Periadventitial fat releases a vascular relaxing factor

MATTHIAS LÖHN,[1] GALYNA DUBROVSKA,[1] BIRGIT LAUTERBACH, FRIEDRICH C. LUFT, MAIK GOLLASCH, AND ARYA M. SHARMA[2]

HELIOS Klinikum Berlin, Franz Volhard Clinic and Max Delbrück Center for Molecular Medicine, Medical Faculty of the Charité, Humboldt University of Berlin, Germany

ABSTRACT **Virtually all blood vessels are surrounded by adventitial fat. Adipocytes produce a host of vasoactive substances that may influence vascular contraction. We tested whether or not perivascular adipose tissue modulates contraction of aortic ring preparations. We studied aortic rings surrounded by periadventitial adipose tissue from adult Sprague-Dawley rats. At a maximum concentration of 300 nM angiotensin II, 6.5 μM serotonin, and 5 μM phenylephrine, the contractile response of intact rings was 95%, 80%, and 30% lower than that of vessels without periadventitial fat. The anticontractile effect of periadventitial fat was reduced by inhibition of ATP-dependent K^+ channels with glibenclamide (3 μM) and by the tyrosine kinase inhibitor genistein (10 μM). Blocking NOS, cyclo-oxygenase, cytochrome P450, or adenosine receptors did not restore the vascular response in intact vessels. The anticontractile effect of perivascular fat was present in Zucker fa/fa rats, suggesting that leptin receptors were not responsible. Transferring the bath solution from intact vessels, isolated periadventitial tissue, and cultured rat adipocytes to precontracted vessels lacking periadventitial fat resulted in a rapid relaxation. We suggest that perivascular adventitial adipose tissue releases a transferable adventitium-derived relaxing factor that acts by tyrosine kinase-dependent activation of K^+ channels in vascular smooth muscle cells.—Löhn, M., Dubrovska, G., Lauterbach, B., Luft, F. C., Gollasch, M., Sharma, A. M. Periadventitial fat releases a vascular relaxing factor. *FASEB J.* 16, 1057–1063 (2002)**

Key Words: fat · adventitium · ion channels · tyrosine phosphorylation

VIRTUALLY ALL BLOOD vessels are surrounded by variable amounts of adventitial adipose tissue (1). Perivascular fat is widely assumed to serve largely as structural support for the vasculature and is routinely removed for contraction studies of isolated blood vessels. Soltis and Cassis demonstrated that perivascular fat significantly attenuates vascular responsiveness of aortic ring preparations to norepinephrine in vitro (2). They suggested that this anticontractile effect was due to uptake of norepinephrine by the surrounding adipose tissue. We reexamined the idea that perivascular adipose tissue modulates vascular responsiveness. Our studies confirmed the inhibitory effect of perivascular adipose tissue on vascular contraction. However, we also found that this effect is most likely mediated by a transferable factor that acts through activation of K^+ channels and tyrosine kinase in vascular smooth muscle cells. We found that the action is not dependent on nitric oxide (NO) synthesis. We suggest that perivascular adventitial adipose tissue releases an 'adventitium-derived relaxing factor' (ADRF). Perturbations could conceivably contribute to hypertension in obese or nonobese individuals.

MATERIALS AND METHODS

All animal procedures were in accordance with institutional guidelines corresponding to The American Physiological Society. Male Sprague-Dawley rats (250–300 g, Charles River, Wilmington, MA) were killed under ether. The thoracic aortas were removed, quickly transferred to cold (4°C) oxygenated (95% O_2/5% CO_2) physiological salt solution (PSS), and dissected into 5 mm rings as described previously (3), whereby perivascular fat and connective tissue were either removed or left intact. In some experiments, the endothelial cell layer was also removed. After 1 h equilibration, aortic ring contractile force with and without perivascular fat tissue was measured isometrically using standard bath procedures as described (3). The composition of PSS (in mM) was 119 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 25 $NaHCO_3$, 1.2 $MgSO_4$, 11.1 glucose, 1.6 $CaCl_2$. The volume of the bath solution was 20 mL. Cumulative response curves were obtained for angiotensin II, phenylephrine, or serotonin in the presence and absence of inhibitors. Tension is expressed as a percentage of the steady-state tension (100%) obtained with isotonic external 60 mM KCl.

In bioassay experiments, we transferred aliquots of the bath solution from either intact preparations or isolated aortic perivascular fat tissue incubated in a donor bath chamber to vessel preparations without periadventitial fat in an acceptor bath chamber (**Fig. 1**). The volume of the solutions in the bath chambers was 20 mL. In most experiments, transfer interval of aliquots was 15 to 20 min; the volume of the aliquots was 3 or 5 mL. Transfer of bath solution aliquots from aortic vessels without perivascular adventitial tissue or fresh PSS did not affect contraction of vessel preparations without periadventitial fat in the acceptor bath chamber. Rat adipocytes (passage 1) and mouse NIH 3T3 fibroblasts (passage 9) were kept in culture medium (Dulbecco's modified Eagle medium/Ham's F12, 1:1; 1%

[1] The contributions of M.L. and G.D. were equal.

