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Dedicated to Professor Geoffrey Burnstock

P2 receptor-mediated responses in pregnant human uterus

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Abstract

The responses of human pregnant and non-pregnant uterus to P2 receptor agonists was studied by the pharmacological organ bath method. On the preparations of isolated pregnant uterus P2 receptor agonists caused concentration-dependent contractions with rank order of α,β-methylene-ATP > UTP > ATP = ADP. The uterus contractions to α,β-methylene-ATP was significantly reduced the P2 receptor antagonist pyridoxalphosphate-azophenyl-2',4'-disulphonic acid. On the preparations of non-pregnant uterus P2 receptor agonists had no effect. It is concluded from this study that there are P2 receptors in the human pregnant uterus, which mediate the contractile responses of the tissues to P2 agonists.

Introduction

Extracellular ATP has numerous effects acting on specific receptors for it - P2 receptors [1,2]. According to the current classification, P2 receptors are divided into two big families - P2X and P2Y receptors, P2X receptors being a ligand-gated ion channels, while P2Y receptors are G-protein-coupled [3]. Both families consist of several subtypes, which were numbered after their molecular structure was identified [4].

The P2 receptors widely represented in many tissues, including uterus of various mammalian species [5-12]. However, there is no data about the presence of P2 receptors in human uterus. The aim of this study was to examine in vitro the presence of P2 receptors in human pregnant and non-pregnant uterus and to give the primary pharmacological characteristic of these receptors.

Material and Methods

Samples of human uterus were taken during planned obstetric and gynecological operations performed in the Republican Clinical Hospital of Tatarstan Republic, Kazan, Russia. Samples of pregnant uterus were taken from the anterior wall of the lower uterine segment (the upper edge of the operation incision) during the planned operation of Cesarean section (39-41 weeks of pregnancy). Samples of non-pregnant uterus were taken during planned hysterectomy because of submucosal-interstitial myomas. In the present study myometrium of 28 pregnant and 8 non-pregnant women has been examined.

The tissue was placed immediately in the modified Krebs solution and used within 2-4 hours for pharmacological organ bath analysis. The Krebs solution was of the following composition (mM): NaCl 133, KCl 4.7, $NaHCO_3$ 16.4, $MgSO_4$ 0.6, $NaH2PO_4$ 0.8, $CaCl_2$ 2.5 and glucose 7.7. Samples of uterus were dissected from connective tissues and strips of smooth muscle, approximately 2 by 10 mm, were prepared and suspended vertically in 10 ml organ baths for isometric recording of mechanical activity. An initial load of 1 g was applied to the strips, which were then allowed to equilibrate for at least 60 min, the bath temperature was kept at 37±1°C. The modified Krebs solution was gassed with 95% O_2 and 5% CO_2 (pH 7.3-7.4). Mechanical activity of the tissues were recorded with a Linton FSG-01 force-displacement transducer, acquired by Biopack MP100WSW Data Acquisition System and displayed on a computer screen.

ATP, ADP, UTP, adenosine and α,β-methylene ATP (α,β-meATP) were added directly to the organ bath and the tissue was washed out several times with fresh Krebs solution after maximal contraction was reached. Care was taken to avoid desensitization of the receptors by leaving 15 min (30 min in case of α,β-meATP) before the next concentration of agonist was added. Responses of the tissue to α,β-meATP were examined before and after incubation with pyridoxalphosphate-azophenyl-2',4'-disulphonic acid (PPADS) at a concentration of 30 μM for at least 25 min.

All contractile responses were calculated as a percentage of the response evoked by KCl at a concentration of 240 mM which was added at the end of the experiments.

Means were compared by Student's paired and unpaired *t* test as well as by two-way ANOVA test. A probability of less than or equal to 0.05 was considered as significant.

The study has been approved by the Ethical Committee of the University Clinics of Kazan State Medical Uni-

versity. Permission has been obtained from each woman who took part in the study by signing a special Informed Consent form.

Results

Histamine (1-300 μM) induced concentration-dependent contractions of the human non-pregnant uterus muscle strips, while ATP and adenosine even in the highest concentration tested (300 μM) were without effect (Table 1).

In the preparations of pregnant uterus responses to histamine and adenosine were similar to those in non-pregnant uterus: histamine caused concentration-dependent contractions while adenosine had no effect. In contrast, ATP at concentrations of 10 μM and higher induced concentration-dependent contractile responses of pregnant uterus.

ADP, UTP and (α,β-meATP also caused concentration-dependent contractile responses of the isolated pregnant uterus preparations. The rank order of potency of the agonists was α,β-meATP > UTP > ATP=ADP (Table 2).

A P2 receptors antagonist, PPADS (30 μM) significantly reduced the contractile response to a P2 agonist, α,β-meATP (Table 3).

The effects of purine derivatives on uterus contractile activity were shown earlier on various animals. It has been described, for example, that agonists of P2 receptors cause concentration-dependent contractions of the isolated non-pregnant uterus of guinea-pig [5-7], rat [8-10], mice [11] and rabbits [12]. It was also shown, that often the contractile activity of P2 agonists has a prostaglandin-mediated mechanism [5,12]. Besides contractions in response to P2 receptor agonists were registered also in a pregnant uterus of rat [9-10] and rabbits [12]. However until our recent abstract the information about presence P2-receptor mediated responses in a human pregnant uterus was absent [13].

