

# Effects of selective agonists of adenosine, P2X, and P2Y receptors on motility of isolated fallopian tube

M. Novakovic<sup>1</sup>, S. V. Jankovic<sup>1</sup>, I. Stojic<sup>1</sup>, J. Djuric, M. Folic, N. Folic<sup>1</sup>, A. Dimitrijevic<sup>1</sup>, G. Babic<sup>1</sup>,  
D. Stojadinovic<sup>1</sup>, S. M. Jankovic<sup>1</sup>

<sup>1</sup>Faculty of Medical Sciences, University of Kragujevac, Kragujevac (Serbia)

## Summary

**Background:** During reproductive life of women, adenosine causes both contraction (with low concentrations) of fallopian tubes and inhibition of their spontaneous motor activity (with high concentrations). **Objective:** The aim of this study was to investigate effects of natural agonists of adenosine, P2X and P2Y receptors on motility of isolated fallopian tubes taken from postmenopausal women. **Materials and Methods:** Isolated preparations of isthmus and ampoule were made from fallopian tubes of 21 women in post-menopause, and then tested for reactivity on increasing concentrations of adenosine and P2X/P2Y selective agonists. **Results:** Adenosine showed concentration-dependent inhibitory effect on spontaneous contraction of both isthmus and ampullary segments of fallopian tubes, while P2X and P2Y agonists (adenosine-5-diphosphate, adenosine-5-triphosphate, uridine-5-diphosphate, and uridine-5-triphosphate) did not influence motility of the isolated preparations. Contractile effect of adenosine was not observed throughout the concentration range used in the experiments. **Conclusions:** Fallopian tubes of postmenopausal women are unresponsive to P2X and P2Y agonists, unlike those of women in reproductive period. Only an inhibitory effect of adenosine on spontaneous contractions of fallopian tubes is maintained in post-menopause, while a contractile effect is observed in younger women at low concentrations is lost.

**Key words:** Adenosine; Fallopian tubes; Spontaneous contractions; P2X agonists; P2Y agonists.

## Introduction

Receptors that bind purines and pyrimidines have numerous roles in human body, and are still classified as A (or adenosine) receptors, that bind adenosine, and P2 receptors, that bind adenosine 5'-triphosphate (ATP), and adenosine 5'-diphosphate (ADP) in the first place, and are further subdivided to P2X (ligand-gated ion channels) and P2Y (G protein coupled) receptors [1]. Adenosine receptors (previously denoted as P1 receptors) are G-protein coupled, existing in four subtypes, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>, while adenine nucleotides cannot bind for these receptors, adenosine acts as full agonist at all four subtypes [2]. Adenosine receptors are widespread in the body and have multiple roles, including pro-inflammatory, cardioprotective, and anti-nociceptive [2]. There are seven subtypes of P2X receptors (P2X<sub>1</sub>–P2X<sub>7</sub>) which are activated by ATP, and eight subtypes of P2Y receptors (P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub>, P2Y<sub>13</sub>, and P2Y<sub>14</sub>) which are activated by ATP (P2Y<sub>2</sub> and P2Y<sub>11</sub>), ADP (P2Y<sub>1</sub>, P2Y<sub>12</sub>, and P2Y<sub>13</sub>), uridine-5-diphosphate (UDP) (P2Y<sub>6</sub> and P2Y<sub>14</sub>) and uridine-5-triphosphate (UTP) (P2Y<sub>2</sub> and P2Y<sub>4</sub>) [3].

Roles of adenosine, P2X, and P2Y receptors in control of fallopian tubes' motility were scarcely investigated. At first Samuelson *et al.* showed that adenosine in low concentrations caused weak tonic contraction of both isolated

fallopian tubes' isthmus (only during proliferative phase of menstrual cycle) and ampulla (during both proliferative and secretory phases), and in high concentrations caused inhibition of spontaneous contractions of both isthmus and ampulla (during whole menstrual cycle). They concluded that there were two types of adenosine receptors (stimulatory and inhibitory) in fallopian tubes, situated on smooth muscle cells [4]. The same research group in another study showed that adenosine also inhibited release of noradrenaline from sympathetic innervation of Fallopian tubes [5, 6]. Later on Ziganshin *et al.* demonstrated potentiation of spontaneous contractions of isolated fallopian tubes by ADP and ATP, while UTP was ineffective [7]. The same group showed in the next study that this response diminishes if the fallopian tubes were exposed to inflammation, and concluded that expression of P2 receptors was suppressed by inflammatory mediators [8, 9]. However, neither of the research groups investigated effects of adenosine, P2X, and P2Y agonists on Fallopian tubes from postmenopausal women; furthermore, they did not test whole palette of natural agonists of adenosine, P2X, and P2Y receptors in the same series of experiments, introducing uncertainty that some of the patients' characteristics (comorbidities, concomitant therapy, etc.) could have influenced the responses, as being present in one and not in another series of experiments.

