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# Visceral Periadventitial Adipose Tissue Regulates Arterial Tone of Mesenteric Arteries

Stefan Verlohren, Galyna Dubrovskaya, Suk-Ying Tsang, Kirill Essin, Friedrich C. Luft, Yu Huang, Maik Gollasch

**Abstract**—Periadventitial adipose tissue produces vasoactive substances that influence vascular contraction. Earlier studies addressed this issue in aorta, a vessel that does not contribute to peripheral vascular resistance. We tested the hypothesis that periadventitial adipose tissue modulates contraction of smaller arteries more relevant to blood pressure regulation. We studied mesenteric artery rings surrounded by periadventitial adipose tissue from adult male Sprague-Dawley rats. The contractile response to serotonin, phenylephrine, and endothelin I was markedly reduced in intact vessels compared with vessels without periadventitial fat. The contractile response to U46619 or depolarizing high  $K^+$ -containing solutions (60 mmol/L) was similar in vessels with and without periadventitial fat. The  $K^+$  channel opener cromakalim induced relaxation of vessels precontracted by serotonin but not by U46619 or high  $K^+$ -containing solutions (60 mmol/L), suggesting that  $K^+$  channels are involved. The intracellular membrane potential of smooth muscle cells was more hyperpolarized in intact vessels than in vessels without periadventitial fat. Both the anticontractile effect and membrane hyperpolarization of periadventitial fat were abolished by inhibition of delayed-rectifier  $K^+$  ( $K_v$ ) channels with 4-aminopyridine (2 mmol/L) or 3,4-diaminopyridine (1 mmol/L). Blocking other  $K^+$  channels with glibenclamide (3  $\mu$ mol/L), apamin (1  $\mu$ mol/L), iberiotoxin (100 nmol/L), tetraethylammonium ions (1 mmol/L), tetrapentylammonium ions (10  $\mu$ mol/L), or  $Ba^{2+}$  (3  $\mu$ mol/L) had no effect. Longitudinal removal of half the perivascular tissue reduced the anticontractile effect of fat by almost 50%, whereas removal of the endothelium had no effect. We suggest that visceral periadventitial adipose tissue controls mesenteric arterial tone by inducing vasorelaxation via  $K_v$  channel activation in vascular smooth muscle cells. (*Hypertension*. 2004;44:271-276.)

**Key Words:** muscle, smooth ■ mesenteric arteries ■ obesity ■ hypertension, obesity

Periadventitial adipose tissue is routinely removed for contraction studies on isolated blood vessels. Soltis and Cassis demonstrated that periadventitial fat significantly attenuates vascular responsiveness of rat isolated aortic rings to norepinephrine.<sup>1</sup> We confirmed the inhibitory action of periadventitial fat on aortic contraction. However, we also found that the effect is antagonized by depolarizing external high  $K^+$  solutions and partly by glibenclamide, suggesting that the anticontractile effects of fat are mediated in part by opening of ATP-dependent  $K^+$  ( $K_{ATP}$ ) channels in aortic smooth muscle cells.<sup>2</sup> The action was not dependent on NO synthesis or endothelium. The anticontractile effects did not require the cyclooxygenase or P450 pathway, activation of adenosine receptors, or functional leptin receptors.<sup>2</sup> However, we found that relaxation was induced by a transferable adipocyte-derived relaxing factor (ADRF) released from periadventitial adipose tissue.<sup>2</sup> The results were not obtained in vessels that contribute to peripheral vascular resistance, and thus, their

relevance to hypertension is unclear. We tested the hypothesis that periadventitial fat modulates contraction of smaller peripheral arteries. We used isolated mesenteric artery rings surrounded by periadventitial adipose tissue from adult male Sprague-Dawley rats and performed isometric contraction measurements. We found that periadventitial fat significantly attenuated vascular responsiveness to several hormonal agonists. In contrast to the findings in aorta, visceral perivascular adipose tissue controlled mesenteric arterial tone by activating voltage-dependent, delayed-rectifier  $K^+$  ( $K_v$ ) channels.

