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REVIEW

Vasodilator signals from perivascular adipose tissue

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Visceral fat has been linked to metabolic disturbances and increased risk for cardiovascular disease and type 2 diabetes. Recent studies propose a paracrine role for periadventitial adipose tissue in the control of arterial vascular tone. This regulation depends on the anatomical integrity of the vessels and involves a transferable mediator(s) (adipokine) released from either periadventitial adipocytes or perivascular adipose tissue. Although a number of adipokines with vasoactive properties have been identified, a still unidentified adipocyte-derived relaxing factor (ADRF) plays a major role in the periadventitial vasoregulation of visceral arteries, such as the aorta and mesenteric arteries. ADRF is released by visceral periadventitial adipocytes and primarily produces endothelium-independent vasorelaxation by opening voltage-dependent (K_v) K^+ channels in the plasma membrane of smooth muscle cells. At least in part, KCNQ (K_v7) channels could represent the subtype of K_v channels involved. Glibenclamide-sensitive K_{ATP} channels are not involved or play a minor role. The 'third gas', namely H_2S, could represent ADRF. Alterations in the paracrine control of arterial tone by visceral periadventitial adipose tissue have been found in animal models of hypertension and metabolic disease. ADRF, or perhaps its putative targets, might represent exciting new targets for the development of drugs for treatment of cardiovascular and metabolic disorders.

LINKED ARTICLES

This article is part of a themed section on Fat and Vascular Responsiveness. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.165.issue-3

Abbreviations

4-AP, 4-aminoyporidine; ADRF, adipocyte-derived relaxing factor; ASP, acylation stimulation protein; BKCa, large-conductance, Ca^{2+}-activated K^+ channel; CSE, cystathionine gamma lyase; SHR, spontaneously hypertensive rats; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; HMG-CoA, hydroxy-3-methylglutaryl-coenzyme A; IGF-1, insulin-like growth factor; PAI-1, plasminogen activator protein; KATP, glibenclamide-sensitive K^+ channel; K_v, voltage-dependent K^+ channel; RAS, renin–angiotensin system; sGC, soluble guanylyl cyclase; ACRP30, adiponectin (ACRP30); XE991, 10,10-bis(4-pyridinylmethyl)-9(10*H*)-anthracenone dihydrochloride

Obesity is one of today's most blatantly visible public health problems. There is major concern that medical progress in reducing coronary heart disease, high cholesterol and hypertension could be reversed by the escalating global epidemic of overweight and obesity – 'globesity' (World Health Organization, 1998). Obesity is a risk factor for hypertension, type 2 diabetes, congestive heart failure, stroke, renal dysfunction, gallbladder disease, obstructive sleep apnea, cancer, osteoarthritis, and impaired quality of life (Sharma, 2003; Flier, 2004). Up to 50% of obese individuals have concomitant hypertension (Sharma, 2003). However, the mechanisms underlying these observations have yet to be fully understood. Increased sympathetic nerve activity, insulin resistance and hyperinsulinaemia, sodium and volume retention, renal abnormalities and ,more recently, hyperleptinaemia have all been implicated in obesity-related hypertension (Hall *et al.*, 2001). The haemodynamic profile of obese hypertensive subjects is characterized by high intravascular volume, high

cardiac output and increased peripheral vascular resistance (Messerli *et al.*, 1983; Fortuno *et al.*, 2003). Increased vascular resistance in obesity is known to be accompanied by structural changes in peripheral resistance vessels (Rocchini *et al.*, 1992) and blunted Ca^{2+} regulation of vascular smooth muscle tone by insulin (Zemel, 1998). However, the role of periadventitial adipocytes in vascular reactivity has received little attention. This is surprising since adipose tissue secretes a number of bioactive substances.

