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The role of perivascular adipose tissue on human saphenous vein vascular tone

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Abstract: Perivascular adipose tissue (PVAT) is situated outside of almost every blood vessel. Recent studies showed that PVAT provides mechanical support for blood vessels and secretes vasoactive adipokines that could regulate vascular tone. However, most of the studies evaluating PVAT effects on vascular tone have been performed with vessels derived from animals. Therefore, we aimed to investigate the role of PVAT surrounding human coronary bypass graft vessels such as saphenous vein (SV). Human SV preparations were set up in an organ bath in the presence or absence of their PVAT. The presence of PVAT significantly attenuated the contractile response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). However, potassium chloride (KCl)-induced concentration-response curve wasn't modified in PVAT-intact SV preparations. On the other hand, endothelium-dependent relaxation induced by acetylcholine (ACh) or endothelium-independent relaxation induced by sodium nitroprusside (SNP) were similar between SV with PVAT versus SV without PVAT preparations. Sensitivity of the SV to contractile agonists (KCl, $PGF_{2\alpha}$) or relaxant agonists (SNP, ACh) were not modified in the presence of PVAT. These results suggest that PVAT could decrease $PGF_{2\alpha}$-induced contractile tone via endothelium-independent mechanisms in SV. Retaining PVAT in SV preparations during bypass graft surgery could prevent graft vasospasm possibly via PVAT derived relaxant factor(s).

Key words: Perivascular adipose tissue, human saphenous vein, vascular tone

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Introduction

Perivascular adipose tissue (PVAT) is situated outside of almost every blood vessel exception of cerebral artery and pulmonary vessels and it is structurally distinct from the adventitia (Szasz et al., 2013). At the beginning, PVAT has been thought to provide only mechanical support for blood vessels, but recent studies showed that PVAT also secretes vasoactive cytokines named adipokines such as, adiponectin, leptin and resistin (Oriowo, 2015; Ozen et al., 2015). These molecules secreted from PVAT have roles on the regulation of vascular tone via their paracrine effects.

First time, Soltis and Cassis demonstrated that PVAT significantly attenuates *in vitro* vascular response of noradrenaline (NA) in rat aorta (Soltis & Cassis, 1991). However, they suggested this attenuation in vascular tone was due to NA uptake by PVAT. Subsequently, Lohn et al. confirmed that PVAT reduced vascular reactivity in response to serotonin, angiotensin II (Ang II) and phenylephrine. It has been confirmed by this study that the anticontractile substances of PVAT namely, adipocytes derived relaxing factors (ADRF) were independent of adrenergic neuronal uptake since these substances were not subjected to reuptake by the adrenergic nerves. Furthermore, it has been verified that this anticontractile effect was not due to the physical presence of PVAT since supernatant of rat adipocytes also induced vasorelaxation (Lohn et al., 2002). The identity of this factor that mediates the anticontractile influence of PVAT is still under debate, however it is known that PVAT regulates vascular tone by releasing different adipokines depending on the anatomic location of the adipose tissue depot and the species being studied. The anticontractile effect of PVAT has been observed mostly in animal tissues such as mesenteric arteries of rats (Galvez et al., 2006), venous rings of rats (Lu et al., 2011), mesenteric arteries of mice (Takemori et al. 2007) and coronary arteries of pig (Bunker & Laughlin, 2010). In humans, there is a few study which evaluates the role of PVAT on the regulation of vascular tone. Most of the studies in humans were performed with coronary artery bypass grafts such as internal mammary artery (IMA) and saphenous vein (SV). Our previous study has showed that vascular contraction induced by NA was significantly decreased in the presence of PVAT in SV preparations. Preincubation with indomethacin, a cyclooxygenase inhibitor, increased NA contraction in SV

preparations with PVAT. This results suggests that PVAT of SV releases vasodilatory prostanoids such as prostaglandin E_2 (PGE_2), prostacyclin (PGI_2) as the anticontractile factors (Ozen et al., 2013).

Generally, SV was stripped of its surrounding PVAT in its conventional preparation during bypass surgery but recently no-touch method has been demonstrated where the PVAT surrounding the vein remained intact (Souza et al., 2001). The studies performed by the bypass grafts with PVAT is quite interesting since they could bring new aspect and perspective in determining the best bypass grafting technique. For this reason, we aimed to investigate the effect of PVAT on vascular contraction or relaxation responses in isolated human SV. The concentration-response curves induced by different agonists were established in PVAT-intact or PVAT-removed SV rings. The endothelium-dependent relaxation responses were induced by acetylcholine (ACh) whereas, endothelium-independent relaxations were produced by sodium nitroprusside (SNP). Contractile responses were obtained with $PGF_{2\alpha}$ which activates a G protein coupled-receptor and with potassium chloride (KCI) which induces the depolarization of the smooth muscle fibers.