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The aim of this study was to investigate effects of natural agonists of adenosine, P2X, and P2Y receptors on motility of isolated fallopian tubes taken from post-menopausal women.

## Materials and Methods

Fallopian tubes were taken from 21 female patients (one tube per patient) during abdominal hysterectomy with adnexectomy. All patients underwent surgery because of extensive uterine fibroids which caused prolonged uterine bleeding. The patients were in the post-menopause and could not recall a regular menstrual bleeding for at least six months prior to hospital admission. The mean age of the patients was  $52.5 \pm 6.6$  years, the range from 39 to 64 years. The Ethics Committee of Clinical Center "Kragujevac", Serbia, approved the study, and the patients signed the informed consent forms.

The patients were operated from 2014 to 2017 in the Gynecological Clinic of Clinical Centre "Kragujevac" in Kragujevac, Serbia. None of the patients received sex hormones for at least two months prior to the operation. The operations were performed under balanced general anesthesia with gas  $N_2O$ , opioid fentanyl, and neuroleptic droperidol. The anesthesia was induced by intravenous injection of thiopental sodium, and muscle relaxation achieved initially by succinyl-choline, and later on by rocuronium. All patients were pre-medicated with 0.5 mg of atropine subcutaneously.

After clamping the blood supply and resecting a fallopian tube, it was placed in 250-ml dish filled with De Jalons solution (154 mM NaCl, 5.95 mM  $NaHCO_3$ , 5.63 mM KCl, 0.54 mM  $CaCl_2 \cdot 2H_2O$ , 2.78 mM glucose) which was gassed (95%  $O_2$  and 5%  $CO_2$ , 5 mL/min) and transported to the laboratory.

Twenty minutes after taking a fallopian tube in the operating room, the isolated preparations were mounted in an isolated organ bath. Two types of fallopian tube preparations were isolated: isthmus and ampulla. The serosa was removed from both the isthmus and the ampullary preparations. The isthmus preparations with following measures were used in the experiments: 4 cm in length, wall thickness 1.2 mm, and the lumen diameter 1 mm. Also, the ampullary preparations with following measures were used in the experiments: 5 cm in length, wall thickness 1.2 mm and the lumen diameter 5-6 mm. Both types of preparations were mounted in an organ bath longitudinally, and opposite walls of the preparation were attached to the bath base and the transducer, respectively.

The isolated preparations were mounted in 75-ml isolated organ bath, filled with De Jalons solution (154 mM NaCl, 5.95 mM  $NaHCO_3$ , 5.63 mM KCl, 0.54 mM  $CaCl_2 \cdot 2H_2O$ , 2.78 mM glucose). The bath solution was maintained at  $37^\circ C$  and aerated with 95%  $O_2$  and 5%  $CO_2$ . One end of the isolated preparation was attached to the bath base, and the other to the lever of the isometric transducer. The tension of the isolated preparations was continuously recorded with the isometric transducer and registered on personal computer using Majk Electronic interface and software. The isolated preparations were given a passive load of 1 mN and allowed to equilibrate for one hour before an experiment began.

Spontaneous contractions of the isolated preparations were measured by area under the curve (AUC). The effects of experimental substances on area under the contraction curve were recorded.

In the beginning of each experiment, at least one hour of spontaneous activity was recorded, in order to observe for spontaneous changes of phasic contractions. The experimental substances were added to the isolated organ bath cumulatively, without washing between the subsequent doses. The interval between two adjacent

doses was always 5-6 minutes. After cumulating all doses of a substance, the bath was washed three times, and the isolated preparation was allowed to rest for further 30 minutes. The effect of each experimental substance was observed on at least four isolated preparations, taken from different individuals.

The following substances were used in the study: adenosine, adenosine-5-diphosphate, adenosine-5-triphosphate, uridine-5-diphosphate, and uridine-5-triphosphate.

The effect of each concentration of a test-substance on spontaneous contractions was expressed as a percentage of the maximum effect obtained with that agonist, and used for construction of concentration-response curves. The concentration-response relationship was determined by linear regression on logarithmically transformed data calculated according to the method of least squares. The range of values used for the linear regression was from 15% to 85% of the maximal response, in the more linear part of the curve. The concentration of an agonist eliciting 50% of its own maximum response ( $EC_{50}$ ) and its confidence limits (1,96 x standard error) were determined graphically for each curve by linear interpolation. The significance of changes in phasic activity of the isolated preparations was tested by one-way analysis of variance.

