,a}$	0.28 ± 0.11$^{\#,*,b}$	0.13 ± 0.10$^{\#,*,c}$

Rate of myometrial contractions (mean number of contractions/10 min ± SD) after the administration of one dose of oxytocin (0.50 IU/ml), the 1st dose of atosiban (0.0015 ng/ml) and the 2nd dose of atosiban (0.0015 ng/ml). *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 12.

Experimental animals	Mean height of contractions			
	Initial value	Oxytocin	1st dose of atosiban	2nd dose of adosiban
Non-pregnant (n = 6)	1.89 ± 0.13$^{\#}$	4.51 ± 0.79$^{\#,*,a}$	2.23 ± 0.24$^{\#,b}$	0.50 ± 0.55$^{\#,*,c}$
Pregnant n = 6	2.36 ± 0.31$^{\#}$	8.80 ± 1.84$^{\#,*,a}$	3.52 ± 1.16$^{\#,b}$	1.67 ± 0.75$^{\#,*,c}$
Total n = 12	2.94 ± 0.30	6.65 ± 2.62^{*,a}	2.87 ± 1.05^{b}	1.08 ± 0.87^{*,c}

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) after the administration of one dose of oxytocin (0.50 IU/ml), the 1st dose of atosiban (0.0015 ng/ml) and the 2nd dose of atosiban (0.0015 ng/ml). *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

the second dose of atosiban caused a further decrease in the rate of myometrial contraction to levels lower to the original (before the administration of oxytocin). The two-way mixed analysis of variance showed that the effect of oxytocin and atosiban on the rate of myometrial contractions was significantly stronger in the pregnant group compared to non-pregnant group (-2.52 ± 0.17 vs -1.17 ± 0.12, $p < 0.001$).

The intensity of the myometrial contractions was increased after the administration of oxytocin in both pregnant ($p = 0.002$) and non-pregnant ($p = 0.002$) experimental animals. Administration of the first dose (0.0015 ng/ml) of atosiban induced a decrease in the intensity of myometrium in the pregnant ($p < 0.001$) and non-pregnant ($p = 0.004$) group. The administration of the second dose of atosiban (0.0015 ng/ml) induced further decrease on the intensity of myometrial contractions on pregnant ($p = 0.039$) and non-pregnant (0.012) groups. As observed in the rate of myometrial contractions, the effects of oxytocin and atosiban on the intensity of myometrial contractions was significantly stronger in the pregnant compared to non-pregnant group ($p < 0.001$).

Table 13.

Experimental animals	Mean number of contractions per min					
	Initial value	Benzoylec-gonine	Oxytocin	1st dose of atosiban	2nd dose of adosiban	Ritodrine
Non-pregnant n = 6	0.80 ± 0.06$^{\#}$	1.05 ± 0.05$^{\#,*,a}$	1.33 ± 0.12$^{\#,b}$	1.13 ± 0.12$^{\#,c}$	1.07 ± 0.12$^{\#}$	0.13 ± 0.05$^{\#,*,d}$
Pregnant n = 6	1.35 ± 0.10$^{\#}$	1.67 ± 0.12$^{\#,*,a}$	1.85 ± 0.29$^{\#,b}$	1.58 ± 0.23$^{\#,c}$	1.53 ± 0.14$^{\#}$	0.28 ± 0.08$^{\#,*,d}$
Total n = 12	1.08 ± 0.30	1.36 ± 0.33^{a}	1.59 ± 0.34^{b}	1.36 ± 0.30^{c}	1.30 ± 0.27	0.21 ± 0.10^{*,d}

Rate of myometrial contractions (mean number of contractions/10 min ± SD) after the administration of one dose of benzoylecgonine (0.0030 ng/ml), one dose of oxytocin (0.50 IU/ml), 1st dose of atosiban (0.0038 ng/ml), 2nd dose of atosiban (0.0038 ng/ml), one dose of ritodrine (0.001 ng/ml) with 10 min interval between the administration of each agent. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Table 14.