[2] Correspondence: Franz-Volhard Klinik, Wiltbergstrasse 50, 13125 Berlin, Germany. E-mail: sharma@fvk-berlin.de or gollasch@fvk-berlin.de

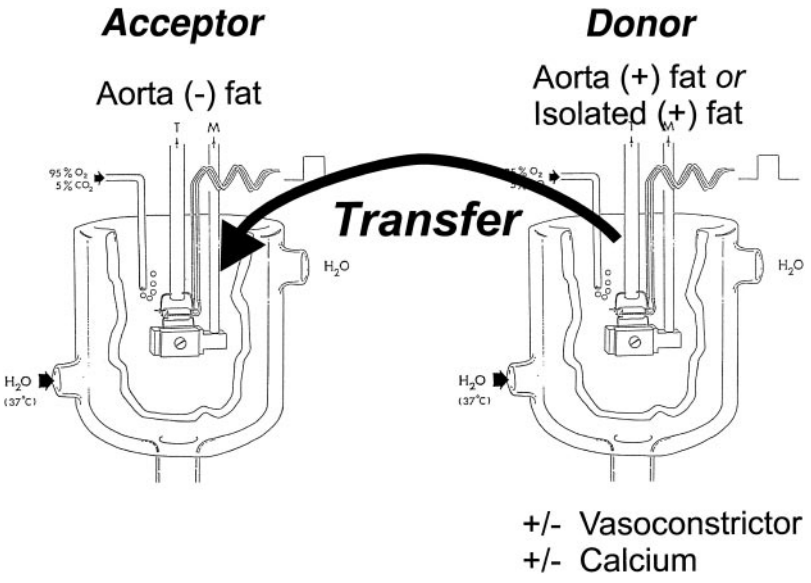

Figure 1. Schematic illustration of the bioassay experiments. Aliquots (3–5 mL) of the bath solution from intact aortic preparations or isolated aortic perivascular fat tissue incubated in a closed donor bath chamber were transferred to vessel preparations without periadventitial fat in a closed acceptor bath chamber. In most experiments, donor preparations were treated with vasoconstrictors (serotonin or phenylephrine). In other experiments, donor preparations were untreated or left in Ca^{2+}-free external solution. The volume of the bath chambers was 20 mL.

fetal calf serum) for 5 days. The cell density was ~150,000 cells/mL.

All values are given as mean and standard error (SE). For group comparisons, paired and unpaired Student's *t* tests or nonparametric Wilcoxon tests were used as appropriate. A value of $P < 0.05$ was considered statistically significant; *n* represents the number of arteries tested.

RESULTS

To test the hypothesis that perivascular fat influences vascular contraction, we generated dose-response curves to angiotensin II (**Fig. 2*A–C***), serotonin (Fig. 2*D*), and phenylephrine (Fig. 2*E*) for both intact (open circles, $n=10$) and aortic rings without periadventitial fat (filled circles, $n=10$). At a maximum concentration of 300 nM angiotensin II, 6.5 μM serotonin, and 5 μM phenylephrine, the contractile response of intact rings was around 95%, 80%, and 30% lower than that of vessels lacking periadventitial fat. These results provide strong evidence for a vasodilatory, or rather anticontractile, effect of periadventitial adipose tissue.

We then tested the hypothesis that K^+ channels are involved in this anticontractile effect. At 3 μM, the

