Original Article

A Reduction in the Amount and Anti-Contractile Effect of Periadventitial Mesenteric Adipose Tissue Precedes Hypertension Development in Spontaneously Hypertensive Rats

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The aim of this study was to determine whether alterations in periadventitial adipose tissue and its anti-contractile effect precede hypertension development. We used 4-week-old male Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR), which were pre-hypertensive. Vascular function was studied in the perfused mesenteric bed (MB, 1.5 mL/min). MB weight was lower in SHR (8.0 ± 0.3 mg/g body weight) than in WKY (9.0 ± 0.3 mg/g body weight) rats. Concentration-response curves to KCl (6 to 75 mmol/L) and to acetylcholine (10^{-9} to 10^{-5} mol/L) were similar between groups. Contractile responses to serotonin (10^{-9} to 10^{-5} mol/L) were significantly higher in SHR compared to WKY. 4-Aminopyridine (4-AP, 2 mmol/L), a blocker of K_v channels, induced a similar increase in perfusion pressure in both strains. However, 4-AP (2 mmol/L) significantly increased the contractile response to serotonin (10^{-9} to 10^{-5} mol/L) only in WKY. The anti-contractile effect of fat was confirmed by a comparison of (+) fat and (–) fat mesenteric arteries, which revealed that 4-AP significantly enhanced contractions only in (+) fat rings from WKY. These results show that alterations in visceral periadventitial fat mass and function in SHR precede hypertension, suggesting a constitutive mechanism independent of age and the hypertensive state. (*Hypertens Res* 2008; 31: 1415–1423)

Key Words: periadventitial adipose tissue, adipocyte-derived relaxing factor, mesenteric arteries, spontaneously hypertensive rat, prehypertension

Introduction

Several recent studies have revealed a paracrine role for periadventitial adipose tissue in the regulation of vascular func-

tion. Soltis and Cassis (*1*) were the first to demonstrate that periadventitial fat significantly attenuates vascular responsiveness of rat aortic rings to norepinephrine. More recent reports have confirmed the inhibitory action of periadventitial fat on the contractile response to a variety of vasoconstrictors

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on rat aortic (2, 3) and mesenteric arteries (4, 5), as well as on human thoracic arteries (6). The anti-contractile action is induced by a still undefined transferable factor released by periadventitial fat, called adipocyte-derived relaxing factor (ADRF) (2). Its anticontractile effect seems to be mediated by two different mechanisms, *i.e.*, an endothelium-dependent relaxation mediated through nitric oxide release and subsequent calcium-dependent K^+ channel activation (3) and an endothelium-independent effect mediated either by H_2O_2 (3) or by the activation of different types of K^+ channels depending on the type of vascular bed and species (2, 4–6). In rat mesenteric arteries, ADRF induces an endothelium-independent relaxation through the activation of voltage-dependent K^+ channels (K_v) (4, 5). Interestingly, the anti-contractile effect in these vessels is directly dependent on the amount of periadventitial fat (4, 5). The mesenteric vascular bed, which significantly contributes to total peripheral vascular resistance, is surrounded by a considerable amount of adipose tissue. Since this tissue inhibits vascular tone of mesenteric arteries *via* ADRF release and K_v channels activation, changes in the amount of fat might have consequences for the regulation of mesenteric artery tone and thus systemic arterial blood pressure. In fact, abdominal visceral fat mass has been correlated with the prevalence of hypertension (7). In this context, our group has recently described that 3-month-old spontaneously hypertensive rats (SHR) have a lower amount of mesenteric adipose tissue (5). This correlates with a reduced anti-contractile effect of periadventitial fat that might be attributed to the reduced release of ADRF (5). The aim of this work was to determine if these changes in periadventitial fat observed in SHR precede hypertension. We used 4-week-old SHR and Wistar Kyoto (WKY) rats, which display little differences in blood pressure at this age (8–12). Under these conditions, we considered that a comparison of the effect of periadventitial adipose tissue on vascular function might reveal functional alterations in SHR previous to the establishment of hypertension. For this purpose, we analyzed 1) the amount of mesenteric adipose tissue and 2) the effect of 4-aminopyridine (4-AP) in the perfused mesenteric bed and in isolated mesenteric artery rings with and without fat from 4-week-old WKY and SHR.

