



Term-dependency of P2 receptor-mediated contractile responses of isolated human pregnant uterus[☆]

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Received 14 April 2005; received in revised form 1 November 2005; accepted 14 November 2005

Abstract

Objective: The aim of the study was to test the hypothesis that in the human uterus, the effectiveness of P2 receptor-mediated contractile responses is up-regulated during pregnancy.

Study design: Experiments were performed on myometrial samples obtained from women undergoing caesarean section at 28–30 weeks of pregnancy (3 women, Group 1), 32–34 weeks of pregnancy (6 women, Group 2) and 38–41 weeks of pregnancy (16 women, Group 3). Concentration–response relationships for a non-selective P2 receptor agonist, adenosine 5'-triphosphate (ATP), a selective P2X receptor agonist, α,β -methylene-ATP (α,β -meATP), and a frequency–response relationship for non-adrenergic non-cholinergic (NANC) electrical field stimulation (EFS) were obtained using routine pharmacological organ bath technique. Effects of pyridoxalphosphate azophenyl-2',4'-disulphonic acid (PPADS, 10^{-5} M), a P2 receptor antagonist, were also evaluated. Parametric Student's *t*-test, non-parametric Wilcoxon *T*-test, Mann–Whitney *U*-test, two-way analysis of variance (ANOVA) and Krushkal–Wallis tests were used for statistical analysis.

Results: ATP (10^{-6} to 3×10^{-4} M), α,β -meATP (10^{-7} to 3×10^{-5} M) and EFS (2–32 Hz) evoked contractions of isolated pregnant uterus in all three groups. Uterus responses to ATP were not correlated with the term of pregnancy while the amplitude of uterine contractions to α,β -meATP and EFS was higher in full term pregnancy than in earlier pregnancy. PPADS antagonized uterus responses to α,β -meATP and EFS, but not to ATP, in all three groups.

Conclusion: P2X receptor-mediated contractions of human pregnant uterus to α,β -meATP and EFS, but not to ATP, are increased with the progression of pregnancy.

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Keywords: P2 receptors; Human myometrium; Terms of pregnancy

1. Introduction

A substantial body of evidence has been accumulated during the last three decades to support the view that extracellular adenosine 5'-triphosphate (ATP) is able to

regulate many cellular functions acting on specific receptors named P2 receptors [1]. According to current classification, P2 receptors are divided into two super families – ionotropic P2X and metabotropic P2Y receptors; seven subtypes of P2X and eight subtypes of P2Y receptors have been cloned and characterized [2,3].

P2 receptors are widely distributed in the animal and human tissue [4]. The presence of some P2 receptors subtypes was shown in the pregnant and non-pregnant uterus of several species [5–13] while the data about human uterus are rather limited. Recently we found that ATP and some other P2

[☆] P2X receptor-mediated contractile responses of isolated human uterus are increased with progression of the pregnancy.

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receptor agonists caused contractions of human full-term pregnant, but not non-pregnant uterus which were antagonized by the antagonist of P2 receptors, pyridoxalphosphate azophenyl-2',4'-disulfonic acid (PPADS) [14]. The present study aims to evaluate the time-dependency of P2 receptor-mediated responses of human uterus during pregnancy. Considering the big ethical problems to perform such type of investigations, we obtained permission from the local Ethical Committee for only a limited period of time, and as such the number of patients included in the study is small.

2. Materials and methods

The study was approved by the Ethical Committee of Kazan State Medical University; documented, informed consent was obtained from each woman who took part in the study.

A full-thickness myometrial sample was obtained from the superior edge of a transverse uterine incision from women undergoing elective caesarean section at 28–30 weeks of pregnancy (3 women, Group 1), 32–34 weeks of pregnancy (6 women, Group 2) and 38–41 weeks of pregnancy (16 women, Group 3). Indications for the pre-term caesarean section were eclampsia, pre-eclampsia, placental abruption, and HELLP syndrome (from H – hemolysis, EL – elevated liver enzymes, LP – low platelets). In full-term group, the indications for the cesarean section were high level of myopia and pathology of the fundus of the eye (3 out of 16 patients), scar on the uterus after the cesarean section (3) or after the conservative myomectomy (1), hypertension-induced prolonged pregnancy (3), breech presentation and big fetus (2), prolonged gestation and not mature maternal passages (2), chronic fetus hypoxia and fetus-placental insufficiency (2).

