

Perivascular Adipose Tissue, Vascular Reactivity and Hypertension

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Key Words

Perivascular adipose tissue · Vascular smooth muscle · Anticontractile effect · Adipocyte-derived relaxing factor · Macrophages · Hypertension

Abstract

Most blood vessels are surrounded by a variable amount of adventitial adipose tissue, perivascular adipose tissue (PVAT), which was originally thought to provide mechanical support for the vessel. It is now known that PVAT secretes a number of bioactive substances including vascular endothelial growth factor, tumor necrosis factor- α (TNF- α), leptin, adiponectin, insulin-like growth factor, interleukin-6, plasminogen activator substance, resistin and angiotensinogen. Several studies have shown that PVAT significantly modulated vascular smooth muscle contractions induced by a variety of agonists and electrical stimulation by releasing adipocyte-derived relaxing (ADRF) and contracting factors. The identity of ADRF is not yet known. However, several vasodilators have been suggested including adiponectin, angiotensin 1–7, hydrogen sulfide and methyl palmitate. The anticontractile effect of PVAT is mediated through the activation of potassium channels since it is abrogated by inhibiting potassium channels. Hypertension is characterized by a reduction in the size and amount of PVAT and this is associated with the attenuated anticontractile effect of PVAT in hyperten-

sion. However, since a reduction in size and amount of PVAT and the attenuated anticontractile effect of PVAT were already evident in prehypertensive rats with no evidence of impaired release of ADRF, there is the possibility that the anticontractile effect of PVAT was not directly related to an altered function of the adipocytes per se. Hypertension is characterized by low-grade inflammation and infiltration of macrophages. One of the adipokines secreted by macrophages is TNF- α . It has been shown that exogenously administered TNF- α enhanced agonist-induced contraction of a variety of vascular smooth muscle preparations and reduced endothelium-dependent relaxation. Other procontractile factors released by the PVAT include angiotensin II and superoxide. It is therefore possible that the loss could be due to an increased amount of these proinflammatory and procontractile factors. More studies are definitely required to confirm this.

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Introduction

It is well established that reactivity of the vascular smooth muscle to vasoconstrictors is affected by a variety of factors including age, gender, affinity, temperature, receptor reserve and pathological states [1–6]. Even though earlier studies have shown that the vascular endothelium

generates a substance, prostacyclin, with vasodilator properties [7, 8], the fact that endothelium played a significant role in modulating basal vascular smooth muscle tone and agonist-induced responses through the release of nitric oxide and endothelium-derived contracting and hyperpolarizing factors was not discovered until the 1980s, led by the pioneering work of Furchgott and Zawadzki [9]. It is now known that the endothelium modulates vascular smooth muscle tone via the release of a number of vasoactive factors including nitric oxide, prostacyclin, yet-to-be-identified endothelium-derived hyperpolarizing and contracting factors, thromboxane and endothelins.

Another source of factors regulating vascular smooth muscle tone has emerged within the past 20 years and is still evolving. With the exception of cerebral vessels, vascular smooth muscles are surrounded by a variable amount of adipose tissue called perivascular adipose tissue (PVAT) because of its location around blood vessels. The PVAT is made up of brown adipose tissue (BAT) and white adipose tissue (WAT). Both BAT and WAT are innervated by the sympathetic nervous system [10–15]. BAT is highly vascularized, metabolically active and associated with energy production via nonshivering thermogenesis. In contrast, WAT is less vascularized and is generally believed to be a storage organ for lipids [16]. The relative distribution of WAT and BAT varies between vessels. PVAT in the abdominal aorta and mesenteric and femoral artery is made up of WAT while PVAT surrounding the aorta is mixed, comprising of both BAT and WAT [17]. PVAT was originally thought to provide a mechanical support for the blood vessels. However it is now known that it functions as a paracrine and endocrine organ secreting a number of bioactive substances including vascular endothelial growth factor, tumor necrosis factor- α (TNF- α), leptin, adiponectin, insulin-like growth factor, interleukin-6, plasminogen activator substance, resistin and angiotensinogen [16]. These substances regulate adipocyte metabolism and other cellular processes including vascular smooth muscle tone.

