Contractile Activity of Human Greater Saphenous Vein Mediated by P2-Receptors

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In vitro experiments were carried out to measure the contractile responses to P2-receptor agonists in the greater saphenous vein isolated from patients with obliterating vascular atherosclerosis and varicose veins of the legs. In patients with varicose veins, the contractile responses of the greater saphenous vein to ATP, α,β-methylene-ATP, and UTP were significantly lower than in patients with obliterating atherosclerosis, while the responses to ADP, adenosine, and 2-methylthio-ATP were similar in both groups. These data attest to the presence of P2-receptor-mediated contraction component in the greater saphenous vein, which are pronouncedly weakened during varicose disease.

Key Words: *human greater saphenous vein*; *P2-receptors; varicose veins*

ATP produces pronounced extracellular effects mediated by specific P2-receptors [1,3] widely presented in the cardiovascular system of various animal species. These receptors participate in the regulation of the vascular tone, cardiac function, and hemostasis [4, 6,7]. However, little is known about the presence and physiological role of these receptors in human organs and tissues. In particular, there are practically no data on pathological role of P2-receptors in cardiovascular diseases.

Our aim was to study P2-receptors in human greater saphenous vein (GSV) and characterize them pharmacologically. The reason to examine GSV is the fact that although the vein diseases in lower extremities are widely spread in human vascular pathology, the changes in innervation and in the spectrum of vascular wall receptors, which are characteristic of this pathology, were little studied.

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MATERIALS AND METHODS

The study was carried out on GSV segments isolated from patients (n=24) with vascular pathology in lower extremities hospitalized at Kazan Hospital No. 6. Written consent were obtained from all patients. The study was approved by Ethic Committee of Kazan State Medical University.

The patients were divided into two groups. The first group comprised patients with arteriosclerosis obliterans (AO), occlusion of superficial femoral arteries, and chronic arterial insufficiency of the lower extremities degree IIB (14 males and 1 female aged 43-64 years). A femoral-popliteal artery saphenous vein bypass with GSV was applied in all patients of this group. The second group comprised 5 females and 4 males (aged 28-53 years) with compensated or subcompensated varicose vein disease (VVD). The disease was localized within the crus. Skin trophics was not disturbed. The patients underwent Troyanov—Babcock—Trendelenburg phlebectomy. All the patients were operated for the first time.

The vascular segments were isolated from GSV orifice and immediately placed in cold (4°C) modified

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Krebs solution. The experiments were started no later than 5 h after isolation. The connective tissue was removed and the vessel was cut into 2-4 rings 5-8 mm width. Two stainless steel wires (diameter 0.5 mm) were inserted into the ring. One wire was fixed, while the other was tied with a silk thread to an isometric tension transducer (Grass FTOC3). Other details of the experiments were described previously [2]. All contractile responses to test agonists were calculated as the percentage of the maximum contraction elicited by 240 mM KCl applied at the end of the experiment.

The results were analyzed statistically using Student's *t* test.

RESULTS

In both groups, norepinephrine, histamine, and carbacholine induced dose-dependent contractions of the isolated GSV rings. In these groups, the contractile responses to histamine and carbacholine in all examined concentrations did not differ significantly. When applied in a maximal concentration of 10^{-4} M, norepinephrine induced more potent contractions in AO group in comparison with those in VVD group. All other concentrations of norepinephrine induced similar responses in both groups (Fig. 1, *a*; Table 1).

ATP (10^{-6}-10^{-4} M) induced dose-dependent contractions of isolated GSV. These responses were more pronounced in AO group compared to VVD group for all examined concentrations (Fig. 1, *b*).

Similar relationship was revealed in experiments with α,β-methylene-ATP (α,β-meATP): in concentrations of 10^{-6} or 10^{-5} M this agonist induced more potent contractions of the isolated GSV in AO group compared to VVD group (Fig. 1, *b*). It is noteworthy that in both groups α,β-meATP was about 100-fold more efficient than ATP.