Vascular effects of adipokines

Recent *in vitro* and *in vivo* evidence identifies adipose tissue as a critical endocrine organ that secretes a variety of bioactive signalling molecules into the circulation. These molecules include vascular endothelial growth factor (VEGF), interleukin 6, interleukin-1, tumour necrosis factor-α (TNF-α), leptin, adiponectin (ACRP30), resistin, omentin, acylation stimulation protein (ASP), apelin, adipsin, agouti, insulin-like growth factor (IGF-1), angiotensinogen, plasminogen activator protein (PAI-1), reactive oxygen species and sex steroids (Fortuno *et al.*, 2003; Havel, 2004; Thalmann and Meier, 2007; Yamawaki *et al.*, 2010). These substances can play an autocrine role in the regulation of adipocyte metabolism. Upon secretion into the bloodstream, they can also play an endocrine role in the regulation of other cellular processes, including vascular function and peripheral resistance. Although a number of adipocyte-derived substances can influence vascular tone (Ohkawa *et al.*, 1994; Loughrey *et al.*, 2003; Salcedo *et al.*, 2007; Yamawaki *et al.*, 2009; Yamawaki *et al.*, 2010), the vasoactive effects of leptin and TNF-α deserve highlighting. Leptin regulates blood pressure by at least two opposing mechanisms, that is release of nitric oxide (NO) and excitation of the sympathetic nervous system (Fruhbeck, 1999; Lembo *et al.*, 2000). Leptin induces direct vasodilation by stimulation of NO and endothelium-derived hyperpolarizing factor (EDHF) (Lembo *et al.*, 2000) but not involving glibenclamide-sensitive (K_{ATP}) and TEA-sensitive (BK) K^+ channels (Kimura *et al.*, 2000; Sahin and Bariskaner, 2007). On the other hand, leptin induces indirect vasoconstriction via central activation of the sympathetic nervous system (Fruhbeck, 1999). TNF-α is also both a vasoconstrictor (Wagner, 2000; Zhang *et al.*, 2002) and a vasodilator (Baudry and Vicaut, 1993; Shibata *et al.*, 1996; Brian and Faraci, 1998; Johns and Webb, 1998). TNF-α-mediated vasorelaxation can involve NO and prostaglandin production (Shibata *et al.*, 1996; Brian and Faraci, 1998) or NAD(P)H oxidase-dependent Ca^{2+} spark activation (Cheranov and Jaggar, 2006). Moreover, TNF-α plays a role in endothelial dysfunction (Park *et al.*, 2011; Zhang *et al.*, 2006; Yang *et al.*, 2009). Notably, a complete renin–angiotensin system (RAS) has been identified in adipose tissue (Engeli *et al.*, 2003; Galvez-Prieto *et al.*, 2008a). Thus, adipose cells play a more dynamic role in regulating cellular processes than previously recognized. However, most of these studies have been focused on the identification of substances released from adipose tissue and their possible humoral vasoactive effects but not on a possible paracrine function for adipose tissue in the regulation of arterial tone and peripheral vascular resistance.

ADRF and the paracrine regulation of arterial tone by periadventitial adipose tissue

Virtually all blood vessels are surrounded by variable amounts of adventitial adipose tissue (Cinti, 2002). Soltis and Cassis (1991) found that periadventitial fat significantly attenuates vascular responsiveness of Sprague–Dawley aortic ring preparations to noradrenaline *in vitro*. The study was aimed to clarify whether vasoconstriction is due to release of noradrenaline from nerves stimulating the vascular smooth muscle directly or from the innervation of the adipose tissue in rat aorta. Using three methods that cause the release of noradrenaline from sympathetic nerve terminals, the authors demonstrated that the innervation to the adipose tissue influences responsiveness of the aortic smooth muscle. The authors suggested that periaortic adipose tissue contains sufficient sympathetic nervous system innervation to influence *in vitro* aortic vascular responsiveness. They suggested that the anticontractile effect on the response to noradrenaline was due to uptake of noradrenaline by the surrounding adipose tissue. However, according to this theory, vessels with perivascular fat are expected to exhibit greater contractile responses to depolarizing external K^+ (60–100 mM) solutions than vessels without fat. However, this appears not to be the case. Therefore, the authors concluded that the reason for the lack of difference in KCl responsiveness between intact vessels and vessels without fat is not known. They appreciated limitations of their adipose tissue innervation theory to explain the reduced vascular responsiveness of intact aortic ring preparations with fat in response to noradrenaline. Lohn *et al.* (2002) reexamined the idea of periadventitial vasoregulation. Their studies on Sprague–Dawley rat aorta confirmed the inhibitory effect of periadventitial adipose tissue on vasoconstriction induced by serotonin, angiotensin II and phenylephrine. In contradistinction to noradrenaline, these substances are not subject to reuptake by adrenergic nerves. The anti-contractile effects did not involve neuronal pre-synaptic N-type Ca^{2+} and Na^+ channels or vanilloid, cannabinoid, and calcitonin gene-related peptide receptors (Dubrovska *et al.*, 2004). Rather, this regulation depends on the anatomical integrity of the vessels and is mediated by a transferable 'adipocyte-derived relaxing factor' (ADRF) (Lohn *et al.*, 2002; Gollasch and Dubrovska, 2004; Verlohren *et al.*, 2004c). Blocking NO synthase by L-NAME, cyclo-oxygenase by indomethacin or cytochrome P450 by miconazole did not affect the effects of ADRF (Lohn *et al.*, 2002). Blocking adenosine receptors or removal of the endothelium did affect the anti-contractile effects of perivascular fat (Lohn *et al.*, 2002). Lack of functional leptin receptors in the Zucker fa/fa rat did not modify the anticontractile effect of periadventitial fat, indicating that the ADRF effects are independent of functional leptin receptors (Lohn *et al.*, 2002). Studies on adiponectin gene knockout mice demonstrated that ADRF is *not* adiponectin (Fesus *et al.*, 2007).