Anticontractile Effect of PVAT

Based on the traditional belief that the primary function of PVAT was to provide mechanical support for the blood vessels, vascular reactivity studies were usually carried out using vessel segments with no attached adipose tissue. The rationale was that the PVAT could affect reactivity either by metabolizing the agonist or preventing ac-

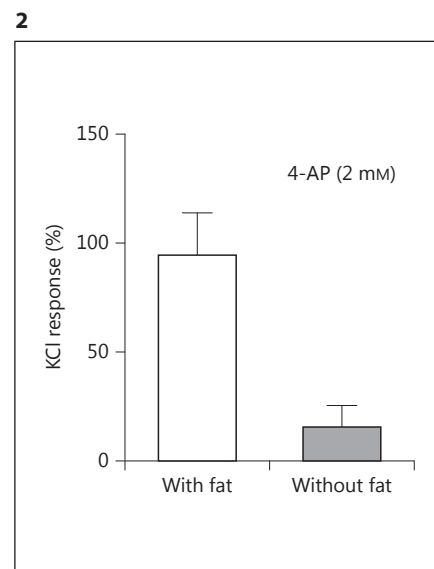
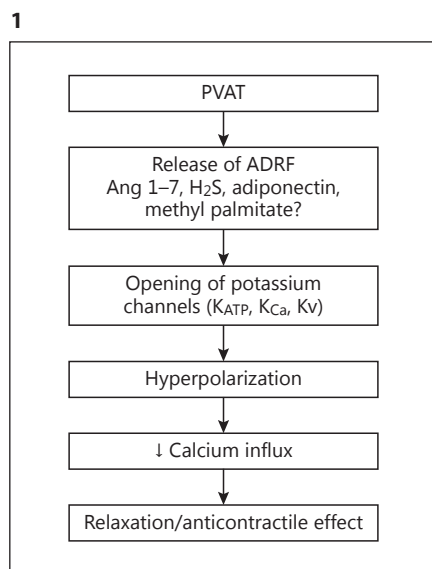
cess of the agonist to the adventitia layer. Interestingly, the demonstration that the mRNA for angiotensinogen (possibly suggesting local generation of angiotensin II) is expressed [18–20] in the PVAT coupled with the fact that there is no barrier between PVAT and the adventitial layer did not change this traditional belief about PVAT or the way vascular reactivity was studied. The first evidence suggesting modulation of vascular smooth muscle tone by PVAT was provided by Soltis and Cassis [21], who reported that PVAT significantly attenuated noradrenaline-induced contraction of the rat aorta. Since PVAT had no effect on contractions induced by phenylephrine and KCl, it has been suggested that the anticontractile effect of PVAT may be due to the uptake of noradrenaline into adrenergic nerves in the fat tissue [21]. However, while confirming the anticontractile effect of PVAT against phenylephrine-induced contractions, Lohn et al. [22] also observed an anticontractile effect of PVAT against 5-HT and angiotensin II as agonists. This observation would suggest that uptake into noradrenaline-containing nerves was not a factor in the anticontractile effect of PVAT since angiotensin II is not a substrate for the uptake process. These early observations of the anticontractile effects of PVAT have been confirmed by several researchers in a variety of vascular smooth muscle preparations [23–31; Oriowo and Oommen, unpubl. data]. PVAT is also not limited to arterial smooth muscles, as its anticontractile effect has been demonstrated in ring segments of the vena cava [32]. The anticontractile effect of PVAT is not a nonspecific event, since not all agonists are uniformly affected even in the same arterial preparation. Thus, noradrenaline-induced but not phenylephrine- or KCl-induced contractions were attenuated by PVAT in the rat aorta [21] while in the rat mesenteric artery, PVAT attenuated ET-1- and 5-HT-induced but not U 46619-induced contractions [25]. Inhibition of ureteral motility by periureteral adipose tissue has recently been reported by Killian and Bund [33].