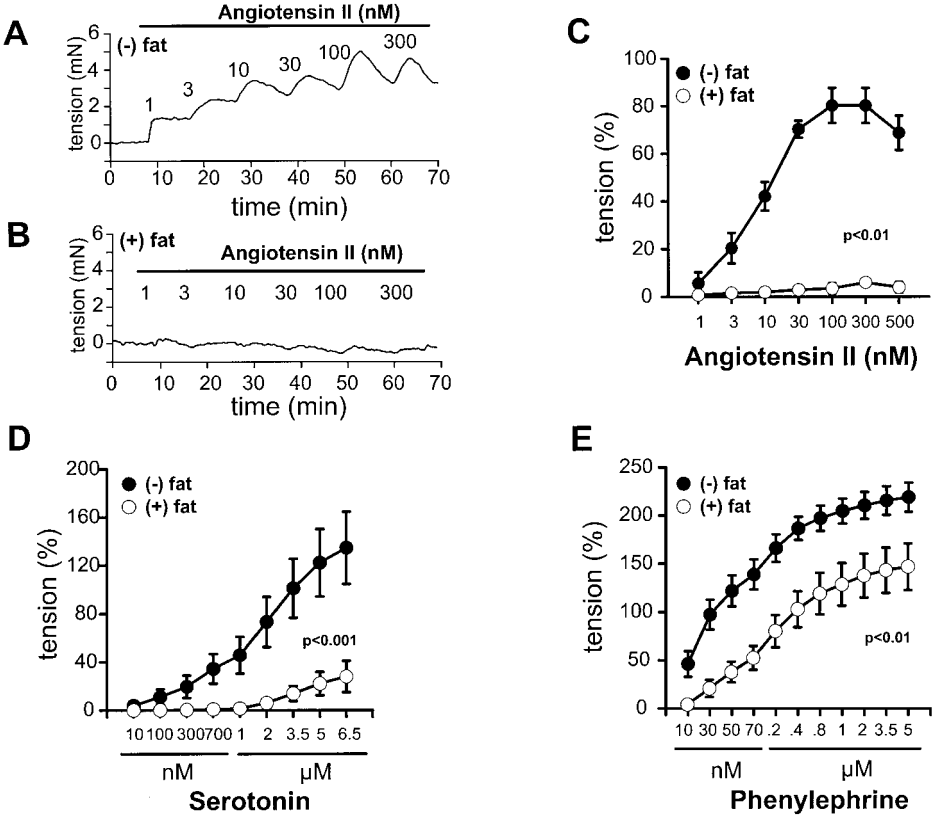

Figure 2. Dose-response-relationship for agonist-induced contractions of aortic rings without periadventitial fat (−fat) and intact (+fat) aortic ring preparations. Representative tracing of contractile response to incremental doses of angiotensin II in the absence (*A*) and presence (*B*) of periadventitial fat. Dose-response curves to angiotensin II (*C*), serotonin (*D*), and phenylephrine (*E*) in intact aortic ring preparations (open circles) and aortic ring preparations without periadventitial fat (filled circles). Thus, the presence of periadventitial fat reduced the contractile response to these agonists.

ATP-dependent K^+ channel blocker glibenclamide virtually inhibited the difference in response between intact vessels and vessels without periadventitial fat (n=6) to serotonin (**Fig. 3*A***). Blockers of other potassium channels, i.e., tetraethylammonium (1 mM) and 4-aminopyridine (2 mM), which inhibit large-conductance Ca^{2+}-activated potassium channels and delayed rectifier K^+ channels, respectively, and Ba^{2+} (100 μM), which blocks the inward rectifying K^+ channels (4), were less or not effective (data not shown). These results suggest that the difference in response to serotonin between intact vessels and vessels lacking periadventitial fat is likely mediated by opening of ATP-dependent K^+ channels.

We next challenged intact aortic rings and aortic rings without periadventitial fat (n=12) with 60 mM KCl. Raising external K^+ would be expected to diminish the effects of any K^+ channel opener by substantially reducing the difference between the K^+ equilibrium potential and the membrane potential. Figure 3*B* shows that the contractile responses of intact vessels and vessels without periadventitial fat to 60 mM KCl were not significantly different. These findings demonstrate that excitation-contraction coupling in intact arteries and arteries lacking periadventitial fat remains functional and that the presence of perivascular fat does not mechanically or otherwise alter the contractility of aortic rings. In addition, the perivascular fat-induced anticontractile effect was absent when the vessels were preincubated with the K^+ channel opener cromakalim at 0.3 μM (Fig. 3*C*, n=6–7), i.e., when ATP-dependent K^+ channels were submaximally prestimulated by blocking their sensitivity to ATP.

To explore the influence of endothelium-derived NO on the fat-modulated response of aortic ring contraction, we measured the contractile response to serotonin in vessels (n=6) incubated in 300 μM N^G-nitro-L-arginine (LNNA). Despite inhibition of NO formation, contractile response to serotonin remained higher in the vessels lacking periadventitial fat (Fig. 3*E*). Mechanical removal of endothelium did not influence the anticontractile effect of perivascular fat (Fig. 3*F*).

The tyrosine kinase inhibitor genistein (10 μM) abolished the anticontractile response to perivascular fat in the absence (n=4) or presence of LNNA (n=12) (Fig. 3*D*) whereas the inactive genistein (daidzein; 10 μM) showed no inhibitory effect (data not shown). These results suggest that the difference in response to serotonin between intact vessels and vessels without periadventitial fat is indeed dependent on functional ATP-dependent K^+ channels and activation of tyrosine kinase. The findings clearly demonstrate that the inhibitory effect of periadventitial fat on vascular contraction is independent of NO production and therefore not dependent on the presence of a functional endothelium.

To demonstrate that the intact aortic preparation releases a substance that can abrogate vascular contraction, we performed bioassay experiments where we transferred aliquots of the bath solution from an intact donor preparation incubated in 2 μM serotonin-containing solution to vessel preparations without periadventitial fat, precontracted with serotonin. This maneuver transferred the factor released by either intact preparations (**Fig. 4*A***, n=14) or isolated perivascular adipose tissue (Fig. 4*B*, n=6) to arteries without perivascular fat. Transfer of the donor bath solution aliquots