## Materials and Methods

An extended Methods section is available online at <http://www.hypertensionaha.org>.

Briefly, superior mesenteric arteries of male Sprague-Dawley rats (200 to 300 g, 6 to 8 weeks) were quickly transferred to cold (4°C) oxygenated (95%  $O_2$ /5%  $CO_2$ ) physiological salt solution, and dissected into 2-mm rings as described previously, whereby periadventitial fat and connective tissue were either removed [(−) fat rings]

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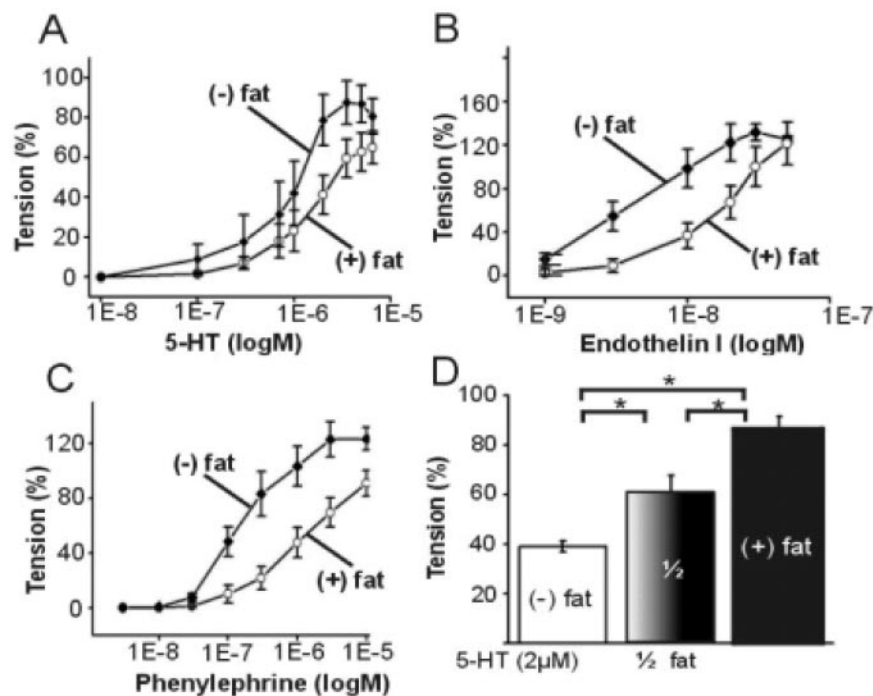
From the Franz Volhard Clinic and Max Delbrück Center for Molecular Medicine (S.V., G.D., K.E., F.C.L., M.G.), Charité University Hospitals, Humboldt University of Berlin, HELIOS Klinikum Berlin, Germany; Louisiana State University Health Sciences Center (M.G.), Department of Physiology, New Orleans; Department of Physiology (S.-Y.T., Y.H.), Chinese University of Hong Kong, Shatin, China.

Correspondence to Maik Gollasch, MD, PhD, Franz Volhard Clinic, Wiltbergstraße 50, 13125 Berlin, Germany. E-mail [gollasch@fvk-berlin.de](mailto:gollasch@fvk-berlin.de) or [mgolla@lsuhsc.edu](mailto:mgolla@lsuhsc.edu)

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**Figure 1.** Dose-response curves to serotonin (A), endothelin I (B), and phenylephrine (C) in intact (+) fat preparations (○) and (–) fat preparations without periadventitial fat (●). Contraction induced by 2 μmol/L serotonin of mesenteric rings without (–) fat, mesenteric (1/2 fat) rings after longitudinal removal of 50% periadventitial adipose tissue along the ipsilateral side, and intact (+) fat mesenteric ring preparations (D). \**P*<0.05.

