Interaction of Perivascular Adipose Tissue and Sympathetic Nerves in Arteries From Normotensive and Hypertensive Rats

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Summary

The inhibitory action of perivascular adipose tissue (PVAT) in modulation of arterial contraction has been recently recognized and contrasted with the prohypertensive effect of obesity in humans. In this study we demonstrated that PVAT might have opposing effect on sympatho-adrenergic contractions in different rat conduit arteries. In superior mesenteric artery isolated from normotensive Wistar-Kyoto rats (WKY), PVAT exhibited inhibitory influence on the contractions to exogenous noradrenaline as well as to endogenous noradrenaline released from arterial sympathetic nerves during transmural electrical stimulation or after application of tyramine. In contrast, the abdominal aorta with intact PVAT responded with larger contractions to transmural electrical stimulation and tyramine when compared to the aorta after removing PVAT; the responses to noradrenaline were similar in both. This indicates that PVAT may contain additional sources of endogenous noradrenaline which could be responsible for the main difference in the modulatory effect of PVAT on adrenergic contractions between abdominal aortas and superior mesenteric arteries. In spontaneously hypertensive rats (SHR), the anticontractile effect of PVAT in mesenteric arteries was reduced, and the removal of PVAT completely eliminated the difference in the dose-response curves to exogenous noradrenaline between SHR and WKY. These results suggest that in mesenteric artery isolated from SHR, the impaired anticontractile influence of PVAT might significantly contribute to its increased sensitivity to adrenergic stimuli.

Key words

Perivascular adipose tissue • Mesenteric artery • Abdominal aorta • Adrenergic contraction • Spontaneously hypertensive rats

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Introduction

Perivascular adipose tissue (PVAT) is a structure surrounding most of the blood vessels; however, until recently, it was largely ignored in *in vitro* studies of vascular function. Together with recognizing the endocrine properties of adipose tissue in general, the (humoral) influence of PVAT on function of the adjacent vascular wall structures has begun to be intensively examined. It is expected that this periadventitial adipose depot can contribute to the regulation of numerous aspects of vascular function such as vasoreactivity, proliferation of vascular cells or inflammation (Szasz and Webb 2012, Matloch *et al.* 2016).

Results obtained in the last two decades indicate that under the physiological conditions PVAT exerts predominantly anticontractile effect (Soltis and Cassis 1991). This was confirmed as inhibitory action on arterial responses to a variety of vasoconstrictors including noradrenaline, serotonin, angiotensin II, thromboxane A_2 agonists or endothelin-1 (Verlohren *et al.* 2004). It was demonstrated that most of this effect is transferable, mediated by PVAT-derived relaxing factor which has not been clearly defined yet (Löhn *et al.* 2002). One of the most conclusive substances in this regard seems to be angiotensin-(1-7) (Lee *et al.* 2009). However, other adipocyte-derived products (adipokines) are also potential candidates (Boydens *et al.* 2012, Szasz and Webb 2012).

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Some authors found also a non-transferable component of PVAT-evoked anticontractile action and this is possibly mediated by some briefly-acting products such as reactive oxygen species (hydrogen peroxide) (Gao *et al.* 2007).

One of the important points in the mediation of PVAT action on vascular function is its interaction with perivascular sympathetic nerves due to their anatomical relationship within external layers of the vessel wall. Moreover, PVAT itself could contain the innervation (Dashwood and Loesch 2011, Bulloch and Dally 2014) which might contribute or possibly overlap the vascular modulatory effect of PVAT. Several authors published some conflicting results demonstrating that the presence of PVAT enhances (Gao *et al.* 2006, Lu *et al.* 2010) or inhibits (Watkins *et al.* 2014) the contractile responses to perivascular nerve stimulation. This is particularly important in pathophysiology because in obesity as well as in hypertension the increased tone of sympathetic nerves on cardiovascular system was documented (Smith and Minson 2012).

The first findings of inhibitory action of PVAT on arterial contraction contrast with the prohypertensive effect of obesity in humans. Indeed, it was confirmed that the protective (anticontractile) action of PVAT is impaired in many pathophysiological states associated with cardiovascular dysfunction (Li *et al.* 2013). Alterations in PVAT function in obesity and metabolic syndrome are accompanied by changes in the release of adipokines, inflammation and oxidative stress (Fernández-Alfonso *et al.* 2013). The above mentioned interaction of PVAT with perivascular sympathetic nerves might also have important consequences in such disease states.