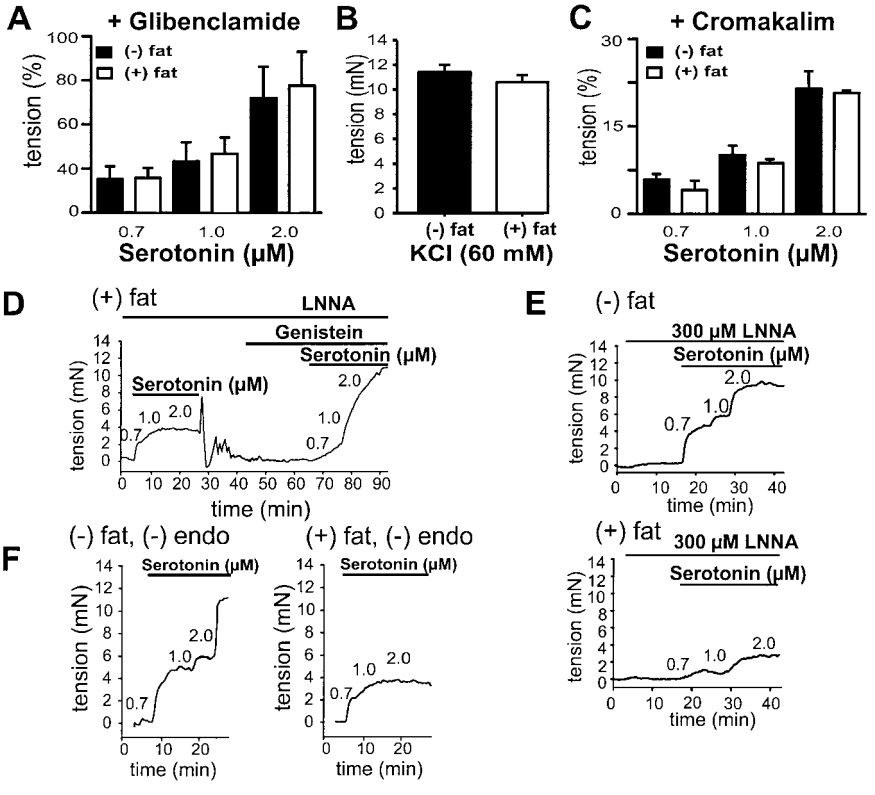

Figure 3. Blocking ATP-dependent K^+ channels with 3 μM glibenclamide (*A*), opening all K^+ channels with 60 mM KCl (*B*), or opening ATP-dependent K^+ channels with 300 nM cromakalim (*C*) abolished the difference in contractile response between aortic rings without periadventitial fat (black bars) and intact (open bars) aortic rings. The anticontractile effect of periadventitial adipose tissue is genistein sensitive. Incubation of (+) fat aortic rings with genistein significantly increased the contractile response to serotonin (*D*). Incubation with 300 μM N^G-nitro-L-arginine (LNNA) did not abrogate the anticontractile effect of periadventitial adipose tissue on the vascular response to serotonin (*E*). The anticontractile effect of periadventitial adipose tissue on the vascular response to serotonin was not affected by removal of endothelium (F). [(+) endo, aortic ring with endothelium, (−) endo, aortic ring without endothelium].

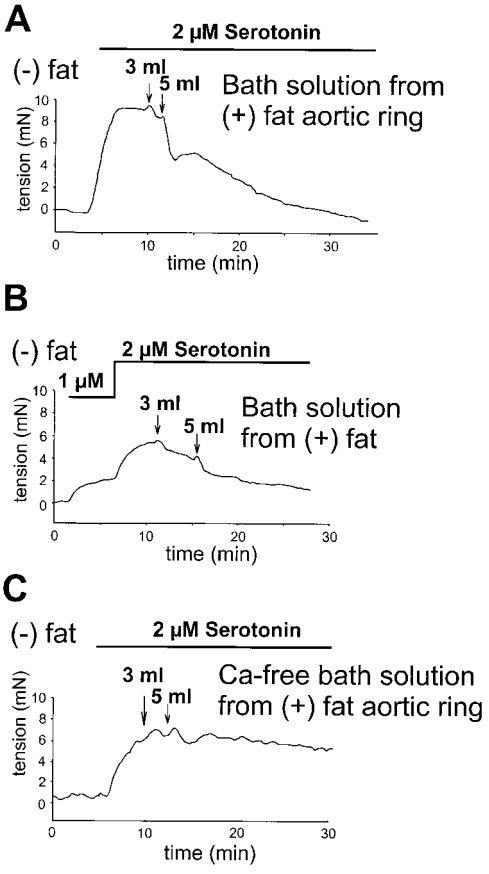

Figure 4. Transfer of aliquots of the bath solution containing the hypothesized adventitial-derived relaxing factor (ADRF) elaborated from a donor aortic ring with perivascular adventitium [(+) fat] to a serotonin precontracted aortic ring without periadventitial fat [(−)fat] resulted in relaxation. Repeated applications of ADRF (5 mL after 3 mL) evoked dose-dependent relaxations in (−)fat aortic rings (*A*). Transfer of aliquots of the bath solution containing the hypothesized ADRF elaborated from isolated aortic perivascular adventitium [(+) fat] to a serotonin precontracted aortic ring without periadventitial fat [(−)fat) resulted in relaxation. Repeated applications of ADRF evoked dose-dependent relaxations in (−)fat aortic rings (*B*). Transfer of aliquots of the bath solution of a donor aortic ring with perivascular adventitium [(+) fat] incubated in Ca^{2+}-free solution to a serotonin precontracted aortic ring without periadventitial fat [(−)fat] did not induce relaxation. Repeated applications of aliquots did not evoke dose-dependent relaxations in (−)fat aortic rings (*C*).