or left intact [(+) fat rings].<sup>3,4</sup> The periadventitial fat was removed with scissors,<sup>2</sup> being careful not to damage the adventitia.<sup>5</sup> In some experiments, 50% of the periadventitial fat was removed longitudinally along the ipsilateral side of the vessel ring with scissors; it was left intact on the contralateral side of the vessel ring. The organ bath was filled with physiological salt solution of the following composition (mol/L): 119 NaCl, 4.7 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 1.2 MgSO<sub>4</sub>, 11.1 glucose, and 1.6 CaCl<sub>2</sub> (95% O<sub>2</sub> plus 5% CO<sub>2</sub>, 37°C, pH 7.4). The rings were placed in a small vessel wire myograph under an optimal resting tension of 2 mN.<sup>3,4</sup> Tension is expressed as a percentage of the steady-state tension (100%) obtained with isotonic external 60 mmol/L KCl.

In the first series of experiments, the rings were exposed to increasing doses of serotonin (10<sup>-8</sup> to 10<sup>-5</sup> mol/L), endothelin I (10<sup>-9</sup> to 10<sup>-7</sup> mol/L), phenylephrine (3×10<sup>-9</sup> to 10<sup>-5</sup> mol/L), or U46619 (10<sup>-9</sup> to 10<sup>-6</sup> mol/L). In some experiments, we measured the concentration of serotonin in the bath solution of (+) fat and (–) fat vessels. After a 10-minute incubation period of (–) fat and (+) fat with 2 μmol/L serotonin, the serotonin concentration was not different in the bath solution between both groups (high-performance liquid chromatography analysis), indicating that the effects of perivascular fat are anticontractile and not because of partial degradation of the vasoconstrictor agent. In the second series of experiments, the effect of serotonin was investigated in rings pretreated with different K<sup>+</sup> channel blockers. The effects were compared with contractions to 2 μmol/L serotonin 10 minutes before addition of the inhibitors. In the third series of experiments, the effects of cromakalim (100 nmol/L) were tested on 2 μmol/L serotonin- or 0.1 μmol/L U46619-induced contraction in rings following 10-minute exposure of serotonin or U46619.

Intracellular membrane potential was measured using sharp intracellular glass microelectrodes as previously described.<sup>6</sup> The glass microelectrodes were prepared by means of a horizontal puller and filled with 3 mol/L KCl (tip resistance in the range of 40 to 60 MΩ). Impalement was from the adventitial side of each vessel.

All values are given as mean±SEM. Paired and unpaired Student *t* tests or ANOVA were used as appropriate. *P*<0.05 was considered statistically significant; *n* represents the number of arteries tested.