ADRF and smooth muscle K_v channels

Though the chemical nature of ADRF(s) is unknown and there might be more than one ADRF, there is evidence that

the substance produces relaxation by opening smooth muscle K^+ channels. The disappearance of the anti-contractile effect of periadventitial adipose tissue in high (60 mM) external K^+ solutions suggested that K^+ channels are critically involved in this effect (Lohn *et al.*, 2002). By donor-acceptor transfer bioassay experiments, we and others showed that the effects are mediated by a transferable factor (Dubrovska *et al.*, 2004; Gao *et al.*, 2005), which opens K_v channels (ADRF-K_v pathway) to produce vasorelaxation (Verlohren *et al.*, 2004c; Fesus *et al.*, 2007). Periadventitial adipose tissue was also reported to inhibit arterial contraction of human mesenteric arteries (Verlohren *et al.*, 2004a). The K_v-subfamily involved in the effects of ADRF was recently explored using a pharmacological approach with XE991 (10,10-*bis*(4-Pyridinylmethyl)-9(10*H*)-anthracenone dihydrochloride) (Schleifenbaum *et al.*, 2010). XE991 inhibited the anti-contractile effects of perivascular fat in mesenteric arteries and in aortas. We also re-examined the effects of glibenclamide (3 μM) in the rat aorta (Lohn *et al.*, 2002) and found that this drug antagonizes the anti-contractile effect of fat by a non-specific, ADRF-independent mechanism (Schleifenbaum *et al.*, 2010). In particular, we found that glibenclamide (3 μM) increased contractile responses of (+) fat aortas to serotonin. However, dose-response curves of (-) fat aortas for serotonin were similarly shifted to the left by glibenclamide. These results indicate that K_{ATP} channels play no or only a minor role in vasorelaxation induced by ADRF. Instead, the pharmacological data suggest that, at least in part, KCNQ channels represent the K_v channel family involved in the effects of ADRF (Figure 1).

KCNQ channels were previously described as targets for vasopressin in aortic smooth A7r5 cells (Brueggemann *et al.*, 2007). Yeung *et al.* (2007) detected mRNA of KCNQ1, 4 and 5 mesenteric arteries, in aorta, carotid arteries and femoral arteries of mice, with KCNQ4 being the most abundant (Yeung *et al.*, 2007). Notably, intracellular tetraethylammonium ions (TEA) are known to block KCNQ current by a mechanism that is distinct from the inhibition by polyvalent cations (Hadley *et al.*, 2000; 2003; Shah *et al.*, 2002; Koyama and Appel, 2006; Schwarz *et al.*, 2006; Suh and Hille, 2007). These effects may explain why some authors have observed that the anti-contractile effects of perivascular fat are inhibited by TEA (Gao *et al.*, 2007; Takemori *et al.*, 2007; Zeng *et al.*, 2009). The role of BK_{Ca} channels has been ruled out using a genetic approach. Accordingly, mice with genetically inactivated large Ca-activated K^+ channels **(**BK_{Ca}) in smooth muscle (Pluger *et al.*, 2000; Sausbier *et al.*, 2005) showed normal anti-contractile effects of ADRF (Fesus *et al.*, 2007). Nevertheless, the KCNQ channel subtype involved in the ADRF effects is unknown. Furthermore, direct evidence for membrane hyperpolarizing effects of ADRF in arterial smooth muscle is shown only for rat mesenteric arteries (Verlohren *et al.*, 2004c). Direct evidence for activation of KCNQ channels is still missing. Further studies with gene-silencing or knock-out technologies might help to confirm these observations and to determine the KCNQ channel subtypes and possibly other K_v channels activated by ADRF (Figure 1). Nonetheless, the data support the novel concept that periadventitial adipose tissue represents a paracrine tissue to produce membrane hyperpolarization and vasorelaxation by opening K_v channels in arterial smooth muscle cells. The effects appear to be mediated largely by opening KCNQ-type K_v channels. These data suggest that a promising candidate for ADRF should fulfill the requirement to mimic ADRF characteristic effects on K^+ channels, i.e. being a potent KCNQ channel opener.