Methods

Animals

Experiments were conducted in 4-week-old (28 ± 2 d) male WKY and SHR (Charles River, Barcelona, Spain) maintained under controlled light (12 h light cycles from 8 AM to 8 PM) and temperature (22–24°C) conditions. The rats were fed with standard rat chow (A.04; Panlab, Barcelona, Spain) and had free access to tap water. The Institutional Animal Care and Use Committee approved all experimental procedures according to the guidelines for ethical care of experimental

animals of the European Community. Systolic blood pressure (SBP) was recorded directly by femoral artery cannulation in a group of animals under anesthesia (sodium pentobarbital 50 mg/kg). Since anesthesia might influence adipose tissue metabolism and plasma lipidic parameters, another group of animals was killed by decapitation. To avoid lipid mobilization and lipolysis induced by fasting, free-feeding animals were sacrificed at 10 AM. Unpublished observations of our group indicate that overnight fasting significantly reduces mesenteric perivascular adipose tissue. Blood was collected in EDTA-coated tubes, then centrifuged and the plasma frozen until biochemical determinations. The mesentery was rapidly separated from the intestine and weighed. The mesenteric bed volume was determined plethysmographically by measuring the displacement of a saline solution (NaCl 0.9%) in a plethysmometer (LI 7500; Cibertec, Madrid, Spain). Wet weight and volume were expressed as body weight ratio. Then, the mesentery was placed in warm (37°C) oxygenated (95% O_2 and 5% CO_2) physiological salt solution (PSS) for functional studies.

Plasma Analysis

Glucose (Glucose Trinder Method; Roche Applied Science, Indianapolis, USA), triglycerides (GPO Method; Biolabo, Maizy, France), cholesterol (CHOD/PAP Method; Roche Applied Science) and glycerol (GPO Trinder Method; Roche Applied Science) were measured by enzymatic colorimetric tests as specified by the manufacturers. Leptin was determined by using a specific ELISA kit for rat leptin (Assay Designs, Inc., Ann Arbor, USA). The obtained values were within the detection range of the assays.

Determination of Adipose Noradrenaline Turn-over

Both noradrenaline (NA) and 3,4-dihydroxymandelic acid (DOMA) were quantified by coulometric determination. Briefly, samples of lumbar adipose tissue (100 mg) were homogenized in 500 μ L of buffer (0.1 mol/L phosphate, pH 2.5, containing 15% methanol). Samples were sonicated 3 times for 5 s at a potency of 30 W (Sonics Vibracell; Sonics, Newton, USA) and then centrifuged at 14,500 rpm for 10 min. The supernatant was collected and loaded (20 μ L) in a chromatograph (Shimadzu SIL-10A; Shimadzu, Kyoto, Japan) coupled to a coulometric detector (ESA Coulochem II; ESA, Bedford, USA) and equipped with a Nucleosil C_{18} column (Nucleosil, 20 $\times$ 0.4 cm; Scharlau, Barcelona, Spain). Catecholamines were eluted by using a buffered solution (0.05 mol/L Na_2HPO_4 , 0.03 mol/L citric acid, 0.1 mmol/L EDTA, 0.035% octyl sodium sulphate, 4.5% methanol). Both NA and DOMA were quantified by comparison with the area of calibrated standards.

