Varicose disease affects the P2 receptor-mediated responses of human greater saphenous vein


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Abstract

The aim of the present study was to investigate in vitro the differences in P2 receptor mediated responses of human greater saphenous vein (GSV) taken from patients with varicose disease and obliterating atherosclerosis. Samples of the inguinal part of the GSV were taken from the patients who underwent phlebectomy operation due to varicose disease (n=9, VD group) or femoropoplitea bypass operation using auto-vein due to obliterating atherosclerosis of lower extremities (n=11, OA group). The mechanical responses of the isolated segments of GSV to P2 receptor agonists were tested using standard organ-bath technique. ATP (10^-6–10^-4 M), ADP (10^-6–10^-4 M) and α,β-methyleneATP (10^-5–10^-3 M) caused concentration-dependent contractions of the veins of both groups, the latter agonist being approximately tenfold more active than first two. ATP at all concentrations tested, α,β-methyleneATP at concentrations of 10^-6 and 10^-5 M and ADP at a concentration of 10^-6 M produced significantly higher contractions of the GSV taken from OA group than from VD group. UTP (10^-6–10^-4 M) caused concentration-dependent contractions of the veins taken from OA group, while in VD group this agonist was virtually without effect. Adenosine (10^-6–10^-4 M) and 2-methylthio-ATP (10^-7–10^-5 M) had no significant contractile activity in this tissue in both groups. It is concluded from this study that there are P2 receptor and adrenoceptor mediated contractions in human greater saphenous veins, which are impaired by varicose disease, in contrast to contractions produced by histamine and carbachol which are, if anything, enhanced.

Keywords: P2 receptors; Human saphenous vein; Varicose disease

1. Introduction

It is well established now that ATP has numerous extracellular actions, which are mediated by specific receptors named P2 receptors divided into two families, P2X ligand-gated ion channels and P2Y G protein-coupled receptors (Ralevic and Burnstock, 1998; Abbracchio and Williams, 2001; Burnstock, 2001). P2 receptors are widely distributed in the cardiovascular system of many species and are involved in regulation of vascular tone, cell proliferation and death, heart activity and haemostasis (Burnstock and Ralevic, 1994; Boarder and Hourani, 1998; Kunapuli and Daniel, 1998; Pelleg and Vassort, 2001; Vassort, 2001; Burnstock, 2002). However, less is known so far about the pathophysiological role of these receptors in the human cardiovascular system.

The involvement of P2 receptors in the responses to stimulation of sympathetic nerves has been shown in dog (Hiraoka et al., 2000) and human (Rump and von Kugelgen, 1994; Racchi et al., 1999) saphenous vein. Since P2 receptors can mediate both vasodilation and vasoconstriction (Burnstock, 1990; Pelleg and Vassort, 2001) it is likely
that they are involved in regulation of vein tonus in physiological and pathophysiological conditions.

Varicose disease of lower extremities, the pathological dilution of subcutaneous veins due to lost of their tonus, is one of the most common vascular pathologies in humans, affecting the ability to work and threatens the quality of life of many thousands of people (Weingarten, 2001). Although the theoretical basis and clinical aspects of the varicose disease are widely discussed in the literature, the role of different receptors in the development of the disease is still not well understood.

Lower extremity atherosclerotic occlusive disease is one of the serious manifestation of systemic atherosclerosis, when peripheral arteries are badly affected by the disease while veins remain relatively intact. The disease often needs surgical treatment of which one of the most common procedures is a bypass operation using patient’s auto-vein.

With the aim to investigate in vitro the P2 receptor mediated responses in human veins in case of chronic vein pathology, in the present study we compare the effects of P2 receptor agonists on human greater saphenous vein (GSV) taken from patients with obliterating atherosclerosis and varicose disease of lower extremities.