Notably, KCNQ channels in arterial smooth muscle cells can contribute to the resting membrane and carry remarkably large outward currents in response to membrane depolarisations (Yeung and Greenwood, 2005; Mackie *et al.*, 2008; Greenwood and Ohya, 2009; Gurney *et al.*, 2010). Thus, KCNQ channels exhibit a large hyperpolarisation reserve and are enabled to fulfil physiological vasodilatory functions in the presence of perivascular fat, i.e. in response to ADRF. Moreover, since ADRF is released from perivascular adipocytes, KCNQ channels seem to play a unique role for vasodilatory signals from outside the vessel ('outside control'). KCNQ channels are not targeted by endothelium-derived relaxing factors (EDRF) ('inside control'), including NO, prostaglandin I_2 (prostacyclin) and endothelium-derived hyperpolarizing factor (EDHF). Although exogenous H_2O_2 as candidate EDHF has been reported to open 4-aminopyridine (4-AP)-sensitive K_v channels in certain vascular beds (Rogers *et al.*, 2007), endogenously produced H_2O_2 induces EDHF-like responses independent of K_v (KCNQ) channels (Hercule *et al.*, 2009). Moreover, superoxide produced by perivascular fat in response to electrical field nerve stimulation does not produce vasorelaxation, but rather vasoconstriction (Gao *et al.*, 2006). Furthermore, H_2O_2 generated by perivascular adipose tissue has been reported to produce vasodilation *via* smooth muscle soluble guanylyl cyclase (sGC) without activation of large-conductance, Ca^{2+} activated K^+ (BK_{Ca}) channels (Gao *et al.*, 2007), which is in contrast to previous findings demonstrating that BK_{Ca} channels are strongly activated by the sGC/cGMP/PKG pathway [for example (Robertson *et al.*, 1993)]. Ang-(1–7) released by perivascular adipose tissue has been reported to cause endothelial release of NO, which leads to vasorelaxation by opening large-conductance, Ca^{2+} activated K^+ (BK_{Ca}) channels (Lee *et al.*, 2009). Together, it can be concluded that KCNQ channels in visceral arteries are not targeted by physiological vasodilators released from the luminal side of arteries, i.e. from endothelial cells ('inside control'), and from perivascular nerve endings. KCNQ channels are also not targeted by other perivascular candidates, such as H_2O_2, superoxide and Ang (1–7). The data support the novel concept that ADRF (hydrogen sulphide, H_2S) is the physiological activator of KCNQ channels, at least in part, in visceral arteries (Figure 1). Should our hypothesis be confirmed, we will have identified a novel role of KCNQ channels in arterial smooth muscle function.

ADRF and small vessels

The aforementioned studies addressed ADRF effects in relatively large vessels, vessels that do not contribute greatly to peripheral vascular resistance. In contrast, smooth muscle tone in small arteries and arterioles of the microcirculation is an important determinant of peripheral vascular resistance and hence blood pressure. In more recent studies, periadventitial vasoregulation was studied in smaller visceral arteries of rats and mice (Verlohren *et al.*, 2004b; Fesus *et al.*, 2007). In

