Pharmacological Characteristics of P2 Receptors in Human Uterus

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We studied contractile responses of isolated smooth muscles from human uterus induced by P2 receptor agonists. In preparations from pregnant uterus all tested P2 receptor agonists caused smooth muscle contractions. The relative activity of P2 receptor agonists decreased in the following order: α,β-methylene-ATP—uridine triphosphate—ATP. Responses induced by ATP and ADP were similar. The amplitude of contractions induced by α,β-methylene-ATP significantly decreased in the presence of P2 receptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid. None of tested P2 receptor agonists induced contractions of isolated myometrium from nonpregnant women. Our results indicate that pregnant human uterus contains P2 receptors mediating the contractile response.

Key Words: *human uterus; pregnancy; P2 receptors*

Various effects of extracellular ATP are realized via specific P2 receptors [1]. Recent studies revealed 10 subtypes of P2 receptors that belong to P2X and P2Y families. The P2X family includes P2 receptors acting as ligand-operated ion channels. G-protein-coupled P2 receptors belong to the P2Y family [4]. The molecular structure of P2 receptors was evaluated. Similar recombinant receptors expressed on frog oocytes were studied. However, under natural conditions recombinant P2 receptors differ from native receptors by their pharmacological properties. Therefore, studies of these receptors in organs and tissues are of considerable importance.

P2 receptors are present in various mammalian organs and tissues, including the uterus [3,5-11]. The contractile effect of purines is realized via a prostaglandin-mediated mechanism [3,11]. Contractile responses to P2 receptor agonists were recorded in the uteri from pregnant rats [6,9] and rabbits [11. Our previous experiments revealed P2 receptors in human uterus [13]. Here we studied P2 receptor-mediated responses in human uterus and pharmacological properties of these receptors.

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MATERIALS AND METHODS

Uterine specimens were obtained from 16 pregnant and 6 nonpregnant women during planned surgeries performed at the Republican Clinical Hospital (Tartar Ministry of Health). Fragments of normal uteri were taken from nonpregnant women during extirpation of the uterus for subserous and interstitial myomas. Fragments of pregnant uteri (38-42 weeks gestation) were obtained during cesarean section. Smooth muscle preparations of the circular layer were isolated. The tissue was immediately placed in modified Krebs solution (4-6°C). Preparations of the circular layer (2×10 mm, n=4-8) were obtained 5-8 h after surgery [2].

Contractile responses of isolated uterine fragments to ATP, ADP, α,β-methylene-ATP (α,β-meATP) and uridine triphosphate (UTP) in concentrations of 10^{-6}-3×10^{-4} M were recorded before and after addition of 3×10^{-5} M pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, P2 receptor antagonist). We evaluated the effect of PPADS on contractile reactions of isolated uterine preparations induced by electrical stimulation (ES, 8-64 Hz) in the presence of 10^{-6} M atropine sulfate (muscarinic receptor blocker) and 3×10^{-6} M phentolamine (α-adrenoblocker). The re-

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TABLE 1. Amplitude of Contractile Responses of Isolated Myometrium from Nonpregnant and Pregnant Women (% of Control, *M*±*m*)

Concentration, M	ATP (*n*=8)		Adenosine (*n*=8)		Histamine (*n*=6)	
	nonpregnant uterus	pregnant uterus	nonpregnant uterus	pregnant uterus	nonpregnant uterus	pregnant uterus
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*
3×10^{-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
3×10^{-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
3×10^{-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

Note. *$p\leq0.05$ compared to the nonpregnant uterus.

sponses to test drugs were compared with those induced by adenosine and histamine in concentrations of 10^{-6}-3×10^{-4} M. Contractions induced by 240 mM KCl recorded by the end of the experiments served as the control. The results were expressed in percents of the control.

RESULTS

Histamine in concentrations of 10^{-6}-3×10^{-4} M produced dose-dependent contractions of smooth muscle preparations from nonpregnant myometrium, while ATP and adenosine were ineffective even in maximum doses (Table 1). The contractile response of pregnant uterine fragments to histamine was similar. However, adenosine did not induce contractions of preparations from the pregnant uterus. ATP in concentrations of 10^{-5} M and higher produced dose-dependent contractions of the myometrium preparations from pregnant women. Contractile response to ATP in a maximum concentration was 82% of the control (Table 1).