## Results

Preparations from all patients showed spontaneous activity which was composed of slow phasic contractions with amplitude of  $11.1 \pm 4.4 \mu N$  ( $4.4 \mu N$  = standard deviation /SD/) and frequency of three to seven cycles per minute. The spontaneous change in phasic activity of isolated ampulla was not observed after two hours of follow-up ( $F = 0.149$ ,  $df_1 = 8$ ,  $df_2 = 28$ ,  $p > 0.05$ ).

Adenosine (from  $4.9 \times 10^{-8}$  M/l to  $7.2 \times 10^{-4}$  M/l) produced concentration-dependent decrease (inhibition) of spontaneous contractions of the isolated ampulla ( $EC_{50} = 4.55 \pm 2.17 \times 10^{-5}$  M/l,  $r = 0.800$ ,  $p < 0.05$ ) (Figure 1). Adenosine-5'-diphosphate (from  $2.7 \times 10^{-8}$  M/l to  $1.2 \times 10^{-4}$  M/l), adenosine-5'-triphosphate (from  $2.4 \times 10^{-8}$  M/l to  $3.5 \times 10^{-4}$  M/l), uridine-5'-diphosphate (from  $3.3 \times 10^{-8}$  M/l to  $1.5 \times 10^{-4}$  M/l), and uridine-5'-triphosphate (from  $2.4 \times 10^{-8}$  M/l to  $1.1 \times 10^{-4}$  M/l) did not affect spontaneous contractions of isolated fallopian tube ampulla ( $F = 0.260$ ,  $df_1 = 7$ ,  $df_2 = 24$ ,  $p > 0.05$ ;  $F = 0.930$ ,  $df_1 = 8$ ,  $df_2 = 27$ ,  $p > 0.05$ ;  $F = 0.429$ ,  $df_1 = 7$ ,  $df_2 = 24$ ,  $p > 0.05$ , and  $F = 0.883$ ,  $df_1 = 7$ ,  $df_2 = 24$ ,  $p > 0.05$  respectively).

Preparations from all patients showed spontaneous activity which consisted of slow phasic contractions with amplitude of  $8.2 \pm 3.4 \mu N$  ( $3.4 \mu N$  = standard deviation /SD/) and frequency of three to eight cycles per minute. The spontaneous change in phasic activity of isolated isthmus was not observed after two hours of follow-up ( $F = 0.271$ ,  $df_1 = 8$ ,  $df_2 = 26$ ,  $p > 0.05$ ).

Adenosine (from  $4.9 \times 10^{-8}$  M/l to  $7.2 \times 10^{-4}$  M/l) produced concentration-dependent decrease (inhibition) of spontaneous contractions of the isolated isthmus ( $EC_{50} = 1.69 \pm 1.51 \times 10^{-5}$  M/l,  $r = 0.930$ ,  $p < 0.05$ ) (Figure 1). Adenosine-5'-diphosphate (from  $2.7 \times 10^{-8}$  M/l to  $1.2 \times 10^{-4}$  M/l), adenosine-5'-triphosphate (from  $2.4 \times 10^{-8}$  M/l to

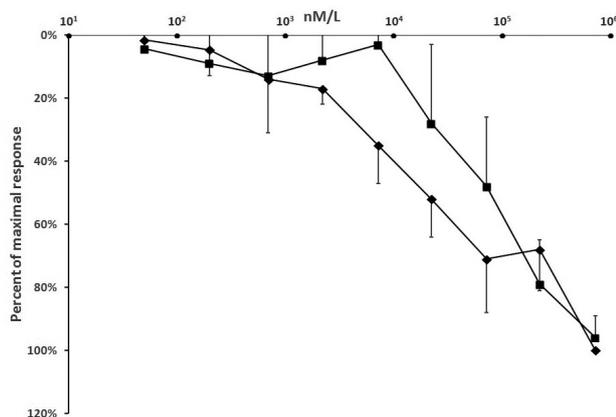


Figure 1. — Inhibition of spontaneous activity of fallopian tube ampulla (■) and isthmus (◆) ( $n=8$ ) caused by adenosine.