Table 1. Animal and Adipose Tissue Characteristics in 4-Week Old (28±2 d) Male WKY and SHR

	WKY	SHR
Rat body weight (g)	69.0±3.2	62.0±2.5
SBP (mmHg)	88±5	99±4
Mesenteric weight (mg)	600±26	485±30*
Mesenteric volume (mL)	0.628±0.025	0.556±0.014*
Mesenteric density (g/L)	0.94±0.10	0.96±0.03
Lumbar adipose tissue (mg)	139.0±3.0	44.0±0.6*

Data are expressed as mean±SEM. Number of data $n=16-18$ animals per strain. * $p<0.05$ between groups. WKY, Wistar Kyoto rat; SHR, spontaneously hypertensive rat; SBP, systolic blood pressure.

Vascular Reactivity in Mesenteric Vascular Beds

The mesenteric vascular bed was perfused as previously described (13). Briefly, the superior mesenteric artery was cannulated at its junction with the abdominal aorta, put on a platinum grid in an organ chamber, and perfused using a peristaltic pump (Ismatec, Wertheim, Germany) at constant flow (1.5 mL/min) with an oxygenated (95% O₂ and 5% CO₂) PSS of the following composition: 115 mmol/L NaCl, 4.6 mmol/L KCl, 2.5 mmol/L CaCl₂, 25 mmol/L NaHCO₃, 1.2 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄, 0.01 mmol/L EDTA and 11 mmol/L glucose, and 3×10^{-6} mol/L dexamethasone to avoid induction of inducible nitric oxide synthase. Perfusion pressure was continuously determined by a pressure transducer (Grass Instruments, Quincy, USA) and recorded on a polygraph (Grass Instruments) for the rest of the experiment. A bubble trap system removed any air bubbles in the perfusate. Since flow was maintained at a constant rate, changes in perfusion pressure were used as an index of changes in the resistance of the arteries, and a rise or a decrease in the perfusion pressure indicated either vasoconstriction or vasodilatation, respectively. After an equilibration period of 60 min and before starting the experiment, the vascular mesenteric bed was contracted with KCl 75 mmol/L to assess its contractility. Subsequently, the mesentery was contracted with phenylephrine (10^{-5} mol/L), and acetylcholine (ACh, 10^{-9} to 10^{-5} mol/L) was added to functionally confirm the presence of the endothelium.

Vascular Reactivity in Isolated Mesenteric Arteries

The superior mesenteric artery was cut into 2-mm segments. Periadventitial fat and connective tissue were left intact, in the case of (+) fat rings, or removed with scissors for (–) fat rings (4, 5). Rings were mounted in PSS buffer at 37°C in a small vessel myograph (Danish Myotechnology, Aarhus, Denmark), at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg (4, 5). Segments were

Table 2. Metabolic and Hormonal Parameters of 4-Week Old (28±2 d) WKY and SHR

	WKY	SHR
Plasma glucose (mg/dL)	142.0±5.8	139.0±9.6
Plasma triglycerides (mg/dL)	42.1±5.8	50.2±54.6
Plasma glycerol (μmol/L)	194±22	195±14
Plasma total cholesterol (mg/dL)	53.1±5.5	55.1±4.0
Plasma leptin (ng/mL)	4.4±0.9	1.9±0.1*

Data are expressed as mean±SEM of 8–10 determinations per strain. * $p<0.05$ between groups. WKY, Wistar Kyoto rat; SHR, spontaneously hypertensive rat.

threaded onto two stainless wires and suspended in 5-mL microvascular myograph baths (Danish Myotechnology) according to Mulvany and Halpern (14). The arteries were equilibrated for 60 min, and then they were exposed to isomolar 60-mmol/L KCl-containing solution (KPSS) to ensure their viability and contractility. KPSS was similar to PSS except that the NaCl was exchanged for equimolar KCl. After washing in PSS, the effects of 4-aminopyridine (4-AP, 2 mmol/L) on basal vessel tone were investigated. Tension was expressed in mN, since the contractile responses to 60 mmol/L KCl (KPSS) were not different among the four groups. The contractile responses to 60 mmol/L KCl were 12.4 ± 1 , 13.8 ± 1 , 11.0 ± 1 and 12.1 ± 1 mN in (–) fat rings of WKY rats, (+) fat rings of WKY rats, (–) fat rings of SHR rats, and (+) fat rings of SHR rats, respectively.

