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Perivascular tissue of internal thoracic artery releases potent nitric oxide and prostacyclin-independent anticontractile factor☆

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Abstract

Objective: It has been recently suggested that perivascular tissue (PVT) releases hypothetic adipocyte- or adventitia-derived relaxing factor. The aim of the study was to assess anticontractile properties of perivascular tissue of human internal thoracic artery (ITA) and to check if this activity is nitric oxide (NO)- or proctacyclin-dependent. We also analyzed the influence of pleural adipose tissue on ITA reactivity. **Methods:** Human ITA rings were studied in vitro. First, skeletonized and pedicled ITA reactivity to serotonin and angiotensin II was compared. In subsequent experiments fragments of ITA were skeletonized and divided into two preparations. One was incubated alone, the other together with PVT or pleural adipose tissue floating freely in the bath. First, concentration–response curves to either serotonin or angiotensin II were constructed. Tissue was then transferred from one bath to the other and concentration–response curves were reconstructed. The same protocol was applied with the inhibition of NO synthase with L-NMMA (10^{-4} M) and cyclooxygenase with indomethacin (10^{-5} M). **Results:** Skeletonization augmented contractile response to serotonin (E_{max} 16.6 ± 1.85 mN vs 43.8 ± 3.87 mN; pedicled vs skeletonized ITA, respectively; $p < 0.001$) and angiotensin II (E_{max} 10.9 ± 1.07 mN vs 26.6 ± 1.45 mN, respectively; $p < 0.001$). PVT presence in the bath caused decrease of E_{max} from 40.8 ± 5.01 to 20.1 ± 2.69 mN for serotonin; $p < 0.001$ and from 31.4 ± 3.75 to 13.0 ± 1.60 mN for angiotensin II, $p < 0.001$ (PVT(−) vs PVT(+), respectively). PVT did not change ITA sensitivity (EC_{50}) to serotonin or angiotensin II. Pleural adipose tissue did not change the contractile response of ITA to serotonin (E_{max} 37.2 ± 4.95 mN vs 36.3 ± 4.83 mN, pleural fat + and pleural fat−, respectively; $p = 0.9$). NO and prostacyclin inhibition failed to abolish anticontractile properties of perivascular tissue. PVT with cyclooxygenase and NO synthase inhibition decreased E_{max} of serotonin from 46.6 ± 3.03 to 28.2 ± 4.02 mN, $p < 0.001$ and E_{max} of angiotensin II from 27.2 ± 2.00 to 16.4 ± 2.10 mN, $p < 0.001$. **Conclusions:** Perivascular tissue of ITA releases potent, soluble, nitric oxide and prostacyclin-independent anticontractile factor. The pleural adipose tissue does not influence ITA reactivity to vasoconstrictors. Preservation of perivascular tissue may protect against vasospasm of ITA graft in clinical settings.

Keywords: Internal thoracic artery; ADRF; Perivascular tissue; Adventitia; Pleural adipose tissue

1. Introduction

Left internal thoracic artery (ITA) is the gold standard graft in coronary artery surgery. It provides the excellent long-term patency [1] and improves survival in patients undergoing CABG [1,2]. Traditionally ITA grafts are harvested as a pedicle with surrounding tissues. However, ITA skeletonization can provide a longer graft with superior flow [3]. Obtaining the skeletonized graft can result in less incidence of postoperative sternal wound infection, decreased chest wall pain [4] and better pulmonary function [5,6]. Although technically more demanding ITA skeletonization can provide a graft with preserved integrity [7,8] and functionally active endothelium [3]. The above makes the skeletonized ITA more and more popular particularly among surgeons advocating the idea of total arterial revascularization. We have shown previously that skeletonized and pedicled ITAs are functionally different vessels having unlike reactivity when studied in vitro [9]. Higher sensitivity of skeletonized versus pedicled artery may be of clinical significance, increasing risk of perioperative ITA vasospasm [10]. Preserving perivascular tissue (PVT) might be therefore beneficial, not only allowing for less surgical trauma to the artery but also enabling its vascular modulatory properties. This is especially the case, as it has been suggested that perivascular tissue releases anticontractile transferable factor, adventitium- or adipocyte-derived relaxing factor (ADRF). Its existence was proposed initially in rat aorta [11–13] and than in rat mesenteric arteries [14]. The action of

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this hypothetic anticontractile factor is mediated by opening of different types of potassium channels in various vascular beds (i.e. voltage dependent K^+ in rat mesenteric arteries [14], K_{ATP} in the rat aorta [12] and K_{ca} in human ITA [15]). ADRF action was initially shown not to depend on endothelium [12]. This fact was however recently challenged in the elegant bioassay study of Gao et al. [16].