Mediators of the Anticontractile Effect of PVAT

Lohn et al. [22] were the first to suggest that the anticontractile effect of PVAT involved the release of a vasodilator (ADRF) from the PVAT, which then diffuses into the adventitia to evoke a relaxation (fig. 1). Using a bioassay system, these researchers demonstrated that the transfer of a small volume of solution from an artery segment with intact PVAT into a bath containing a precontracted artery segment without PVAT resulted in relaxation, in-

Fig. 1. Schematic representation of the effect of PVAT on the vascular smooth muscle. PVAT releases a relaxing factor which activates potassium channels on the vascular smooth muscle membrane leading to hyperpolarization, reduced influx of calcium and relaxation. Ang = Angiotensin; H₂S = hydrogen sulfide.

Fig. 2. Bar chart showing 4-AP-induced contractions of rat thoracic aorta ring segments with and without attached PVAT.



dicating the release of a vasorelaxing factor from the artery segment with intact PVAT. This has subsequently been confirmed by others [25, 34–36]. The identity of ADRF is not yet known. However, it is known that:

- Release of ADRF is calcium dependent.
- ADRF produces relaxation by endothelium-dependent (via nitric oxide) and endothelium-independent mechanisms involving activation of potassium channels [25].
- The effect of ADRF can be attenuated by genistein, a tyrosine kinase inhibitor, suggesting mediation via tyrosine kinase [22].

Adiponectin is one of the vasodilators released by PVAT [36, 37]. Others include angiotensin 1–7 [26, 31, 38], methyl palmitate [39] and hydrogen sulfide [40, 41]. Available evidence suggests that ADRF may vary between tissues [26, 31, 36–41].

PVAT and the Regulation of Resting Vascular Smooth Muscle Tone

Many studies have shown that activation of voltage-dependent potassium channels (Kv channels) modulates basal vascular smooth muscle tone. Since the anticontractile effect of PVAT is mediated via activation of potassium channels, there is the possibility that PVAT, through activation of these channels, could play a role in regulating resting vascular smooth muscle tone. In the mesenteric artery, Verlohren et al. [25] have shown that the resting membrane potential was hyperpolarized and that the hy-

perpolarization was more in artery segments with intact PVAT than in those without PVAT, suggesting a role for PVAT in regulating resting vascular smooth muscle tone. Abolition of the difference in membrane potential between artery segments with and without PVAT by 4-aminopyridine (4-AP) would confirm a role for Kv channels in PVAT-induced regulation of membrane potential in this artery. Using the increase in perfusion pressure in the presence of 4-AP as an index of regulation of vascular smooth muscle tone, Galvez et al. [28] concluded that PVAT was involved in regulating basal artery tone since 4-AP induced a greater increase in perfusion pressure in a mesenteric vascular bed with intact PVAT. This has been confirmed by Galvez-Prieto et al. [42] using ring segments of the superior mesenteric artery. Zavaritskaya et al. [43] have recently reported more hyperpolarized resting membrane potential in the rat gracilis artery with intact PVAT that was abolished in the presence of XE 991, a selective inhibitor of Kv7 potassium channels. XE 991 produced a depolarization that was accompanied by an increase in basal smooth muscle tone. 4-AP increased resting tension (contraction) of the rat aorta in artery segments with and without intact PVAT. The contraction was significantly greater in artery segments with intact PVAT (fig. 2), confirming a role for PVAT in regulating Kv potassium channel activity and resting tone of the rat aorta [Oriowo and Oommen, unpubl. data]. It would therefore seem that PVAT, through the release of ADRF, is involved in regulating resting membrane potential and vascular smooth muscle tone by a mechanism involving the activation of Kv7 channels.