Materials and Methods

Vascular preparations

The Institutional Review Board of the Istanbul University Faculty of Medicine Ethic Committee approved the study plan. All patients voluntarily participated in the study and gave their written informed consent for human vessels. The study was performed on isolated segments of human SV with intact endothelium, obtained from patients (14 male, 6 female) who had undergone coronary artery bypass surgery in Gaziosmanpaşa Avrupa Şafak Hospital. The SV fragments for the experiments where placed in the cold Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118.5; KCl 4.8; NaHCO3 25; MgSO4·7H2O 1.2; CaCl2 1.9; KH2PO4 1.2; glucose 10.1; disodium ethylenediaminetetraacetic acid (EDTA) 0.026; pH 7.4 and transferred immediately to the laboratory.

Functional studies

Segments of SV from one patient, were cut into 2 rings. Depending on the quantity of PVAT, one preparation was left intact with PVAT and the other was dissected free of PVAT. Preparation with or without PVAT from same patient were studied in parallel. Rings were suspended between two stainless steel L-shaped hooks in 10 mL jacketed organ baths containing Krebs-Ringer bicarbonate solution at 37◦C and gassed with a mixture of 95% O_2 and 5% CO_2. One hook was fixed at the bottom of the organ bath, while the other was connected to a force displacement transducer (Grass Model FT03). The force displacement transducer was fixed to a manipulator, allowing adjustments in the resting tension of the rings. Each ring was initially stretched to an optimal load (2 g) as described previously (Ozen et al., 2013). Subsequently, preparations were allowed to equilibrate for 90 min with bath solution changes taking place every 15 min. After equilibration period, the viabilities of the vessel specimens were checked by KCl (40 mM). Two consecutive KCl responses were obtained in each ring for the standardization of the preparations. After the last KCl contraction, the preparations were washed until the basal tone was reestablished (Ozen et al., 2013). Thereafter, the vessels were contracted with increasing concentrations of KCI (20-120 mM) or $PGF_{2\alpha}$ (10^{-9}–10^{-4} M) in a cumulative manner to establish the concentration-effect relationship. Endothelium capacity of SV preparations was checked by ACh. For this purpose, SV preparations were precontracted with NA (10^{-6}-10^{-5}M) and afterwards cumulative concentration-response curve for ACh (10^{-8}–10^{-4} M) was established. Endothelium-independent vascular relaxation capacity of the vessel was tested by SNP (10^{-8}–10^{-4} M) in precontracted SV preparations at the end of each experiment. Relaxation responses were calculated as a percentage of the precontration induced by NA.

Chemicals

NA, KCl, SNP, ACh were purchased from Sigma–Aldrich (St. Louis, MO, USA). A stock solution of NA was prepared in 0.001 N HCI and ascorbic acid was added to prevent oxidation. Other drugs were prepared in distilled water. $PGF_{2\alpha}$ were diluted from Dinolytic® produced by Pfizer.

Statistical analysis

Data obtained from each patient (n) was expressed as mean ± standard error of the mean (SEM). Changes in $PGF_{2\alpha}$ induced responses were given as relative (% of 40 mM KCl) contractions whereas absolute force was measured from isometric recordings in grams (g). Relaxation to SNP or ACh were expressed as the percentage of NA-induced precontraction. The maximal contraction or relaxation (E_{max} value) responses produced by $PGF_{2\alpha}$, KCI, SNP or ACh and the half-maximum effective concentration (EC50 value) were interpolated from the individual concentration-effect curves. The pEC_{50} values were calculated as the negative log EC_{50} values. Statistical analysis was performed by Student's paired t-test or RM two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison *post hoc* tests. P value less than 0.05 was considered statistically significant. All analyses were performed using GraphPAD PRISM version 5.00.

Results

Patient characteristics

Clinical characteristics of the patients undergoing coronary artery bypass operations and their drug therapies are given in Table 1. The mean age was 63.2±3.01 years. Most of patients were hypertensive and using beta-blocker drug therapies.

Table 1. Clinical characteristics of patients from whom saphenous vein (SV) specimens were obtained.