Our study was designed to prove anticontractile properties of PVT of human ITA and confirm that these properties depend on the release of transferable relaxing factor. In the view of recent controversies regarding endothelium contribution to the action of ADRF we examined if typical endothelium derived relaxants, i.e. nitric oxide (NO) and prostacyclin are the mediators of ADRF anticontractile properties.

Because it was shown that cultured adipocytes can be the origin of putative ADRF we also checked whether the other than perivascular, clinically relevant human adipose tissue, can modulate the function of ITA.

2. Materials and methods

The study was performed on isolated segments of left human ITA discarded after the conduit had been trimmed to the length necessary for grafting. The Local Research Ethics Committee agreed to the use of human tissue for the experimental work and patient informed consent was waived. The study arterial segments were obtained from patients undergoing surgery for stable isolated coronary artery disease. All grafts where harvested pedicled in standard fashion using electrocautery. The ITA fragments for the experiments where next placed in the cold (4 °C) calcium-free modified Krebs—Henseleit solution (NaCl, 123.0; KCl, 4.70; $MgSO_4$, 1.64; $NaHCO_3$, 24.88; KH_2PO_4, 1.18; glucose, 5.55; sodium pyruvate, 2.0 [mM] (pH 7.4) and transferred immediately to the laboratory. The vessels were divided into 3 mm long segments. The arterial rings were suspended on stainless steel wire hooks in the organ bath chamber filled with oxygenated (95% O_2, 5% CO_2) Krebs—Henseleit solution of the following composition: NaCl, 119.0; KCl, 4.70; $CaCl_2$, 1.6; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; glucose, 11.01; sodium pyruvate, 2.0 [mM] (pH 7.4). The temperature was maintained at 37 °C. The Schuler isolated organ bath (Hugo Sachs Elektronik (HSE); March-Hugstetten, Germany) was used. Vessel wall tension was measured with isometric force transducer F 30 (HSE) with bridge amplifier (HSE) and the signal was enhanced with bridge amplifier Type 336 (HSE) and recorded using PowerLab/4SP system and Chart software (AD Instruments, Chalgrove, Oxfordshire, UK). After the short period of initial incubation the vessel wall tension and diameter were normalized in a standardized procedure as described by Mulvany and Halpern [17]. This way every vessel ring was set to the 90% of diameter it would have had in vivo, when relaxed and under the transmural pressure of 100 mmHg using the Laplace law; $P = 2T/d$. After equilibration the vessel was left for 30 min to stabilize, during which period the tissue was thoroughly washed. Then, appropriate experimental protocol was applied.

In all experiments we elected to use serotonin and angiotensin II as vasoconstrictors as they seem to be clinically relevant mediators of vascular tone. In this study we decided not to use norepinephrine as a vasoconstrictor as it is subject to reuptake by adrenergic nerve endings present in perivascular tissue and may therefore make inferences on paracrine role of PVT difficult.

2.1. Experiment 1

We first compared the reactivity of skeletonized and pedicled ITA to serotonin and angiotensin II. To do so, ITA specimen from one patient was divided into two 3mm segments. One was left intact with surrounding tissue (pedicled — PVT(+)) and the other was dissected free of surrounding tissue (skeletonized — PVT(−)). Than, the arteries were gradually contracted with serotonin starting from 10^{-9} M and rising in negative logarithm half molar cumulative steps up to 10^{-4} M to establish the concentration—effect relationship. Next, the preparation was washed and second concentration—response curve for serotonin was performed. In the same fashion in other preparations the concentration-response curves were constructed for angiotensin II (10^{-9}—$10^{-5.5}$ M).