Role of Potassium Channel Activation in the Anticontractile Effect of PVAT

In addition to regulating resting vascular smooth muscle tone, activation of potassium channels has also been shown to modulate agonist-induced contractions in a variety of vascular smooth muscles. When activated, these channels cause hyperpolarization accompanied by reduced influx of extracellular calcium into the smooth muscle cells, leading to reduced contractions (fig. 1). Earlier studies have shown that the anticontractile effect of PVAT was attenuated by increasing potassium concentration to between 60 and 80 mM. This would suggest a role for potassium channel activation in the anticontractile effect of PVAT [22, 25]. However, available evidence (described below) tends to suggest that the type of potassium channels involved in the anticontractile effect varies with the arterial preparation under investigation. In the rat aorta, blockade of ATP-dependent potassium channels (K_{ATP} channels) with glibenclamide abolished the anticontractile effect of PVAT [22]. On reexamination, however, it was found that glibenclamide increased the contractile responses to 5-HT similarly in aorta segments with or without PVAT, suggesting that activation of K_{ATP} channels may not explain the effect of PVAT on 5-HT-induced contractions of the rat aorta [44]. Oriowo and Oommen [unpubl. data] have observed that blockade of calcium-activated potassium channels with tetraethylammonium enhanced 5-HT-induced contraction of rat aorta segments with or without PVAT and that the enhancement was less in artery segments with intact PVAT, suggesting that tetraethylammonium-sensitive K_{Ca} channel activation was not involved in the anticontractile effect of PVAT in the aorta. However, recent studies have shown that an anticontractile effect of PVAT on noradrenaline-induced contractions in small mesenteric mouse arteries was not observed in the presence of BK_{Ca} channel inhibitor or in BK_{Ca} knockout mice [45]. In addition, it was also observed that PVAT from BK_{Ca} knockout mice had no effect on noradrenaline-induced contractions [46]. This would suggest that activation of the BK_{Ca} channel was involved in the anticontractile effect of PVAT in small mesenteric mouse arteries, thus indicating that the role of the BK_{Ca} channel in the anticontractile effect of PVAT could be vessel and/or species specific.

In the superior mesenteric artery preparation, treatment with 4-AP and 3,4-diaminopyridine (both blockers of Kv channels) abolished the anticontractile effect of PVAT, confirming that the difference in reactivity to 5-HT in artery segments with and without PVAT resulted

from activation of Kv channels [25]. A role for these channels in the anticontractile effect of PVAT has also been demonstrated in small mesenteric arteries [45]. Iberiotoxin and tetraethylammonium at concentrations that specifically block K_{Ca} channels enhanced 5-HT-induced contractions in artery segments with and without PVAT and did not discriminate between the two types of preparations, indicating that activation of K_{Ca} channels modulated 5-HT-induced contractions of the mesenteric artery but was not responsible for the anticontractile effect of PVAT. A recent study [43] in the gracilis muscle artery has confirmed a role for Kv channels in mediating the anticontractile effect of PVAT. These investigators observed that XE 991 and linopirdine (both selective inhibitors of Kv7 channels) abolished the anticontractile effect of PVAT against 5-HT-induced contractions in the gracilis muscle artery, thus confirming the involvement of the Kv7 channel isoform in the anticontractile effect of PVAT [43]. It could therefore be concluded that there is a regional/species variation in the role of potassium channel isoforms in the anticontractile effect of PVAT. This is supported by the observation [26, 32] that ADRF release from vessel segments with intact PVAT and which is thought to be angiotensin 1–7 (the proposed transferable factor) relaxed ring segments of the rat aorta and inferior vena cava through activation of the BK_{Ca} channel and Kv channels, respectively.