Parameters	**n**
Women	6
Men	14
Age (mean±SEM)	63.2±3.01
Chronic Diseases	
Angina pectoris	10
Hypercholesterolemia	8
Hypertension	11
Diabetes mellitus	9

Medications	
Ca^{+2} channel blokers	7
β-blokers	8
Nitrovasodilators	7
ACE inhibitors	5
Diuretics	4
Statins	2
Oral antidiabetics	8

n : indicates the number of patients.

Effects of PVAT on Contraction Responses

In the presence of PVAT, vascular contraction induced by KCI were not modified (Figure 1). E_{max} and pEC_{50} values derived from the concentration-effect curves induced by KCI were similar in SV preparations without PVAT in comparison with those of control with PVAT (Table 2). On the other hand, vascular contraction response induced by $PGF_{2\alpha}$ was significantly decreased particularly at higher concentrations in the presence of PVAT. In addition, the whole curve with PVAT was significantly lower than that of without PVAT (Figure 2). E_{max} of the concentration-effect curves induced by $PGF_{2\alpha}$ were significantly decreased in SV preparations with PVAT versus without PVAT, while pEC50 values were indifferent (Table 2).

Table 2. Effects of perivascular adipose tissue (PVAT) on potassium chloride (KCI) or Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induced contractions in isolated human saphenous vein (SV).

	E_{max}	pEC_{50}	n
KCl			
SV without PVAT	12.09 ±1.04	39.58±0.35	13
SV with PVAT	12.92±1.42	36.57±0.36	12
$PGF_{2\alpha}$			
SV wihout PVAT	157.87±13.33	5.54±0.20	4
SV with PVAT	118.82±18.85*	5.43±0.08	7

Changes in KCl responses were given in grams. Contractions to $PGF_{2\alpha}$ were expressed as the percentage relaxation of KCI (40 mM) induced contraction. Values are means ± SEM. n: indicates number of patients.* $p<0.05$ when compared to SV without PVAT.

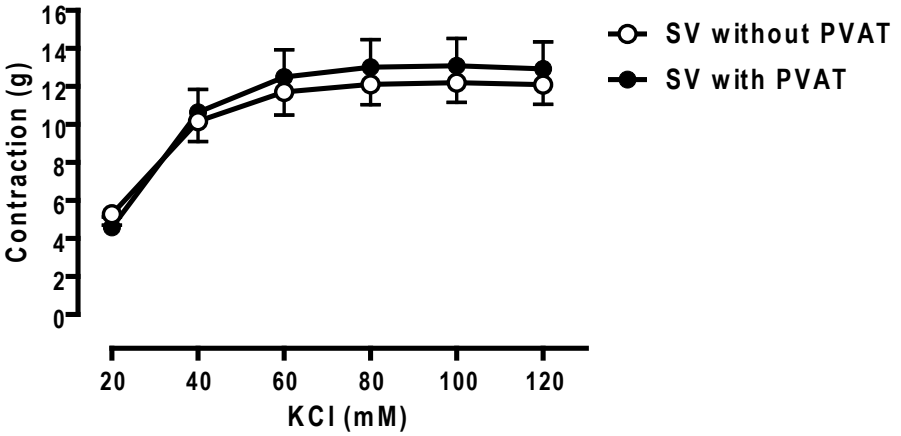

Figure 1. Effects of perivascular adipose tissue (PVAT) on vasoreactivity of saphenous veins to potassium chloride (KCI). Changes in KCl induced contractions are given in grams (g). Values are given as mean ± SEM.

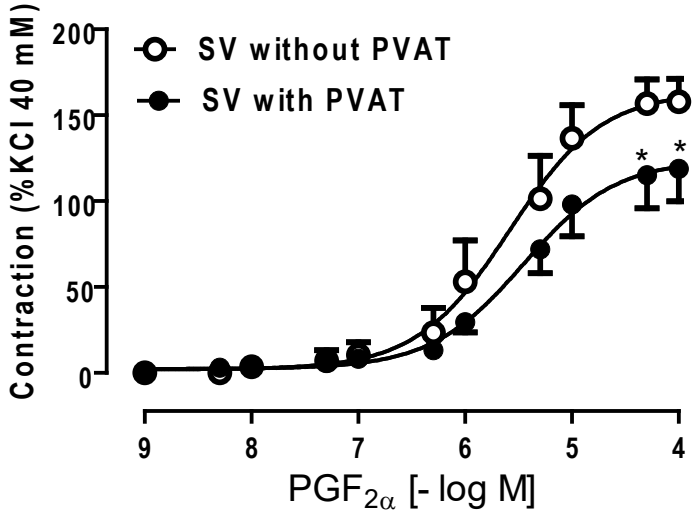

Figure 2. Effects of perivascular adipose tissue (PVAT) on prostaglandin F2α ($PGF_{2\alpha}$)-induced contractions in isolated human saphenous veins (SV). Contraction responses to $PGF_{2\alpha}$ were expressed as the percentage of KCI (40 mM) induced contraction. * indicates $P<0.05$ compared to SV without PVAT. In addition, the whole curves with and without PVAT are significantly different. Values are given as mean ± SEM.