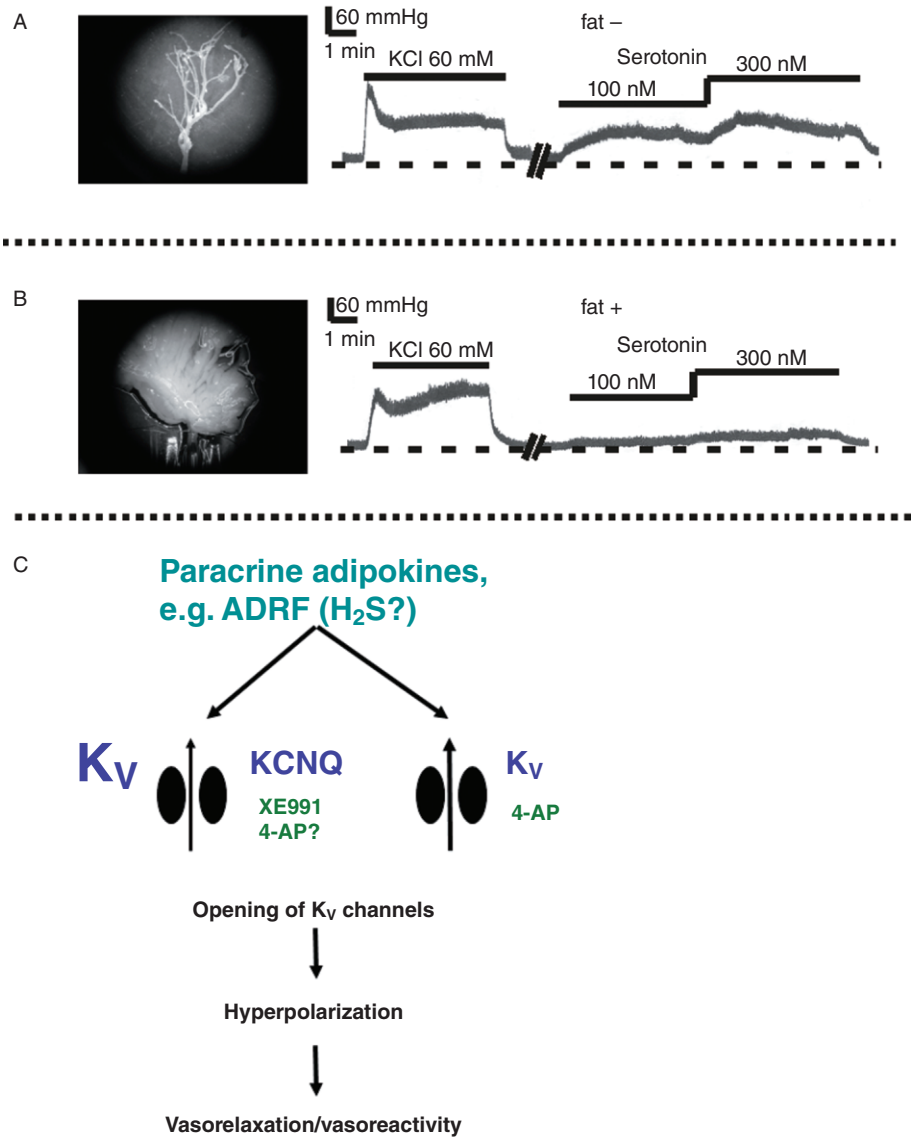

Figure 1

Perivascular fat limits vascular reactivity to serotonin in mouse mesenteric vascular beds. Shown are original recordings of perfusion pressure for perfused isolated mesenteric vascular beds in the absence (fat –) and presence (fat +) of perivascular fat (modified from Fesus *et al.*, 2007). Dashed lines represent 30 mmHg. Horizontal bars show the presence of the drugs. The perivascular fat was carefully removed in fat – beds (A) or left intact (B). C. Periadventitial vasoregulation of visceral arteries by ADRF. ADRF is released by periadventitial adipocytes in response to vasoconstrictors (such as serotonin) and primarily produces endothelium-independent vasorelaxation by opening voltage-dependent (K_v) K^+ channels in the plasma membrane of smooth muscle cells. At least in part, KCNQ (K_v7) channels could represent the subtype of K_v channels involved. KCNQ channels are blocked by XE991. It is possible that additional subtypes of K_v channels play a role in the effects of ADRF, which are sensitive to 4-AP. H_2S could represent ADRF.

these studies, periadventitial adipose tissue significantly attenuated vascular responsiveness of small mesenteric arteries to several hormonal agonists, including serotonin, phenylephrine and endothelin-1. Inhibition of the contractile response to serotonin by fat depended on the amount of fat on each ring but did not depend on the presence of endothelium. The anti-contractile effect of periadventitial fat was abolished by inhibition of K_v channels using 4-AP or 3,4-diaminopyridine. It was largely inhibited by XE991 (Schleifenbaum *et al.*, 2010). However, it was not abolished by pharmacological inhibition of BK_{Ca} channels, K_{ATP} channels, small-conductance calcium-activated K^+ channels and inward rectifying K^+ channels (Verlohren *et al.*, 2004b; Fesus *et al.*, 2007). Experiments on knockout mice lacking the β1 subunit of BK_{Ca} channels confirmed no involvement of BK_{Ca} channels in ADRF effects (Fesus *et al.*, 2007). The resting membrane potential of smooth muscle cells in intact mesenteric artery rings surrounded by fat was more hyperpolarized than rings without periadventitial fat; this difference was abolished by K_v channel inhibition with 4-AP (Verlohren *et al.*, 2004b).

Collectively, these data provide strong evidence that the anti-contractile effects of adipose tissue in small visceral arteries are mediated by activation of smooth muscle K_v channels, leading to membrane hyperpolarization, reduced Ca^{2+} influx into smooth muscle cells and vasorelaxation. KCNQ-type K_v channels seem to play a major role in these effects.

