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Pharmacological Characterization of P2-Receptors in Human Fallopian Tubes

A. U. Ziganshin, Z. R. Vafina, and I. F. Fatkullin

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In women with various gynecological disorders, ATP, ADP, and 2-methylthio-ATP dose-dependently potentiated spontaneous contractility of isolated fallopian tubes, while α,β-methylene-ATP and uridine triphosphate were little efficient. Pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid, a P2-receptor antagonist, inhibited responses to 2-methylthio-ATP, produced no effect on responses to ATP, and potentiated ADP-induced responses in fallopian tubes. During inflammation, sensitivity of fallopian tubes to P2 agonists and antagonists decreased. The data attest to the presence of functionally active P2 receptors in human fallopian tubes probably involved in the regulation of their mechanical activity.

Key Words: *fallopian tubes; P2 receptors; agonists; antagonists*

In mammals, P2 receptors activated by endogenous agonist purine and pyrimidine nucleotides, are involved in various neuronal and non-neuronal mechanisms. Specifically, they participate in the regulation of inflammation, immune response, hemostasis, function of endocrine and exocrine glands, and contractile activity of smooth muscles and tissues [5]. Considerable recent attention was focused on the study of physiological and pathological role of P2-receptors in human organism, which is important in the search for novel efficient drugs with original mechanisms of action [8]. In our previous studies we found functionally active P2 receptors are located in some blood vessels [2] and uterus of pregnant women [1,13].

ATP regulates fluid secretion in fallopian tubes (FT) [7]. However, little is known on availability and functional activity of P2 receptors in human FT. Our aim was to assess availability and functional activity of P2 receptors in human FT of patients subjected to surgery for some gynecological diseases.

MATERIALS AND METHODS

The study was carried out on FT of women aged 41-54 years (n=42), in whom hysterectomy or adnexectomy were performed for myoma, uterine cancer, cervical cancer, serous ovarian cystadenoma, dermoid ovarian cyst, or ovarian endometriosis. According to pathohistological examination of FT, all women were subdivided into two groups. The fist group comprised patients with chronic salpingitis (n=20), and the second group included women without sighs of inflammation (n=22). Women with other pathomorphological pictures were excluded from the study.

Tissue specimens were immediately placed in cold (4°C) Krebs solution containing (in mM): 133 NaCl, 4.7 KCl, 16.3 $NaHCO_3$, 0.6 $MgCl_2$, 1.35 Na_2HPO_4, 2.5 $CaCl_2$, 7.8 glucose and delivered to the lab for examination within 2-4 h. Longitudinal smooth muscle preparation with endosalpinx (~2×10 mm) were prepared from the ampullar subdivision of FT and placed

Department of Pharmacology, Department of Obstetrics and Gynecology, Kazan State Medical University. ***Address for correspondence:*** airatz@auz.kcn.ru. Ziganshin A. U.

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vertically into thermostabilized tubes (10 ml) filled with aerated Krebs solution (95% O_2+5% CO_2). The preparations were adapted to the medium for 1 h under an initial load of 1 g. To the end of this period regular spontaneous undulating contractions of isolated FT preparation were usually observed. These contractions were recorded using an FSG-01 isometric transducer (Linton). The signals were fed into PC with Biopack interface and software. P2 receptor agonists ATP, ADP, 2-methylthio-ATP, α,β-methylene-ATP (α,β-meATP), and uridine triphosphate (UTP) were added in various concentrations directly to the tube, thereafter changes in spontaneous contractions were assessed. Since amplitude and frequency of initial spontaneous contractions greatly varied, the effect of agonists on contractile activity was assesses by the area under the curve per unit time. This integral parameter made it possible to take into account simultaneous changes in the amplitude, frequency, and baseline level of these contractions. The criterion for evaluation of changes in contractile activity of FT preparation was the difference between areas before and after application of P2 agonists for 60 sec. The initial contractile activity was taken as 100%. P2-receptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) was added directly to the tube to a concentration of 10 μM for at least 30 min, thereafter the effect of P2 agonist was assessed again. The results were analyzed statistically using Student's *t* test for independent and nonindependent groups at $p<0.05$.

The study was approved by Ethic Committee of Kazan State Medical University. All patients provided written consent on participation in the study.

