Abstract—Perivascular adipose tissue has been recognized unequivocally as a major player in the pathology of metabolic and cardiovascular diseases. Through its production of adipokines and the release of other thus far unidentified factors, this recently discovered adipose tissue modulates vascular regulation and the myogenic response. After the discovery of its ability to diminish the vessel’s response to vasoconstrictors, a new paradigm established adipose-derived relaxing factor as a paracrine smooth muscle cells’ potassium channel opener that could potentially help combat vascular dysfunction. This review will discuss the role of adipose-derived relaxing factor in vascular dysfunction in obesity and hypertension, the different potassium channels that can be activated by this factor, and describes new pharmacological tools that can mimic the adipose-derived relaxing factor effect and thus can be beneficial against vascular dysfunction in cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: adipose tissue◼ KCNQ potassium channels

Blood vessels are dynamic miniorgans, which have several mechanisms of regulation. The old paradigm described the endothelium as a main regulator of vascular homeostasis through the release of vasoregulators.1 Twenty-five years ago, Soltis and Cassis2 introduced a new prototype of vessel regulation by demonstrating a decreased response of rat thoracic aorta rings to the vasoconstrictor norepinephrine in the presence of the perivascular fat. These pioneering studies, which reversed the trend to disregard adipose tissue around vessels, led to the eventual discovery of adipocyte-derived relaxing factor (ADRF).3–5 ADRF has been shown to inhibit the vasoconstriction of several humoral agents; however, its identity is still under study and it is likely that >1 factor (molecule) represents ADRF. In obesity and hypertension, the actions of ADRF seem to be disturbed, thus contributing to a periadventitial vascular dysfunction.6–8 This is indeed important in humans because severely obese patients who undergo bariatric surgery have a great improvement in the function of their perivascular adipose tissue (PVAT).9 The mechanism of action of ADRF relies on the opening of smooth muscle K+ channels, in particular, members of the voltage-gated (Kv) K+ family.1 Furthermore, recent studies have helped to further delineate subclasses of the Kv family as the potential critical mediators of the ADRF effects, namely Kv channels, which display pharmacological properties of (Kv7) KCNQ channels.5 In addition, pharmacological openers of KCNQ channels mimic the effects of ADRF in a hypertensive rat model (spontaneously hypertensive rats) as well as in the New Zealand Obese mouse model of metabolic syndrome.6 This short review will highlight the different mediators released from PVAT, which have an influence on the vascular tone of the arterial blood vessels. It will also give an update on ADRF, the channels downstream of its release, and discuss the pharmacological tools that could represent a future therapy for improvement of periadventitial vascular dysfunction.

PVAT Displays Anticontractile Properties Through ADRF

Adipose tissue is now recognized as an endocrine organ, which releases several adipokines and cytokines to metabolically regulate different cells and tissues.10 PVAT plays a role in nonshivering thermogenesis and metabolism of fatty acids,11 inflammation, vascular morphology, and smooth muscle cell proliferation.12–14 PVAT, similarly to white and brown adipose tissues, also secretes many adipokines in the circulation, which potentially participate in the regulation of vascular tone of certain blood vessels11,15 (see Figure). A few of these adipokines, notably adiponectin,16 angiotensin-1 to 7, and methyl
Adipokines secreted by perivascular adipose tissue and their vasoactive properties in organ bath experiments. Ang 1-7 indicates angiotensin-1 to 7; ROS, reactive oxygen species; and TNF-α, tumor necrosis factor-α.

PVRF: Potential Candidates

Several studies have tried to pinpoint the nature of PVRFs. The role of adiponectin as PVRF has been thus far inconclusive. Vessels from the aorta and mesenteric arteries of adiponectin-knockout mice have been initially shown to maintain their anticontractile properties. In contrast, recent studies in mesenteric vessels of wild-type mice advance that the vasorelaxing and hyperpolarizing effects of adiponectin rely on the opening of maxi Ca²⁺-activated K⁺ (BKCa) channels. In addition, a factor that might be adiponectin is released after stimulation of rat and murine mesenteric arteries with β3 adrenoceptor agonist. This latter factor seems to exert its paracrine effect by activation of AMPK and opening of BKCa channels. Similarly to adiponectin, leptin has also been proposed as a candidate for PVRF in a hypertension mouse model. Leptin, however, is perhaps not ADRF in the systemic circulation because the PVAT of Zucker fa/fa rats that lack the leptin receptor still display anticontractile properties. As mentioned above, angiotensin-1 to 7 and methyl palmitate are also proposed as PVRF. A study using aortas from Mas (angiotensin-1–7 receptor)–knockout mice observed reduced anticontractile effects of PVAT in rat aorta and vena cava and thus highlighted the importance of angiotensin-1 to 7 as a potential PVRF. The angiotensin-1 to 7 effect was, however, blocked by the nonspecific BKCa channel blocker tetraethylammonium or a combination of charybdotoxin and apamin (IKCa/SKCa channels), suggesting that KCa channels are involved. However, interpretation of data with tetraethylammonium is difficult because tetraethylammonium is also a relatively potent inhibitor of KCNQ channels. An interesting recent study using a new superfusion bioassay cascade technique demonstrated that palmitic acid methyl ester could also be PVRF. This molecule released from the PVAT of Wistar Kyoto rats caused relaxation of precontracted Sprague–Dawley rat aortic rings in a calcium-dependent manner. This relaxation is inhibited by the KCa channel blocker 4-aminopyridine, which indicates a critical role of voltage-gated K⁺ channels on smooth muscle cells in the action of PVRF1 vide infra. Of note, 4-aminopyridine partially inhibits PVAT-dependent relaxation of human internal thoracic artery. A recent study identified an indoleamine 2,3-dioxygenase metabolite (kynurenine) primarily in brown (but not white) fat surrounding the thoracic aorta to depress aortic contractility. One of the best candidates described thus far for PVRF/ADRF is the cystathionine-γ-lyase-derived H₂S, although it exhibits differential effects in the mouse and the rat. In effect, our group and Fang et al. have demonstrated that inhibitors of cystathionine-γ-lyase significantly reduced the anticontractile effect of PVAT in rat aortic rings, whereas murine mesenteric arteries and aorta were not affected. Interestingly, vasoconstrictors as well as the statin atorvastatin seem to increase the release of H₂S from the PVAT in rats, thus increasing its anticontractile effect. Importantly, NaHS, a H₂S donor, can cause a dose-dependent relaxation that is inhibited by treatment with the KCNQ (Kv7) channel blocker XE991. The cystathionine-γ-lyase inhibitor β-cyano-γ-alanine had no effect on coronary flow, which might be explained by organ differences or low-dose β-cyano-γ-alanine used in this study. Altogether, these results paint an interesting picture of PVRF, which most likely is a combination of several different molecules.