Effect of Hypertension on the Anticontractile Effect of PVAT

Several studies [23, 28, 31, 38, 39, 42, 45–48] have examined the effect of hypertension on the anticontractile effect of PVAT. The general agreement in all these studies is that the anticontractile effect of PVAT is attenuated in artery segments from hypertensive rats irrespective of the experimental model used. The mechanism responsible for the loss of anticontractile effect of PVAT is however not yet known. It could be due to impaired release of ADRF resulting from the reduced amount and size of PVAT, reduced expression and function of potassium channels in the adipose tissue or the vascular smooth muscle or increased production of contractile factor(s) (fig. 3).

Prehypertensive Rats

Galvez-Prieto et al. [42] reported that 5-HT-induced contraction was greater in the mesenteric vascular bed from prehypertensive 4-week-old spontaneously hyper-

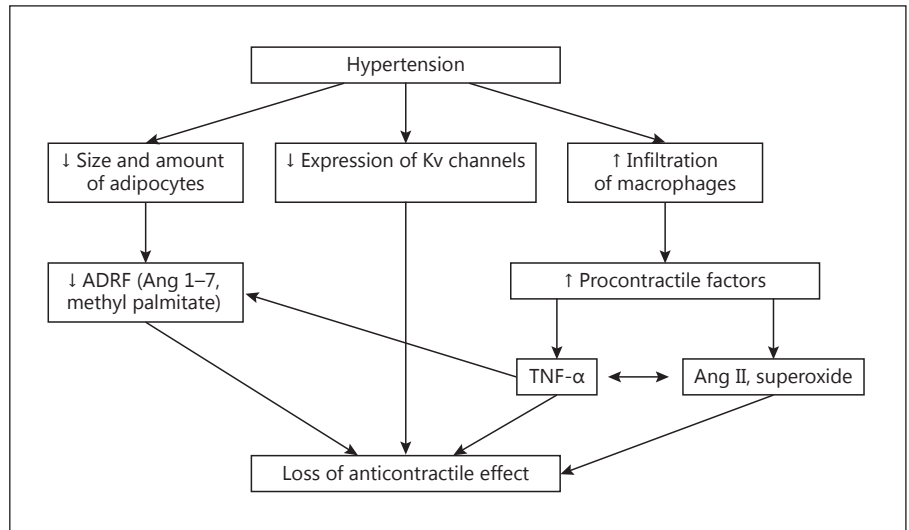


Fig. 3. Schematic presentation of the possible mechanisms underlying the loss of anticontractile effect of PVAT in hypertension. Ang = Angiotensin.

tensive rats (SHR) compared with Wistar-Kyoto (WKY) rats. These authors also reported a reduction in the amount and function of PVAT in these animals, leading to the suggestion that a reduction in the amount and function of PVAT preceded the development of hypertension. Even though it would appear that loss of anticontractile activity of PVAT correlated with a reduced amount of PVAT [28, 42], no cause and effect relationship has been established. Using the pressor response to 4-AP as an index of ADRF release, Galvez-Prieto et al. [42] observed that the increase in perfusion pressure induced by 4-AP was not different between WKY and SHR, suggesting that basal release of ADRF was not different at this prehypertensive stage. However, since Kv channels are also involved in the relaxing effect of ADRF on vascular smooth muscle, similar 4-AP-induced vasoconstriction of the perfused mesenteric vascular bed in WKY and prehypertensive 4-week-old SHR would also rule out a dysfunction of the Kv7 potassium channels located on the vascular smooth muscle. This would imply that the loss of anticontractile effect of PVAT in the mesenteric vascular bed of prehypertensive SHR was not due to impaired ADRF release or impaired vascular smooth muscle Kv channel function.