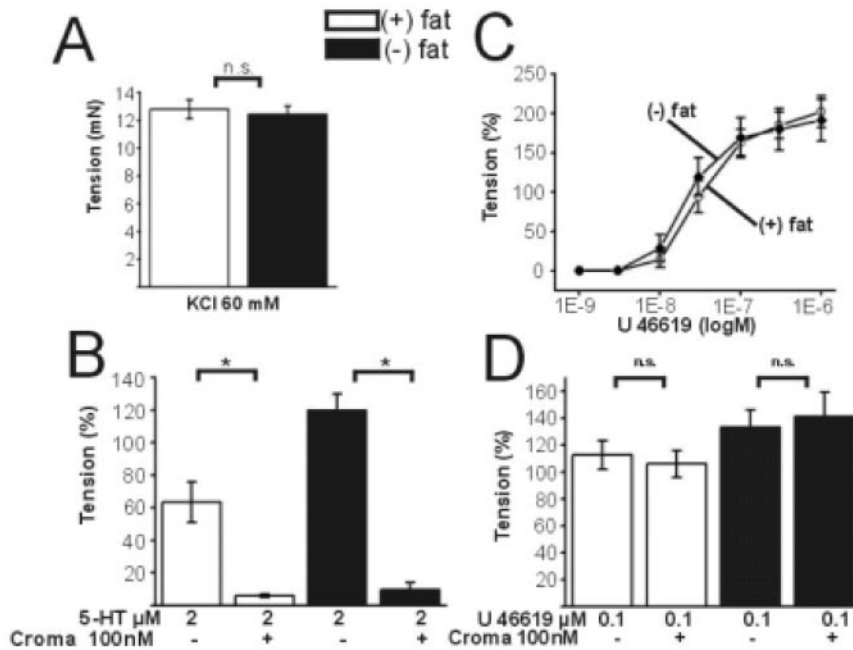
## Results

### Anticontractile Effect of Perivascular Adipose Tissue

To test the hypothesis that periadventitial fat influences vascular contraction, we generated dose-response curves to serotonin (Figure 1A), endothelin I (Figure 1B), and phenylephrine (Figure 1C) for both mesenteric artery rings with (+) fat and without (–) fat. At a concentration of 2 μmol/L serotonin, 10 nmol/L endothelin I, and 300 nmol/L phenylephrine, the contractile response of intact rings was 50%, 60%, and 80% lower than that of vessels without periadventitial fat. Endothelial removal did not influence the anticontractile effects of periadventitial fat (Figure 1A online). These results provide evidence for a vasodilatory, or rather anticontractile, effect of periadventitial adipose tissue. We next tested the hypothesis that the anticontractile effect of periadventitial fat depends on the amount of fat on each ring. Figure 1D shows contractile responses to 2 μmol/L serotonin of mesenteric artery rings without (–) fat, mesenteric artery rings after longitudinal removal of 50% periadventitial adipose tissue (1/2 fat), and intact (+) fat mesenteric artery ring preparations. Thus, the inhibition of the contractile response to serotonin by fat depends on the amount of fat on each ring.

### Involvement of K<sup>+</sup> Channels

We next tested the hypothesis that K<sup>+</sup> channels are involved in this anticontractile effect. We challenged (+) fat rings and (–) fat rings (*n*=26) with 60 mmol/L KCl and 45 mmol/L KCl. Raising external K<sup>+</sup> would be expected to diminish the effects of any K<sup>+</sup> channel opener by substantially reducing the difference between the K<sup>+</sup> equilibrium potential and the membrane potential.<sup>6,7</sup> Figure 2A shows that the contractile responses of (+) fat vessels and (–) fat vessels to 60 mmol/L



**Figure 2.** Reducing the difference between the  $K^+$  equilibrium potential and the membrane potential by application of isotonic bath solution containing 60 mmol/L KCl (A) abolished the difference in contractile response between (+) fat (■) and (-) fat (□) rings. Opening of  $K_{ATP}$  channels by cromakalim (Croma, 100 nmol/L) induced complete relaxation of serotonin (5-HT)-precontracted (+) fat mesenteric rings and (-) fat mesenteric rings (B). Dose-response curves to U46619 (C) in intact (+) fat preparations (○) and (-) fat preparations without fat (●). The presence of fat did not reduce the contractile response to this agonist. Opening of  $K_{ATP}$  channels by cromakalim did not induce relaxation of U46619-precontracted (+) fat (■) mesenteric rings and (-) fat (□) mesenteric rings (D). \* $P < 0.05$ ; n.s. indicates not significant.