Experimental animals	Mean height of contractions					
	Initial value	Benzoylec-gonine	Oxytocin	1st dose of atosiban	2nd dose of adosiban	Ritodrine
Non-pregnant n = 6	1.91 ± 0.16$^{\#}$	4.92 ± 0.26$^{\#,*,a}$	9.45 ± 0.378$^{\#,b}$	4.61 ± 0.297$^{\#,c}$	4.54 ± 0.317$^{\#}$	1.25 ± 0.27$^{\#,*,d}$
Pregnant n = 6	2.30 ± 0.27$^{\#}$	5.62 ± 0.47$^{\#,*,a}$	10.94± 0.457$^{\#,b}$	5.83 ± 0.49$^{\#,c}$	5.56 ± 0.40$^{\#}$	1.81 ± 0.41$^{\#,*,d}$
Total n = 12	2.10 ± 0.295	5.27 ± 0.51$^{\#,*,a}$	10.19 ± 0.875^{b}	5.22 ± 0.75^{c}	5.052 ± 0.63	1.527 ± 0.44^{*,d}

Intensity of myometrial contractions (mean height of contractions/10 min ± SD) after the administration of one dose of benzoylecgonine (0.0030 ng/ml), one dose of oxytocin (0.50 IU/ml), 1st dose of atosiban (0.0038 ng/ml), 2nd dose of atosiban (0.0038 ng/ml), one dose of ritodrine (0.001 ng/ml) with 10 min interval between the administration of each agent. *: $p < 0.05$ versus initial value, #: $p < 0.05$, non-pregnant versus pregnant. Significant differences ($p < 0.05$) within each group (non-pregnant or pregnant) over time are shown by letters.

Oxytocin, benzoylecgonine, atosiban and ritodrine

Administration of benzoylecgonine (0.003 ng/ml) caused a significant rise in the rate of myometrial contractions in both pregnant ($p < 0.001$) and non-pregnant ($p < 0.001$) groups. The following oxytocin administration (0.5 IU/dose) further increased the rate of myometrial contractions ($p = 0.012$ for pregnant, $p = 0.001$ for non-pregnant). When the first dose of atosiban (0.0038 ng/ml) was administrated, the rate of contractions was significantly decreased ($p = 0.009$ for pregnant and $p = 0.01$ for non-pregnant). After the second dose of atosiban (0.0038 ng/ml) a decrease in the rate of contractions was observed but not at a significant level. Noticeably, the rate of contractions reached similar levels to those after administration of benzoylecgonine. After the following administration of ritodrine (0.001 ng/ml), the rate of contractions was significantly decreased to levels lower than the initial, in both pregnant ($p = 0.001$) and non-pregnant ($p < 0.001$) groups. The effect of ritodrine was more intense in the pregnant compared to non-pregnant group (-1.25 ± 0.14 vs -0.93 ± 0.14, $p = 0.003$) (Table 13).

The intensity of myometrial contraction was increased after the administration of benzoylecgonine ($p < 0.001$ for pregnant and $p < 0.001$ for non-pregnant). The subsequent administration of oxytocin further increased the intensity of contractions ($p < 0.001$ for pregnant and $p < 0.001$ for non-pregnant groups). After the administration

of the first dose of atosiban the intensity of contractions was reduced in both pregnant ($p = 0.01$) and non-pregnant rats ($p < 0.001$). After the second dose of atosiban the reduction of the intensity of contractions was not significant but it reached levels similar to those after the administration of benzoylecgonine. The administration of ritodrine induced a significant decrease in the intensity of contractions, to levels lower that the initial (before the administration of oxytocin) ($p < 0.001$ for pregnant, $p < 0.001$ for non-pregnant). The effect of ritodrine was shown to be stronger in the pregnant compared to non-pregnant animals (-3.76 ± 0.58 vs -3.29 ± 0.16, $p = 0.096$) (Table 14).

Discussion

As shown in our results, the administration of benzoylecgonine, a cocaine metabolite and oxytocin increases myometrial contractility, while the administration of atosiban and ritodrine induce myometrial relaxation. In particular, atosiban was shown to be able to revoke the action of oxytocin but not the action of benzoylecgonine. Ritodrine was shown to be able to induce muscle relaxation in both oxytocin and benzoylecgonine administration.

The effects of oxytocin on myometrial contractility are well known [40-50]. In our experiments the administration of oxytocin caused an increase of the rate and the strength of myometrial contractions, whereas the subsequent administration of benzoylecgonine further increased those parameters. This additional increase of contractility is attributed to the different mechanisms of actions of oxytocin and benzoylecgonine.