2. Materials and methods

Samples of human GSV have been taken from 20 patients who were undergoing in-patient surgical treatment in Kazan Center of Cardiovascular Surgery. The patients were divided into two groups. The first group consisted of 11 patients (10 male and 1 female age of 43–64 y.o.) with the diagnosis of obliterating atherosclerosis, the occlusion of arteries of lower extremities, chronic arterial insufficiency of lower extremities (OA group); the patients underwent femoropoplitea bypass operation using their own GSV. The second group consisted of 9 patients (4 male and 5 female, age of 28–53 y.o.) with the diagnosis of varicose disease of lower extremities at the stage of compensation or subcompensation (VD group); the pathological process was localized within shank; skin trophic disturbances were absent, these patients underwent phlebectomy operation.

Segments of inguinal part of GSV were taken during the operation and were immediately placed in cooled Krebs’ solution. The samples were transported to the laboratory and used for organ bath experiments within no more than 4 h from the operation. Segments of the vein without valves approximately 5–8 mm long were mounted horizontally under isometric tension in 10 ml organ bath by inserting two tungsten wires into the lumen. An initial tension of 1000 mg was applied to the vessels which were then allowed to equilibrate for at least 60 min. The tissue was bathed in modified Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaHCO3 16.3, MgCl2 0.6, NaH2PO4 1.35, CaCl2 2.5, glucose 7.8 and gassed with 95% O2 and 5% CO2 (pH 7.3–7.4). The solution was changed every 10–15 min by overflow methods throughout the experiment. Mechanical activity of the tissue was recorded by a Linton FSG-01 (Great Britain) force-displacement transducer, acquired by Biopack MP100WSW Data Acquisition System (Great Britain) and displayed on a computer screen. All contractile responses were calculated as a percentage of the response evoked by KCl at a concentration of 240 mM which was added at the end of the experiments.

All data are expressed as means±S.E.M. Differences between mean values were assessed using Student’s paired and unpaired t-tests and considered significant at P<0.05. The study has been approved by the Ethical Committee of Kazan State Medical University. Permission has been obtained from every patient who took part in the study by signing a special Informed Consent form.

3. Results

Noradrenaline, histamine and carbachol (10−7–10−4 M) caused concentration-dependent contractions of isolated human GSV taken from both groups of patients. Contractions to carbachol and histamine were statistically similar in both groups at all concentrations of agonists tested. At the highest concentration tested (10−3 M), noradrenaline caused significantly smaller contractions of the vein in the VD group compare to those of the VD group (Fig. 1). With lower concentrations of noradrenaline, the vein contractions were statistically identical in both groups.

ATP (10−6–10−4 M), α,βmethyleneATP (α,βmeATP, 10−6–10−5 M) and ADP (10−6–10−4 M) all caused concentration-dependent contractions of the isolated human GSV of both patient groups (Fig. 2). At all ATP concentrations tested, contractions of the vein taken from the VD group were significantly lower than those from the OA group (Fig. 2a). α,βmeATP at concentrations of 10−6 and 10−5 M caused significantly less prominent contrac-

![Fig. 1. Comparative activity of agonists at a concentration of 100 µM to induce contractions of human greater saphenous vein taken from the patients with obliterating atherosclerosis (OA group) and varicose disease (VD group). Data are presented as means±S.E.M. (n=5–6). *P<0.05 against OA group.](image-url)
tions of isolated saphenous vein from the VD group, than from the OA group (Fig. 2b). In both groups, $\alpha, \beta$meATP was almost tenfold more active agonist than ATP in evoking contractions (Fig. 3). At the lowest concentration tested ($10^{-6}$ M), ADP caused statistically smaller contractions of the vein in group with VD than those in group with OA, although at two higher concentrations this agonist evoked statistically identical contractions of the vein of both patient groups (Fig. 2c). UTP ($10^{-6}$–$10^{-4}$ M) caused concentration-dependent contractions of the vein taken from OA patients, while this agonist, even at the highest concentration tested ($10^{-4}$ M), had little, if any, contractile action on the vein taken from the VD group (Fig. 2d).

Adenosine ($10^{-6}$–$10^{-4}$ M) and 2-methylthio-ATP (2meSATP, $10^{-6}$–$10^{-4}$ M) had little contractile activity on human saphenous vein; at the highest concentration tested ($10^{-4}$ M), these agonists caused contractions which were no more than 6% of those evoked by 240 mM KCl (Fig. 3).