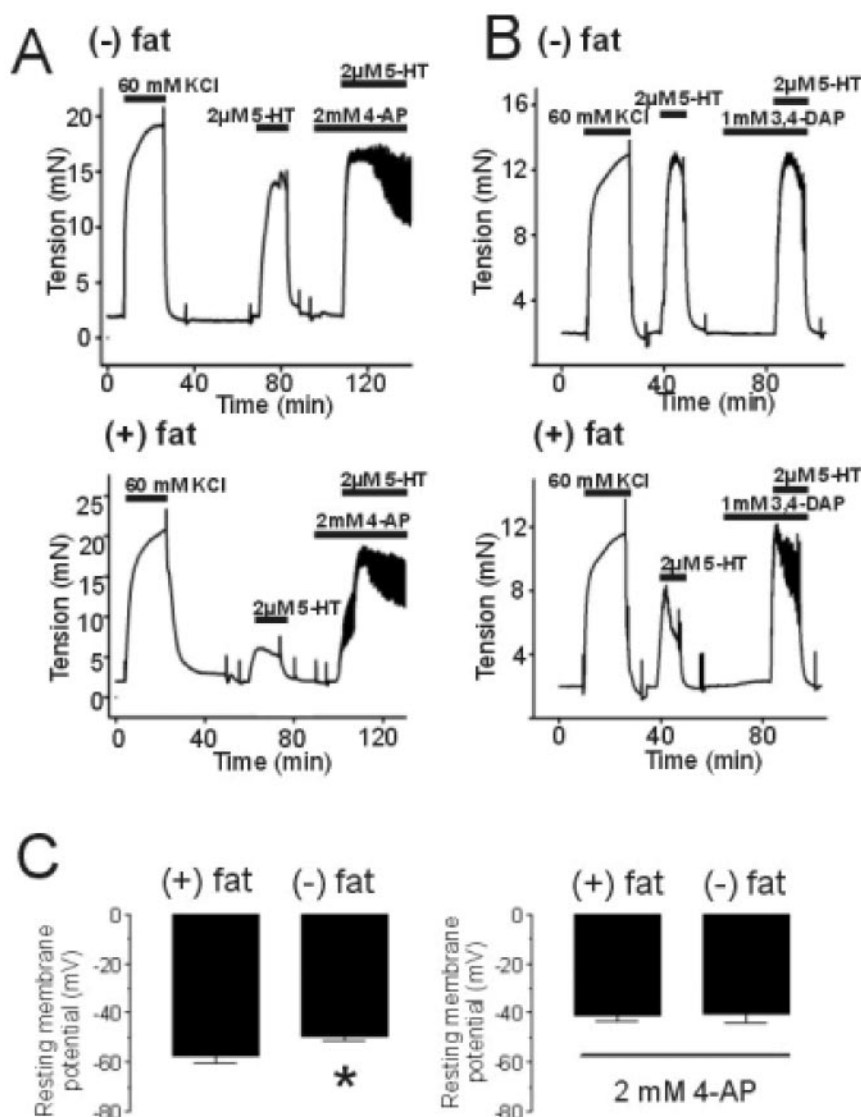
KCl were not significantly different. Figure 1B shows that 45 mmol/L KCl induced smaller submaximal contractions, but the contractile responses of (+) fat vessels and (-) fat vessels to 45 mmol/L KCl were not significantly different. These findings demonstrate that excitation-contraction coupling in intact arteries and arteries lacking periadventitial fat remain functional and that the presence of periadventitial fat does not mechanically or otherwise alter the contractility of artery rings. In addition, the synthetic  $K^+$  channel opener cromakalim<sup>7,8</sup> at 0.1  $\mu$ mol/L ( $n=5$ ) did not reduce 60 mmol/L KCl-induced contractions in (+) fat vessels and (-) fat vessels (not shown). However, cromakalim at 0.1  $\mu$ mol/L almost completely relaxed contractions of (+) fat vessels and (-) fat vessels to 2  $\mu$ mol/L serotonin (Figure 2B). Thus,  $K_{ATP}$  channels are functional and membrane hyperpolarization of the smooth muscle cells can reverse serotonin-dependent contractions. The results suggest that the difference in response to serotonin between intact vessels and vessels without periadventitial fat is dependent on opening of  $K^+$  channels.

We next challenged (+) fat rings and (-) fat rings ( $n=6$ ) with the thromboxane A2 analogue U46619. U46619 induces vascular contraction that is largely independent of vascular smooth muscle cell membrane potential changes. Thus, U46619-dependent contractions are resistant to opening of  $K^+$  channels in vascular smooth muscle cells.<sup>9</sup> We generated dose-response curves to U46619 for intact (+) fat,  $n=6$ , and mesenteric artery rings without (-) fat,  $n=6$ . Figure 2C shows that the contractile responses of (+) fat vessels and (-) fat vessels to U46619 were not significantly different. In addition, cromakalim at 0.1  $\mu$ mol/L had no effect on the contractile responses of (+) fat vessels and (-) fat vessels to 0.1  $\mu$ mol/L U46619 (Figure 2D). These results are in line with our previous data indicating that the difference in contractile response to serotonin between intact vessels and

vessels without periadventitial fat is dependent on opening of  $K^+$  channels.