Previous studies have shown that cocaine augments myometrium contractility by both adrenergic and non-adregenergic mechanisms [51]. Regarding the adrenergic receptors, it is possible that cocaine acts on a post-receptor level [52]. On the other hand, ritodrine is a β-2 adrenergic receptor agonist. Our results showing that ritodrine is a potent inhibitor of the action of cocaine metabolites on myometrium confirm the opinion that the effects of cocaine on adrenergic mechanisms are responsible to a large part for cocaine actions on myometrium.

In conclusion, cocaine metabolites seem to act on the myometrium through different pathways compared with oxytocin. After comparing two widely used tocolytic agents: atosiban and ritodrine, it is indicated that only ritodrine, a β-2 adrenergic receptor agonist, can inhibit the action of cocaine metabolites on myometrial tissue. This finding indicates that the actions of cocaine on adrenergic mechanisms are responsible to a large part for its effects on myometrium contractility. The use of β-2 adrenergic receptor agonists seems to be preferable for the treatment of myometrial contractions induced by cocaine consumption in non-pregnant ($p < 0.001$) rats.

References

[1] Newman B.R.: "Uterine contraction Assessment". *Obstet. Gynecol. Clin. North Am.,* 2005, *32*, 341.
[2] Lopez B.A.: "Mechanisms of labour-biochemical aspects". *BJOG*, 2003, 110, *13* (suppl. 20), 39.
[3] Blanks A.M., Thornston S.: "The role of oxytocin in parturition". *BJOG*. 2003, *110* (suppl. 20), 46.
[4] Kiss A., Mikkelsen J.D.: "Oxytocin – anatomy and functional assignments: a mini review". *End. Regulat*., 2005, *39,* 97.
[5] Plessinger M.A., Woods J.R.: "Cocaine in pregnancy. Recent data on maternal and fetal risk". *Obstet. Gynecol. Clin. North Am.,* 1998, *25*, 99.
[6] Savitz D.A., Henderson L., Dole N., Harring A., Wilkins D.G., Rollins D., Thorp J.H. *et al*.: "Indicators of cocaine exposure and preterm birth". *Obstet. Gynecol*., 2002, *99,* 458.
[7] Buscher U., Chen F.C., Riesenkampff E., von Dehn D., David M., Dudenhausen J.W.: "Effects of oxytocin receptor antagonist atosiban on pregnant myometrium in vitro". *Obstet. Gynecol.,* 2001, *98,* 117.
[8] Merkatz I.R., Peter J.B., Barden T.P.: "Ritodrine Hydrochloride: A betamimetic agent for use in preterm labor. Evidence of efficacy". *Obstet. Gynecol*., 1980, *56*, 7.
[9] Jones R.T.: "The pharmacology of cocaine". *NIDA Res. MONGR,* 1984, *50,* 34.
[10] Monga M.: "The effect of cocaine on myometrial contractile activity: basic mechanisms". *Semin. Perinatol*., 1996, *20,* 140.
[11] Rosenak D., Diamant Y.Z., Yaffe H., Hornstein E.: "Cocaine: Maternal use during pregnancy and its effect on the mother, the fetus and the infant". *Obstet. Gynecol. Surv*., 1990, *45,* 348.