2.2. Experiment 2

Bioassay experiment was performed to analyze the anticontractile properties of perivascular tissue. The experimental set-up is shown in Fig. 1. Both ITA rings prepared from one segment were skeletonized. One segment was incubated alone, the other together with perivascular tissue remaining from skeletonization. We randomly chose which segment of the vessel was incubated first with PVT. The perivascular tissue from skeletonization (mean weight 333 ± 70 mg) was always floating freely in the bath. Both preparations were simultaneously contracted with cumulative concentration of either serotonin (10^{-9}—10^{-4} M) or angiotensin II (10^{-9}—$10^{-5.5}$ M). Then the preparations were thoroughly washed and the loose PVT was transferred from one bath to the other. Afterwards,

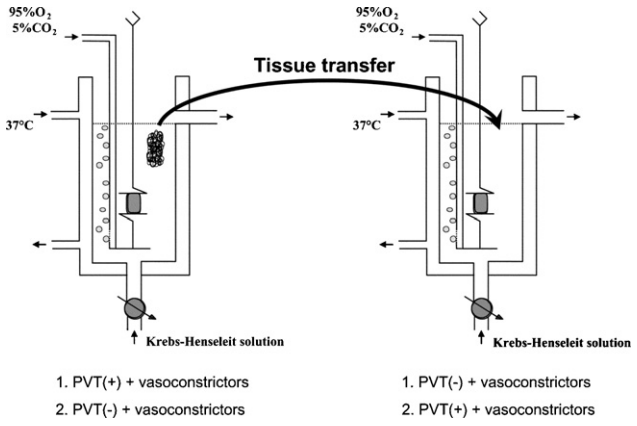

Fig. 1. Schematic illustration of experimental setup and bioassay protocol. The experiments were always performed in two tissue baths using two 3 mm ITA rings obtained from the same patient. Perivascular tissue (PVT) remaining from skeletonization floated freely in the bath in one of two experimental vessels. After the first concentration—response curve was constructed PVT was transferred from one bath to the other. Then, concentration—response relationship to vasoconstrictors was assessed again. In Experiment 3 (see text) pleural adipose tissue was used instead of PVT. Serotonin or angiotension II were used as vasoconstrictors.

concentration–response curves with the same contractile factor were reconstructed. This allowed for obtaining two consecutive concentration–response curves in each ITA ring: in one preparation first curve with PVT in the bath and second without, while in another preparation first without and second with PVT in the bath.

2.3. Experiment 3

To evaluated if adipose tissue of other than perivascular origin modulates contraction of human ITA the same bioassay protocol as described in experiment 2 was applied using pleural fat (mean weight 566 ± 79 mg) instead of ITA's PVT.

2.4. Experiment 4

In this series of experiments we investigated the influence of NO and prostacyclin on the anticontractile properties of PVT. Following the stabilization period arterial segments (one with and one without PVT in the bath) were incubated for 30 min in the presence of 10^{-4} M of N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase inhibitor, and 10^{-5} M of indomethacin, cyclooxygenase inhibitor. Thereafter, still in the presence of L-NMMA and indomethacin, the concentration–effect relationship for serotonin or angiotensin II was assessed. After the washout, perivascular tissue of ITA (mean weight 337 ± 50 mg) was transferred from one bath to the other, L-NMMA and indomethacin were reapplied and concentration–effect relationship for serotonin or angiotensin II was assessed again.

The artery contraction was always measured as an increase in the vessel wall tension above the resting tension and expressed in mN. The responses at each concentration were presented as a mean ± SEM and compared using the paired *t*-test. The concentration–effect relationships were obtained from a regression analysis to the general logistic equation of Hill and Langmuir:

$$E = \frac{E_{max} \times D^n}{D^n + K_D^n},$$

where E is effect, E_{max} is maximal effect, D is concentration, K_D is drug-receptor complex dissociation constant equal to the concentration causing half-maximal effect (EC_{50}) and n is the Hill coefficient. The estimated EC_{50} was subsequently log transformed and presented as $pD_2 = -\log(EC_{50})$. As described in experimental protocols in all experiments we chose to perform two concentration–response curves on every preparation. Acquired data from two concentration–response curves were pooled into one regression analysis. We therefore obtained one concentration–response relationship for skeletonized and one for non-skeletonized preparations (Experiment 1). Similarly, one averaged concentration–response regression was obtained for arteries with perivascular or pleural tissue in the bath and one when the tissue was not present in the bath (Experiment 2–4). The parameters of regression analysis within every experiment were compared using Student's *t*-test. One-way analysis of variance (ANOVA) was used to determine the difference in maximal response to vasopressors between skeletonized ITA (data pooled from all experiments where skeletonized ITA ring with no PVT was studied), and rings of ITA with PVT tissue present (i.e. non-skeletonized, with PVT in the bath and with PVT and indomethacin + L-NMMA in the bath).