Interestingly, ADRF effects occur also in mesenteric resistance vessels exhibiting myogenic tone. Fesus *et al*. (2007) perfused the isolated mesenteric vascular bed at a constant flow in the absence and presence of perivascular fat. KCl (60 mM) induced similar increases in perfusion pressure in the presence and absence of perivascular fat. However, serotonin induced stronger increases in perfusion pressure in isolated mesenteric vascular beds without fat, compared with mesenteric vascular beds with fat of wild-type mice. K_v channel inhibition with 4-AP abrogated the anti-contractile effects of perivascular fat. The experiments with fat-removed mesenteric vascular beds showed that these effects were solely dependent on the presence of perivascular fat (Figure 1). Adiponectin was not involved. This report was the first to show a direct vasodilating action of perivascular fat on resistance vessels exhibiting myogenic tone in a perfused organ and under flow (Fesus *et al*., 2007). These results did also demonstrate that the anti-contractile effects of perivascular fat occur through access of ADRF from outside the vessel ('outside control'). This consideration is important because most studies on periadventitial vasoregulation rely on open-ended ring preparations, which are commonly used in wire myography. In wire myography, substances released from the adipose tissue could have had their effects simply as a result of their diffusion to the intraluminal side of the vessel through the open ends ('inside control'). In a normal *in vivo* situation, this could not occur. Thus, the results of Fesus *et al*. indicate that periadventitial vasoregulation is not only a phenomenon of open-ended isolated arteries in wire myography but is also present in intact visceral mesenteric beds *in vivo*.

Candidates for ADRF

Protein bands with relative masses of 74.0, 59.8, 54.4, 28.7 and 13.8 kDa have been identified as candidates for ADRF in the rat aorta (Yang *et al*., 2005). ADRF is released by periadventitial adipose tissue in a Ca^{2+}-dependent manner. Although half-maximal release of ADRF occurred at relatively high Ca^{2+} concentrations (4.7 mM), the Hill coefficient of ~1 indicates that the release can be modulated by changes of the plasma Ca^{2+} concentrations *in vivo* (Dubrovska *et al*., 2004). This release is also inhibited by blockers of tyrosine kinase and protein kinase A (Dubrovska *et al*., 2004). Moreover, bioassay experiments showed that ADRF is inactivated or caused to disappear by heating (65°C) and is not adsorbed by essentially fatty acid-free serum albumin (Lohn *et al*., 2002). Interestingly, hypoxia aggravates ADRF effects in the aorta (Maenhaut *et al*., 2010). We explored the possibility that ADRF might be a volatile, gaseous mediator that disappears into the atmosphere during purification (Schleifenbaum *et al*., 2010). Previously, we demonstrated that NO is not ADRF (Lohn *et al*., 2002; Verlohren *et al*., 2004c). We tested the possibility that ADRF might be another biological active gaseous mediator, such as H_2S or carbon monoxide (CO). H_2S is a newly discovered physiologic vasorelaxant generated by cystathionine gamma lyase (CSE) (Yang *et al*., 2008). This enzyme is expressed in perivascular adipose tissue and endogenously generates H_2S (Feng *et al*., 2009). Lack of CSE induces systemic hypertension in mice (Yang *et al*., 2008) (but see Ishii *et al*., 2010). We found that inhibitors of CSE inhibited the anti-contractile effects of perivascular fat (Gollasch and Dubrovska, 2009; Schleifenbaum *et al*., 2010). Moreover, we found that the effects of ADRF can be mimicked by synthetic KCNQ channel openers in conditions of reduced H_2S release from perivascular adipose tissue. Furthermore, the H_2S donor NaHS produced dose-dependent vasorelaxation, which was blocked by the KCNQ channel blocker XE991 (Gollasch and Dubrovska, 2009; Schleifenbaum *et al*., 2010). In contrast, the heme oxygenase inhibitors tin mesoporphyrin and zinc deuteroporphyrin IX had no effects on the anti-contractile effects of perivascular fat. Hemin, a substrate of heme oxygenase, induced vasorelaxation of tibial and mesenteric arteries. The effects were not inhibited by XE991 (Schleifenbaum *et al*., 2010). The effects were also neither inhibited by the K_v channel blocker 4-AP nor by the Ca^{2+}-activated large-conductance BK_{Ca} channel blocker iberiotoxin or K_{ATP} channel blocker glibenclamide (Essin *et al*., 2008). Notably, CO generated by heme oxygenase in astrocytes can induce cerebral vasodilation by activating smooth muscle BK_{Ca} channels (Li *et al*., 2008). Nonetheless, the data indicate that H_2S represents a candidate or modulator of ADRF (Figure 1).