### Role of $K_v$ Channels

To explore the nature of  $K^+$  channels involved in the fat-modulated response of mesenteric ring contraction, we tested different blockers of  $K^+$  channels present in rat mesenteric arteries. At 2 mmol/L, the  $K_v$  channel blocker 4-aminopyridine (4-AP)<sup>10-12</sup> virtually abolished the difference in response between intact (+) fat vessels and vessels without (-) fat ( $n=6$ ) to serotonin (Figure 3A and Figure IIA online). The  $K_v$  channel blocker 3,4-diaminopyridine (3,4-DAP)<sup>13</sup> at 1 mmol/L had similar effects ( $n=5$ , Figure 3B and Figure IIB). The small-conductance,  $Ca^{2+}$ -activated  $K^+$  channel blocker apamin<sup>4</sup> (1  $\mu$ mol/L,  $n=6$ ; Figure 4A and Figure IIC) and the  $K_{ATP}$  channel blocker glibenclamide<sup>10,14</sup> (3  $\mu$ mol/L,  $n=8$ ; Figure 4B and Figure IID) were not effective. The inward rectifier  $K^+$  channel blocker  $Ba^{2+}$  (3  $\mu$ mol/L)<sup>11</sup> did not influence the anticontractile effect of fat ( $n=6$ , Figure III online). These results suggest that the difference in response to serotonin between intact vessels and vessels lacking periadventitial fat is most likely mediated by opening of  $K_v$  channels in vascular smooth muscle cells. Blockers of large-conductance,  $Ca^{2+}$ -activated potassium channels, that is, iberiotoxin<sup>10,14-16</sup> (100 nmol/L,  $n=6$ , Figure IVA online) and tetraethylammonium<sup>10,14,16</sup> (TEA; 1 mmol/L,  $n=6$ , Figure IVB), and tetrapentylammonium,<sup>17</sup> which also blocks  $K_{ATP}$  channels,<sup>18</sup> (2  $\mu$ mol/L,  $n=6$ , not shown) enhanced serotonin-dependent contractions by 20% to 30% in both (-) fat and (+) fat rings. These results suggest that large-conductance  $Ca^{2+}$ -activated  $K^+$  channels limit serotonin-dependent contractions of both (+) fat and (-) fat rings. However, the data also indicate that the difference in response to serotonin between intact (+) fat vessels and (-) fat vessels is not mediated by opening of large-conductance  $Ca^{2+}$ -activated  $K^+$  channels in vascular smooth muscle cells.



**Figure 3.** Blockers of voltage-dependent, delayed-rectifier  $K^+$  channels inhibited the anticontractile effect of perivascular fat on the response to serotonin. A, Representative experiment showing that 4-AP (2 mmol/L) enhanced the contractile response to serotonin (5-HT, 2  $\mu$ mol/L) in (+) fat rings but not in (-) fat rings. B, Representative experiment showing that 3,4-DAP (1 mmol/L) enhanced the contractile response to serotonin (5-HT, 2  $\mu$ mol/L) in (+) fat rings but not in (-) fat rings. The intracellular membrane potential of vascular smooth muscle cells in (+) fat rings is hyperpolarized in comparison to (-) fat rings (C, left); 4-AP (2 mmol/L) inhibited the difference (C, right). \* $P < 0.05$ .