containing the proposed ADRF to serotonin precontracted aortic rings without periadventitial fat [(−)fat)) resulted in a dose-dependent relaxation. There was no change in biological activity when transfer intervals were increased up to 45 min. However, the relaxation was completely abolished if acceptor vessels were pretreated with glibenclamide (3 µM) or genistein (10 µM) (data not shown). Moreover, the relaxation did not occur when donor aortic rings with periadventitial fat were incubated in a Ca^{2+}-free solution (PSS containing 0 mM Ca^{2+} and 0.5 mM EGTA) (Fig. 4*C*, n=6). However, transfer of donor bath solution aliquots of intact donor preparations (or isolated donor perivascular adipose tissue) not treated with serotonin (2 µM) or phenylephrine (100 nM) induced relaxation of acceptor vessels even in the presence of the sodium channel blocker tetrodotoxin (1 µM) (data not shown). These findings suggest Ca^{2+}-dependent, continuous release of the factor by periadventitial adipose tissue. **Figure 5** shows that heating (65°C, 10 min) largely inhibited relaxation (Fig. 5*A*, n=8) whereas addition of 0.1% essentially fatty acid-free human serum albumin did not prevent relaxation (Fig. 5*B*, n=6). These data suggest that the factor is inactivated by heating but not adsorbed by essentially fatty acid-free serum albumin, consistent with a peptide rather than a lipid.

To determine the putative cellular origin of this factor, we next performed bioassay experiments using cultured rat adipocytes and fibroblasts. **Figure 6** shows that addition of cultured rat adipocytes (~750,000 cells) and culture medium (5 mL) to the acceptor bath solution (20 mL) resulted in a relaxation of intact aortic rings without perivascular adventitial tissue compared with control conditions. In contrast, addition of cultured fibroblasts (~800.000 cells) and culture medium (5 mL) to the acceptor bath solution did not induce relaxation of intact aortic rings without perivascular adventitial fat (n=4).

The anticontractile effect of perivascular fat was also tested in obese Zucker fa/fa rats that lack functional leptin receptors. In the presence of 300 µM LNNA, serotonin evoked markedly reduced aortic contractions in the presence of perivascular fat (**Fig. 7**, n=8). This reduced contraction was again reversed by incubation of the vessels with 10 µM genistein (not shown).

We next examined the hypothesis that a cyclooxygenase or cytochrome P450-dependent vasoactive substance mediates adventitial modulation of aortic ring contraction to serotonin. However, inhibition of cyclooxygenase by preincubation of intact aortic rings and aortic rings without periadventitial fat over 20 min with 3 µM indomethacin still resulted in a significantly greater response to serotonin in the vessel without periadventitial fat than in vessels with intact periadventitial fat (n=5; 53±7% vs. 28±6%, P<0.01). Similarly, inhibition of cytochrome P450 by 10 nM 17-ODYA (17-octadecynoic acid, an inhibitor of de novo synthesis of epoxyeicosatrienoic and epoxyeicosatetraenoic acid provided by W. H. Schunck, MDC, Berlin, Germany) did not abrogate the increased response of vessels lacking perivascular fat to serotonin compared with intact vessels (n=5; 60±4% vs. 20±6%, P<0.01). In these experiments, 17-ODYA was added for 10 min to the organ bath, then washed out after short-term application of serotonin (5 µM, 5 min). After another 60 min, the vessels were restimulated with serotonin (5 µM). In addition, inhibition of cytochrome P450 using 3 µM miconazole for ~90 min did not block the anticontractile effect of perivascular fat (n=4, data not shown).

Finally, inhibition of adenosine receptors did not affect the vasodilatory influence of periadventitial fat. Neither preincubation for 10 min with CGS (9-chloro-