Notably, Fang *et al*. (2009) obtained similar results with CSE inhibitors in the rat aorta. In an elegant series of experiments, they measured endogenous H_2S production in the aorta and found that serotonin, phenylephrine and angiotensin II increase H_2S release from periadventitial adipose tissue. Release of H_2S is decreased with aging of rats and can be blocked by CSE inhibitors (Fang *et al*., 2009). We are aware that the H_2S data summarized here are preliminary, and that several weaknesses remain in the methodological approaches (Tangerman, 2009). There are no data on genetically engineered mice available that might define the importance of H_2S that might open KCNQ channels. More studies are needed to demonstrate KCNQ channel activation by H_2S and to determine the molecular mechanisms of channel activation.

Perivadventitial regulation in human arteries

The first demonstration that perivascular adipose tissue mediates an anti-contractile effect in human arteries compared to K^+-induced contraction comes from studies on isolated small mesenteric arteries (Verlohren *et al*., 2004a). Although K_v channel blockade was not tested in this study, the aforementioned data indicate critical roles of K_v channels and H_2S in the periadventitial vasoregulation of this type of arteries. Subsequently, periadventitial adipose tissue was reported to inhibit arterial contraction of human internal thoracic arteries (Gao *et al*., 2004). Significantly, blocking the BK_{Ca} channel by TEA or iberiotoxin attenuated the anti-contractile effects of periadventitial fat in human internal thoracic arteries, suggesting activation of large-conductance, calcium-activated

potassium (BK_{Ca}) channels is functionally relevant, and that there are regional vascular or species differences (Gao *et al.*, 2004). However, there is no direct evidence that membrane hyperpolarization and BK_{Ca} channel opening occurred by the transferable factor released from perivascular fat in internal thoracic arteries. Greenstein *et al.* (2009) were the first to demonstrate a complete loss of the anti-contractile effect of perivascular fat in patients with obesity and metabolic syndrome. In their study, periadventitial vasoregulation has been studied in small arteries taken from subcutaneous gluteal fat biopsy samples (Greenstein *et al.*, 2009). This study showed that healthy adipose tissue around small arteries secretes factors that influence vasodilation by increasing NO bioavailability. Adiponectin was suggested to play a major role in this effect (Greenstein *et al.*, 2009). Notably, in subcutaneous arteries from obese subjects with metabolic syndrome, the dilator effect was lost, suggesting malfunction of adiponectin. Epicardial perivascular fat-derived leptin has been found to exacerbate coronary endothelial dysfunction in a swine model of metabolic syndrome (Payne *et al.*, 2010). These latter findings have been implicated to play a role in 'outside to inside' signalling for perivascular adipose tissue-derived factors in the regulation of coronary tone and pathogenesis of coronary artery disease. Of note, previous studies on adiponectin gene-deficient mice and Zucker *fa/fa* rats (that lack functional leptin receptors) ruled out that adiponectin and leptin are ADRFs in visceral arteries of rats and mice (Fesus *et al.*, 2007; Lohn *et al.*, 2002), despite the fact the adiponectin exhibits humoral vasodilatory properties possibly via activation of 4-AP-sensitive K_v channels (Fesus *et al.*, 2007). Interestingly, no correlation could be detected between perivascular adipose tissue and local endothelial function in conduit skeletal arteries (i.e. the brachial artery) of humans (Rittig *et al.*, 2008), arguing against 'outside to inside' signalling as universal paradigm of perivascular vasoregulation. Together, these data indicate that there are regional vascular and species differences in periadventitial vasoregulation.

Animal models of hypertension and metabolic diseases

Aging and obesity are independent risk factors for hypertension. Spontaneously hypertensive (SHR) rats and New Zealand obese mice are models of lean and obese hypertension respectively. Fang *et al.* (2009) recently showed that in aging normotensive rats, endogenous H_2S production in aortic tissues and isolated perivascular adipose tissue were decreased, while CSE protein expression levels increased. In SHR rats, gene expression of CSE and activity of CSE were suppressed in thoracic aorta, whereas plasma levels of H_2S were decreased (Yan *et al.*, 2004). ADRF also malfunctions in visceral arteries of SHR rats and New Zealand obese mice with increased age (Galvez *et al.*, 2006; Fesus *et al.*, 2007; Galvez-Prieto *et al.*, 2008b). This malfunction has been confirmed by others (Marchesi *et al.*, 2009; Zeng *et al.*, 2009). It does not involve adiponectin (Fesus *et al.*, 2007) but may be improved by atorvastatin, which inhibits the mevalonate pathway or 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase pathway (Zeng *et al.*, 2009). Although CSE expression and endogenous H_2S production have not been studied in perivascular adipose tissue of those animal models, the findings raise the notion that a malfunctioning CSE/H_2S pathway is likely involved in the pathogenesis of hypertension. With the advent of NO, a precedent for novel gas molecules as pivotal signalling pathway regulators is now routinely accepted. We predict the same for H_2S. ADRF, or perhaps its putative targets, might represent exciting new targets for the development of drugs for treatment of cardiovascular and metabolic disorders.