In all instances of statistical analysis $p < 0.05$ was considered significant. All analyses were performed using SigmatPlot 10.0 and Sigma Stat 3.5 Software (Systat Inc., San Jose, CA).

The following substances were used in the study: 5-Hydroxytryptamine hydrochloride (serotonin), angiotensin II, indomethacin, N^G-monomethyl-L-arginine (all Sigma-Aldrich Corp., St. Louis, MO).

3. Results

3.1. Experiment 1

ITA rings devoid of PVT contracted stronger to serotonin in comparison to pedicled ITA: maximal response 43.8 ± 3.87 mN versus 16.6 ± 1.85 mN; respectively ($p < 0.001$), $n = 11$ for each – Fig. 2A. Similarly E_{max} was significantly higher in skeletonized arteries when angiotensin II was used to construct concentration–response relationship 26.6 ± 1.45 mN versus 10.9 ± 1.07 mN, respectively ($p < 0.001$), $n = 10$ for each – Fig. 2B. Neither did pD_2 differ for serotonin: 6.6 ± 0.23 versus 6.0 ± 0.23, (skeletonized vs pedicled ITA, respectively, $p = 0.1$), nor was it different for angiotensin II: 8.2 ± 0.12 versus 7.8 ± 0.21 skeletonized versus pedicled ITA, respectively, $p = 0.1$.

3.2. Experiment 2

The initial presence of PVT in the tissue bath caused significantly lower maximal response to serotonin and angiotensin II comparing to preparation without PVT. PVT removal resulted in the augmentation of contraction to vasopressors in the donor vessel, whereas the transfer induced the decrease of E_{max} in the recipient preparation (Fig. 3). Concentration–response relationship pooled from two curves showed that the presence of PVT in the bath caused two-fold decrease of E_{max} to serotonin from 40.8 ± 5.01 to 20.1 ± 2.69 mN (PVT(−) vs PVT(+), respectively, $p < 0.001$; $n = 10$ for each) - Fig. 4. Similarly, concentration–response analysis for contraction to angiotensin II revealed augmented maximal response when there was no PVT in the bath: 31.4 ± 3.75 mN versus 13.0 ± 1.60 mN (PVT(−) vs PVT(+), respectively, $p < 0.001$; $n = 11$ for each) – Fig. 5. EC_{50} was not changed by PVT presence either for serotonin or angiotensin II. For serotonin pD_2 equaled 6.2 ± 0.29 versus 5.8 ± 0.26 ($p = 0.3$); and for angiotensin II: 7.8 ± 0.26 versus 7.9 ± 0.26 ($p = 0.7$) – (PVT(−) vs PVT (+), respectively).

3.3. Experiment 3

We failed to find modulatory effect of pleural adipose tissue on ITA contraction to serotonin. The maximal response equaled 37.2 ± 4.95 mN versus 36.3 ± 4.83 mN; fat versus no fat present, respectively ($p = 0.9$). Pleural adipose tissue presence in the bath did not influence ITA sensitivity to