[12] Monga M., Weisbrodt N.W., Andres R.L., Sanborn B.M.: "The acute effect of cocaine exposure on pregnant myometrial contractile activity". *Am. J. Obstet. Gynecol.,* 1993, *169*, 782.
[13] Plessinger M.A., Woods J.R.: "Cocaine in pregnancy. Recent data on maternal and fetal risk". *Obstet. Gynecol. Clin. North Am*., 1998, *25*, 99.
[14] Mirsha A., Landzberg B.R., Parente J.T.: "Uterine rupture in association with alkaloidal (crack) cocaine abuse". *Am. J. Obstet. Gynecol*.,1995, *173,* 243.
[15] Wang H.Y., Runyan S., Yadin E., Friedman E.: "Prenatal exposure to cocaine selectively reduces D1 dopamine receptor-mediated activation of striatal Gs proteins". *J. Pharmacol. Exp., Ther.,* 1995, *273*, 492.
[16] Monga M., Weisbrodt N.W., Andres R.L., Sanborn B.M.: "Cocaine acutely increases rat myometrial contractile activity by mechanisms other than potentiation adrenergic pathways". *Am. J. Obstet. Gynecol.,* 1993, *169*, 1502.
[17] Hurd W.W., Betz A.L., Dombrowski M.P., Fomin V.P.: "Cocaine augments contractility of the pregnant human uterus by both adrenergic and non-adrenergic mechanisms". *Am. J. Obstet. Gynecol*., 1998, *178*, 1077.
[18] Blanks A.M., Smygol A., Thorton S.: "Regulation of oxytocin receptors and oxytocin receptor signalling". *Semin. Reprod. Med*., 2007, *25,* 52.
[19] Gimpl G., Fahrenholz F.: "The oxytocin receptor system: structure, function, and regulation". *Physiol. Rev.,* 2001, *81,* 629.
[20] Kiss A., Mikkelsen J.D.: "Oxytocin – anatomy and functional assignments: a mini review". *End. Regulat*., 2005, *39*, 97.
[21] Blanks A.M., Thornston S.: "The role of oxytocin in parturition". *BJOG*, 2003, *110* (suppl. 20), 46.
[22] Ivell R., Bathgate R.A., Walther N., Kimura T.: "The molecular basis of oxytocin and oxytocin receptor gene expression in the reproductive tissues". *Adv. Exp. Med. Biol.,* 1998, *449*, 297.
[23] Bossmar T., Akerlund M., Szamatowicz J., Laudanski T., Fantoni G., Maggi M.: "Receptor-mediate uterine effects of vasopressin and oxytocin in nonpregnant women". *Br. J. Obstet. Gynecol.,* 1995, *102*, 907.
[24] Brenninkmeijer C.B.A.P., Price S.A., Bernal A.L., Phaneuf S.: "Expression of G-protein-coupled receptor kinases in pregnant term and non pregnant human myometrium". *J. Endocrinol*., 1999, *162,* 401.
[25] Mamamtavrishvili I.D., Kintraia P.I., Kintraia N.P., Gotsirizde E.G., Bakradze M.M.: "Molecular mechanisms of regulation uterine contractile activity during pregnancy and labor in mammals". *Georgian Med. News*, 2006, *138*, 16.
[26] Shmigol A.V., Eisner D.A., Wray S.: "Simultaneous measurements of changes in sarkoplasmic reticulum and cytosolic". *J. Physiol.,* 2001, *531,* 701.