$3.5 \times 10^{-4}$  M/l), uridine-5'-diphosphate (from  $3.3 \times 10^{-8}$  M/l to  $1.5 \times 10^{-4}$  M/l), and uridine-5'-triphosphate (from  $2.4 \times 10^{-8}$  M/l to  $1.1 \times 10^{-4}$  M/l) did not affect spontaneous contractions of isolated fallopian tube isthmus ( $F = 0.273$ ,  $df_1 = 7$ ,  $df_2 = 24$ ,  $p > 0.05$ ;  $F = 0.860$ ,  $df_1 = 8$ ,  $df_2 = 27$ ,  $p > 0.05$ ;  $F = 0.178$ ,  $df_1 = 7$ ,  $df_2 = 24$ ,  $p > 0.05$ , and  $F = 0.153$ ,  $df_1 = 7$ ,  $df_2 = 23$ ,  $p > 0.05$  respectively).

## Discussion

In the present study only adenosine showed clear inhibitory effect on spontaneous contraction of both isthmus and ampullary segments of Fallopian tubes taken from postmenopausal women, while P2X and P2Y agonists did not influence motility of the isolated preparations. Contractile effect of adenosine was not observed throughout the concentration range used in the experiments.

Effects of adenosine on activity of oviduct smooth muscle cells was not replicated in neither in vitro nor in vivo studies on other species. However, several studies investigated effects of adenosine on ciliary activity in oviductal ciliated cells, showing that this mediator increases the ciliary beat frequency in cultures of oviductal cells with cilia. This effect in ciliated cells was linked with activation of adenosine  $2_A$  receptors and consequent increase in intracellular cyclic adenosine mono-phosphate (cAMP) [10]. Elevated cAMP enhances the ATP-induced  $Ca^{2+}$  influx in ciliated oviductal cells through the activation of protein kinase A (PKA) [11]. It was also shown in estrous rats that increase in adenylyl cyclase activity and rise of intracellular cAMP levels resulted with enhanced transport of oocytes [12]. Whether intracellular elevation of cAMP and enhancement of the ATP-induced  $Ca^{2+}$  influx are what occurs in fallopian tubes' smooth muscle cells when contracted by low doses of adenosine remains to be established in future studies, but its inhibitory effect on spontaneous contractions of isthmus and ampullary parts certainly was not as-

sociated with rise in intracellular calcium ions [13].

There are no published studies on effects of P2X and P2Y agonists on oviduct motility in animal species, but a few studies investigated activity of adenosine tri-phosphate (ATP) on ciliary movements of oviduct epithelial ciliated cells. ATP increases  $Ca^{2+}$  influx in the ciliated cells and enhances ciliary movements [11], and this effect could be result of activating either P2X or P2Y<sub>2</sub> and P2Y<sub>11</sub> receptors [3]. Increase of intracellular  $Ca^{2+}$  in epithelial cells of fallopian tubes was also demonstrated when in vitro cultures of these cells were exposed to ATP, and furthermore, changes in membrane potential were also registered [14]. Similar intracellular changes could be expected to occur in oviduct smooth muscle cells when they are exposed to exogenous ATP, leading to contraction and stimulation of spontaneous motility, but for the time being we are missing experimental proofs of this assumption.

In the present study the authors could not replicate stimulatory effects of ATP and ADP on motility of fallopian tubes, although the present patients who donated the fallopian tubes were not suffering from inflammatory diseases [7, 8]. This was not surprising, since decreased expression of receptors for other mediators (e.g. progesterone receptor A) was already demonstrated in fallopian tubes of postmenopausal women [15], probably as a result of aging. Transcriptional regulators aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) are less expressed in genital tract of postmenopausal women [16], suggesting that consequent changes in expression could be expected for wide array of receptors, including P2X and P2Y.

There are several limitations of the present study. First, due to limited number of available isolated preparations the authors were not able to test effects of selective antagonists of certain receptor subtypes and therewith have additional confirmation of the results obtained with selective agonists. Second, for the same reason as above, the authors could not test the influence of sodium channel blockers on effects observed with adenosine, leaving exact location of the receptors (on membranes of smooth muscle cells or on nerve fibers) unconfirmed.

In conclusion, fallopian tubes of postmenopausal women are unresponsive to P2X and P2Y agonists, unlike those of women in reproductive period. Only inhibitory effect of adenosine on spontaneous contractions of fallopian tubes is maintained in post-menopause, while contractile effect observed in younger women at low concentrations is lost, probably due to changes in expression of adenosine receptors associated with aging.

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Corresponding Author:  
 S.M. JANKOVIC, M.D., DSC, MSC, PRIM.  
 Faculty of Medical Sciences  
 University of Kragujevac  
 Svetozara Markovica Street, 69  
 34000 Kragujevac (Serbia)  
 e-mail: slobnera@gmail.com