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Reduced anti-contractile effect of perivascular adipose tissue on mesenteric small arteries from spontaneously hypertensive rats: Role of Kv7 channels

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ABSTRACT

Perivascular adipose tissue (PVAT) has been shown to produce vasoactive substances and regulate vascular tone. This function of PVAT has been reported to be altered in hypertension. However, the underlying mechanisms are not fully understood. In this study we used age-matched normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) as well as Sprague-Dawley rats and tested effects of PVAT on mesenteric small arteries. Vessels were mounted in a Mulvany–Halpern myograph and cumulative concentration–response relations to noradrenaline were determined in the presence or absence of PVAT. We found that PVAT has an anti-contractile effect on mesenteric small vessels, irrespective of strains. A reduced effect of PVAT was observed in SHR compared to WKY rats; the difference between strains was eliminated by 10 μM XE991, a blocker of Kv7 (KCNQ) voltage-dependent potassium channels. The anti-contractile effect of PVAT was not affected by depolarizing smooth muscle cells with high K^+ solution. Sensitivities to exogenous vasodilators acetylcholine or sodium nitroprusside were not potentiated but reduced in vessels with PVAT. Our results suggest that the reduced anti-contractile effect of PVAT in SHR correlates with a deficiency in Kv7 channels. Diffusion hindrance of PVAT is also a factor that should be considered in investigations on rat mesenteric small arteries.

1. Introduction

Adipose tissue is an active endocrine organ that may produce a variety of vasoactive substances and thus regulate vascular tone (Gollasch and Dubrovska, 2004; Maenhaut, 2011). The first evidence of an anti-contractile effect of perivascular adipose tissue (PVAT) was obtained 20 yrs ago on rat aortic rings by Soltis (1991). More recent studies have suggested that a transferable adipocyte-derived relaxing factor (ADRF) causes vasorelaxation by activating K^+ channels on the smooth muscle cells (Löhn et al., 2002; Verlohren et al., 2004; Lee et al., 2011). This appears to be a direct action, not involving NO synthesis or endothelium (Löhn et al., 2002). In aorta the effect of PVAT (which contains brown fat; Gálvez-Prieto et al., 2008) seems to be mediated primarily via ATP-dependent K^+ channels (Löhn et al., 2002), while in mesenteric small arteries (surrounded by white adipose tissue; Gálvez-Prieto et al., 2008) the effect has been shown to depend on hyperpolarization due to activation of voltage-dependent K^+ channels (Kv channels) (Verlohren et al., 2004; Mackie et al., 2008; Greenwood and Ohya, 2009). Furthermore, different mediators released from perivascular fat have been suggested (Lee et al., 2009; Schleifenbaum et al., 2010; Lee et al., 2011) possibly dependent on the vascular bed under investigation. Recent studies on mesenteric arteries suggest that the ADRF from mesenteric PVAT is hydrogen sulfide (H_2S) (Cheng et al., 2004; Tang et al., 2006). This gaseotransmitter targets a specific subtype of Kv channels in vascular muscle, Kv7 (related to the KCNQ gene) (Fang et al., 2009; Schleifenbaum et al., 2010; Gollasch, 2012).

An anti-contractile effect of PVAT opens the possibility that alteration of this function, whether primary or secondary, may contribute to the increased blood pressure in hypertension. It has been reported that the size of adipocyte in mesenteric bed is significantly smaller in the spontaneously hypertensive rat (SHR) than age matched normotensive Wistar-Kyoto (WKY) rats with no change in number and type of adipocyte between strains (Gálvez et al., 2006). Furthermore, the anti-contractile effect of PVAT seems reduced in SHR compared to WKY rats (Gálvez et al., 2006; Lu et al., 2011). Since a recent study has indicated a reduction in expression and function of vascular Kv7 channels in SHR (Jepps et al., 2011) we have here examined the anti-contractile effect of PVAT in mesenteric small arteries from normotensive and hypertensive rats with special emphasis on Kv7 function.

2. Materials and methods

2.1. Materials and preparation

Weight-matched (250–400 g, male) normotensive Wistar-Kyoto (WKY rats, Charles River, Germany and Taconic, USA) and

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spontaneously hypertensive rats (SHR, Charles River, Germany and Taconic, USA) as well as Sprague-Dawley rats (Charles River, Germany) were used. Where a direct comparison between strains was not necessary, experiments were performed on Sprague-Dawley rats. Animal handling complied with the guidelines of the Swedish Central Council for Laboratory Animals and was approved by the local ethical committee for animal research. Animals were euthanized with carbon dioxide. The mesenteric bed was quickly removed and transferred into cold physiological saline solution (PSS) with composition (in mM): NaCl, 119; KCl, 4.7; $NaHCO_3$, 25; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; ethylenediaminetetraacetic acid, 0.026; glucose, 5.5. Segments of 2 mm length were taken from the first- or second-order branches of superior mesenteric arteries. For clean vessels, all PVAT and connective tissues were carefully dissected away from arteries. For intact PVAT vessels, about 40% of boundary PVAT was removed from each side of the vessels to facilitate following experimental procedure. In loose PVAT experiments, perivascular fat was longitudinally removed along the arteries and stored in 37 °C PSS bubbled with 5% CO_2 in O_2 (for later use). In these experiments, care was taken to use artery segments of equal length (close to 2 mm) and the amount of PVAT applied was estimated from blotted weights at the end of experiments. These weights were 9.3 ± 0.56 mg (WKY rats) and 8.7 ± 0.63 mg (SHR).