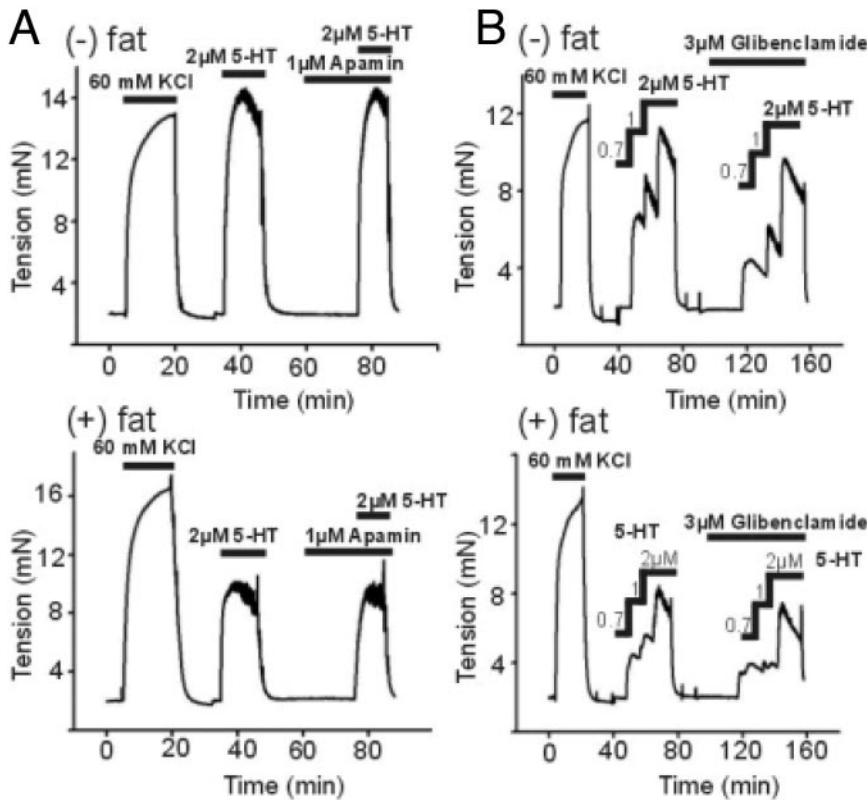
To confirm the conclusion that 4-AP-sensitive, voltage-dependent  $K^+$  channels in arterial smooth muscle cells are involved in the anticontractile effects of fat, we measured the intracellular vascular smooth muscle cell membrane potential of (+) fat rings and (-) fat rings. Figure 3C shows that the intracellular membrane potentials were more hyperpolarized in (+) fat rings than in (-) fat rings ( $n=12$  each). At 2 mmol/L, 4-AP virtually abolished the difference in intracellular membrane potential between intact (+) fat vessels and vessels without (-) fat ( $n=12$  each).

### Discussion

We found that perivascular fat significantly attenuates vascular responsiveness of mesenteric arteries to several hormonal agonists, including serotonin, phenylephrine, and endothelin I. Our data suggest that visceral perivascular adipose tissue controls mesenteric arterial tone by activating voltage-dependent, delayed-rectifier  $K^+$  ( $K_v$ ) channels that hyperpolarize the vascular smooth muscle cell membrane. The mechanisms we describe here are distinct from those we described earlier from aortic prep-

arations, where  $K_{ATP}$  channels were primarily involved. Because we studied superior mesenteric arteries that contribute to the regulation of mesenteric blood flow and to peripheral resistance, perturbations in  $K_v$  channel regulation could conceivably contribute to blood pressure elevation in obesity-related hypertension.

Inhibition of the contractile response to serotonin by fat depended on the amount of fat on each ring. The effects were not dependent on the endothelium. We studied the possible involvement of plasma membrane  $K^+$  channels in detail. We found that the anticontractile effect of perivascular fat was not abolished by inhibition of  $K_{ATP}$  channels, small-conductance  $Ca^{2+}$ -activated  $K^+$  channels, and inward rectifying  $K^+$  channels. However, we found that the anticontractile effect of perivascular fat was abolished by inhibition of  $K_v$  channels. The resting membrane potential of smooth muscle cells in intact mesenteric artery rings was more hyperpolarized than in mesenteric artery rings without perivascular fat, a difference that was abolished by  $K_v$  channel inhibition with 4-AP. Together, these results suggest that visceral perivascular adipose tissue controls mesenteric arterial tone



