EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of PPADS on P2X Receptor-Mediated Responses of Human Blood Vessels

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In vitro experiments showed that pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid almost completely suppressed contractile responses of the gallbladder artery to α,β-methylene-ATP, while α,β-methylene-ATP-induced contractions of the major subcutaneous vein of patients with varicose disease did not change under the effect of the antagonist. Pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid significantly reduced contractions of the major subcutaneous veins induced by α,β-methylene-ATP (in two highest concentrations) in patients without varicosity. These results indicate different sensitivity of human blood vessels to the studied P2 receptor agonist and antagonist.

Key Words: *human gallbladder artery; human femoral major subcutaneous vein; P2 receptors; pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid*

P2 receptors (main endogenous ligand ATP) are extensively presented in the cardiovascular system in many animal species. In animals P2 receptors are involved in the regulation of vascular tone, local circulation, cardiac activity, and hemostasis [5,9]. P2X receptors (ligand-operated ionic channels) and metabotropic P2Y receptors conjugated with G protein are present in vascular tissues. P2X receptors on smooth muscle cells mediate vasoconstrictor influences of nucleotides, while P2Y receptors on the endothelium provide vasodilatation [11].

The presence and physiological role of P2 receptors in human organs and tissues are less studied. The data on the role of P2 receptors in human cardiovascular function are scanty. For example, we recorded P2 receptor-mediated contractions in human major femoral subcutaneous vein (MSV) and these contractions markedly decreased in varicose disease [3].

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The effect of ATP as P2 receptor agonist is very short-lasting because of rapid destruction by extracellular nucleotidases [14]. Therefore the use of enzyme-resistant ATP analog α,β-methylene-ATP is more convenient for experiments; this agents showed a 100-fold higher efficiency as P2X receptor agonist than ATP in comparative pharmacological experiments [7].

We compared the effects of α,β-methylene-ATP on human peripheral artery and vein and evaluated the efficiency of P2 receptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) on these vessels. Experiments were carried out on MSV and gallbladder artery (GBA) of surgical patients.

MATERIALS AND METHODS

Segments of blood vessels were obtained during surgery from patients treated in hospitals of Kazan for lower limb diseases (n=13; MSV was taken) or calculous cholecystitis (n=7; GBA was taken). All patients gave written consent to participation in experiments.

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The patients were divided into 3 groups:

— group 1 (n=6) with obliterating atherosclerosis of the lower limb vessels, occlusion of surface femoral arteries, and IIB degree chronic arterial insufficiency of the lower limbs. Autovenous femoropopliteal bypass using MSV was carried out in all patients;

— group 2 (n=7) with varicose disease of the lower limb veins during compensation or subcompensation stages. The process was located on the shin. None of the patients had trophic disorders of the skin. Phlebectomy after Troyanov—Beblung—Trendelenburg was carried out. All patients were operated for the first time;

— group 3 (n=7) with chronic calculous cholecystitis. Planned laparoscopic cholecystectomy was carried out; a 1-2-cm GBA segment was collected from the removed material.

MSV segments (1.5-2 cm) at the mouth of the vein were collected in groups 1 and 2. The vascular segments were immediately placed into cold (4°C) modified Krebs solution. The experiment was started no latter than 3 h after collection. Two-four vascular preparations were made from the collected material: vascular segments were cleansed from connective tissues and 5-8 mm-wide rings were cut. Two stainless wires (0.5 mm) were inserted into the lumen of the vessel, one of these wires was fixed and the other was connected (by means of a silk thread) to an isometric mechanical transducer. Subsequent procedures were described previously [2].

Contractile responses of vessels to α,β-methylene-ATP (10^{-7}-3×10^{-5} M) were evaluated by the cumulative method before and after incubation with PPADS in a concentration of 10^{-5} M for at least 25 min. All responses to the agonist were calculated as the percentage of the maximum contraction in response to KCl in a concentration of 240 mM, added at the end of the experiment.

The results were statistically processed using Student's t test for bound and unbound variables and by unifactorial dispersion analysis (ANOVA). The difference was considered significant at $p<0.05$.

The study was permitted by the Ethic Committee of Kazan State Medical University.

RESULTS

α,β-Methylene-ATP in concentrations of 10^{-7}-3×10^{-5} M induced concentration-dependent contractions of isolated vessels from patients of all 3 groups. The maximum contraction response of MSV in groups 1 and 2 was observed at agonist concentration of 3×10^{-5} M, while the contractions of GBA in response to α,β-methylene-ATP reached the peak at a one order of magnitude lower concentration (Fig. 1). Analysis of dispersions showed highly significant differences in responses of the vessels to this agonist in the three groups ($p<0.001$).