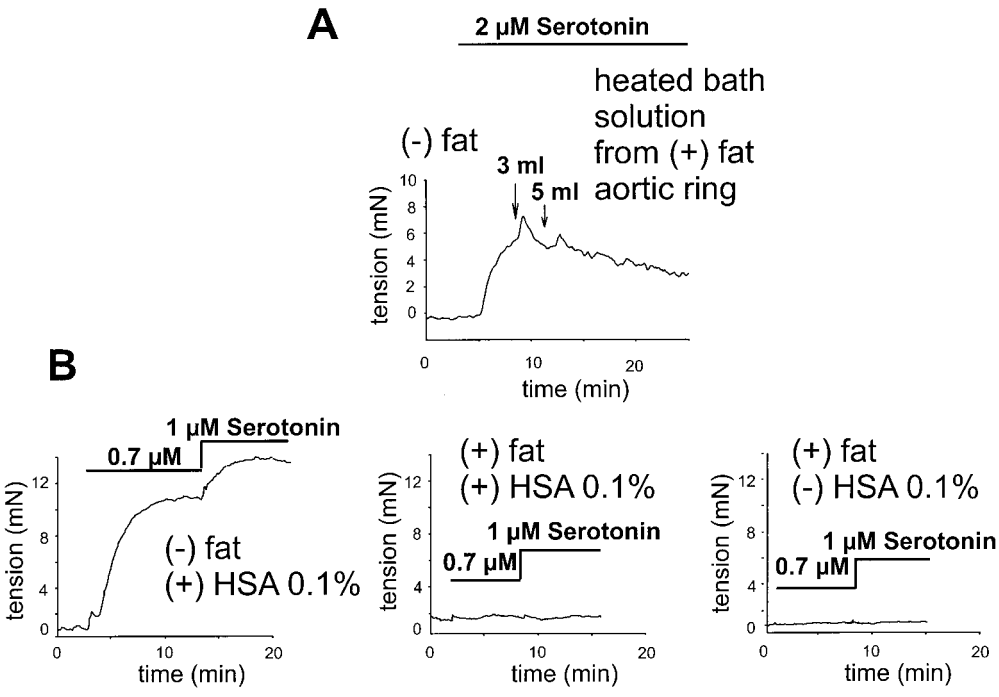

Figure 5. Transfer of heated aliquots (65°C, 10 min) of the bath solution of a donor aortic ring with perivascular adventitium [(+) fat] to a serotonin precontracted aortic ring without periadventitial fat [(−)fat] did not induce relaxation. Repeated applications of aliquots did not evoke dose-dependent relaxations in (−)fat aortic rings (*A*). Nonheated aliquots of this bath solution induced relaxation of acceptor aortic rings without perivascular adventitium [(−) fat] preconstricted with serotonin (0.7 and 1 µM) (not shown). Addition of 0.1% essentially fatty acid-free human serum albumin (HSA) to the bath solution did not abolish the anticontractile effects of periadventitial fat (*B*). Vessels responded with similar contractions to external application of 60 mM KCl (not shown).

2-(2-furyl)(1,2,4)triazol(1,5-c)quinazolin-5-amine), a highly potent nonselective adenosine receptor antagonist, nor preincubation for 10 min with 1 µM of the A1 antagonist DCPCX (8-cyclopentyl-3,7-dipro-1,3-dipropyl-1H-purine-2,6-dione) or 1 µM of the A2 antagonist DMPX (3,7 dimethyl-1-proparglylxanthine), attenuated the vasodilatory influence of periadventitial fat (data not shown). Thus, the vasodilatory effect of periadventitial fat is not mediated by NO or adenosine receptors.

DISCUSSION

We demonstrate that periadventitial adipose tissue markedly attenuates the contractile response to angiotensin II and other vasoactive compounds in aortic ring preparations. Periadventitial fat appears to release a substance into the organ bath that acts via ATP-dependent K^+ channels and activates tyrosine kinase. In bioassay experiments, we showed that this substance(s) is relatively stable and can be transferred to smooth muscle tissue, where it likewise exerts an anticontractile effect. Synthesis or action of this substance is not dependent on the cyclooxygenase or P450 pathway, the formation of NO, activation of adenosine receptors, or the presence of functional leptin receptors. The substance is released by perivascular adventitial tissue in a Ca^{2+}-dependent manner. It is inactivated by heating and not adsorbed by essentially fatty acid-free serum albumin, suggesting that the substance is more likely a protein than a lipid.

Soltis and Cassis were the first to test the hypothesis that perivascular adventitial fat might be important for vascular regulation (2). They observed a diminished response to norepinephrine in intact vessels compared

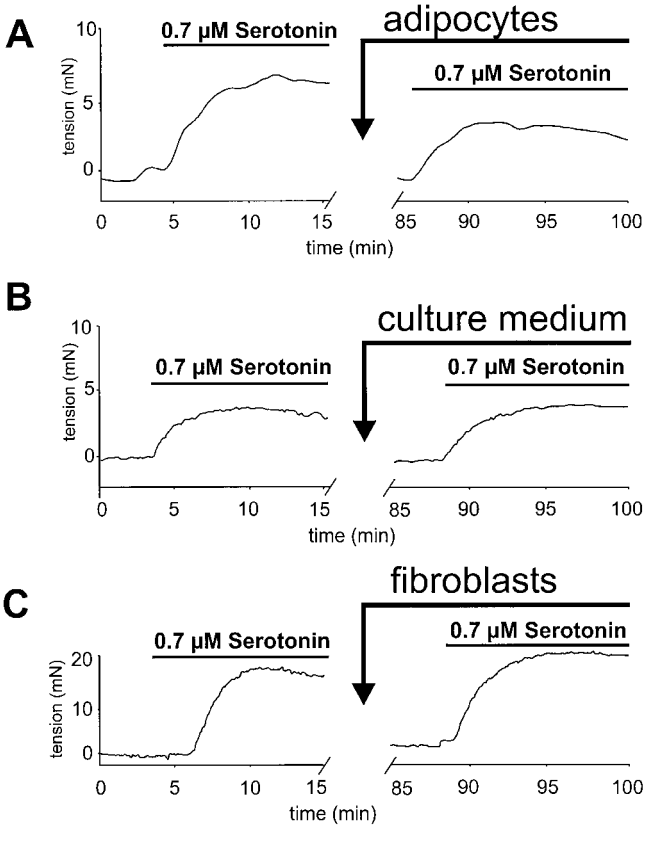

Figure 6. Transfer of cultured rat adipocytes (~750.000 cells) in culture medium (5 mL) to serotonin precontracted acceptor aortic rings without periadventitial fat [(−)fat] resulted in reduced contraction responses to serotonin (*A*). Transfer of culture medium (5 mL, *B*) or fibroblasts in culture medium (~ *800*.000 cells in 5 mL, *C*) to a serotonin precontracted acceptor aortic rings without perivascular adventitium [(−)fat] did not inhibit serotonin-dependent contraction in [−) fat aortic rings.