[27] Molnar M., Rigo J., Hertelendy F.: "Signal transduction in human myometrial cells". *Acta Physiol. Hung*., 1996, *84,* 89.
[28] Myatt L., Lye S.J.: "Expression localization and function of prostaglandin receptors in myometrium Prostaglandins". *Leukot Essent. Fatty Acids,* 2004, *70,* 137.
[29] Karteris E., Zervou S., Pang Y., Dong J., Hillhouse E.W., Randeva H., Thomas P.: "Progesterone signalling in human mtometrioum through two novel membrane G protein-coupled receptors: Potential role in functional progesterone withdrawal at term". *Mol. Endocrinol.,* 2006, *20*, 1519.
[30] Merkatz I.R., Peter J.B., Barden T.P.: "Ritodrine Hydrochloride: A betamimetic agent for use in preterm labor. Evidence of efficacy". *Obstet. Gynecol.,* 1980, *56*, 7.
[31] Ikeda S., Tamaoki H.: "Pharmacological investigation of ritodrine hydrochloride, a beta 2-adrenoceptor stimulant". *Jpn. J. Pharmacol*., 1984, *36*, 477.
[32] Barden T.P., Peter J.B., Merkatz I.R.: "Ritodrine hydrochloride: a betamimetic agent for use in preterm labor; pharmacology, clinical history, administration, side effects and safety". *Obstet. Gynecol.,* 1980, *56*, 1.
[33] Ikeda S., Tamaoki H., Akahane M., Nebashi Y.: "Effects of ritodrine hydrochloride, a beta 2-adrenoceptor stimulant, on uterine motilities in late pregnancy". *Jpn. J. Pharmacol.,* 1984, *35,* 319.
[34] Dubin N.H., Ghodgaonkar R.B.: "Effect of ritodrine hydrochloride and dibutyryl cyclic AMP on contractile activity and prostanoid production of uteri from pregnant rats in vitro". *Prostaglandins Leucot Med*., 1984, *13,* 119.
[35] Izumi H., Kishikawa T.: "Effects of ritodrine hydrochloride, a beta 2-adrenoceptor agonist, on smooth muscle cells of the rat myometrium of pregnant rats". *Br. J. Pharmacol*., 1982, *76,* 463.
[36] Gyetvai K., Hannah M.E., Hodnett E.D., Ohisson A.: "Tocolytics for preterm labor: a systematic review". *Obstet. Gynecol.,* 1999, *94,* 869.
[37] Fung Lam M.D., Pamela G.: "β - Agonist Tocolytic Therapy". *Obstet. Gynecol. Clin. N A.,* 2005, *32,* 457.
[38] Caughey A.B., Parer J.T.: "Tocolysis with beta-adrenergic receptor agonists". *Semin. Perinatol*., 2001, *25*, 248.
[39] Lamont R.F.: "The development and introduction of anti-oxytocic tocolytics". *BJOG*, 2003, *110* (suppl. 20), 108.
[40] Wilson R.J., Allen M.J., Nandi M., Glies H., Thornton S.: "Spontaneous contractions of myometrial from humans, non-human primate and rodents are sensitive to selective oxytocin receptor antagonism in vitro". *BJOG*, 2001, *108*, 960.
[41] Manning M., Miteva K., Pancheva S., Stoev., Wo N.C., Chan W.Y.: "Design and synthesis of highly selective in vitro and in vivo uterine receptors antagonists of oxytocin: comparisons with atosiban". *Int. J. Pept. Protein Res*., 1995, *46,* 244.
[42] Buscher U., Chen F.C., Riesenkampff E., von Dehn D., David M., Dudenhausen J.W.: "Effects of oxytocin receptor antagonist atosiban on pregnant myometrium in vitro". *Obstet. Gynecol.,* 2001, *98,* 117.
[43] Gaspar R., Gal A., Galik M., Ducza E., Minorics R., Kolarovszki-Sipiczki Z. *et al*.: "Different roles of alpha(2)-adrenoreceptor subtypes in non-pregnant and late-pregnant uterine contractility in vitro in the rats". *Neurochem. Int.,* 2007, *51,* 311.
[44] Engstrom T., Bratholm P., Vilhardt H., Christensen N.J.: "Effect of oxytocin receptor and β_2-adrenoreceptor blockade on myometrial oxytocin receptors in parturient rats". *Biol. Reprod*., 1999, *60,* 322.
[45] Hemler H., Brunbauer M., Rohrmeister K.: "Exploring the role of tractocile in everyday clinical practice". *BJOG*, 2003, *110,* 113.
[46] Husslein P.: "Development and clinical experience with the new evidence – based tocolytic atosiban". *Acta Obstet. Gynecol. Scand.,* 2002, *81,* 633.
[47] Moutquin J.M., Sherman D., Cohen H., Mohide P.T., Hochner-Clnikier D., Fejgin M. *et. al*.: "Double blind, randomized, controlled trial of atosiban and ritodrine in the treatment of preterm labor: a multicenter effectiveness and safety study". *Am. J. Obstet. Gynecol*., 2000, *182,* 1191.
[48] "Worldwide Atosiban versus Beta – agonists Study group; Effectiveness and safety of the oxytocin antagonist atosiban versus beta-adrenergic agonists in the treatment of preterm labour". *BJOG*, 2001, *108*, 133.
[49] Alfirevic Z., Kelly A.J., Dowswell T.: "Intravenous oxytocin alone for cervical ripening and induction of labour". *Cochrane Database Syst. Rev.,* 2009, *7,* CD003246.
[50] Blanks A.M., Smygol A., Thorton S.: "Regulation of oxytocin receptors and oxytocin receptor signalling". *Semin. Reprod. Med.,* 2007, *25,* 52.
[51] Hurd W.W., Betz A.L., Dombrowski M.P., Fomin V.P.: "Cocaine augments contractility of the pregnant human uterus by both adrenergic and non-adrenergic mechanisms". *Am. J. Obstet. Gynecol.,* 1998, *178,* 1077.
[52] Hurd W.W., Gauvin J.M., Dombrowski M.P., Hayashi R.H.: "Cocaine selectively inhibits beta-adrenergic receptor binding in pregnant human myometrium". *Am. J. Obstet. Gynecol*., 1993, *169,* 644.

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