The tissue samples were placed immediately in the modified Krebs solution and used within 2–4 h for pharmacological organ bath studies. The Krebs solution was of the following composition (mM): NaCl 133, KCl 4.7, NaHCO₃ 16.4, MgSO₄ 0.6, NaH₂PO₄ 0.8, CaCl₂ 2.5 and glucose 7.7, gassed continuously with 95% O₂ and 5% CO₂, (pH 7.3–7.4). Strips of smooth muscle, approximately 2 mm × 10 mm, were prepared without the endometrium. The preparations were suspended vertically in 10 ml tissue baths for isometric recording of mechanical activity; the bath temperature was kept 37 ± 0.5 °C by a TE-8A water pump (Techne, Cambridge, UK). An initial load of 1 g was applied to the strips, which were then allowed to equilibrate for at least 60 min. Electrical field stimulation (EFS) was provided by a Grass S9 (USA) stimulator and was applied via two platinum-wire rings 2.5 mm in diameter, 10 mm apart, through which the strips were threaded. Mechanical activity of the tissues were recorded isometrically with a FSG-01 force-displacement transducer (Linton Instrumentation, Norfolk, UK), data were recorded and stored digitally by a MP100WSW Data Acquisition System (BIOPACK Systems Inc., Santa Barbara, USA), and displayed on a computer screen.

Concentration–response curves for adenosine 5'-triphosphate (10⁻⁶ to 3 × 10⁻⁴ M) and α,β-methylene-ATP (α,β-meATP, 10⁻⁷ to 3 × 10⁻⁵ M) were obtained by adding the agents directly to the organ bath, and the tissue was washed with a fresh Krebs solution after a maximum amplitude of contraction (peak or plateau) or null-response (within 2–3 min) had been observed. Intervals of 10 min were allowed between additions of successive concentrations of ATP, while intervals of 25–30 min were allowed between contractions elicited by α,β-meATP in order to prevent desensitization of the P2X receptors. The second concentration–response curve for a given agonist was constructed after incubation of the tissues with pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (10⁻⁵ M) for at least 25–30 min.

EFS applied at a given frequency (2–32 Hz) with a pulse width of 0.5 ms and supramaximal voltage, until a maximal contraction was reached and the tone of the tissue declined by approximately 10–20%; usually the period of the EFS was not more than 10 s. Intervals of 2 min were maintained between consecutive stimulations. After an initial frequency–response relationship had been obtained, a second relationship was established after incubation of the tissue with PPADS (10⁻⁵ M, 30 min). Atropine (3 × 10⁻⁷ M) and phentolamine (10⁻⁶ M) were present in Krebs solution throughout the experiments with EFS.

All experiments were performed in quadruplicate or in triplicate using four (or three) parallel channels, thus the number of variables used for statistical analysis (*n*) was equal to number of women in the group (*N*) multiplied by four (or by three). Only one agonist or only EFS (before and after PPADS) was tested on each given tissue preparation. All responses were expressed as a percentage of the contractions evoked by 240 mM KCl, which was applied in the very end of each experiment.

For evaluation of concentration–effect relationships in vitro, experimental curves were fitted to a non-linear regression analysis. Differences between mean values of groups with normally distributed variables were assessed using Student's paired and unpaired *t*-test as well as by two-way analysis of variance (ANOVA) test. To evaluate normality of variances, Kolmogorov–Smirnov and Shapiro–Wilk normality tests were used. Differences between variables that were not normally distributed were evaluated by Wilcoxon *T*-test, Mann–Whitney *U*-test as well as by Kruskal–Wallis test. *P* ≤ 0.05 was considered significant. The statistical analyses of the data were carried out by using GraphPadPrism software. Data in the text are presented as mean ± standard error of the mean (*n*).