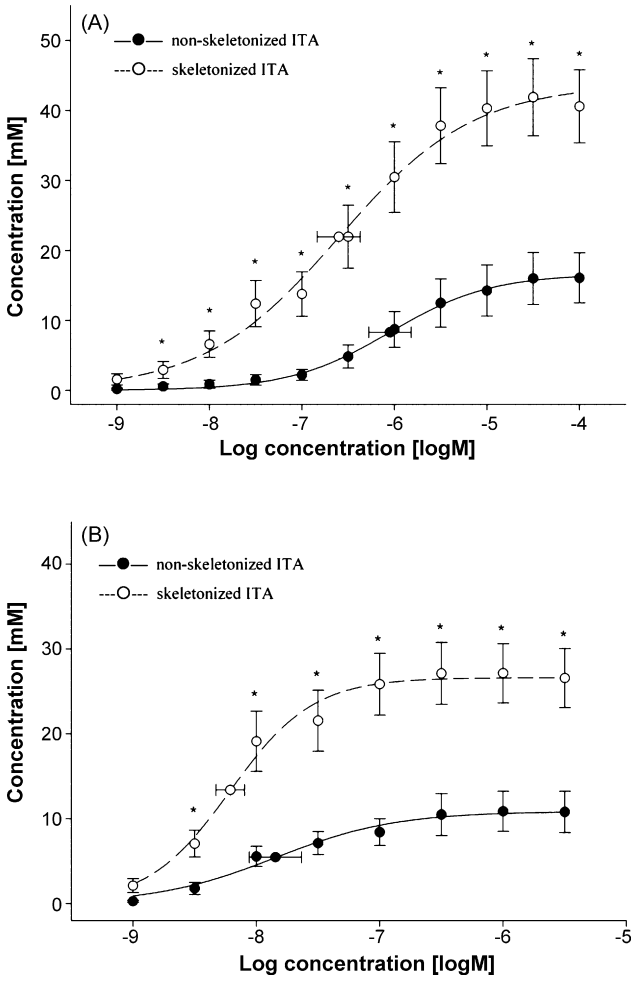

Fig. 2. Concentration–response curves (logistic regression lines) for contraction to serotonin (A) and angiotensin II (B) for skeletonized and pedicled ITA. Symbols represent mean effect at the given concentration ± SEM. EC_{50} ± SEM is depicted as well. $^{*}p < 0.05$ skeletonized versus non-skeletonized ITA as per paired *t*-test.

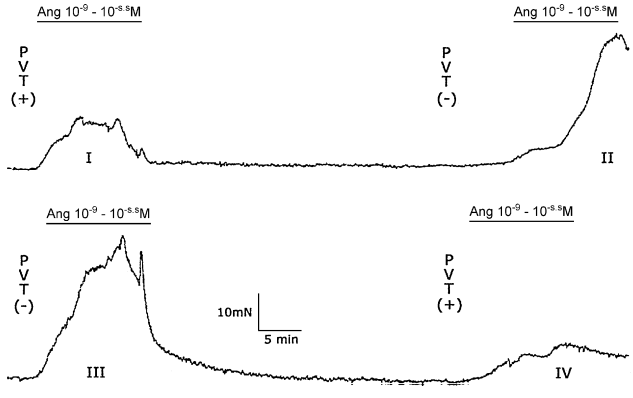

Fig. 3. The example of original recording of internal thoracic artery concentration–response contraction to angiotensin II. Transferring of perivascular tissue (PVT) from one bath to the other caused significant increase of contractility in one preparation (top panel) while the response to angiotensin II was reduced in the other (bottom panel). For regression analysis data from concentration–response curves I and IV (PVT+) as well as II and III (PVT−) were pooled together.

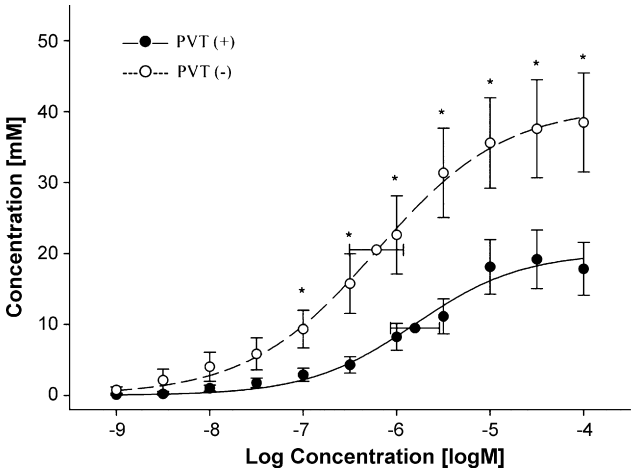

Fig. 4. Concentration–response curves (logistic regression lines) for ITA contraction to serotonin with and without PVT in the tissue bath. Symbols represent mean effect at the given concentration ± SEM. EC_{50} ± SEM is depicted as well. $^{*}p < 0.05$ PVT(+) versus PVT(−) as per paired *t*-test, PVT − perivascular tissue.

serotonin: $pD_2 = 6.6 \pm 0.36$ versus 6.6 ± 0.36, respectively ($p = 0.9$) ($n = 9$ for each).