In both groups, the ADP- and ATP-produced contractions of GSV did not differ significantly. In AO group, UTP induced dose-dependent contractions of isolated GSV, while in VVD group it did not modulate GSV tone even in the maximum concentrations

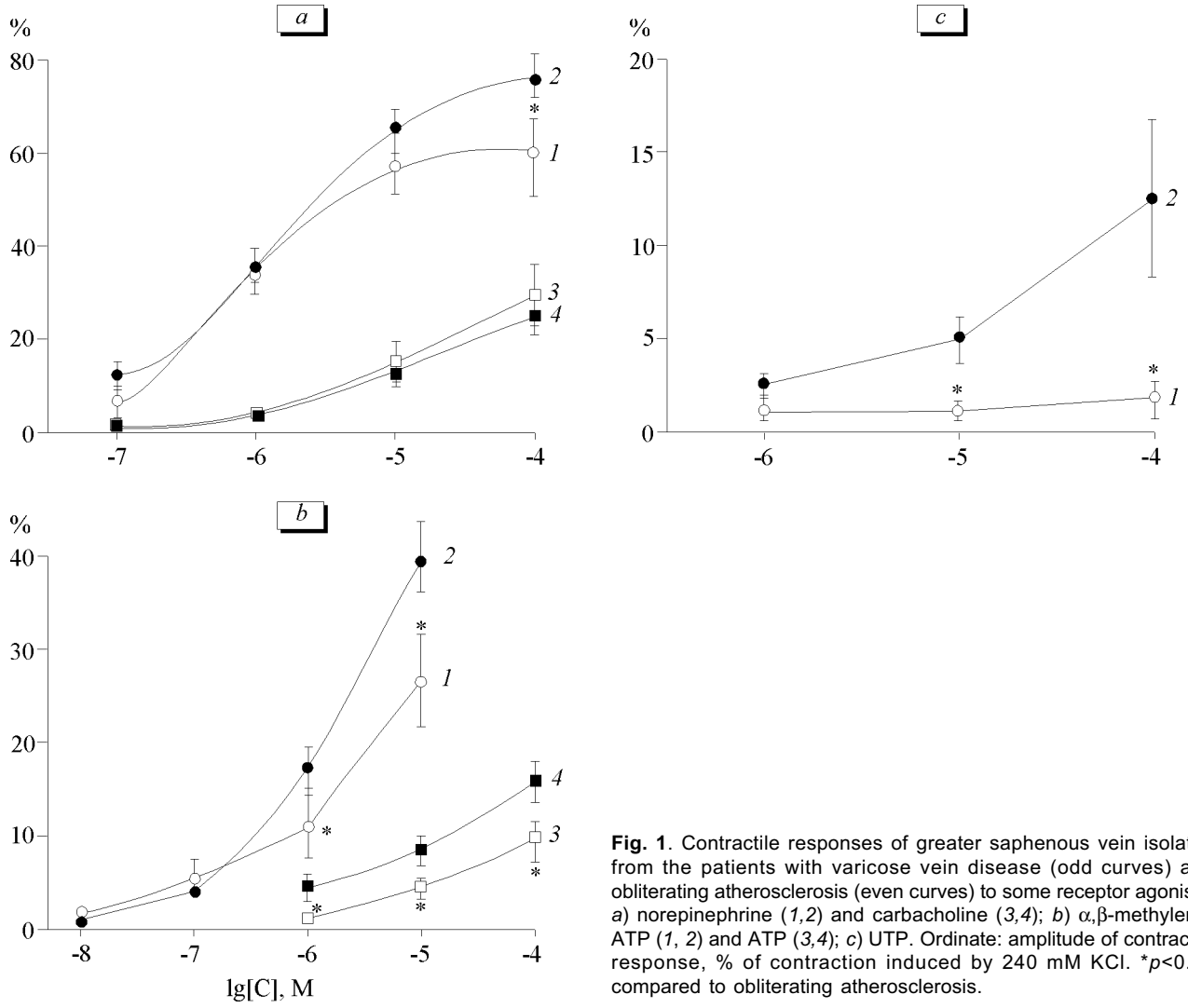

Fig. 1. Contractile responses of greater saphenous vein isolated from the patients with varicose vein disease (odd curves) and obliterating atherosclerosis (even curves) to some receptor agonists. *a*) norepinephrine (*1,2*) and carbacholine (*3,4*); *b*) α,β-methylene-ATP (*1*, *2*) and ATP (*3,4*); *c*) UTP. Ordinate: amplitude of contractile response, % of contraction induced by 240 mM KCl. *p<0.05 compared to obliterating atherosclerosis.