In this study we analyzed the effect of PVAT on contractile responses to exogenous and endogenous noradrenaline (released from perivascular sympathetic nerves as a neurotransmitter) in isolated rat superior mesenteric artery and abdominal aorta. Moreover, we compared these reactions in arteries from normotensive Wistar-Kyoto rats and spontaneously hypertensive rats to examine the effect of high blood pressure and enhanced vascular sympathetic tone on this PVAT modulatory action.

Methods

The animal protocols were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health, and they were approved by the Animal Health and Welfare Division of the State Veterinary and Food Administration of the Slovak Republic.

Experiments were conducted in 12-week-old male Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). The rats were housed under standard laboratory conditions and maintained under a 12-h light-dark cycle and they had a free access to drinking water and food. Systolic blood pressure was measured in conscious animals at the end of 12th week by the non-invasive tail-cuff method. Then the rats were sacrificed under CO_2 anesthesia, and abdominal aorta and superior mesenteric artery were isolated for isometric tension studies in organ chambers. The relative heart weight was determined as a ratio of heart weight and tibia length.

Functional studies

The rat aorta and mesenteric artery were collected in cold modified Krebs solution with the following composition (in mmol/l): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, $CaNa_2EDTA$ 0.03. For measurement of contractile activity, arteries were cut into rings 3.0-3.5 mm of length. Paired arterial rings, one with PVAT intact and the other with PVAT removed, were prepared from each artery. PVAT removal was carried out with fine scissors under microscope with extra caution not to damage the adventitial layer. The rings were fixed on stainless steel hooks and suspended in an organ bath containing modified Krebs solution which was continuously aerated with a mixture of 95 % O_2 + 5 % CO_2 and maintained at 37 ºC. One side of the ring was connected by a thread to a force-displacement transducer Sanborn FT 10 (Baltimore, USA) to measure changes in isometric tension which were recorded with a polygraph TZ 4200 (Labora, Czech Republic). The resting tension of arterial rings was 10 mN. The preparations were allowed to equilibrate for 60-90 min before experimental observation. During this period, the Krebs solution was changed at 15-min intervals.

Adrenergic contractions were determined in abdominal aorta and mesenteric arteries as the responses to cumulatively applied exogenous noradrenaline or as the neurogenic responses elicited by electrical stimulation of periarterial sympathetic nerves. The increasing concentrations of noradrenaline were added to incubation bath in a cumulative fashion, with sufficient time necessary to reach a plateau in response to a particular

concentration. For transmural electrical stimulation (TES), arterial rings were mounted between two platinum electrodes placed on either side of the preparation and connected to an electrostimulator ST-3 (Medicor, Hungary). Frequency-response curves to electrical stimuli were obtained using square pulses of 0.5 ms in duration, at supramaximal voltage (> 40 V), 1-32 Hz, for a period of 20 s. The contractions of rat abdominal aortas and mesenteric arteries elicited by TES were blocked by guanethidine or tetrodotoxin, indicating that they were induced mainly by nerve-released (endogenous) noradrenaline.

In separate group of measurements we compared the reactions to single dose of noradrenaline (10^{-6} mol/l), tyramine (10^{-4} mol/l) and single frequency of TES (4 Hz). Tyramine, an indirect sympathomimetic drug, was used as a tool to test whether a functional pool of catecholamines (mainly noradrenaline) exists in PVAT.

Contractile responses were expressed as the active wall tension in mN and normalized to the length (in mm) of the particular preparation. Area under curve (AUC, in arbitrary units) was calculated from concentration- (frequency)-response curves using the rectangular rule for numerical integration (according to Pruessner *et al.* 2003).

The chemicals used were purchased from Sigma-Aldrich (Germany). All drugs were dissolved in distilled water and concentration was expressed as final concentration in the incubation chamber.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) or by Student's t-test. The differences were considered as significant when $P<0.05$.

Table 1. General characteristics of experimental animals.

	Wistar-Kyoto rats	**Spontaneously hypertensive rats**
Body weight (g)	291.7±3.28	254.64±5.65 ***
Heart weight / tibia length (mg/mm)	29.70±0.32	31.54±1.01
Systolic blood pressure (mm Hg)	116.23±1.90	163.80±3.83 ***
Heart rate (bpm)	346.67±10.36	446.34±10.16 ***

Values represent mean ± SEM of 10 rats. *** P<0.001 SHR vs. WKY.