Hypertensive Rats

In contrast to no change in the 4-AP-induced increase in perfusion pressure recorded in the mesenteric vascular bed of prehypertensive rats, 4-AP-induced increase in perfusion pressure was significantly reduced in the mesenteric vascular bed from hypertensive rats. This could

suggest reduced release of ADRF or impaired expression and function of vascular smooth muscle Kv channels or both. An increased release of contracting factors cannot be ruled out. The reduced anticontractile effect of PVAT in established hypertension has been shown to be associated with a reduced release of vasodilators, leptin [28, 47] and methyl palmitate [39], from the PVAT. However, the observation that the direct dilator effect of leptin on angiotensin II-induced vasoconstriction was attenuated in artery segments from SHR [49] would indicate that whatever mechanism mediates leptin-induced vascular smooth muscle relaxation is also impaired in vessels from SHR. This would suggest that the loss of vasodilator response to leptin in hypertension occurs at the level of PVAT and vascular smooth muscle. It has been reported that the loss of anticontractile effect of PVAT in hypertension could be due to impaired functioning of the Kv7 channels [46]. This is consistent with the demonstration of a significant reduction in the expression of Kv channels in SHR [50] and the observation that 4-AP potentiated 5-HT-induced contraction only in WKY artery segments with intact PVAT. These observations are, however, in contrast to those reported by Zavaritskaya et al. [43] in the gracilis artery. These authors showed that the anticontractile effect of PVAT was attenuated in gracilis muscle arteries of SHR even though the inhibitory effects of potassium channel openers (measure of channel function) were similar in artery segments from SHR and Wistar rats. This would probably suggest that the reduced anticontractile effect of PVAT in the gracilis artery from SHR is associated with a reduced release of ADRF but not

the expression and function of vascular smooth muscle Kv7 potassium channels. Thus, hypertension is associated with reduced release of ADRF, irrespective of the vascular smooth muscle preparation studied, while its effect on the expression and function of Kv7 potassium channels appeared to be vessel specific.

Infiltration of Macrophages as a Possible Reason for the Loss of Anticontractile Effect of PVAT

It is clearly evident that the loss of anticontractile effect of PVAT in prehypertensive and hypertensive rats appeared to be mediated via different mechanisms [43, 46, 49]. Even though both stages of hypertension are characterized by a similar reduction in the amount and size of the adipose tissue, the release of ADRF and the function of the membrane potassium channels were impaired in artery segments from hypertensive but not prehypertensive SHR [28, 39, 42, 47]. This raises the question as to what common mechanisms mediate the loss of anticontractile effect of PVAT in these animals. It is being postulated here that the loss of anticontractile effect of PVAT could be due to the release of contractile factors from the PVAT. This is based on the fact that in addition to ADRF, PVAT also produces contracting factors including angiotensin II and superoxide ions. An attenuated anticontractile effect of PVAT is commonly observed in artery segments from hypertensive and obese rats even though these pathological states are characterized by a reduction (hypertension) and increase (obesity) in the amount and size of PVAT. These observations would suggest that the loss of anticontractile effect of PVAT is not dependent on changes in the size and amount of adipocytes per se. Infiltration of macrophages into adipose tissues has been observed in a variety of pathological states including obesity, inflammation and hypoxia, and could be responsible for the loss of anticontractile activity (as described below). In a recent study, Withers et al. [51] concluded that activation of macrophages is responsible for the loss of anticontractile effect of PVAT in inflamed perivascular fat. This was based on the observation that noradrenaline-induced contractions of artery segments isolated from wild-type and macrophage-deficient mice was not different, with or without fat, indicating loss of anticontractile effect of PVAT in the aorta. Vascular inflammation is a prominent feature of hypertension [52–55] and it has been reported that macrophages accumulate in the vascular wall during hypertension [52, 55–57]. Wenzel et al. [52] have shown that depletion of monocytes, which