Effects of PVAT on Relaxation Responses

In the presence of PVAT, the endothelium-dependent and –independent relaxation responses induced by ACh and SNP, respectively were not changed (Figures 3-4). In addition, E_{max} and pEC_{50} values derived from the concentration-effect curves induced by these substances were found similar between preparations with PVAT versus without PVAT (Table 3).

Table 3. Effects of perivascular adipose tissue (PVAT) on sodium nitroprusside (SNP) and acetylcholine (ACh) induced relaxations in isolated human saphenous vein (SV).

	E_{max}	pEC_{50}	n
ACh			
SV without PVAT	23.5±2.76	6.9±0.32	6
SV with PVAT	26.56±1.79	6.66 ± 0.23	6
SNP			
SV without PVAT	109.01±2.51	6.50±0.11	6
SV with PVAT	116.35±2.15	6.5±0.07	6

Endothelium-dependent relaxations induced by ACh and endothelium-independent relaxation induced by SNP were expressed as the percentage of the precontraction induced by NA. Values are given as mean ± SEM. n : indicates the number of patients.

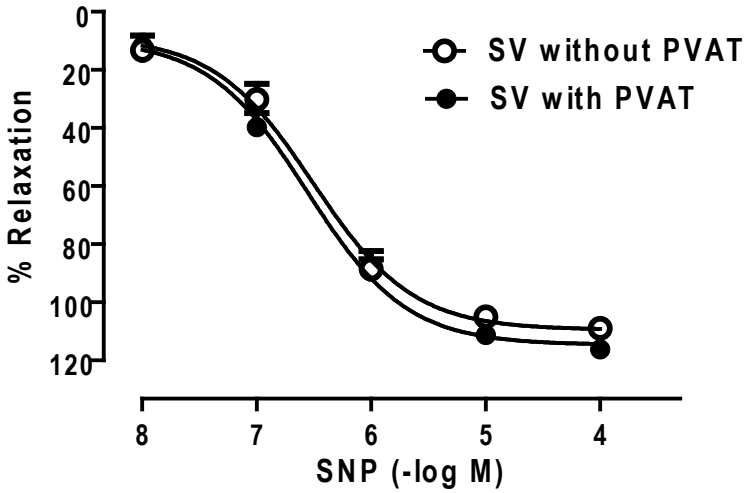

Figure 3. Effects of perivascular adipose tissue (PVAT) on endothelium-independent relaxation induced by sodium nitroprusside (SNP) in isolated human saphenous veins (SV). Relaxations to SNP were expressed as the percentage of NA-induced precontraction. Values are given as mean ± SEM.

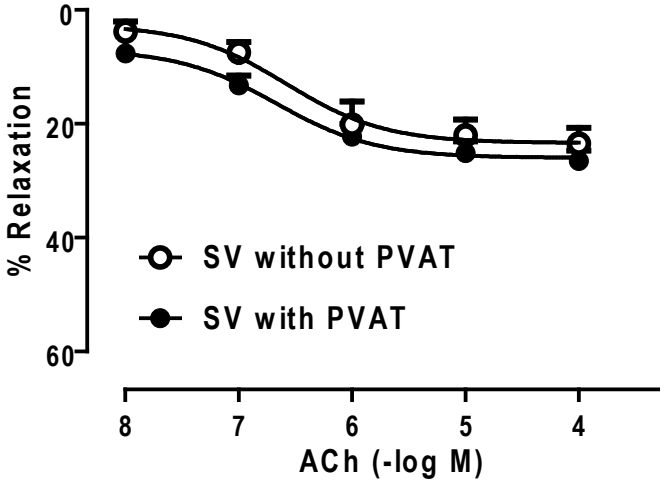

Figure 4. Effects of perivascular adipose tissue (PVAT) on endothelium-dependent relaxation responses induced by acetylcholine (ACh) in isolated human saphenous veins (SV). Relaxations to ACh were expressed as the percentage of NA-induced precontraction. Values are given as mean ± SEM.