TABLE 1. Effect of Various Agonists in Maximum Concentrations (10^{-6} M for α,β-meATP and 2-Methylthio-ATP and 10^{-4} M for Other Agents) on Contractile Response of Greater Saphenous Vein Isolated from Patients with Obliterating Atherosclerosis and Varicose Vein Disease (*M*±*m*)

Agonist	Obliterating atherosclerosis	Varicose vein disease
Histamine	53.8±7.2	67.6±7.2
Norepinephrine	76.7±4.5	59.1±8.2*
Carbacholine	25.0±4.1	29.4±6.5
α,β-meATP	39.8±3.8	26.5±4.9*
ATP	15.6±2.7	9.3±2.2*
ADP	17.0±2.6	15.9±4.0
UTP	12.5±7.3	1.7±1.0*
2-methylthio-ATP	4.4±1.6	2.2±1.5
Adenosine	3.9±0.9	5.9±1.7

Note. *$p<0.05$ compared to obliterating atherosclerosis. Contractile response is expressed as percent of that induced by 240 mM KCl.

(10^{-4} M, Fig. 1, *c*). Adenosine and 2-methylthio-ATP induced weak contractions of GSV (no more than 6% of that produced by KCl) only in the highest concentrations, and these contractions did not significantly differ in both groups of patients (Table 1).

Thus, the isolated human GSV responded to most agonists used in this study, and these responses strictly depended on the agonist concentration.

The contractile responses to epinephrine, histamine, and carbacholine confirmed the presence of adrenergic, histaminergic, and cholinergic receptors in the venous wall. It should be noted that in contrast to large arterial vessels, where carbacholine induces endothelium-dependent relaxation, in GSV this agonist induced a contractile response, although the amplitude of this response was 2-2.5-fold lower than vascular response to histamine and norepinephrine.

Of particular importance are the data on the efficiency of P2-agonists. According to modern classification, P2 receptors are subdivided into two families (P2X and P2Y), and each family is further subdivided into 6-8 subtypes. P2X-receptors are associated with ligand-operating non-selective ionic channels, while P2Y-receptors are typical G-protein-mediated receptors [5]. In most vessels of animals, stimulation of P2X-receptors induces constriction, while stimulation of P2Y-receptors induces endothelium-dependent relaxation [7]. In pharmacological tests, the most potent agonist of P2X- and P2Y-receptors are α,β-meATP and 2-methylthio-ATP, respectively, while ATP can activate receptors of both types [4,6].

In AO group, the contraction-inducing activity of P2-agonists decreases in the following series: α,β-meATP>>ATP=ADP=UTP>2-methylthio-ATP. The highest contraction-inducing activity of α,β-meATP attests to involvement of P2X-receptors in contractile response of GSV to P2-agonists, and the most probable participants among this population of receptors are $P2X_1$ and $P2X_2$ receptors, because they are most frequently present in vessels of various animal species. At the same time, equal contractile efficiency of ATP and UTP suggests involvement of some subtypes of P2Y-receptors in the vascular response, specifically of $P2Y_2$ and $P2Y_4$, which were also found in many vessels of animals [7].

When subdividing the patients into the groups according to diagnosis, we tried to compare GSV responses in individuals with and without chronic venous pathology *e.g.* VVD. Evidently, GSV of AO patients cannot be considered as purely normal controls. Atherosclerosis is a system disease, which does not affect the venous bed, therefore the state of GSV in AO patients can be considered as normal in this comparative study.

Thus, it can be concluded that VVD significantly disturbs P2-receptor-mediated contraction of GSV: venous responses to ATP and α,β-meATP markedly decrease, while those elicited by UPT completely disappear. These changes can contribute to a decrease in venous tone during VVD and can play a role in the pathogenesis of this disease.

Therefore, our study revealed the presence of P2-receptors in human GSV, whose stimulation produces the venous contractile response. P2-receptor agonists induce weaker contractions in GSV from VVD patient compared to those from AO patients, which indicates possible involvement of P2X-receptor-mediated mechanisms in the pathogenesis of varicose vein disease in humans.

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