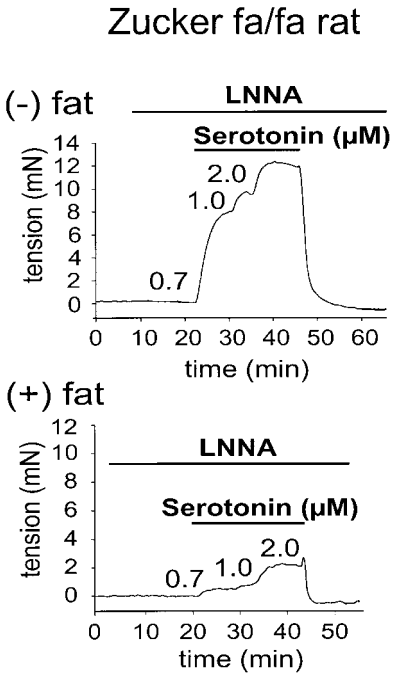

Figure 7. Contractile response to serotonin was markedly attenuated in (+) fat aortic rings of Zucker fa/fa rats, suggesting that the effect of ADRF is not dependent of functional leptin receptors.

to vessels without periadventitial fat. This difference was eliminated by the administration of the neuronal reuptake inhibitor desipramine plus deoxycorticosterone. They also found that intact vessels responded more readily to tyramine than vessels without periadventitial fat and that electrical stimulation resulted in a response only in intact vessels (2). They concluded that perivascular adventitial fat tissue influences vascular responses in vitro and attributed this effect to the dense sympathetic innervation in periadventitial adipose tissue.

We found that the response to angiotensin II, serotonin, and phenylephrine was reduced by 95, 80, and 30%, respectively, in intact vs. vessels without periadventitial fat. In contradistinction to norepinephrine, these substances are not subject to reuptake by adrenergic nerves. Therefore, our results show that the anticontractile effect of perivascular adipose tissue is independent of adrenergic neuronal reuptake. Rather, we present evidence that regulation of ATP-dependent K^+ channels in vascular smooth muscle cells and activation of tyrosine kinase are involved. Inactivating the regulation of these channels, either with the strong channel opener cromakalim or the channel blocker glibenclamide, successfully inhibited the anticontractile effect of periadventitial fat. A role for sulfonylurea receptor 2-regulated, glibenclamide-sensitive K^+ channels in regulating membrane potential in rat aorta has been clearly established (5, 6).

Renewed interest in the role of adipose tissue has led to the recognition that adipocytes produce and respond to a host of vasoactive substances including angiotensin II, NO, adenosine receptor agonists, tumor necrosis factor alpha, endothelin, prostanoids, and a variety of nonesterified fatty acids (7). Adipose tissue-derived candidates exerting a vasodilatory effect include epoxyeicosatrienoic acids, which are cytochrome P450-derived epoxides believed to mediate hyperpolarization by activating potassium channels (8). However, neither pretreatment with the cyclo-oxygenase inhibitor indomethacin nor P450 inhibitors 17-ODYA or miconazole restored the contractile effects of serotonin on intact vessels. Likewise, inhibition of adenosine receptors, known to affect vascular tone by inducing membrane hyperpolarization via ATP-dependent K^+ channel activation (5), did not mitigate the vasorelaxing effect of perivascular adipose tissue. The adipocyte-derived cytokine leptin was recently shown to activate the ATP-dependent K^+ channel in a variety of tissues, an effect that was completely blocked by application of the sulfonylureas tolbutamide or glibenclamide (9, 10). Preservation of the anticontractile effect of perivascular adipose tissue in obese Zucker fa/fa rats argues against leptin as a mediator of this effect.

Attempts to establish a bioassay to clearly demonstrate that the source of the relaxing factor is indeed the adipocyte and not other cellular components of the adventitium are hampered by the difficulty in isolating sufficient amounts of homogenous perivascular adipocyte preparations from the vessel wall. Although our bioassay data with cultured adipocytes and fibroblasts suggest that adipocytes produce a relaxing factor, we cannot rule out that the adventitial-derived relaxing factor is released by components of the adventitium other than adipocytes.

Vascular ATP-dependent K^+ channels are activated by numerous endogenous substances released under conditions of increased blood demand or hypoxia (11). They are also believed to be involved in the regulation of basal tone, particularly in certain vascular beds such as the coronary circulation (12) and mesenteric arteries (6). Reactive hyperemia is attenuated by glibenclamide in coronary and skeletal muscle vascular beds (13, 14), and inhibition of ATP-dependent K^+ channels by glibenclamide disrupts coronary (15) and cerebral (16) autoregulation. In fact, potential detrimental cardiovascular effects of sulfonylureas have long been suspected (17). If similar effects are imparted by perivascular adipose tissue of smaller arteries, this tissue may regulate vascular blood flow in accordance with metabolic demand by releasing vasoactive substances that can counteract the action of vasoconstrictive factors. Perturbations of this system could conceivably contribute to hypertension in obese or nonobese individuals. We cannot but help wonder whether some of the abnormalities in vascular response attributed to changes in endothelial function are indeed mediated by hitherto unrecognized 'adventitial' dysfunction.