Discussion

Our results showed that vascular contractile responses induced by $PGF_{2\alpha}$ in isolated human SV were significantly decreased in the presence of PVAT. This finding supported our previous observation demonstrating that PVAT surrounding the human SV attenuated the contractions induced by noradrenaline (Ozen et al., 2013). In addition, in this study we showed that endothelium-dependent and independent relaxation responses induced by Ach and SNP, respectively, were not modified in the presence PVAT. Thus, these results suggest that PVAT of SV releases anticontractile factor(s) that can modify contractile tone possibly via endothelium-independent mechanisms.

ACh induced endothelium-dependent relaxations in precontracted vessels are known to be related to the release of NO from the endothelium. The similarity in ACh induced relaxations in SV rings with PVAT compared to the rings without PVAT suggested that the acting mechanism of PVAT-derived relaxant factor is independent of the endothelium. Likewise, Soltis and Cassis have showed similar results in the isolated rat aorta preparation (Soltis & Cassis, 1991). In addition some studies which performed in endothelium-denuded isolated vessels also revealed that PVAT-derived relaxant factor is still present (Lohn et al., 2002). Moreover, pretreatment with the NOS inhibitor failed to abolish vasorelaxant effect of PVAT either in IMA (Malinowski et al., 2008) or in rat aorta (Lohn et al., 2002). In contrast, some other studies suggested that PVAT- derived relaxant effect is endothelium-dependent and possibly related to the release of NO (Gao et al., 2007; Lu et al., 2011).

In this study, we also evaluated the effect of PVAT on contractions induced by KCl as well as on endothelium-independent relaxations induced by SNP. Our results show that PVAT did not modify the cumulative concentration responses to both KCl and SNP, supporting our previous study (Ozen et al., 2013). These results are also in line with another study performed on rat aorta (Lohn et al., 2002). Lohn et al. showed comparable contractile responses of intact vessels with PVAT and vessels without PVAT to KCl (60 mM) while the contraction induced by serotonin was significantly decreased. These findings demonstrated that excitation-contraction coupling in arteries with or without PVAT remains functional and that the

presence of PVAT does not mechanically alter the contractility of aortic rings (Lohn et al., 2002). In addition, an other group has also demonstrated an attenuation in the maximal contraction to U46619 and the contraction to phenylephrine in the presence of PVAT while, the contraction to KCI was not modified in isolated IMA rings. (Gao et al., 2005). In our study, we showed that $PGF_{2\alpha}$-induced vascular contraction was significantly decreased in the presence of PVAT. These results are in accordance with our previous study showing a decreased contraction to noradrenaline in PVAT-intact vessels. Moreover, current study also showed that the relaxant influence of PVAT is still present, although the endothelial capacity of SV was weak (about % 20-25). This result also supports that PVAT mediated relaxant effect is independent of the endothelium layer.

Until recently, PVAT was often removed from the graft material during the coronary artery bypass grafting. Since PVAT of SV possibly releases vasorelaxant substances, retaining PVAT around the vessel might be helpful in reducing the occurrence of vasospasm of these graft vessels in either the perioperative or the early postoperative period. During cardiopulmonary bypass surgery, it has been previously documented that the levels of thromboxane (TxA_2) and endothelin, which are known to induce vasoconstriction, were increased (Pearl et al., 1999). Thus, preserving of PVAT could limit the vasocontractile effect that may be initiated from the increased levels of TxA_2 and endothelin. In this regard, Souza and coworkers evaluated a no-touch technique to dissect the SV with surrounding perivascular adipose tissue and suggested that this newtechnique could have beneficial effects such as, the prevention of graft spasm, proliferation, platelet agregation as well as the protection of the vascular wall from the injury (Souza et al., 2001; Dashwood & Tsui, 2013). Other groups also reported a superior graft patency when no-touch SV harvesting technique was employed (Sepehripour et al., 2011). Indeed, endothelial cells preserved better in their normal morphology and their structure when the no-touch technique was used for the vein graft harvesting (Sen et al., 2013).

In conclusion, the present study originally demonstrated the anticontractile influence of PVAT on isolated human SV tone evoked by $PGF_{2\alpha}$, a spasmogen acting on receptor- operated mechanism. Indeed, this finding well confirms with the previous studies reporting the regulatory

role of PVAT on the arterial tone and also displays its apparent importance in vessels that even have a weak endothelial capacity. Herein, we also suggested that this anticontractile influence is independent from the endothelium since the endothelium-dependent relaxation induced by ACh was not modified in the presence of PVAT. Thereby, retaining of PVAT in SV during coronary artery bypass grafting could contribute to the improvement of patency rate via suppressing its contractility to spasmogens by a relaxing factor (s) derived from PVAT.

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