3. Results

In the preliminary series of the experiments we found that the contractility of isolated human full-term pregnant uterus did not significantly change during the experimental procedure; repeated concentration–response curves for either

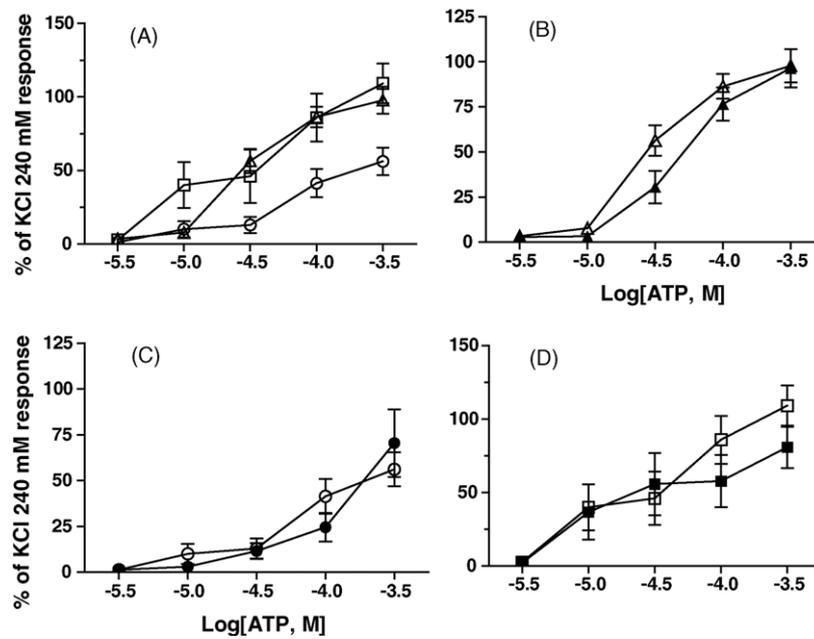


Fig. 1. Effects of the gestation term (A) and PPADS, a P2 receptor antagonist (B, C and D), on the contractions of the isolated human pregnant uterus preparations induced by ATP. ATP-evoked contractions were registered at 28–30 weeks (Group 1, triangles), 32–34 weeks (Group 2, circles) and 38–41 weeks (Group 3, squares) registered before (open symbols) and after incubation with PPADS (10^{-5} M, closed symbols). Data shown are means and vertical bars indicate standard error of the mean, $n = 10-22$.

of the P2 agonists used as well as repeated frequency–response curves for EFS were statistically identical ($P > 0.05$, two-way ANOVA test, data not shown). Another set of preliminary experiments were performed with EFS; incubation of the tissue with tetrodotoxin (10^{-6} M) abolished the contractile responses of the pregnant uterus to EFS thus indicating that the given parameters of the EFS provided the

nerve-mediated responses and did not directly stimulate the muscle cells.

ATP at concentrations of higher than 10^{-6} M caused concentration-dependent contractions of isolated human uterus preparations of all three groups of women (Fig. 1A). Concentration–response curve for ATP in Group 1 was almost identical to that in Group 3; however in Group 2, the