Results

The body weight of WKY at the end of the 12th week was higher than that of SHR. Systolic blood pressure and heart rate were higher in SHR compared to WKY. Relative heart weight did not differ between these two groups (Table 1).

The presence of PVAT significantly reduced the contractions to exogenous noradrenaline in isolated mesenteric arteries of both WKY and SHR (Fig. 1A). However, the arterial dose-dependent responses to noradrenaline expressed as AUC were significantly reduced due to the presence of PVAT only in WKY (13.51±1.78 vs. 23.81±1.88 in arteries with intact vs. removed PVAT, P<0.01) and not in SHR (17.07±1.41 vs. 21.53±1.84 in arteries with intact vs. removed PVAT, P>0.05). In preparations with intact PVAT the sensitivity to noradrenaline was significantly higher in SHR when compared to WKY (pEC_{50} 5.86±0.11 in WKY vs. 6.22±0.09 in SHR, P<0.05). After removing PVAT, the difference in sensitivity to noradrenaline between SHR and WKY mesenteric arteries was abolished (pEC_{50} 6.76±0.21 in WKY vs. 6.74±0.14 in SHR, P>0.05). The presence of intact PVAT significantly decreased the sensitivity to noradrenaline in both WKY and SHR mesenteric arteries.

Contraction to 100 mM KCl was measured as a reference value and it was not influenced by PVAT neither in WKY (2.40±0.17 mN/mm vs. 2.71± 0.06 mN/mm in arteries with intact vs. removed PVAT, P>0.05) nor in SHR (2.79±0.13 mN/mm vs. 2.96± 0.16 mN/mm in arteries with intact vs. removed PVAT, P>0.05).

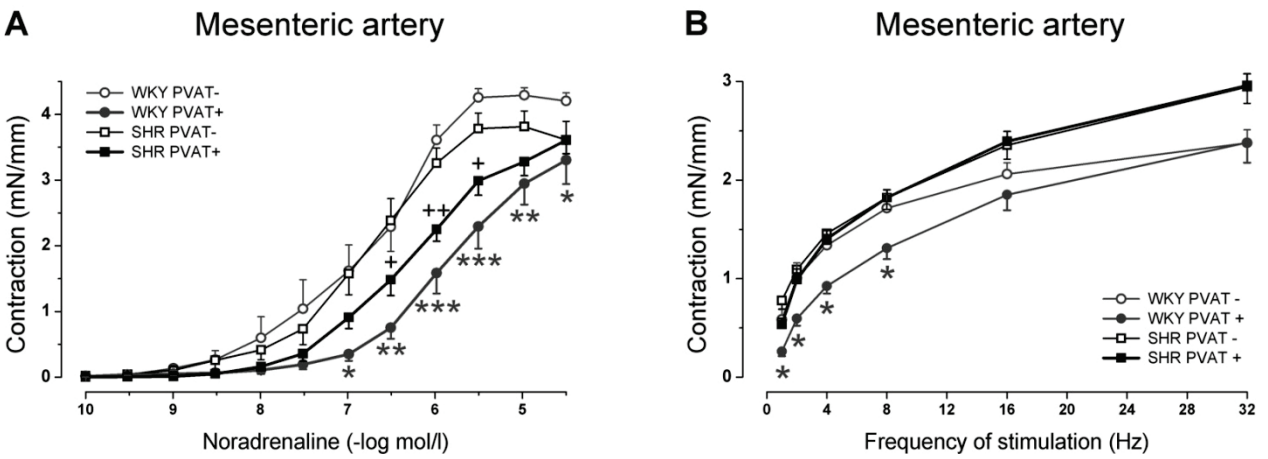

Fig. 1. Dose-response curves to exogenous noradrenaline **(A)** and frequency-response curves to transmural electrical stimulation **(B)** in mesenteric arteries with intact (+) and removed (–) perivascular adipose tissue (PVAT) from Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Values represent mean ± SEM of 10 rats. * P<0.05, ** P<0.01, *** P<0.001 WKY PVAT+ vs. WKY PVAT–, $^{+}$ P<0.05, $^{++}$ P<0.01 SHR PVAT+ vs. SHR PVAT–.