KCNQ Channels as Putative Downstream Targets of PVRF

Although the research is still undergoing to fully uncover the nature of PVRF, its mechanism of action through smooth muscle K⁺ channels has been well documented. In large vessels, Löhn et al. had demonstrated a suppression of the anticontractile effect of PVAT in the presence of high extracellular K⁺, suggesting a requirement for the opening of vascular smooth muscle K⁺ channels. Pharmacological manipulations using glibenclamide indicated involvement of both ATP-dependent K⁺ channels (KATP) and Kᵣ channels. Recent
quantitative reexamination of this data, however, showed that glibenclamide uses a nonspecific, PVRF-independent mechanism to inhibit the anticontractile effect of PVAT. The new paradigm suggests a major role of K,–KCNQ family of K+ channels as putative downstream targets of ADRF and possibly other PVRFs. This hypothesis is supported by the blockade of the anticontractile effects of PVAT by XE991 (KCNQ blocker) in rat aortas. Similar to the aorta, smaller visceral and skeletal muscle arteries, which are important in the regulation of peripheral vascular resistance, rely on opening of XE991-sensitive K+ (KCNQ) channels to mediate the paracrine effect of ADRF. In both mice and rat models of cardiovascular disease, notably obesity and hypertension, inhibition of K+ channels using 4-aminopyridine or 3,4 aminopyridine also abrogated the anticontractile effects of PVAT. Interestingly, recent data showed that XE991 also blocked this effect in rat and mouse mesenteric arteries, suggesting an important role of KCNQ channels in the small vessels as well. It is important to note that mechanisms of perivascular dysfunction could be different in obesity and hypertension. In obesity, inflammation and adipocyte hypertrophy leading to hypoxia are reported to function as main drivers of perivascular dysfunction. In hypertension, however, a reduction of perivascular fat around arteries, accompanied with reduced adipocytes size, and decreased total lipid as well as leptin content seem to be the catalysts. These factors seem to contribute to the reduced anticontractility of PVAT on the mesenteric arterial tone via K+ channels. A recent study by Zavaritskaya et al illustrated the importance of KCNQ-type K+ channels in the anticontractile effects of PVAT in skeletal muscle arteries. This study showed that the KCNQ blockers XE991 and linopirdine significantly blocked the anticontractile effects of PVAT as well as K+ currents in isolated smooth muscle cells of spontaneously hypertensive rats’ Gracilis muscle arteries. Consistent with this concept, therapeutic use of KCNQ channel openers (Table) was successful in restoring the diminished anticontractile effects of PVAT in the spontaneously hypertensive rats and New Zealand Obese mice. These openers also lowered systemic arterial blood pressure in both animal models. Interestingly, these openers had been previously shown by Schleifenbaum et al to cause relaxation of aortic and mesenteric artery vessels despite H,SO, blockade using β-cyano-Lalanine. This study thus illustrates that the perivascular tissue can become dysfunctional in different cardiovascular disease states and that XE991-sensitive K+ (KCNQ) channels are important players in this process.

Conclusions
Perivascular dysfunction is now recognized as an important player in the pathology of hypertension and obesity. The discovery of transferable PVRFs as anticontractile factors in small and large vessels provides an opportunity to resolve this dysfunction. The recent description of KCNQ channel openers as mimics of the PVRF effect is a new approach to target vascular dysfunction in hypertension and will hopefully lead soon to the development of drugs to provide relief to millions of patients with chronic vascular disease and could potentially be important in other diseases such as atherosclerosis.

Significance
ADRFR and other similar factors released by the PVAT have been shown to mediate the contractility of vessels of different sizes and vascular beds. Perivascular tissue similarly to the endothelium can undergo perivascular dysfunction, which contributes to the pathophysiology of vascular diseases. Uncovering the pathways downstream of these factors and new pharmacological tools to improve perivascular dysfunction is thus imperative in the search for cure of vascular and vasculature-related diseases.

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