The results of this study clearly indicate that P2 receptor agonists caused contractions of human pregnant uterus, while in non-pregnant human uterus we did not register such responses. Since in most tissues contraction to

Table 1: The contractile responses of isolated human pregnant and non-pregnant uterus caused by several receptor agonists. Results are a percentage of response of the same tissue to KCl at a concentration of 240 mM, Mean ± S.E.M.

Conc.	ATP (n=8)		Adenosine (n=6)		Histamine (n=6)	
M	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
10^{-6}	1,5 ± 0,1	2,5 ± 0,3*	1,4 ± 0,4	1,5 ± 0,3	57,1 ± 10,2	25,1 ± 8,2*
$3x10^{-6}$	1,9 ± 0,3	3,3 ± 0,8	1,2 ± 1,1	1,9 ± 0,5	68,4 ± 8,1	43,8 ± 10,2
10^{-5}	2,8 ± 1,2	32,4 ± 9,7*	2,6 ± 0,4	1,9 ± 0,5	79,8 ± 8,1	67,0 ± 10,3
$3x10^{-5}$	1,8 ± 0,2	33,3 ± 11,5*	3,2 ± 1,3	1,5 ± 0,3	81,9 ± 6,3	84,1 ± 8,8
10^{-4}	2,6 ± 1,6	70,5 ± 8,1*	2,8 ± 0,6	1,7 ± 0,3	81,1 ± 6,2	88,2 ± 8,4
$3x10^{-4}$	4,1 ± 0,4	82,1 ± 5,9*	3,3 ± 0,8	1,6 ± 0,2	93,1 ± 4,4	102,0 ± 8,4

* - *p ≤ 0.05 from non-pregnant uterus*

Table 2: The contractile responses of isolated human pregnant uterus caused by P2 receptor agonists. Results are a percentage of response of the same tissue to KCl at a concentration of 240 mM, Mean ± S.E.M.

Conc., M	α,β-meATФ	УТФ	АТФ	АДФ
10^{-7}	2,0 ± 1,6	-	-	-
$3x10^{-7}$	2,1 ± 1,3	-	-	-
10^{-6}	31,4 ± 12,5	6,4 ± 2,4	2,5 ± 0,3	2,9 ± 0,4
$3x10^{-6}$	59,1 ± 11,5	12,3 ± 4,3	3,3 ± 0,8	9,4 ± 6,0
10^{-5}	77,4 ± 9,5	54,7 ± 10,9	32,4 ± 9,7	28,3 ± 14,9
$3x10^{-5}$	112,8 ± 8,1	103,2 ± 17,4	33,3 ± 11,5	32,4 ± 12,3
10^{-4}	-	-	70,5 ± 8,1	55,8 ± 17,0
$3x10^{-4}$	-	-	82,1 ± 5,9	118,0 ± 12,4

Table 3: The effects of PPADS ($3x10^{-5}$ M) on contractile responses of isolated human pregnant uterus caused by α,β-meATP. Results are a percentage of response of the same tissue Mean ± S.E.M (n=8).to KCl at a concentration of 240 mM,

Concentration, M	Controls	PPADS
10^{-6}	31,4 ± 12,5	16,0 ± 11,7*
$3x10^{-6}$	59,1 ± 11,5	18,9 ± 10,9*
10^{-5}	77,4 ± 9,5	45,3 ± 15,1*
$3x10^{-5}$	112,8 ± 8,1	36,8 ± 11,9*

* - *p < 0.05 from controls*

P2 receptor agonists mediated via subtypes of P2X receptor family we suggest that P2X receptors can be involved also in human pregnant uterus. This is supported by our findings that the most active agonist in this tissue was α,β-meATP, which is known to be a potent $P2X_1$ and $P2X_3$ receptor agonist. In addition PPADS, known as a relatively selective P2X receptor antagonist in organ bath experiments [14], had good antagonistic activity against effects of α,β-meATP. Thus it seems possible that a subtype of the P2X receptor family, possibly $P2X_1$ mediates P2 receptor agonists evoked contractions of human uterus, since $P2X_3$ receptors are largely present on sensory nerves and have not been shown to be present on smooth muscle [3]. However, the rank order of potency of P2 receptor agonists we established in this tissue is not entirely consistent with those for $P2X_1$ receptors [4]. Therefore it seems likely that there may be co-existence of several subtypes of P2X receptors in human pregnant uterus, for example $P2X_1$ and $P2X_2$. Besides, due to relatively high potency of UTP in this tissue we cannot discard the possibility of a $P2Y_2$ or $P2Y_4$ receptor being involved. The spectrum of P2 receptors involved in response to P2 agonists is yet to be investigated, as well as RT-PCR and immunohistochemical studies of P2 mRNA and receptor protein in both pregnant and non-pregnant uterus.

Thus it is evident from our study that there is an upregulation of P2 receptors in human uterus at a late stage of pregnancy. The appearance of these receptors in uterus at near term time may have a physiological meaning to increase the contractility of the uterus during labor.

In conclusion, in the present study we have shown that P2 receptors agonists evoked contractile responses, which were significantly reduced by P2 receptor antagonist PPADS. In contrast, such responses were not found in human non-pregnant uterus**.**

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