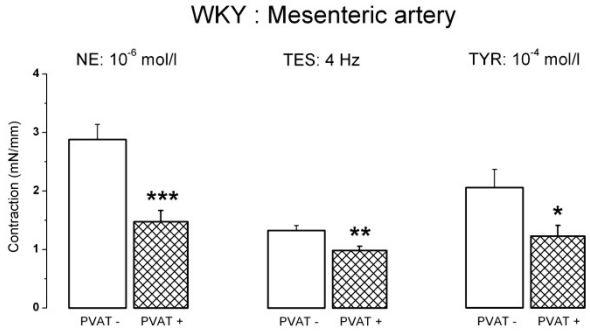

Fig. 2. Comparison of contractile responses in mesenteric artery with intact (+) and removed (–) perivascular adipose tissue (PVAT) from Wistar-Kyoto rats (WKY) to exogenous noradrenaline (NE) and to endogenous noradrenaline released during transmural electrical stimulation (TES) or after application of tyramine (TYR). Values represent mean ± SEM of 10 rats. * P<0.05, ** P<0.01, *** P<0.001 vs. PVAT–.

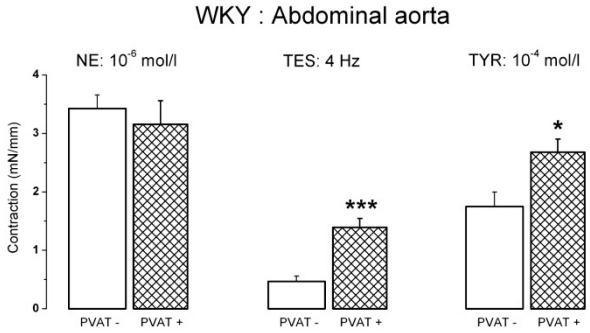

Fig. 3. Comparison of contractile responses in abdominal aorta with intact (+) and removed (–) perivascular adipose tissue (PVAT) from Wistar-Kyoto rats (WKY) to exogenous noradrenaline (NE) and to endogenous noradrenaline released during transmural electrical stimulation (TES) or after application of tyramine (TYR). Values represent mean ± SEM of 10 rats. * P<0.05, *** P<0.001 vs. PVAT–.

As shown in Figure 1B, the presence of PVAT caused significant inhibition of TES-induced contractions in mesenteric arteries from WKY (at frequencies 1-8 Hz) but not in SHR. In arterial preparations with intact PVAT, the frequency-dependent responses to TES expressed as AUC were significantly higher in SHR as compared to WKY (5.99±0.50 in WKY vs. 8.04±0.47 in SHR, P<0.05) and this difference was not seen between WKY and SHR arterial rings without PVAT (7.46±0.60 in WKY vs. 8.19±0.60 in SHR, P>0.05).

More detailed analysis of the effect of PVAT on neurogenic contractions showed different tendencies in mesenteric artery and abdominal aorta of WKY. PVAT exerted inhibitory effect on contractions to TES (4 Hz) in mesenteric artery (Fig. 2). However, in abdominal aorta, the presence of intact PVAT caused enlargement of these responses (Fig. 3). Similar trend in PVAT effect was found when tyramine-induced contractions were examined in these arteries (Figs 2 and 3). In response to exogenously applied noradrenaline, the contractions of the mesenteric arterial rings with intact PVAT were decreased when compared to those with removed PVAT (Fig. 2); however, in abdominal aortas no difference in this response was detected between the preparations with intact and removed PVAT (Fig. 3).

In SHR, there was no change in responses to TES and tyramine between preparations with intact and removed PVAT from both types of arteries (Figs 4 and 5). PVAT inhibited noradrenaline-induced contractions in mesenteric arterial rings (Fig. 4), but no change due to PVAT presence was seen in preparations of abdominal aorta from these rats (Fig. 5).

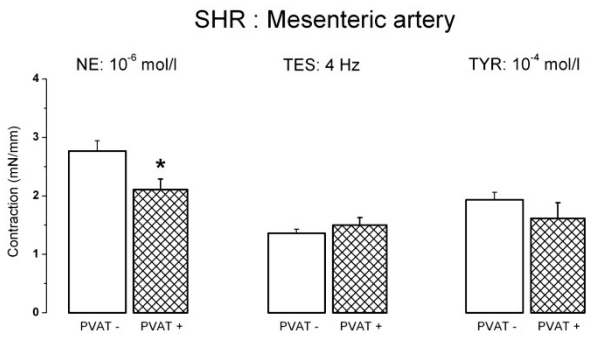

Fig. 4. Comparison of contractile responses in mesenteric artery with intact (+) and removed (–) perivascular adipose tissue (PVAT) from spontaneously hypertensive rats (SHR) to exogenous noradrenaline (NE) and to endogenous noradrenaline released during transmural electrical stimulation (TES) or after application of tyramine (TYR). Values represent mean ± SEM of 10 rats. * P<0.05 vs. PVAT–.