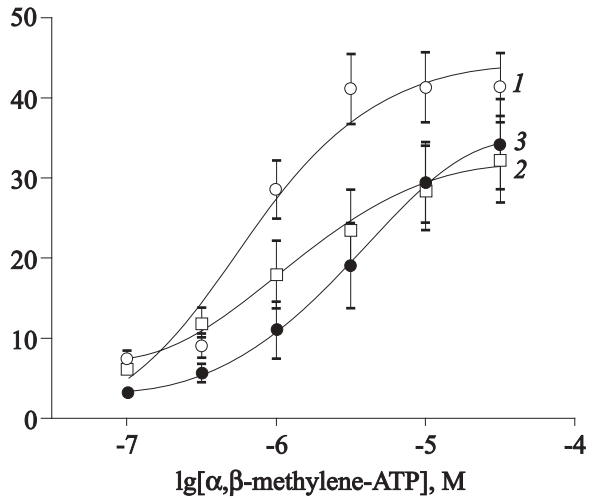

Fig. 1. Contractions of isolated gallbladder artery (*1*), major subcutaneous vein of patients with varicose disease (*2*), and major subcutaneous vein of patients with atherosclerosis obliterans (*3*) induced by α,β-methylene-ATP. Ordinate: percentage of contractile response to 240 mM KCl.

Estimation of the mean effective concentration for α,β-methylene-ATP showed that GBA and MSV of patients with atherosclerosis obliterans were virtually equally sensitive to this agonist: pD_2 values (negative EC_{50} logarithm) for α,β-methylene-ATP were 6.28±0.03 (n=7) and 6.16±0.09 (n=6) for GBA and MSV of patients with atherosclerosis obliterans, respectively; these values are statistically the same. pD_2 value for α,β-methylene-ATP estimated for MSV of patients with varicose disease was 5.72±0.06 (n=7), which was significantly lower in comparison with both above values ($p<0.05$ according to Student's t test for free values).

Incubation with PPADS in a concentration of 10^{-5} M virtually completely prevented contractile responses of MSV induced by α,β-methylene-ATP; some contractions were recorded only at two highest concentrations of the agonist (Fig. 2).

PPADS in the same concentration did not modify the reaction of MSV from patients with varicose disease to α,β-methylene-ATP (the concentration-effect curves for this agonist before and after incubation with PPADS virtually coincided, Fig. 3, *a*).

The reaction of MSV from patients without varicose disease was different: the concentration-effect curve for α,β-methylene-ATP notably sloped down, attesting to antagonistic effect of the substance. However, statistical analysis using Student's t test for bound values revealed significant differences in MSV responses to α,β-methylene-ATP before and after PPADS for only two highest concentrations of the agonist (Fig. 3, *b*).

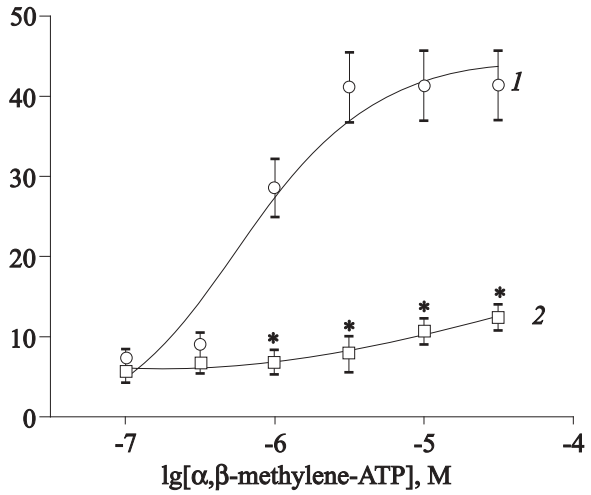

Fig. 2. Contractions of isolated gallbladder artery induced by α,β-methylene-ATP before (*1*) and after incubation with pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) in a concentration of 10^{-5} M (*2*). Ordinate: percentage of contractile response to 240 mM KCl. Here and in Fig. 3: **p*<0.05 compared to the values before PPADS addition.

The study showed that isolated human blood vessels are sufficiently sensitive to exogenous agonist of P2X receptors (α,β-methylene-ATP). pD_2 value for this agonist calculated on the basis of our experiments on GBA and MSV of patients without varicose veins was comparable to pD_2 for α,β-methylene-ATP determined previously in experiments on rabbit and rat vessels [6,8] and on human umbilical artery and vein [12]. Since these vessels have P2X receptors [10], we hypothesized that the recorded contractile responses of human blood vessels to α,β-methylene-ATP are mediated by P2X receptors.