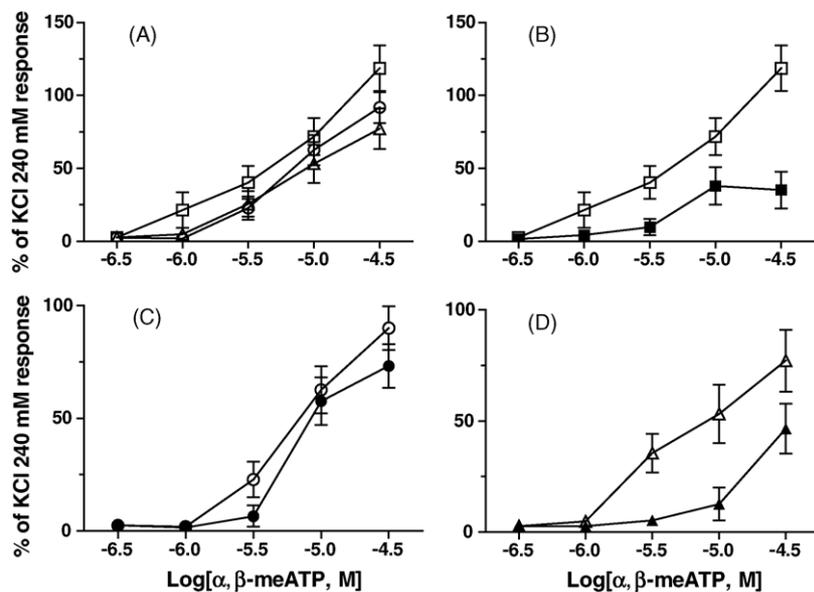


Fig. 2. Effects of the gestation term (A) and PPADS, a P2 receptor antagonist (B, C and D), on the contractions of the isolated human pregnant uterus preparations induced by α, β -meATP. α, β -meATP-evoked contractions were registered at 28–30 weeks (Group 1, triangles), 32–34 weeks (Group 2, circles) and 38–41 weeks (Group 3, squares) registered before (open symbols) and after incubation with PPADS (10^{-5} M, closed symbols). Data shown are means and vertical bars indicate standard error of the mean, $n = 10-22$.

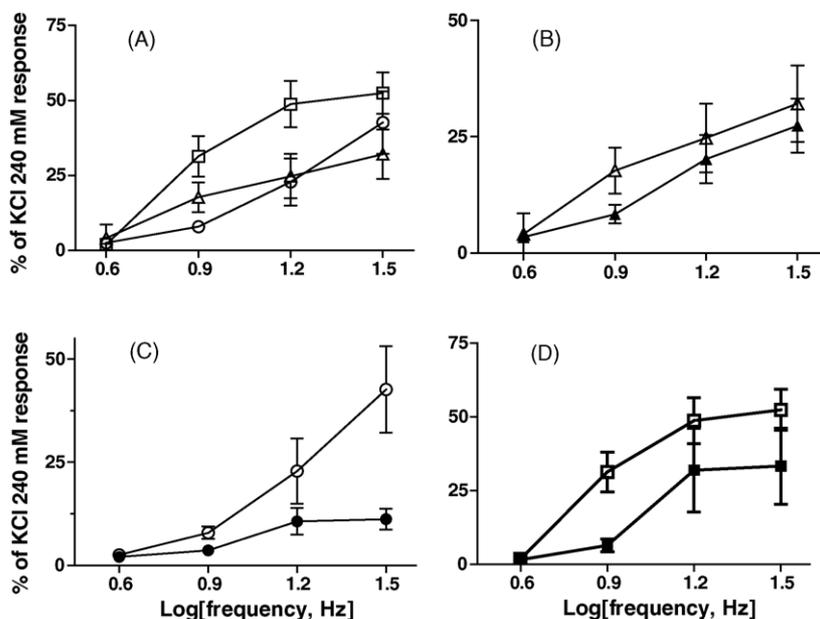


Fig. 3. Effects of the gestation term (A) and PPADS, a P2 receptor antagonist (B, C and D), on the contractions of the isolated human pregnant uterus preparations induced by EFS. EFS-evoked contractions were registered at 28–30 weeks (Group 1, triangles), 32–34 weeks (Group 2, circles) and 38–41 weeks (Group 3, squares) registered before (open symbols) and after incubation with PPADS (10^{-5} M, closed symbols). Data shown are means and vertical bars indicate standard error of the mean, $n = 12$ –28.

concentration–response curve for ATP was shifted to the right comparing with the corresponding curves of the two other groups ($P < 0.05$, two-way ANOVA and Kruskal–Wallis tests).