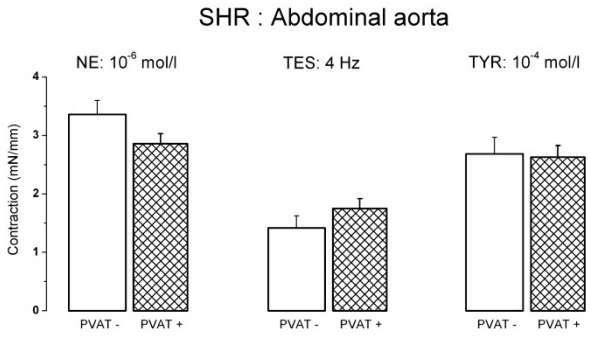

Fig. 5. Comparison of contractile responses in abdominal aorta with intact (+) and removed (–) perivascular adipose tissue (PVAT) from spontaneously hypertensive rats (SHR) to exogenous noradrenaline (NE) and to endogenous noradrenaline released during transmural electrical stimulation (TES) or after application of tyramine (TYR). Values represent mean ± SEM of 10 rats.

Discussion

Perivascular adipose tissue seems to be a new element found in the vascular biology which in cooperation with other regulatory systems is importantly involved in the physiological and pathophysiological processes in blood vessels. It was found that in healthy individuals PVAT exerts inhibitory impact on *in vitro* vascular responses to numerous constrictor substances. In this study we have confirmed that in normotensive WKY rats visceral PVAT considerably attenuates the contractions of mesenteric arteries to exogenous noradrenaline which was expressed by their smaller absolute contractions, as well as by the reduced sensitivity and AUC of their dose-response curves. Such inhibitory influence was detected also in responses to endogenous noradrenaline released during the electrical stimulation of the perivascular sympathetic nerves, particularly at lower frequencies of TES which are nearer to the physiological conditions. Our results on mesenteric arteries from WKY are thus in accordance with the well-documented anticontractile effect of PVAT (Soltis and Cassis 1991, Verlohren *et al.* 2004). It is possible to imagine that PVAT releases some substance(s) that act on mesenteric arterial smooth muscle and decrease its responsiveness to vasoconstrictors. The hyperpolarizing effect of PVAT-derived relaxing factor(s) (PVRF) (Löhn *et al.* 2002, Gao *et al.* 2007, Gollasch 2012) is well applicable in such conception.

In SHR mesenteric arteries, the dose-dependent responses to noradrenaline expressed as AUC were not decreased due to PVAT; however, the inhibitory influence of PVAT was partially detected at particular noradrenaline concentrations. Several authors confirmed that the decreased anticontractile effect of PVAT in arteries from SHR associated with altered functional and structural properties of this adipose tissue (Lu *et al.* 2011, Li *et al.* 2013). These findings might elucidate our observations showing weaker inhibitory influence of intact PVAT on exogenous noradrenaline-induced contractions and no PVAT effect on the responses to TES in mesenteric arteries from SHR. In previous measurements we found that in older SHR (aged 20 weeks) the anticontractile effect of PVAT is even more reduced (Török and Zemančíková 2014).

In the present study, SHR mesenteric arteries with intact PVAT responded more sensitively to exogenous noradrenaline when compared to those of WKY. Similarly, the frequency-response curves to TES demonstrated increased neurogenic contractions in SHR mesenteric arteries with intact PVAT in comparison to WKY arteries. It is interesting that all the differences in adrenergic responses between mesenteric arteries of WKY and SHR were manifested only in preparations with PVAT and they were not detected when PVAT was removed. This indicates that besides many well-confirmed disturbances in SHR arteries among which endothelial dysfunction is one of the most studied (Vanhoutte 1996, Puzserova *et al.* 2014), the altered properties of PVAT could be another important source of pathological changes in vascular system of these rats.