Targeting the amount of perivascular fat

There is evidence that changes in lifestyle, including diet, exercise and nicotine, can affect the paracrine regulation of arterial tone by perivascular fat. Porcine coronary perivascular adipose tissue exhibits blunting of vasoconstrictor responses to endothelin-1 similar to those reported previously in rat aorta and visceral arteries (Reifenberger *et al.*, 2007). This effect is not influenced by diet. However, exercise training abolished the effect of perivascular adipose tissue on endothelin-1-induced vasoconstriction in pigs on normal fat diet but not in the pigs on high fat diet (Reifenberger *et al.*, 2007). Moreover, periadventitial vasoregulation was also studied in Wistar rats on a high-fat diet for 6 months. The results show that rats on high-fat diet had higher periaortic fat mass compared with rats on a chow diet. Interestingly, obesity induced impairment of endothelium-dependent relaxation of the aorta, which was markedly attenuated by temporary periaortic fat removal. (Ma *et al.*, 2010). Furthermore, periadventitial fat can also promote endothelial dysfunction in diet-induced obese C57Bl/6 mice via mechanisms that are linked to increased NADPH oxidase-derived oxidative stress and increased production of pro-inflammatory cytokines (Ketonen *et al.*, 2010). Finally, fetal and neonatal exposure of Wistar–Kyoto rats to nicotine increased the number of adipocytes in aortic perivascular adipose tissue. This increase was associated with blood pressure elevation and reduced periadventitial vasodilation in response to vasoconstrictors (Gao *et al.*, 2008). These data may indicate that there is a reciprocal relationship between the amount of perivascular fat and periadventitial vasorelaxation by perivascular adipose tissue. However, this appears not to be the case. Takemori *et al.* studied using lipoatrophic A-ZIP/F1 transgenic mice. Only a small amount of brown fat was found around the aorta but not around mesenteric arteries. Blood pressure was measured by the tail-cuff method. Blood pressure of A-ZIP/F1 mice became higher than wild-type mice from 10 weeks of age. The presence of perivascular fat reduced the contraction of wild-type aorta to phenylephrine and serotonin, whereas this effect was either absent or less prominent in A-ZIP/F1 aorta. These results suggest that the absence of perivascular fat tissue, which enhances the contractile response of the blood vessels to agonists, and an up-regulation of vascular Ang II type 1 receptors in A-ZIP/F1 mice are some of the mechanisms underlying the blood pressure elevation in these lipoatrophic mice (Takemori *et al.*, 2007). Together, the data indicate that a certain amount of

perivascular adipose tissue is required for optimum periadventitial vasoregulation and blood pressure regulation. Notably, targeting existing vessels in white adipose tissue by a pro-apoptotic peptide to prohibitin caused ablation of white fat in obese mice and rapid obesity reversal and metabolic normalization (Kolonin *et al.*, 2004). Targeted ablation of the vasculature that feeds adipose tissue might not only cause weight loss but also represent a novel therapeutic approach to treat periadventitial vasodysregulation and blood pressure.

Concluding remarks

The focus on vascular research has changed over the past years. Most early functional studies characterized the effects of various agents on arterial smooth muscle and the involved receptor subtypes. The identification of NO as EDRF in the 1980s reoriented vascular research. The endothelium is now considered a paracrine tissue that controls the homeostasis of the underlying smooth muscle cell layer by production of a number of substances. Endothelial dysfunction plays an important role in atherosclerosis. Recent studies proposed the concept of paracrine role for periadventitial adipose tissue in the regulation of vascular tone (Gollasch and Dubrovska, 2004). This regulation involves ADRF, which is released by periadventitial adipocytes and produces vasorelaxation by opening K_v channels in smooth muscle cells. The H_2S findings as candidate ADRF warrant diligent pursuit. We believe that there is more to ADRF than H_2S; further research is necessary. Adipocyte-derived factors and their targets, including KCNQ channels, could represent novel drug targets for regulating vascular tone and for the treatment of obesity-associated cardiovascular disorders.

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Conflicts of interest

There is no conflict of interest.

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