PPADS did not affect contractile responses of the full-term pregnant uterus evoked by ATP (Group 3)—none of the corresponding points in the concentration–response curves before and after PPADS were significantly different (Fig. 1D; $P > 0.05$, Student's paired t -test, two-way ANOVA test). Similarly, in Groups 1 and 2, concentration–response curves for ATP were not markedly different before and after incubation with PPADS (Fig. 1B and C).

An enzymatically stable analogue of ATP, α,β -meATP, also caused concentration-dependent contractions of uterus preparations, but was approximately 10-fold more active to induce uterine contractility than ATP (Fig. 2A). The effectiveness of this agonist was higher in Group 3 than in Group 1 ($P < 0.05$, Kruskal–Wallis test), while statistical difference was not seen between concentration–response curves for α,β -meATP in Groups 1 and 2, or in Groups 2 and 3. PPADS markedly lowered the concentration–response curves for α,β -meATP in Groups 1 and 3 and slightly shifted the curve to the right in Group 2 (Fig. 2B, C and D).

In the presence of a muscarinic, cholinergic blocker, atropine and an α -adrenoceptor blocker, phentolamine, EFS caused frequency-dependent contractions of the uterus preparations of all three groups (Fig. 3A). However, the amplitude of contractions in Group 3 at a given frequency was significantly higher than corresponding value in Groups 1 and 2 ($P < 0.05$, two-way ANOVA and Kruskal–Wallis tests). The frequency–response curves for EFS in Groups 1 and 2 were statistically identical (Fig. 3). The $\log EF_{50}$

values (i.e. the frequency that cause a 50% contraction of the maximum response produced by EFS) were 0.93 ± 0.06 ($n = 12$), 1.10 ± 0.07 ($n = 18$) and 0.92 ± 0.06 ($n = 24$) in Groups 1, 2 and 3, respectively, all these figures were not significantly different from each other ($P > 0.05$, Student's unpaired t -test and Mann–Whitney U -test).

PPADS inhibited the uterine contractile responses evoked by EFS (Fig. 3B,C and D)—the frequency–response curves in the presence of this antagonist in all three groups were significantly lowered comparing with those without PPADS ($P < 0.05$, two-way ANOVA and Kruskal–Wallis tests). The $\log EF_{50}$ values were 1.01 ± 0.07 ($n = 12$), 0.94 ± 0.05 ($n = 18$) and 1.01 ± 0.05 ($n = 24$) in Groups 1, 2 and 3, respectively; only figure of Group 2 was significantly different from its corresponding value without PPADS ($P < 0.05$, Mann–Whitney U -test).

4. Discussion

In this paper we have shown that the effectiveness of P2X receptor-mediated contractile responses of isolated human uterus evoked by α,β -meATP and EFS, but not by ATP, are increased during second half of the pregnancy.

The effects of purine compounds on uterine contractile activity have been shown earlier in several animal species. P1 (adenosine) and/or P2 receptor-mediated responses were registered in isolated non-pregnant uterus of guinea-pigs [5,6], rats [7], mice [8] and rabbits [9] as well as in the pregnant uterus of rats [10,11] and rabbits [9]. It was shown that contractile responses of the uterus to purine compounds are mediated, at least partly, by prostaglandins [9,12,13].

The effect of pregnancy on P2 receptor-mediated processes has been also reported. Responses of the rabbit [15] and rat [16] urinary bladder to ATP were increased during pregnancy, while responses to cholinergic agonists was reduced [17]. It is thought that these changes are consequences of fluctuation of the sex hormones during pregnancy [18]. However, all these effects were shown on animal tissues, while similar data about human uterus are limited.

Recently, we demonstrated for the first time the presence of P2 receptor mediated contractions in the human pregnant uterus [14]. We found that indomethacin, an inhibitor of prostaglandin synthesis, reduced, while L-NAME, an inhibitor of nitric oxide-synthase, enhanced contractile responses of the uterus caused by ATP, suggesting the involvement of prostaglandins and NO in the ATP-induced responses [19,20]. We suggested that P2 receptors may play a physiological role being involved in the regulation (potentiation) of the contractility of the uterus during labor.