Our results illustrating the anticontractile effect of PVAT on mesenteric arteries from WKY seem to be contradictory to some studies documenting the enhancement of arterial contractions to electrical

stimulation of sympathetic nerves by PVAT. The authors explain this potentiation of TES-induced contractions by the release of adipocyte-derived angiotensin II (Lu *et al.* 2010) and by production of superoxide during electrical stimulation (Gao *et al.* 2006). To explore this issue in more detail, we made some separate measurements in two arterial types (superior mesenteric artery and abdominal aorta) from WKY and SHR, in which the responses to single doses of exogenous noradrenaline or tyramine, and to 4 Hz of TES were compared in preparations with intact or removed PVAT. Tyramine was used as an indirectly acting sympathomimetic drug that induces a carrier-mediated transport of axoplasmic noradrenaline out of the perivascular neurons (Graefe *et al.* 1999). As documented above, in mesenteric arteries from WKY, PVAT caused smaller contractions in response to exogenous as well as to endogenous noradrenaline (induced by TES or tyramine application). These results indicate that PVAT could inhibit the contractions mostly postsynaptically through the mediation of PVRF. However, in WKY abdominal aortas, we did not observe the inhibitory effect of PVAT on contractions evoked by exogenous noradrenaline. Moreover, the contractile responses to endogenous noradrenaline induced by either TES or tyramine were even larger in the presence of intact PVAT when compared to the preparations with removed PVAT (Fig. 3). From these observations it seems that PVAT in abdominal aorta might not influence the contractile responses of vascular smooth muscle cells to noradrenaline itself but probably it could interfere with noradrenaline release from endogenous neural sources. It may be supposed that some substance(s) continuously produced in PVAT influence vascular neurotransmission, or that they are released during TES along with noradrenaline and affect the respective contractile responses, e.g. angiotensin II or superoxide, as suggested by Lu *et al.* (2010) and Gao *et al.* (2006).

Another possible explanation is that in abdominal aorta, PVAT itself could be the source of endogenous noradrenaline which participates in the neurogenic contractions in response to TES. This part of noradrenaline sources could be eliminated when PVAT was removed from the aortal surface, and its absence was manifested by the smaller contractile responses to TES when compared to the preparations with intact PVAT. Vargovic *et al.* (2011) demonstrated that the adipocytes have the capacity to produce noradrenaline. In spite of that we consider predominantly the neural noradrenaline sources in PVAT because we presume that the contractile responses evoked by TES were largely of neurogenic origin (see Methods). However, the direct evidence of innervation in PVAT is limited, with exception of several works demonstrating clear innervation of perivascular fat in rat mesenteric artery (Diculescu and Stoica 1970), human saphenous vein (Dashwood and Loesch 2011), and mouse mesenteric arteries (Bulloch and Dally 2014). Interestingly, from our results it seems that PVAT of rat mesenteric artery might not contain a significant amount of neurally-derived noradrenaline because the contractile responses to TES were even reduced in the presence of intact PVAT. Such observation could also be explained by the large production of PVRF (seen also in response to exogenous noradrenaline), the anticontractile effect of which extensively overlaps the potential contribution of PVAT innervation to neurogenic responses of mesenteric artery. On the contrary, in abdominal aorta, the production of PVRF seems to be less important and the influence of sympathetic nerves from PVAT could prevail. Brown *et al.* (2014) showed that abdominal aorta is surrounded by PVAT with mixture of white and brown adipocytes ("beige" adipose tissue, or "browning" white adipose tissue), whereas PVAT of mesenteric arteries contains only white adipocytes. It was documented that sympathetic nerve fiber density correlates positively with the number of brown adipocytes within adipose tissue (Bartness and Ryu 2015) and this could also support our observations and idea of the different distribution of sympatho-neural supply in the mentioned rat arteries: in contrast to white PVAT in mesenteric artery, "beige" PVAT surrounding abdominal aorta might contain a substantial amount of the sympathetic nerves which are responsible for the most part of the observed responses to TES. After removal of aortal PVAT, such contractions are significantly reduced. In contrast, the source of endogenous noradrenaline which induced the neurogenic contractions in mesenteric artery during TES, could be located largely within the vascular wall (adventitia, adventitio-medial junction) (Birch *et al.* 2008) and when PVAT is removed, the majority of the innervation remains in the arterial preparation; however, the inhibitory influence of PVAT is absent so the observed contractile responses are greater after PVAT removal.