are precursors of macrophages, prevented experimentally induced hypertension in mice. This has been confirmed using a chemokine antagonist in deoxycorticosterone/salt hypertension in mice [55]. It is therefore quite possible that the loss of anticontractile effect of PVAT in hypertension could be due to an accumulation of proinflammatory factors released by the macrophages. TNF- α is produced by macrophages and the level is elevated in hypertension [58–61]. The increase in TNF- α was observed even at the prehypertensive stage [62]. Previous studies [63–65] have shown that TNF- α increased agonist-induced contractions in a variety of vascular smooth muscle preparations. Therefore, there is the possibility that the loss of the anticontractile effect of PVAT in hypertension could be due to a release of TNF- α , which then exerts a procontractile effect by a mechanism involving generation of reactive oxygen species and angiotensin II [62, 66]. The observations that treatment with TNF- α antagonist reduced angiotensin-induced hypertension [67, 68] and that chronic blockade of angiotensin AT₁ receptor significantly reduced circulating levels of TNF- α [69] are consistent with the existence of cross-talk between angiotensin II and TNF- α (fig. 3). Increased secretion of TNF- α could also lead to a reduction in the secretion of vasodilator cytokines by the PVAT. Hajri et al. [66] have reported that TNF- α reduced the secretion of adiponectin. A similar observation was reported by Fain et al. [70]. TNF- α has also been reported to interfere with nitric oxide-mediated vasodilation [64, 71–73].

Summary of Experimental Findings

PVAT, in addition to providing mechanical support for the blood vessels, regulates vascular smooth muscle tone through the release of ADRF and adipocyte-derived contracting factors. The identity of ADRF is unknown, but its release is calcium dependent and involves activation of potassium channels. The anticontractile effect of PVAT has been demonstrated in arteries (conduit and resistance) and veins. This anticontractile effect is abolished by blocking potassium channels (mostly voltage-gated channels). Hypertension is associated with an impaired anticontractile effect of PVAT, suggesting a role for PVAT in the pathogenesis of hypertension. Studies in SHR have shown that the loss of anticontractile effect of PVAT is associated with a reduction in the size and amount of PVAT. However, the fact that the loss of anticontractile effect of PVAT was already present in prehypertensive SHR at a time when no reduction in the release

of ADRF could be demonstrated would suggest that altered size and amount of the adipocytes per se was not responsible for the impaired anticontractile effect of PVAT in hypertension. More studies are needed to examine the role of adipocytokines released by macrophages that have been shown to accumulate in the PVAT in hypertension.

Clinical Perspectives

PVAT is now well recognized as a modulator of vascular smooth muscle tone and its anticontractile effect has been demonstrated in both arteries and veins. This anticontractile effect of PVAT is attenuated in hypertension, a condition that is associated with remodeling of the smooth muscle and increased peripheral vascular resistance. The exact mechanism underlying the loss of anticontractile effect is not known. Recent studies [51–57] have shown that hypertension is characterized by a low-grade inflammation associated with infiltration of the adipose tissue by macrophages and an increase in the release of TNF- α , angiotensin II and reactive oxygen species. It has been reported that cross-talk exists between the renin/angiotensin system and TNF- α release. Thus, treatment with TNF- α antagonist reduced angiotensin-induced hypertension while chronic blockade of angiotensin AT₁ receptor significantly reduced circulating levels of TNF- α . Treatment with ACE inhibitors and AT₁ receptor antagonists is associated with increased circulating

levels of angiotensin 1–7 [74]. Recent studies [75, 76] have shown that angiotensin 1–7 decreased the production of proinflammatory cytokines by the adipose tissue. It is therefore possible that the increase in adipose TNF- α levels in hypertension could be due to reduced release of angiotensin 1–7, which has been reported to occur in hypertension. Increased secretion of TNF- α could also lead to a reduction in the secretion of vasodilators adiponectin, nitric oxide and angiotensin 1–7 by PVAT. Thus PVAT would play a significant role in the pathogenesis of hypertension by increasing the release of procontractile factors while at the same time reducing the generation of vasodilators. In addition, these studies would suggest that treatment with ACE inhibitors and AT₁ receptor antagonists would prevent the inflammation and restore the balance between vasoconstrictor and vasodilator factors produced by PVAT and hence its anticontractile effect.

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Disclosure Statement

The author declares no conflict of interest.

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