Statistical Analysis

All values are given as the mean±SEM. Student's *t*-tests or ANOVA were used as appropriate. A value of $p<0.05$ was considered statistically significant; *n* represents the number of animals

Results

Animal and Adipose Tissue Characteristics

Characterization of the animals (Table 1) showed that body weight and SBP (measured under pentobarbital anesthesia) were similar in 4-week-old WKY and SHR, confirming that at this age hypertension is not yet established in SHR. The mesenteric bed wet weight and volume were significantly lower in SHR compared to WKY, whereas the mesenteric bed density was similar between strains. The lumbar fat pad was also significantly lighter in SHR, revealing a reduced amount of visceral adipose tissue in SHR rats.

Biochemical Characteristics

Table 2 summarizes the plasma biochemical values in WKY and SHR. Plasma extraction was performed in the morning in

Fig. 1. Effect of A: 2 mmol/L 4-aminopyridine (4-AP) and B: KCl (6 to 75 mmol/L) on basal perfusion pressure in the perfused mesenteric bed from WKY and SHR. Contraction is expressed as an increase in perfusion pressure in mmHg. Data are shown as the mean $\pm$ SEM of 6 determinations per strain.

free-feeding animals, and it can be assumed that the values obtained are in the range of postprandial concentrations. As shown, plasma analysis revealed similar concentrations of glucose, triglycerides, glycerol, and cholesterol in both strains. However, plasma leptin was significantly reduced in SHR, showing a positive correlation with the amount of visceral adipose tissue ($p < 0.05$).

Determination of Adipose Noradrenaline Turn-over

Because a reduction in the amount of adipose tissue detected in SHR might be linked to an elevated sympathetic outflow (15, 16) and thereby to enhanced lipolysis (17, 18), NA turnover was determined. There was no significant difference in the DOMA/NA ratio between WKY and SHR (WKY: 0.24 ± 0.04 vs. SHR: 0.22 ± 0.04 ; $n = 5$ each).

Vascular Reactivity of the Perfused Mesenteric Bed

We first used the whole perfused mesenteric bed to assess whether the reduced amount of mesenteric adipose tissue found in prehypertensive SHR influences mesenteric tone. Basal perfusion pressure at 1.5 mL/min was similar between strains (WKY: 6.9 ± 0.6 mmHg; SHR: 7.1 ± 0.6 mmHg). The increase in perfusion pressure induced by 75 mmol/L KCl was similar in both groups (WKY: 38 ± 5 mmHg; SHR: 42 ± 5 mmHg), suggesting that there was no difference in the contractile capacity of the vascular bed.

Since mesenteric fat regulates arterial function by means of ADRF release and a subsequent activation of K_v channels (4, 5), we first analyzed the effect of 4-aminopyridine (4-AP), a K_v channel inhibitor, on basal mesenteric perfusion pressure.

The increase in perfusion pressure induced by 2 mmol/L 4-AP was not different between WKY and SHR (Fig. 1A), indicating that there are no differences in basal release of ADRF at low transmural pressure. To discard an eventual difference in the ability of arterial smooth muscle to respond to a putative hyperpolarizing factor released from adipose tissue, we also analyzed contractions elicited by KCl (6 to 75 mmol/L). Concentration-dependent responses to KCl were similar between the strains (Fig. 1B), suggesting that the basal activity of potassium channels is not different between SHR and WKY at this age.