**Figure 4.** A, Representative experiment showing that apamin (1  $\mu\text{mol/L}$ ) did not affect the contractile response to serotonin (5-HT, 2  $\mu\text{mol/L}$ ) in (+) fat rings and (-) fat rings. B, Representative experiment showing that glibenclamide (3  $\mu\text{mol/L}$ ) did not affect the contractile response to serotonin (5-HT, 2  $\mu\text{mol/L}$ ) in (+) fat rings and (-) fat rings.

locally. We suggest that vascular smooth muscle cell  $K_v$  channels are involved in the anticontractile effects of periaortadventitial adipose tissue.

Patch-clamp studies have shown that slowly-inactivating  $K_v$  channels are expressed in smooth muscle cells of our preparation. These channels are sensitive to 4-AP and 3,4-DAP<sup>19–22</sup> but not to TEA.<sup>22,23</sup> In contrast to smooth muscle cells,  $K_v$  channels in adipocytes are rapidly-inactivating, sensitive to TEA,<sup>24–26</sup> and relatively resistant to 4-AP.<sup>27–29</sup>  $\text{Ba}^{2+}$ -sensitive inward rectifying  $K^+$  have not been detected in adipocytes. In addition, we found that the intracellular membrane potential was more negative in smooth muscle cells of (+) fat rings compared with (-) fat rings, a difference that was abolished by 4-AP. We tried to measure the intracellular membrane potential in adipocytes of (+) fat rings. Unfortunately, this was technically impossible; this might also be the reason that there is no publication on intracellular membrane potential measurement in perivascular adipocytes. Taken together, the data are consistent with the idea that  $K_v$  channels in smooth muscle cells are involved in the anticontractile effect of adipose tissue.

In contrast to aorta,  $K_{\text{ATP}}$  channels were not involved in the mesenteric artery periaortadventitial fat effect. A possible explanation is that  $K_{\text{ATP}}$  channels in aortic and mesenteric smooth muscle are different, as large-conductance  $K_{\text{ATP}}$  channels ( $\approx 130$  pS, in symmetric high  $K^+$ ) sensitive to intracellular [ATP] have been found in aorta but not in mesenteric arteries.<sup>30–32</sup> In rat mesenteric arteries, small-conductance  $K_{\text{ATP}}$  ( $K_{\text{NDP}}$ ) channels ( $\approx 25$  pS, in symmetric high  $K^+$ ) have been identified that are regulated by nucleoside diphosphates (UDP or GDP) and ATP acting from the inside.<sup>33,34</sup> The

molecular identity of the aortic and mesenteric  $K_{\text{ATP}}$  channels is not absolutely established. Two Kir6.x subunits have been cloned to date: Kir6.1 and Kir6.2 that differ in their single channel conductance. Three major types of sulfonylurea receptor have been cloned, SUR1, SUR2A, and SUR2B, although other splice variants also exist. The properties of a particular  $K_{\text{ATP}}$  channel are dependent on its subunit composition.  $K_{\text{NDP}}$  channels seem to be equivalent to the Kir6.1/SUR2B channel,<sup>35</sup> whereas the molecular composition of aortic  $K_{\text{ATP}}$  channels is less clear.<sup>36–38</sup> Based on their molecular diversity, it is possible that aortic and mesenteric  $K_{\text{ATP}}$  channels respond differently to ADRF. Alternatively, visceral periaortadventitial adipose tissue may produce a number of ADRFs or involve different ADRF receptors and intracellular second messengers to control vascular tone in different vascular beds.

### Perspectives

We demonstrate an important functional role of periaortadventitial adipose tissue on mesenteric artery tone. We suggest that vascular smooth muscle cell  $K_v$  channels regulate the process. Identification of the putative ADRF may shed light on obesity-related hypertension and could possibly be of therapeutic importance.

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