4. Discussion

The present study showed that most of the agonists we tested caused contractions of the isolated human saphenous vein which were concentration-dependent in manner. The contractions registered to noradrenaline, carbachol and histamine were consistent with earlier reports about the presence of the adreno-, cholino- and histamine-receptors in the vein wall (Hiraoka et al., 2000; Brunner et al., 2001). It is interesting that unlike most arterial vessels, where carbachol caused endothelium-dependent vasodilation, in the saphenous vein, carbachol evoked contractions, although they were 2–3-fold smaller in amplitude than those produced by noradrenaline or histamine.

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Fig. 2. Contractile responses of human greater saphenous vein taken from the patients with obliterating atherosclerosis (closed symbols) and varicose disease (open symbols) evoked by ATP (a), $\alpha, \beta$meATP (b), ADP (c) and UTP (d). Data are presented as means±S.E.M. ($n=7–9$). *$P<0.05$ against varicose group.

Fig. 3. Comparative activity of P2 receptor agonists at a concentration of 10 $\mu$M to induce contractions of human greater saphenous vein taken from the patients with obliterating atherosclerosis (OA group) and varicose disease (VD group). Data are presented as means±S.E.M. ($n=5–9$). *$P<0.05$ against OA group.
It has been shown that in animal vessels, stimulation of the P2X receptors causes vasoconstriction, while activation of P2Y receptors usually leads to endothelium-dependent vasodilatation (Ralevic and Burnstock, 1998; Pelleg and Vassort, 2001), although P2Y receptors on smooth muscle in some vessels lead to endothelium-independent vasodilatation or vasoconstriction (see Burnstock and Knight, in press). The results of the present study showed the following rank order of P2 agonists: \(\alpha,\beta\text{meATP} \gg \text{ATP} = \text{ADP} = \text{UTP} = \text{2meSATP}\) for contractions of OA veins. The highest potency of \(\alpha,\beta\text{meATP}\) supports the view that P2X1 and/or P2X3 receptors are probably involved in vein contractility, since this agonist is selective to these subtypes of P2 receptors (Burnstock, 2001, 2003). We suggest that most likely the P2X1 receptors were mainly responsible for the contractions of GSV induced by P2 receptor agonists in our study since they are typically present in vascular smooth muscle cells (Pelleg and Vassort, 2001).

UTP is a potent agonist at P2Y2 and P2Y4 receptors and has been shown to produce both relaxation and contractions of some vessels (Saig et al., 1990; Eltze and Ullrich, 1996; Miyagi et al., 1996; Rubino and Burnstock, 1996). The contractions of GSV to UTP in this study were similar to those of ATP, suggesting the possible involvement of P2Y2 or P2Y4 subtypes, which have been described in the vessels of several animal species (Kunapuli and Daniel, 1998).

In the present study, the efficacy of P2 receptor agonists to cause contractions of GSV was studied for the first time in two different pathological conditions. Our patients were divided into two groups according to their diagnosis and we compared the vein contractile activity. The patients of the VD group showed all symptoms of chronic vein pathology with typical pathomorphological changes of their veins, while the saphenous vein of the patients with the arterial atherosclerosis showed no obvious visual pathological changes (Cyplakov et al., unpublished data). Although we recognize that veins of OA group cannot be regarded as ‘healthy controls’, they formed a useful condition for comparison with the GSV of VD group.

We found that the varicose disease markedly affects the P2 receptor-mediated responses of saphenous vein; there were significantly lowered contractions evoked by ATP and \(\alpha,\beta\text{meATP}\), while responses to UTP were virtually absent. It is possible that these changes are involved in pathogenesis of varicose disease, in particular in relation to the loss of the tonus of subcutaneous veins.

It is concluded from this study that there are P2 receptors in the human saphenous vein which mediate contractile responses and that there were significantly lower P2X1 receptor-mediated contractions in saphenous vein taken from the patients with varicose disease than those of patients with obliterating atherosclerosis. Further, contractions produced by UTP via P2Y2 or P2Y4 receptors were virtually absent in varicose vein. It is suggested that P2 receptors may be involved in the pathogenesis of varicose vein disease.

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References