Other P2 receptor agonists also produced dose-dependent contractions of myometrium preparations from pregnant uterus. The amplitude of induced contractions decreased in the following order: α,β-meATP—UTP—ATP. The responses induced by ATP and ADP were similar (Table 2).

The P2 receptor antagonist PPADS added to the medium markedly inhibited contractions of pregnant uterus induced by ES or most potent agonist α,β-meATP (Table 3).

Our results suggest that pregnant uterus expresses P2 receptors enhancing its contractile activity in the prelabor period. Contractile reactions of smooth muscles are mediated by P2X receptors [1,4]. In our experiments selective P2X receptor agonist α,β-meATP exhibited maximum activity. It can be hypothesized that P2 receptors in pregnant uterus belong to the P2X family. Pharmacological studies showed that PPADS, a highly selective P2X receptor antagonist [12], considerably decreases the amplitude of contractions in human uterus produced by agonists and ES. The order of activities of test agonists in human uterus is characteristic for none of described P2X receptors [4], which can be explained by coexistence of various subtypes of these receptors in the uterus.

It remains unclear whether contractions of pregnant uteri result from direct effect of ATP on P2

TABLE 2. Amplitude of Contractile Reactions of Isolated Myometrium from Pregnant Women to P2 Receptor Agonists (% of the Control, *M*±*m*, *n*=8)

Concentration, M	ATP	ADP	UTP	α,β-meATP
10^{-7}	—	—	—	2.0±1.6
3×10^{-7}	—	—	—	2.1±1.3
10^{-6}	2.5±0.3	2.9±0.4	6.4±2.4	31.4±12.5
3×10^{-6}	3.3±0.8	9.4±6.0	12.3±4.3	59.1±11.5
10^{-5}	32.4±9.7	28.3±14.9	54.7±10.9	77.4±9.5
3×10^{-5}	33.3±11.5	32.4±12.3	103.2±17.4	112.8±8.1
10^{-4}	70.5±8.1	55.8±17.0	—	—
3×10^{-4}	82.1±5.9	118.0±12.4	—	—

TABLE 3. Effect of PPADS (3×10^{-5} M) on Amplitude of Contractile Reactions of Isolated Myometrium from Human Pregnant Uterus to α,β-meATP and ES (*M*±*m*, *n*=8)

Conditions	Without PPADS	PPADS
α,β-meATP, M		
10^{-6}	31.4±12.5	16.0±11.7*
3×10^{-6}	59.1±11.5	18.9±10.9*
10^{-5}	77.4±9.5	45.3±15.1*
3×10^{-5}	112.8±8.1	36.8±11.9*
ES, Hz		
8	32.3±7.7	14.5±7.7*
16	45.2±8.6	23.9±11.3*
32	56.4±8.1	33.4±12.9*
64	56.2±6.1	47.9±9.3*

Note. *$p<0.05$ compared to contractions without PPADS.

receptors or are mediated through prostaglandin synthesis (as it was reported for the uterus in guinea pigs [3] and rabbits [11]). It is interesting to evaluate subtypes of P2 receptors in human uterus and to study their expression during pregnancy. Studies of the interaction between ATP, other mediators (norepinephrine, acetylcholine, and histamine), and medicinal uterotonic preparations (oxytocin, prostaglandins, and β-adrenoblockers) are of considerable importance.

Our findings suggest that P2 receptor agonists can be used as stimulators of uterine contractions. However, ATP cannot be used as uterotonic drug because of its short half-life after parenteral administration and rapid degradation with extracellular ATPases.

Pharmacological study on isolated preparations of the myometrium showed that ATP and other P2 receptor agonists induce contractions of human pregnant uterus (but not nonpregnant uterus). Our findings suggest that human uterus during late pregnancy expresses P2 receptors, which potentiates uterine contractions during labor.

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