RESULTS

In both groups, ATP (10^{-6}-10^{-4} M) dose-dependently increased the amplitude and frequency of contractions in isolated FT preparations (Fig. 1, *a*). The maximum effect was observed at a concentration of 10^{-4} M, which increased the area under the curve by 15% and 20% in groups 1 and 2, respectively. There was no

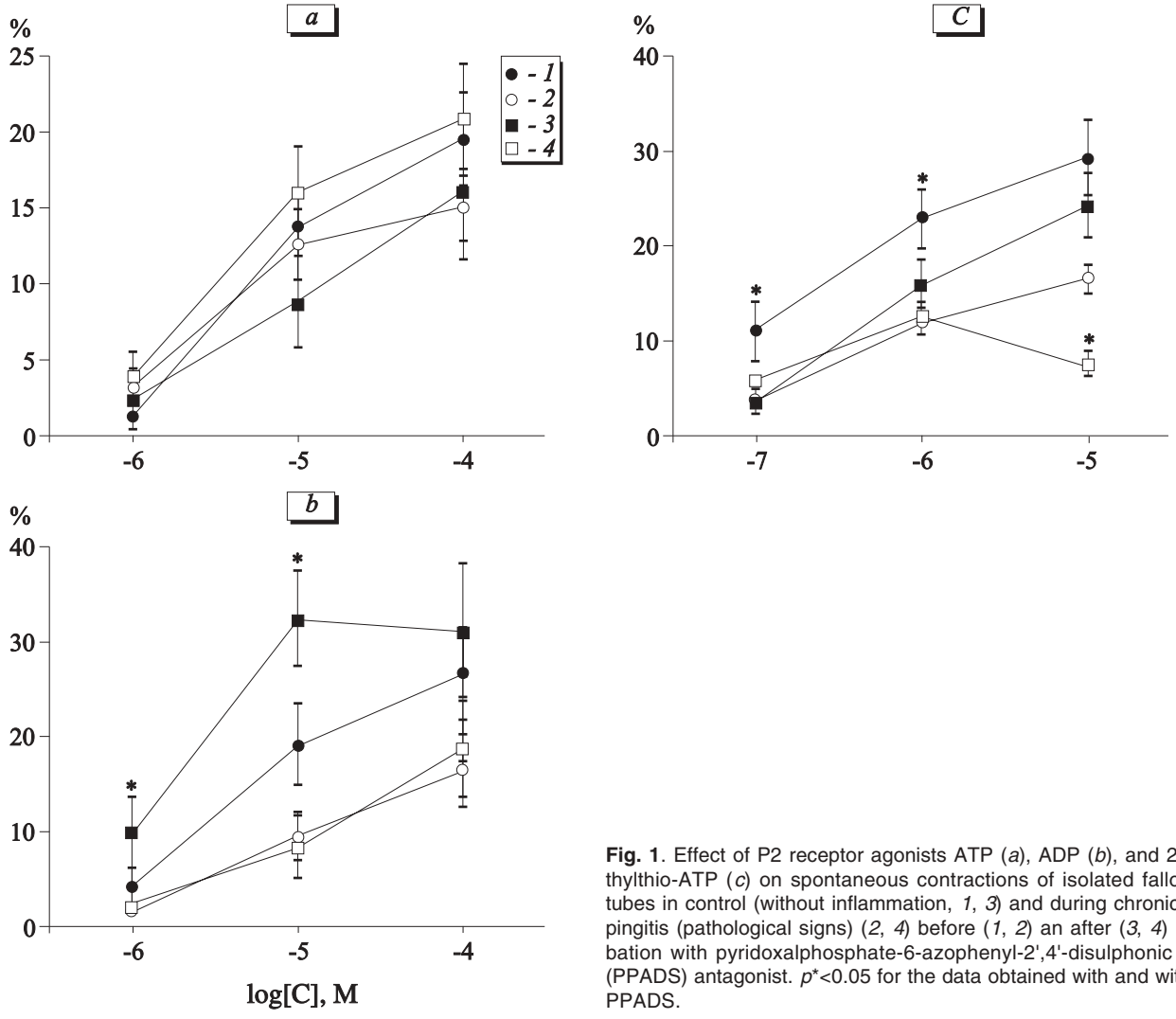

Fig. 1. Effect of P2 receptor agonists ATP (*a*), ADP (*b*), and 2-methylthio-ATP (*c*) on spontaneous contractions of isolated fallopian tubes in control (without inflammation, *1*, *3*) and during chronic salpingitis (pathological signs) (*2*, *4*) before (*1*, *2*) an after (*3*, *4*) incubation with pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) antagonist. $p^*<0.05$ for the data obtained with and without PPADS.

significant difference in the responses in these groups. P2 receptor antagonist PPADS (10^{-5} M) had no reliable effects on FT responses to ATP in both groups. However, it should be noted that in group 2 preparations PPADS slightly moderated contractions induced by ATP, while in group 1 we observed an opposite trend.