The question which remained was about the term-dependency of the appearance of P2 receptors in the human pregnant uterus, since earlier we found little or no contractile reaction to ATP of human non-pregnant uterus [14]. In the present study we have demonstrated that although both agonists of P2 receptors caused concentration-dependent contractions of isolated human uterus preparations of all three developmental stages of pregnant women, there were some specific differences between them. Contractions of the uterus evoked by ATP were similar at 38–41 and 28–30 weeks of pregnancy; concentration–response curves for ATP in these two groups of women were almost overlapping. However, the concentration–response curve for ATP in Group 2 (32–34 weeks) was shifted to the right comparing with that of the two other groups. This indicates that contractile activity of ATP in the uterus at 32–34 weeks of pregnancy is markedly lower than that at two other terms of pregnancy tested.

The possible explanation of the results with ATP could be the following. ATP is a universal agonist of P2X and P2Y receptors, except P2Y₁₂ receptors where ATP is a specific antagonist [1,3]. Analyzing our previous papers [14,19] and the present study, we suggest that there are both P2X and P2Y receptors in human uterus during pregnancy; ATP causes contraction of the uterus activating P2X receptors while acting on P2Y receptors the agonist evokes relaxation. The final registered response of the tissue to ATP is a resultant of these two contrary effects, and as such depends on the number of the receptors involved. If we assume that the expression of P2X receptors slowly but progressively increased during pregnancy being maximal at labor, while the expression of P2Y receptors has a bell-shape profile with the top of near 34–36 weeks of pregnancy and decreasing at labor, then it would explain the results with ATP. This suggestion based on possible physiological meaning of the presence of subtypes of P2 receptors in uterus – P2Y (“relaxant”) receptors ensure the quiescence and safety of

the fetus and preserve the pregnancy at the late stages, while P2X (“contractile”) receptors helped to raise a proper uterine contraction during labor. To complicate the story, it should be recognized that ATP-evoked responses in the uterus have prostaglandin- and NO-mediated components [19]. Thus, it is most likely that the effects of ATP on the uterus have several components and the contribution of each component could be different at different stages of gestation.

The results with an enzymatically stable analogue of ATP, α,β -meATP, support the above suggestion. α,β -meATP is a selective agonist of P2X₁ and P2X₃ receptors, having little or no effects on P2Y receptors [21]. In our experiments concentration–response curves for this agonist were generally displaced to the left with progression of the pregnancy, which indicates the increase of the agonist’s activity during pregnancy. Thus, elevation of the effectiveness of α,β -meATP in the pregnant uterus towards full-term could be a reflection of the increase of the number of the P2X receptors involved in the action of the agonist.

In the presence of M-cholinoceptor blocker atropine and α -adrenoceptor blocker phentolamine, EFS evoked contractions of the pregnant uterus, which have higher amplitude in Group 3 (full-term pregnancy) than in two other groups. Contractions were clearly nerve-mediated since the pre-incubation of uterine preparation with tetrodotoxin, a selective blocker of Na⁺ channels in excitable tissues [22], completely abolished EFS-induced contractile responses of the uterus. The identity of the transmitter(s) for the remaining atropine- and phentolamine-insensitive neurotransmission, i.e. non-adrenergic non-cholinergic (NANC) transmission is not known. However, here we have shown that NANC neurotransmission in the pregnant uterus has a definite purinergic component; the antagonist of P2 receptors, PPADS, significantly reduced the uterine contractile responses evoked by EFS. A purinergic component of sympathetic and parasympathetic co-transmission has been established in the bladder, gut and blood vessels [23].