Basal nitric oxide (NO) production was analyzed using a nonspecific inhibitor of NO synthase, L-NAME. L-NAME (0.1 mmol/L) induced an increase in perfusion pressure that was not different between the two groups of animals (WKY: 0.54 ± 0.1 mmHg [$n = 5$]; SHR: 0.82 ± 0.3 mmHg [$n = 6$]). Application of acetylcholine (10^{-9} to 10^{-5} mol/L) produced a similar decrease in perfusion pressure in WKY and SHR mesenteric beds in the absence (Fig. 2A) and presence of L-NAME (Fig. 2B). This indicates that endothelium-dependent relaxation in the perfused mesenteric bed is mainly due to the release of endothelium-derived hyperpolarizing factor (EDHF) (19–22) and that the sensitivity to EDHF is not different between the two strains. In addition, the level of endothelium-independent vasodilatation induced by sodium nitroprusside (10^{-9} to 10^{-5} mol/L) was similar in the two groups (Fig. 2C).

In order to assess the anti-contractile effect of periaortic fat on the response to contractile agents, concentration-response curves to serotonin (5-HT) were generated in the absence and in the presence of 2 mmol/L 4-AP. In the absence of 4-AP, the contraction to 5-HT was significantly higher in SHR ($E_{\max} = 12 \pm 2$ mmHg) than in WKY ($E_{\max} = 2.0 \pm 0.5$ mmHg). Preincubation with 2 mmol/L 4-AP significantly

Fig. 2. Vasodilatory response to A: ACh (10^{-9} to 10^{-5} mol/L) alone, B: ACh in the presence of L-NAME (10^{-4} mol/L) and C: sodium nitroprusside (SNP, 10^{-9} to 10^{-5} mol/L) in the perfused mesenteric bed from WKY and SHR. Relaxation is expressed in percentage of the previous contraction elicited by phenylephrine (10^{-5} mol/L). Data are shown as the mean $\pm$ SEM of 6 determinations per strain.

enhanced contractions to 5-HT in WKY but not in SHR (Fig. 3). This increase was observed starting at a concentration of 10^{-6} mol/L 5-HT.

Vascular Reactivity of Isolated Mesenteric Arteries

Contractile responses were analyzed in isolated superior mesenteric artery rings with (+) fat and without (–) fat. Under the stereomicroscope, periadventitial adipose tissue was almost abolished in the rings of SHR compared to WKY. However, this difference could not be statistically demonstrated by tissue weighting due to the small size of the rings and the relatively large attached drops of bath solution, which were variable in size and could not be removed in a uniform manner by blotting-paper (data not shown). The contractile responses to 60 mmol/L KCl and 2 mmol/L 4-AP were studied at a tension equivalent to that generated at 0.9 times the

diameter of the vessel at 100 mmHg. 4-AP (2 mmol/L) induced a significantly higher contraction in (+) fat rings of WKY rats compared to (–) fat rings. This (+) fat-dependent effect was not observed in SHR (Fig. 4), since there was no difference in the effect of 4-AP (2 mmol/L) on isometric force between the (+) fat rings and (–) fat rings of SHR. The effects of 5-HT were also analyzed on the isolated mesenteric arteries. At 0.7 μ mol/L 5-HT, the anti-contractile effect of fat was observed in WKY but was almost completely absent in SHR. However, the response to 5-HT (0.7 μ mol/L) in SHR was not different from that elicited in WKY (not shown).

Discussion

In this study, we found that 4-week-old SHR have less mesenteric periadventitial adipose tissue than WKY, although this fact does not influence basal perfusion pressure or contractile responses to KCl in the whole perfused mesenteric bed. Nev-

Fig. 3. Effect of 2 mmol/L 4-aminopyridine (4-AP) on the contractile response elicited by serotonin. Cumulative concentration-response curves to (10^{-9} to 10^{-5} mol/L) serotonin (5-HT) in the perfused mesenteric bed of A: WKY and SHR rats in the absence of 2 mmol/L 4-AP, B: WKY in the absence and presence of 2 mmol/L 4-AP, and C: SHR in the absence and presence of 2 mmol/L 4-AP. *Significant at $p < 0.05$. Data are shown as the mean $\pm$ SEM of 6 determinations per strain.

ertheless, the anti-contractile effect of fat in response to vasoconstrictors, such as 5-HT, is markedly reduced in SHR. These results are likely due to a diminished paracrine regulation of periadventitial adipose tissue on mesenteric arteries by ADRF via voltage-dependent, delayed rectifier K^+ (K_v) channels. The main finding in our study is that alterations in visceral periadventitial fat mass and function in SHR precede hypertension, suggesting a constitutive mechanism independent of age and the hypertensive state.