3.4. Experiment 4

Incubation of ITA rings with L-NMMA and indomethacin failed to abolish anticontractile effect of PVT. Maximal response to serotonin was almost 60% lower when PVT was present in the bath: E_{max}: 46.6 ± 3.03 mN versus 28.2 ± 4.02 mN (PVT(−) vs PVT(+), respectively, $p < 0.001$, $n = 10$ for each) – Fig. 6. The same magnitude of inhibition was observed when angiotensin II was used; E_{max}: 27.2 ± 2.00 versus 16.4 ± 2.10 mN (PVT(−) vs PVT(+), respectively, $p < 0.001$, $n = 7$ for each) – Fig. 7. The estimated EC_{50} for serotonin with and without PVT and incubation with L-NMMA and indomethacin did not differ significantly: pD_2: 6.4 ± 0.15 versus 6.5 ± 0.40, respectively ($p = 0.9$). There was also no

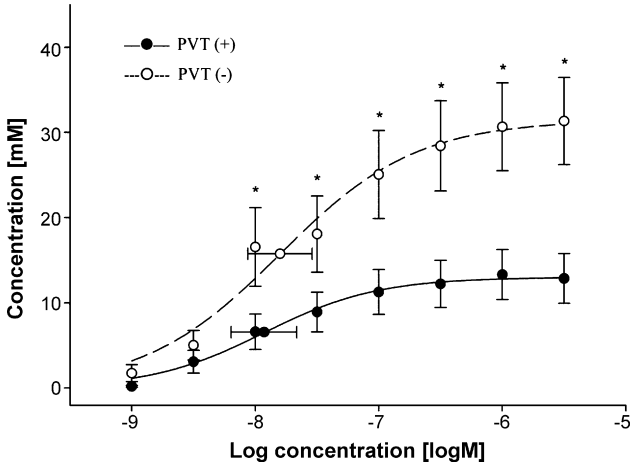

Fig. 5. Concentration–response curves (logistic regression lines) for ITA contraction to angiotensin II with and without PVT in the tissue bath. Symbols represent mean effect at the given concentration ± SEM. EC_{50} ± SEM is depicted as well. $^{*}p < 0.05$ PVT(+) versus PVT(−) as per paired *t*-test, PVT − perivascular tissue.

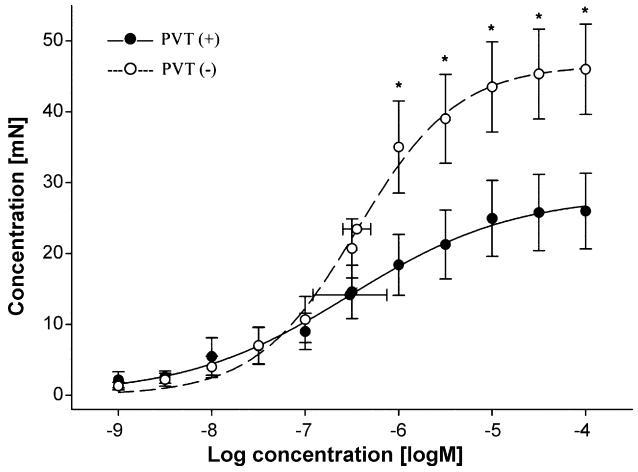

Fig. 6. Concentration–response curves (logistic regression lines) for ITA contraction to serotonin with and without PVT in the bath and inhibition of NO synthase with L-NMMA (10^{-4} M) and cyclooxygenase with indomethacin (10^{-5} M). Symbols represent mean effect at the given concentration ± SEM. EC_{50} ± SEM is depicted as well. $^{*}p < 0.05$ PVT(+) versus PVT(−) as per paired *t*-test, PVT − perivascular tissue.

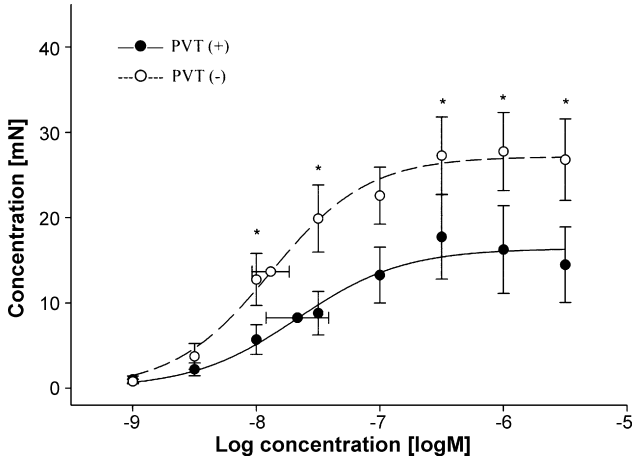

Fig. 7. Concentration–response curves (logistic regression lines) for ITA contraction to angiotensin II with and without PVT in the bath and inhibition of NO synthase with L-NMMA (10^{-4} M) and cyclooxygenase with indomethacin (10^{-5} M). Symbols represent mean effect at the given concentration ± SEM. EC_{50} ± SEM is depicted as well. $^{*}p < 0.05$ PVT(+) versus PVT(−) as per paired *t*-test, PVT − perivascular tissue.

difference for angiotensin II: pD_2: 7.7 ± 0.25 versus 7.9 ± 0.15, respectively ($p = 0.5$).