By the present time more than 20 compounds are described as P2 receptor antagonists, but we have to admit that none of these compounds is characterized by sufficient selective activity towards P2 receptor subtypes [7]. One of the compounds most widely used as P2 antagonist is PPADS, which exhibits pronounced antagonistic effect towards peripheral P2X receptors without modulating the effects mediated by P2Y, muscarinic cholinergic, α-adrenergic, and histamine receptors [1,13]. Though later studies revealed certain tropism of PPADS to P2Y receptors [4], the substance exhibits high selective activity towards P2X receptors in pharmacological experiments on isolated tissues [7,10].

In our experiments on isolated human vessels PPADS showed different efficiency: in the same concentration it virtually completely suppressed the contractile responses of GBA to α,β-methylene-ATP, did not change the contractile responses of MSV from patients with varicose veins, and slightly decreased the responses of MSV from patients without varicose disease. Presumably, $P2X_1$ or $P2X_3$ receptors are extensively presented in human GBA, because, on the one hand, α,β-methylene-ATP is a selective agonist of these subtypes of P2 receptors and on the other hand, they are most sensitive to PPADS [10].

Our findings confirm our previous conclusion that varicose disease notably disorders P2 receptor-mediated responses [3]. We found that pD_2 estimated for α,β-methylene-ATP in experiments on MSV from patients with varicose disease was significantly lower in comparison with that found for MSV of patients without varicosity. Differences in responses of MSV from patients with and without varicose disease can be mainly due to a decrease in the number of P2X recep-

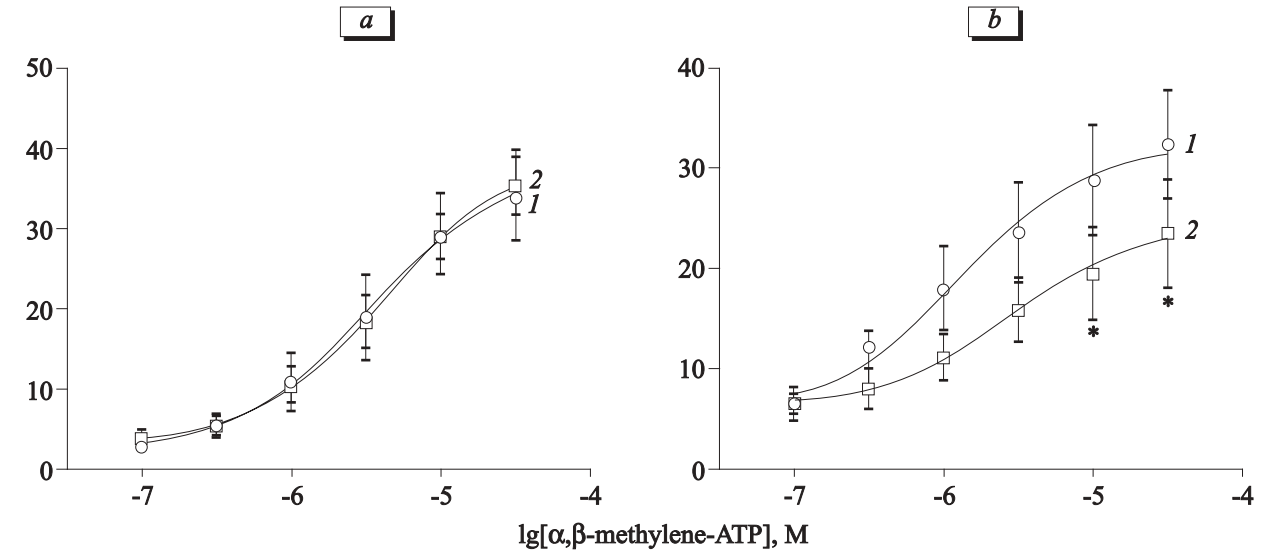

Fig. 3. Contractions of isolated major subcutaneous vein of patients with varicose veins (*a*) and atherosclerosis obliterans (*b*), induced by α,β-methylene-ATP before (*1*) and after incubation with PPADS in a concentration of 10^{-5} M (*2*). Ordinate: percentage of contractions in KCl in a concentration of 240 mM.

tors sensitive to α,β-methylene-ATP in varicose disease. On the other hand, this disease presumably is associated with rearrangement of the P2 receptor system, for example appearance (or increase in the number) of $P2X_4$ receptors not sensitive to PPADS [10]. One more explanation of the absence of PPADS effect on MSV in varicose disease can be the appearance of allosteric regulatory sites in P2X receptors modulating their sensitivity to agonists and antagonists.

Hence, our study provided new data on different efficiency of P2X agonist α,β-methylene-ATP and P2 antagonist PPADS in human arteries and veins. These results attest to possible involvement of P2 receptors into the regulation of vascular tone and local circulation in humans.

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