Adipose tissue is now regarded as an endocrine organ that produces and releases numerous factors with a broad range of biological activities. In recent years, several studies have demonstrated a paracrine role for periadventitial adipose tissue in the regulation of vascular function through the release of vasoactive relaxing factors, such as ADRF (1–6). At least

in the mesenteric arteries (4) and aortas of rats (*cf.* Online Fig. 1), the anti-contractile effect of ADRF is strongly dependent on the amount of perivascular fat. In this context, we have recently described that 3-month-old SHR have a lower amount of mesenteric adipose tissue, which correlates with a reduced anti-contractile effect of ADRF in response to vasoconstrictors (5). In the present study, to assess whether or not the alterations observed in adult animals are dependent on blood pressure, we used in this study 4-week old SHR rats, which show normal systemic blood pressure levels, compared to 4-week old WKY (8–12). We found that SHR at this age already show a reduction in the amount of mesenteric adipose tissue, by approximately $19 \pm 1\%$. This value is similar to that observed in 3-month-old SHR ($17 \pm 1\%$) with established hypertension (5). The reduction in the amount of adipose tis-

Fig. 4. Effects of 4-aminopyridine (4-AP, 2 mmol/L) on the isometric force of mesenteric arterial rings isolated from WKY and SHR. The periadventitial fat and connective tissue were intact in (+) fat rings, and removed in (-) fat rings. The effects of 4-AP were analyzed at 3 min after application of the drug. Data are shown as the mean $\pm$ SEM of 6–10 rings from > 6 animals per strain. * $p < 0.05$.

sue also extended to the lumbar fat pad, suggesting a general reduction in visceral fat in this strain. This conclusion is also supported by the fact that plasma leptin levels, which are known to be closely correlated with the amount of fat (23), were lower in SHR. One possible explanation for the reduction in adipose tissue might be the increased sympathetic outflow in SHR (15, 16), which could lead to enhanced lipolysis (17, 18). However, the noradrenaline turnover, as determined by the DOMA/NA index in lumbar adipose tissue, was not different between the strains. In addition, the plasma lipid parameters (Table 2) are also similar between WKY and SHR. These results suggest that other possible mechanisms, such as genetic changes, may determine the amount of adipose tissue. In conclusion, the reduction of periadventitial fat mass seems to be related to the hypertensive strain but is not dependent on the hypertensive state.

Similarly, the modulation of vascular tone by periadventitial fat seems to be a constitutive mechanism independent of age, since the anti-contractile effect of fat previously observed in adult animals (1–6) is also present in the mesenteric arteries of young WKY. In order to assess whether differences in the amount of fat modulate the response to vasoconstrictors, we studied contractile responses to 5-HT in the presence of 4-AP. Contractile responses to 5-HT were significantly increased by 4-AP in the perfused mesenteric bed of WKY but not in that of SHR. Changes in vascular reactivity between SHR and WKY were not mechanically caused by differences in the mesenteric fat mass, as the responses elic-

ited by KCl 75 mmol/L were similar between the strains. We also excluded possible differences in the sensitivity of vascular smooth muscle cells in responding to membrane depolarization. An interesting finding previously observed in 3-month-old animals is that the anti-contractile effect of perivascular fat seems to be dependent on wall tension and is more evident at wall tensions equivalent to $0.9 \times$ the diameter of the vessel, which are closer to the wall tension “*in vivo*.” This could explain the difference in the responses to 4-AP in the perfused mesenteric bed compared to the isolated superior mesenteric artery. However, we believe that wall tension plays a modulatory role for the release of ADRF for two reasons: First, 4-AP-sensitive effects occurred in the mesenteric beds of WKY rats at lower perfusion pressures than in SHR rats (Fig. 3). Second, bioassay experiments with transfer of ADRF from donor vessels to acceptor rings showed that ADRF is actively released by 5-HT. In contrast, only a small fraction of ADRF is spontaneously released in the absence of 5-HT (cf. Online Fig. 2).