Similarly to ATP, ADP and 2-methylthio-ATP dose-dependently potentiated FT contraction in both groups. However, in group 1 (chronic inflammation) both agonists induced less pronounced changes in FT contractions than in group 2 (Fig. 1, *b*, *c*). In this case, the effect of 2-methylthio-ATP was at least one order of magnitude more potent than the effects of ADP or ATP. For example, 2-methylthio-ATP (10^{-5} M) changed the contractile responses in group 2 by 29.4±4.1% (n=9), while ATP and ADP produced less pronounced changes in this group: 13.8±1.9% (n=21) and 19.2±4.3% (n=11), respectively.

PPADS moderated the effect of 2-methylthio-ATP on FT contractions in both groups, but potentiated the effect of ADP in group 2 and produced no changes in ADP effect in group 1 (Fig. 1, *b*, *c*).

UTP and α,β-meATP induced only minor contractions in both groups, which were not significantly changed by PPADS.

In this study, it was established that spontaneous contractions of human FT are modified by P2 receptor agonists, which suggests that these receptors are involved in the regulation of mechanical activity of FT.

According to modern classification, there are two large families of P2 receptors: P2X receptors, which are ligand-operated ionic channels, and metabotropic P2Y receptors with G-protein-mediated action. Each family comprises several subtypes with individual molecular structure and sensitivity to various derivatives of purine and pyrimidine nucleotides. Seven and 8 subtypes of P2X and P2Y receptors, respectively, are known [3].

Various subtypes of P2 receptors are widely spread in organs and tissues. However, the data obtained in experiments on animals not obligatorily coincide with those for human specimens. In this respect, experiments with human tissues *in vitro* are of special importance. Several types of receptors were found in human FT, including adenosine P1 receptor [10,11]. However, we found no data on the presence of P2 receptors in human FT. Therefore, in this study we provide primary characterization of P2 receptors in human FT with the help of 5 typical agonists and one of the most efficient antagonists.

ATP is a basic endogenous ligand of P2 receptors, which stimulates virtually all subtypes of these receptors except $P2Y_{12}$, whose agonist is ADP, while ATP plays a role of competitive antagonist [5]. It is noteworthy that in most experiments on isolated tissues ATP is not the strongest agonist; it is less efficient than 2-methylthio-ATP (if $P2Y_1$ receptors prevail), α,β-meATP (if $P2X_1$ or $P2X_3$ receptors are available), or UTP (if $P2Y_2$, $P2Y_4$, or $P2Y_6$ receptors are involved in the response). Our data obtained on FT without signs of inflammation, on the one hand, and comparison of the effects of agonists (in equimolar concentrations), on the other hand, yielded the following activity sequence: 2-methylthio-ATP>ADP≥ATP>>α,β-meATP=UTP. In human FT, potentiation of spontaneous contractions induced by P2 agonists can be mediated predominantly by $P2Y_1$ receptor subtype. Although in most cases metabotropic P2Y receptors mediate relaxation of smooth muscle organs and tissues, there are examples of contractile responses produced by these receptors [9]. Minimal efficiency of α,β-meATP and UTP attests to the absence of functionally active $P2X_1$, $P2X_3$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors in this tissue. Moderate and equal action of ATP and ADP on FT tissues is an argument in favor of possible involvement of other subtypes of P2 receptors with the members from both P2X and P2Y families.

Originally, PPADS was considered as an efficient antagonist of P2 receptors with pronouncedly higher affinity to P2X receptors [12]. However, next studies revealed antagonistic effect of this substance towards certain subtypes of P2Y receptors cloned in frog ovocytes [4]. In our experiments with noninflammatory tissue, PPADS inhibited the responses to 2-methylthio-ATP and potentiated those to ADP, but had no significant effect on the responses to ATP. PPADS is an established antagonist for all receptors from P2X family except the $P2X_4$ and $P2X_6$ subtypes; moreover, in high concentrations it inhibits $P2Y_1$ receptors as well [6]. The features of PPADS-induced modification of FT responses to examined agonists do not indicate predominant involvement of some subtype of P2 receptors.

Inflammation in FT tissue produces different effects on the action of P2 agonists: it significantly inhibits responses to 2-methylthio-ATP and ADP, demonstrates inhibiting tendency towards the responses to ATP, and does not modify responses to α,β-meATP and UTP. These peculiarities can be explained by desensitization of the corresponding subtypes of P2 receptors, decrease in their density, or by some other effects. Rearrangement of the receptor apparatus during inflammation is rather typical, and in many cases, it leads to changes in the efficiency of medical preparations.

Thus, our study revealed new facts about possible involvement of various subtypes of P2 receptors into the regulation of mechanical activity of human FT under normal or inflammatory conditions. The data on modification of P2 receptor-mediated responses in FT during inflammation are important for prediction of

the efficiency of medical preparations interacting with P2 receptors.

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