All chemicals were purchased from Merck KGaA (Darmstadt, Germany) and drugs were from Sigma Chemicals (St. Louis, MO, USA). High K^+ physiological saline (KPSS) had a similar composition as PSS except that NaCl was exchanged with equimolar KCl.

2.2. Isometric contractions

Vessels were threaded onto two stainless-steel wires, mounted in a Mulvany–Halpern myograph (Danish Myo Technology, Aarhus, Denmark), and stored in chambers filled with 5 ml oxygenated PSS (95% O_2–5% CO_2) at 37 °C for 45 min before further manipulation (Mulvany and Halpern, 1976). Pretension for arteries was then set to close where the vessel produced maximal active tension (Mulvany and Halpern, 1977). After 60 min equilibration, the arteries were activated three times by KPSS and 10 μM noradrenaline, 2 min each time with 5 min interval. The tension caused by the third activation was considered as maximum tension (100%), and all tensions measured during experiments were expressed as percentage of this reference value. Sensitivities to vaso-activators were determined as cumulative concentration–response relations with each concentration being applied for 2 min.

Pairs of animals were used in loose PVAT experiments where two clean vessels from WKY rats and two from SHR were mounted on the myograph. Noradrenaline sensitivity was first determined in the two types of vessel. Adipose tissue from WKY rats and SHR was then carefully applied around arteries to provide the combinations: WKY rat fat+WKY rat vessel; WKY rat fat+SHR vessel; SHR fat+WKY rat vessel; SHR fat+SHR vessel. Noradrenaline sensitivity was determined again and compared with the first one. In intact PVAT experiments, two clean vessels and two intact vessels were taken from the same animal. Noradrenaline sensitivity was compared between the two groups of vessel.

All drugs were applied by adding them to the bath solution. A concentration of 10 μM was chosen for 10,10-bis(4-pyridinylmethyl)-9(10 H)-anthracenone (XE991), because its specific effect on Kv7 channels is maximal at 10–20 μM and a higher concentration will no longer be selective for Kv7 channels (Wladyka and Kunze, 2006; Greenwood and Ohya, 2009; Zhong et al., 2010; Ipavec et al., 2011). Corticosterone was used at a concentration of approximately fourfold its IC_{50} (Martel et al., 1993). XE991 and corticosterone were applied 30 min and 15 min, respectively, before determining the second noradrenaline concentration–response relations and maintained in the bath for the rest of experiments.

Denervation was conducted in vitro by applying 6-hydroxydopamine, 450 μg/ml for 15 min, followed by 2 h rest in PSS (Aprigliano and Hermsmeyer, 1976). After three times of preactivation, KPSS was applied to all vessels to induce depolarization and noradrenaline sensitivity was then determined.

Measurement of H_2S production was performed using H_2S-selective electrodes (ISO-H_2S-2, WPI Inc., Sarasota FL, USA) and an Apollo 1000 amplifier (WPI Inc.) The electrode was calibrated according to the manufacturer's instructions. For measurements in adipose tissue, mesenteric perivascular fat was dissected free, suspended in 0.5 ml oxygenated PSS containing 10 mM HEPES and heated to 37 °C for 20 min, after which the H_2S concentration in the supernatant was measured. The tissue was subsequently blotted and its wet weight determined.

To test concentration–response relations to exogenous vasodilators, acetylcholine and sodium nitroprusside, a thromboxane receptor agonist 9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619), 500 nM, was used to establish the precontraction reference level (100%), which usually produced a more stable baseline than noradrenaline activation.

2.3. Statistics

All values were given as mean ± SEM Student's *t*-tests or ANOVA were used for statistical analysis as appropriate (Graphpad Prism). A value of $P < 0.05$ was considered as statistical significance; *n* represents the number of arteries.

3. Results

3.1. Effect of intact PVAT on vessels from SHR and WKY rats

We first examined the responses of arteries from SHR and WKY rats with PVAT in place (intact vessels) and compared to arteries with PVAT removed (clean vessels). The data are shown in Fig. 1A. Vessels with intact PVAT had a markedly reduced sensitivity to exogenous noradrenaline compared to clean vessels as revealed by a right-shift of the concentration–response relations. This shift signifies an anti-contractile effect of PVAT in both rat strains. Moreover, the effect was less pronounced in SHR than in WKY rat vessels: Δ(log EC50) was 1.09 ± 0.08 ($n=4$) in SHR vs. 1.54 ± 0.13 ($n=5$) in WKY rats ($P=0.008$) (Fig. 1B). Thus the anti-contractile effect of intact PVAT was smaller in SHR than in WKY rats.

3.2. Effect of XE991 on anti-contractile effect of intact PVAT

As seen in Fig. 2A, 10 μM Kv7 channel blocker XE991 did not affect the concentration–response relation to noradrenaline of clean vessels: the changes of log EC50 before and after treatment with XE991, Δ(log EC50), were in WKY rats 0.019 ± 0.06 and in SHR 0.046 ± 0.01. In intact vessels from WKY rats, however, the anti-contractile effect of PVAT was partially antagonized: Δ(log EC50) was 0.38 ± 0.14 ($n=5$, $P=0.03$), while no blocking effect of XE991 was seen in intact vessels from SHR: Δ(log EC50) was 0.13 ± 0.11 ($n=4$, $P=0.46$). Thus, XE991 eliminated the difference in anti-contractile effect of PVAT between SHR and WKY rats (Fig. 2B).