In our experiments we also observed that mesenteric arteries responded by contraction immediately after initiation of TES; however, in abdominal aortas the contractions to TES started with the delay of about 15 s. This indicates that in aortal preparations the sources of endogenous noradrenaline could be more distant from the

muscular layer (probably within PVAT, as indicated above) whereas in mesenteric artery they are probably closer and that is why the diffusing noradrenaline reaches smooth muscle in a shorter time.

Besides the diversity in distribution of sympathetic innervation within arterial and periarterial structures, the presence of other types of nerves and transmitters could also participate in the different effects of PVAT on noradrenergic contraction. Neuropeptide Y (NPY), which is released as a co-transmitter from sympathetic nerve terminals, could act as a modulator on arterial neurogenic responses. Moreover, it was found to be produced also in adipocytes (Yang *et al.* 2008). Burnstock (2008) documented that the potentiating action of NPY on neurogenic contraction increases with the junctional cleft width. Such effect could be expected in aorta where the increasing occurrence of sympathetic terminals distant from medial layer might enhance the pro-contractile action of co-released NPY and this could contribute to the increase in neurogenic responses observed in aortic preparations with preserved perivascular structures. Regarding other types of nerve mediators, Dubrovska *et al.* (2004) reported that in aorta the sensory neurotransmitters activating vanilloid, cannabinoid, and CGRP receptors do not participate in the modulatory effect of PVAT on arterial contraction. However, in rat mesenterial bed, the function of peptidergic (sensory) nerves seems to be of greater importance and it was shown that their activation inhibits the contractile responses of these arteries. The immunoreactivity and release of calcitonin gene-related peptide (CGRP), one of the sensory neurotransmitters, were detected also within the PVAT region. CGRP was shown to hyperpolarize vascular smooth muscle by activating potassium channels (Dunn *et al.* 2003) and this effect is similar to PVRF. Therefore, one can speculate whether this compound could participate in the anticontractile effect of PVAT in mesenteric arteries.

Some important differences can be presumed in arteries from SHR in comparison to the presented findings on arteries from WKY. As documented by many authors (Head 1989, Zicha *et al.* 2014), the sympathetic tone in SHR is augmented and their vascular contractile responses to sympatho-neural stimulation are increased (Zemančíková and Török 2015). In SHR mesenteric arteries the anticontractile effect of PVAT was observed during the response to exogenous noradrenaline (although it was weaker than that seen in WKY preparations). However, it was not detected when the contractions were evoked by TES or tyramine application. This might indicate that in preparations from SHR the inhibitory influence of PVAT on neurogenic contractions was not sufficient to exceed the potential impact of endogenous noradrenaline originated from neural sources in PVAT which could contribute to these contractions. On the other hand, in WKY mesenteric arteries, the stronger anticontractile effect of PVAT overlapped any possible contribution of sympatho-neural supply from PVAT to the neurogenic contractile responses.

In SHR abdominal aortas, similarly to those from WKY, the inhibitory effect of PVAT on contractile response to exogenous noradrenaline was not detected. However, in aortas from SHR we did not find potentiation of the response to endogenous noradrenaline (induced by TES or by tyramine application) due to PVAT presence that was clearly shown in WKY abdominal aortas with intact PVAT. In contrast to WKY, it seems that in SHR aortas the majority of the sympathetic neural supply is concentrated within the proper arterial wall and the neurogenic contractions are not significantly influenced by PVAT removal.

Conclusions

We confirmed the anticontractile effect of PVAT in mesenteric arteries from normotensive WKY rats. However, in the abdominal aorta, the increase in neurogenic contractions was detected in the presence of PVAT. Our results thus indicate that the inhibitory influence of PVAT on contractile responses might not be necessarily manifested when the contractions are induced by endogenous noradrenaline released from perivascular nerves. Besides several proposed influences and factors originating from PVAT that could interfere with the neurotransmission in vascular preparations, our results show that PVAT itself might contain further sources of endogenous noradrenaline which is released during neural stimulation and could significantly contribute to the observed contractile responses.

Moreover, we suppose that the altered properties of PVAT might be an important source of pathological changes observed in arteries from SHR. The reduced anticontractile influence of PVAT together with the possible increased sympathetic nerve supply within PVAT could significantly contribute to the enhanced sensitivity to vasoconstrictors detected in SHR mesenteric arteries. These results indicate that the presence of PVAT should not be ignored in *in vitro* studies on arterial preparations.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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