The increase in perfusion pressure elicited by 5-HT in the absence of 4-AP was significantly higher in the perfused bed of SHR. Some investigators have also encountered increased contractile responses in mesenteric arteries from prehypertensive SHR (routinely cleaned of periadventitial adipose tissue) (12, 24), whereas others found no difference between SHR and WKY (9). Increased contractile responses in SHR at this age have been related to structural alterations in the mesenteric vascular wall (8, 12, 24, 25). These alterations include an increase in the volume of the medial layer and an increase in the number of smooth muscle cell layers, but no change in lumen diameter in the relaxed state (12). However, despite the presence of medial wall hypertrophy and an increase in the media-lumen ratio in the mesenteric arteries and arterioles, there are no differences in basal perfusion pressure between strains (25), as we also observed in this study.

Gao *et al.* (3) have recently shown that the anti-contractile effect of periadventitial adipose tissue in the rat aorta is due to both endothelium-dependent and endothelium-independent mechanisms. The endothelium-dependent relaxation seems to be mediated by NO release and the subsequent activation of calcium-dependent K^+ channels. This mechanism seems to have been absent in the rat mesenteric arteries used in this study, since the basal release of NO appeared to be the same in both strains and to be independent of the amount of adipose tissue. Moreover, although the relaxation in response to acetylcholine is mediated mainly by EDHF in the mesenteric arteries, the minor contribution of NO, as assessed by the relaxation in response to ACh in the presence of L-NAME, was also not different between the strains. On the other hand, the endothelium-independent anti-contractile effect of peri-aortic adipose tissue reported by Gao *et al.* (3) is due to generation of H_2O_2 and subsequent stimulation of guanylyl cyclase (3). Our present results suggest that in rat mesenteric arteries a possible contribution of guanylyl cyclase, as determined by the vasodilatory response to SNP, is unlikely, since

the vasodilatory response to SNP was not different between the strains and was independent of the amount of fat. Therefore, although the mechanisms suggested by Gao *et al.* cannot be excluded in mesenteric arteries from WKY and SHR, they seem to be similar between the strains. All these findings are in accordance with the results previously observed in 3-month-old animals (5) and consistent with the idea that periadventitial adipose tissue has a paracrine anti-contractile effect through ADRF release and activation of K_v channels.

It is well known that abdominal visceral fat mass is correlated with the prevalence of hypertension (7). Thus, in light of the present and previous findings (5), there seems to be a contradiction between the inhibitory effect of perivascular adipose tissue and obesity-related hypertension. An attractive hypothesis is that under normal weight conditions it might be beneficial to have more perivascular fat, because this means increased release of adipose vasodilator factors such as ADRF. Once perivascular adipose tissue reaches a certain amount, which needs to be determined, there might be a shift in the balance between adipose tissue-derived vasodilator and vasoconstrictor factors leading to hypertension. In fact, regarding ADRF, this factor has been shown to be lost in a model of obesity (26).

In conclusion, we have demonstrated that the anti-contractile effect of perivascular fat previously observed in adult animals is also present in mesenteric arteries of young WKY, suggesting that the modulation of vascular tone by periadventitial fat is a constitutive mechanism independent of age. Moreover, we found that functional alterations in the mesenteric fat of 4-week-old prehypertensive SHR are associated with a lower amount of periadventitial adipose tissue. The finding that these abnormalities precede hypertension suggests that changes in periadventitial adipose tissue in SHR might be, among other well-described mechanisms, a causal factor for the development of hypertension due to a reduced release and anti-contractile effect of ADRF.

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