We obtained some very interesting results with PPADS: this antagonist did not significantly affect ATP-induced contractions of the uterus at all stages of pregnancy, while inhibiting the responses evoked by α,β -meATP and EFS. One of the possible explanations of why PPADS blocks the effect of EFS (i.e. endogenous ATP) but not the action of exogenous ATP can be the following. It might be that with the given parameters of EFS we induced the release of ATP from nerve endings at relatively small concentrations which was easily blocked by PPADS, while exogenously applied ATP was used at rather high concentrations (10⁻⁵ M and above) which was not readily antagonized by PPADS. Alternative explanation of non-effectiveness of PPADS against ATP-evoked contractions could be a low P2 receptor selectivity of PPADS in human myometrium. It is known that PPADS is a P2 receptor antagonist which shows a selectivity to P2X receptors in some pharmacological experiments [24,25] but not in the others [26,27]. It is

possible that in human uterus PPADS shows the absence of selectivity to P2 receptors, i.e. it blocks both P2X and P2Y receptors, so the blocking of the stimulant P2X receptors is counterbalanced by blocking the relaxant P2Y receptors, therefore, the net effects evoked by ATP is the same before and after PPADS. On the other hand, when P2X receptors were stimulated alone by α,β -meATP, the antagonistic profile of PPADS was clearly detected. We suggest that effectiveness of PPADS on EFS-evoked contractions is also mostly due to interaction with P2X receptors. Although detected at higher concentrations than we used in this study, known ability of PPADS to inhibit ecto-ATPases [28] could even more complicate the situation. It is noticeable that although in all experiments PPADS significantly inhibited effects of α,β -meATP, the profile of its inhibition looks rather different in three tested groups (see Fig. 2B, C and D). We cannot explain this at present, and we have to do more experiments to find out whether these differences are truly term-dependent in pregnancy or it is a result of relatively small number of samples in Groups 1 and 2.

The results of this and our previous studies have not clearly indicate about the subtypes of P2X and P2Y receptors expressed in the uterus; for this the pharmacological organ bath experiments need to be supplemented by immunohistochemistry experiments. At present we can only suggest that P2 receptor-mediated contractions are most likely due to dominant involvement of P2X₁ receptor subtype on the basis of the high activity of α,β -meATP in this tissue and since these subtypes are widely present in other smooth muscle tissues [1,4]. However, in immunohistochemical experiments a predominance of immunoreactivity for P2X₂ rather than P2X₁ receptors has been reported in the virgin rat uterus [29], while all seven known subtypes of P2X receptors were found in this tissue during early pregnancy [30]. Earlier we suggested the presence of “pyrimidinergic” subtypes of P2Y receptors (P2Y₂, P2Y₄ and P2Y₆) in the human uterus taking in account the high activity of the agonist of these receptors, uridine 5'-triphosphate (UTP) [14,31]. Thus, several subtypes of P2X and P2Y receptors could be expressed in the pregnant uterus and the expression of the subtypes might depend on the concentrations of estrogens and gestagens in the woman's body at different terms of the pregnancy [18].

Many potential clinical implications of P2 receptor agonists and antagonists have been discussed elsewhere [32,33], however at present, ATP is the only P2 receptor agonist used in clinical practice [34], apart from UTP for dry eye and cystic fibrosis [35]. Recently in our preliminary clinical study we have found that intravenous infusions of ATP could be a useful additional tool to increase uterine contractility during childbirth [20]. Results of the current study taken together with our earlier findings open up a possible new area for developing and introducing new drugs, acting via P2 receptors to regulate uterine contractility during pregnancy and labor.

In conclusion, in the present paper we demonstrated for the first time that P2X receptor-mediated contractions of isolated human pregnant uterus evoked by a selective agonist of these receptors, α,β -meATP, are term-dependently increased during pregnancy. Different profiles of agonist and EFS activity could be related to up- and down-regulation of the subtypes of the P2 receptors involved and may have a physiological significance earlier in maintaining the pregnancy and later in potentiation of contractility in labor. Further experiments are needed to clarify the subtypes of receptors which are present at different stages of gestation in pregnant uterus.

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