One-way ANOVA confirmed the difference between maximal response to serotonin and angiotensin II between arteries with PVT (i.e. non-skeletonized ITA, PVT(+) and PVT(+) with indomethacin + L-NMMA) and skeletonized ITA ($p < 0.001$ for serotonin; $p < 0.001$ for angiotensin), while there were no statistically significant differences in post hoc multiple comparisons between maximal contractions obtained in rings with PVT.

4. Discussion

The main finding of our study is that perivascular tissue of human internal thoracic artery releases anticontractile factor. PVT appears to release a substance that significantly attenuates contractile response to serotonin and angiotensin II. We decided to choose the bioassay experiments with loose tissue transfer to prove that the hypothetic factor is being secreted to the bath. Although this approach clearly enables to show the effect of released substance, it has some limitations. To rule out the influence of the carry-over effect, time and tachyphylaxis we decided to pool the data from curves with PVT in the bath into one regression analysis. The same was done for curves without PVT in the bath. The bioassay experiments we used showed that the factor is being diffused to the tissue bath regardless of connection of PVT to the vessel wall. To our knowledge it is the first report that presents that PVT-derived anticontractile factor works even if there is no complete continuity of vessel wall (smooth muscle cell layer-adventitia-perivascular fat). Our data support the previous findings that skeletonized ITA is the functionally different artery than non-skeletonized one [9]. This difference is possibly due to the presence of active perivascular tissue that not only works as structural support but may also actively participate in the regulation of vascular tone. The novel finding of the present study is that it is not every type of adipose tissue that secretes anticontractile factor. We chose to study pleural adipose tissue, as in clinical settings it is the adipose tissue closest to ITA. Pleural tissue is also the place where in situ ITA graft heals and one may suspect that it would change ITA reactivity over time. The bioassay study of Lohn et al. [12] first demonstrated that the source of relaxing factor is the adipocyte. They used cultured rat adipocytes, transfer of which resulted in reduced contraction responses of aortic rings to serotonin [12]. Human white adipose tissue has been proved to have significant endocrine activity [18]. Several adipocyte-derived substances influence vascular tone (e.g. leptin, angiotensinogen, IL6, TNF alfa, adiponectin, etc.) but their action is mostly endocrine and affects the whole body vasculature. Our results show that various adipose depots may differ in their paracrine activity. White adipose tissue, even if releasing endocrine factors, may not necessarily release ADRF. In fact, it has been actually shown that perivascular adipose tissue differs from subcutaneous fat by the increased angiogenesis [18]. Why different types of human fat have diverse paracrine vasoactive properties requires however further studies. The above suggests that in humans, the analyzed relaxing factor is either exclusively secreted by perivascular adipocytes or it is also partially released by the adventitia of the vessel. The latter may be true, as Gonzalez, even before the identification of ADRF existence, demonstrated that adventitia-denuded vessels contract stronger to norepinephrine [19]. Recently, Laflamme et al. [20] have shown in the elegant study that tissue-engineered vascular adventitia reconstructed with vascular fibroblasts has the relaxing capacity. Moreover, ADRF was initially described as adventita- or adipocyte-derived factor [12,13]. It is thus probable that some parts of adventitia we removed during skeletonization participate in the secretion of this hypothetic anticontractile factor. As we skeletonized ITA in standard technique by removing perivascular adipose tissue and some part of the adventitia (see Fig. 1 in [9]) it might be difficult to distinguish which part of arterial wall complex is responsible for ADRF release. It would require having the artery with no

adventitia but perivascular fat present. This may only be possible using tissue engineering techniques. Thus, it is still not clearly explained which part of perivascular tissue secretes this putative relaxing factor.