In summary, we have shown that periadventitial fat has a profound anticontractile effect on aortic ring preparations. This effect is independent of NO formation and appears to be mediated by a substance released from intact aortic rings into the organ bath. We

suggest that perivascular adventitial adipose tissue elaborates an adventitium-derived relaxing factor that acts at least in part by activation of ATP-dependent K^+ channels and tyrosine kinase. We have not yet identified the factor(s) responsible. However, we suggest that they function independent of NO and sympathetic nervous system vascular innervation. Perturbations could conceivably contribute to hypertension in obese or nonobese individuals. FJ

We thank Jean B. Margot and Victoriya Sagach for kindly providing fibroblasts and adipocytes and Kirill Essin for help in data analysis. This work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Crandall, D. L., Hausman, G. J., and Kral, J. G. (1997) A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. *Microcirculation* **4,** 211–232
2. Soltis, E. E., and Cassis, L. A. (1991) Influence of perivascular adipose tissue on rat aortic smooth muscle responsiveness. *Clin. Exp. Hypertens. A* **13,** 277–296
3. Gollasch, M., Bychkov, R., Ried, C., Behrendt, F., Scholze, S., Luft, F. C., and Haller, H. (1995) Pinacidil relaxes porcine and human coronary arteries by activating ATP-dependent potassium channels in smooth muscle cells. *J. Pharmacol. Exp. Ther.* **275,** 681–692
4. Gollasch, M., Ried, C., Bychkov, R., Luft, F. C., and Haller, H. (1996) K^+ currents in human coronary artery vascular smooth muscle cells. *Circ. Res.* **78,** 676–688
5. Matsushita, Y., Henmi, S., Muraki, K., Imaizumi, Y., and Watanabe, M. (2000) Cromakalim-induced membrane current in guinea-pig tracheal smooth muscle cells. *Eur. J. Pharmacol.* **389,** 51–58
6. Nelson, M. T., and Quayle, J. M. (1995) Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.* **268,** C799–C822
7. Engeli, S., and Sharma, A. M. (2000) Role of adipose tissue for cardiovascular-renal regulation in health and disease. *Horm. Metab. Res.* **32,** 485–499
8. Quilley, J., and McGiff, J. C. (2000) Is EDHF an epoxyeicosatrienoic acid? *Trends Pharmacol. Sci.* **21,** 121–124
9. Spanswick, D., Smith, M. A., Groppi, V. E., Logan, S. D., and Ashford, M. L. (1997) Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature (London).* **390,** 521–525
10. Harvey, J., McKenna, F., Herson, P. S., Spanswick, D., and Ashford, M. L. (1997) Leptin activates ATP-sensitive potassium channels in the rat insulin-secreting cell line, CRI-G1. *J. Physiol. (London)* **504,** 527–535
11. Daut, J., Maier-Rudolph, W., von Beckerath, N., Mehrke, G., Gunther, K., and Goedel-Meinen, L. (1990) Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science* **247,** 1341–1344
12. Samaha, F. F., Heineman, F. W., Ince, C., Fleming, J., and Balaban, R. S. (1992) ATP-sensitive potassium channel is essential to maintain basal coronary vascular tone in vivo. *Am. J. Physiol.* **262,** C1220—C1227
13. Clayton, F. C., Hess, T. A., Smith, M. A., and Grover, G. J. (1992) Coronary reactive hyperemia and adenosine-induced vasodilation are mediated partially by a glyburide-sensitive mechanism. *Pharmacology* **44,** 92–100
14. Vanelli, G., and Hussain, S. N. (1994) Effects of potassium channel blockers on basal vascular tone and reactive hyperemia of canine diaphragm. *Am. J. Physiol.* **266,** H43–H51
15. Narishige, T., Egashira, K., Akatsuka, Y., Katsuda, Y., Numaguchi, K., Sakata, M., and Takeshita, A. (1993) Glibenclamide, a putative ATP-sensitive K+ channel blocker, inhibits coronary autoregulation in anesthetized dogs. *Circ. Res.* **73,** 771–776
16. Hong, K. W., Pyo, K. M., Lee, W. S., Yu, S. S., and Rhim, B. Y. (1994) Pharmacological evidence that calcitonin gene-related peptide is implicated in cerebral autoregulation. *Am. J. Physiol.* **266,** H11—H16
17. Hu, S., Wang, S., and Dunning, B. E. (1999) Tissue selectivity of antidiabetic agent nateglinide: study on cardiovascular and beta-cell K(ATP) channels. *J. Pharmacol. Exp. Ther.* **291,** 1372–1379

Received for publication January 10, 2002.
Revised for publication March 5, 2002.