Initially it was suggested that PVT-derived anticontractile factor acts through endothelium independent mechanisms as the contraction of PVT(+) rings was diminished even in the endothelium denuded vessels [12]. Similarly adding L-NMMA had no influence on diminished contractility of PVT (+) rings with preserved endothelium [12]. Recently Gao et al. [16] questioned this finding showing that perivascular tissue aliquots exerted anticontractile properties when transferred to skeletonized arterial ring only if endothelium was preserved in the acceptor vessel. Accordingly L-NMMA presence in the acceptor vessel bath decreased anticontractile properties of transferred PVT aliquots. In our experiments the anticontractile property of PVT was not abolished by NO synthase and cyclooxygenase inhibitors. It confirms the initial finding that action of putative ADRF is largely independent of two major clinically relevant relaxing compound released by endothelium. At the same time it stays in contrast to the results of the study of Gao et al. [16]. The apparent discrepancy may be partially explained by the fact that unlike Gao group we transferred PVT (not the aliquots) to the acceptor vessel. It may for instance have something to do with half-life of PVT released factor. It may even be that there are actually at least two ADRFs as in fact suggested by Gao. As all above citied studies and our experiments were performed in different experimental setups further studies are necessary to reconcile these apparently diverse results.

L-NMMA did not affect basal tension of the studied ITAs in our experiments (data not shown). Adding L-NMMA and indomethacin slightly increased contractile responses to serotonin and angiotensin irrespective of PVT presence in the bath. The difference was not statistically significant by ANOVA and we attribute it to the inhibition of endothelium dependant action of both vasopressors used [21,22]. On the other hand, lack of significant difference of maximal response to vasopressors between preparation with PVT, as assessed by ANOVA, confirmed similar contractile properties irrespective of whether the PVT is a part of artery (non-skeletonized ITA) or is just freely floating in the bath.

Up to now there is no data on ADRF existence and activity in vivo. The in vitro set-up with all its limitations does not resemble clinical situation with continuous blood flow, stable oxygen supply, continuous removal of metabolic wastes and various vasoactive compounds interacting together. These may limit the chance of easy in vivo identification of this paracrine relaxing factor. Nonetheless the studies on mesenteric bed perfusion confirm that PVT is truly involved in the regulation of vascular tone [23]. It suggests that skeletonization of the artery may produce the vessel with higher reactivity in vivo. This may be important in the early postoperative period when we are afraid of vasospasm of arterial grafts. Similarly, inability of skeletonized ITA to regulate blood flow as suggested by Del Campo may be clinically relevant [24]. The results of ours and previous studies definitely suggest that skeletonized ITA has significantly different vasoreactive profile than pedicled artery [9,15]. All the above can influence the surgical practice. Skeletonization becomes more and more popular especially when performing total arterial revascularization. Even radial artery, much more muscular and prone to spasm, is often harvested skeletonized [25]. One using this technique must be aware of possible drawbacks of removing PVT. On the other hand, there is no data on the influence of PVT removal on the function of other vessels used for coronary bypass grafting such as saphenous vein, radial or gastro-epiploic arteries. The fact that for the time being ADRF existence was proved only in two vascular beds in animals (i.e. aorta and mesenteric artery) and in human ITA, and that potassium channels involved are different in every vessel studied, does not allow for straight generalization on PVT role in other vessels used for grafting.

What is interesting, perivascular tissue might contribute to the progression of atherosclerosis. It may be due to its chemotactic properties [26]. This is especially important as we know that the high fat diet increases the amount of perivascular fat [26] and in consequence may speed up the atherosclerotic changes in obese patients. However, there are also contrary arguments showing that leaving the surrounding tissue (especially veins) enables to slow down artery remodeling, possibly due to faster removal of metabolic wastes [24]. Whether the faster progression of local atherogenesis applies to the ITA, known as an artery 'resistant' to atherosclerosis, will require long-term follow up and patency comparisons of skeletonized and non-skeletonized ITAs.

In conclusion, our results confirm the existence of soluble anticontractile factor released by PVT of human internal thoracic artery. This factor — non-NO, non-prostacyclin dependant — is continuously secreted by PVT as was proved by tissue transfer. Other than perivascular adipose tissue does not necessarily release ADRF. Although skeletonizaton has a role in coronary surgery one must realize the potential deleterious effect of removing perivascular tissue, even if its clinical significance remains unknown and might be difficult to prove. The precise pharmacology, mechanisms of action of ADRF and the role of perivascular tissue in various pathophysiological conditions require further studies. There is also a need to analyze the relaxing properties of PVT in other vessels used in coronary surgery.

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