Perivascular adipose tissue modulates vascular function in the human internal thoracic artery

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Objective: Recent studies have shown that perivascular adipose tissue from the rat aorta secretes a substance that can dilate the aorta. The purpose of the present study was to examine whether this vasodilator is also present in human internal thoracic arteries.

Methods: Vascular function of human internal thoracic arteries with and without perivascular adipose tissue was assessed with wire myography, and morphology was examined with light microscopy.

Results: The presence of perivascular adipose tissue attenuated the maximal contraction to U 46619 and the contraction to phenylephrine (1 μmol/L) by 37% and 24%, respectively. Transfer of the solution incubated with a perivascular adipose tissue–intact vessel (donor) to a vessel without perivascular adipose tissue (recipient) induced a significant relaxation (36%) in the recipient artery precontracted with phenylephrine. Transfer of incubation solution with perivascular adipose tissue alone also induced a relaxation response in the recipient vessel (37%). The relaxation of the recipient artery induced by the transfer of incubation solution from the donor (artery with intact perivascular adipose tissue or perivascular adipose tissue alone) was absent in vessels precontracted by KCl (60 mmol/L) and was prevented by calcium-dependent potassium channel blockers (tetraethylammonium chloride, 1 mmol/L; iberiotoxin, 100 nmol/L), but not by the voltage-dependent potassium channel blocker 4-aminopyridine (1 mmol/L) and the adenosine triphosphate–dependent potassium channel blocker glibenclamide (10 μmol/L).

Conclusions: Perivascular adipose tissue in human internal thoracic arteries releases a transferable relaxation factor that acts through the activation of calcium-dependent potassium channels. Because perivascular adipose tissue is often removed in coronary artery bypass grafting, retaining perivascular adipose tissue might be helpful in reducing the occurrence of vasospasm of the graft vessels.

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This study was supported by the Heart and Stroke Foundation of Ontario, Canada, and the Canadian Institutes of Health Research. Dr Y.-J. Gao is the recipient of the New Investigator Award from the Canadian Institutes of Health Research/Canadian Hypertension Society.

Received for publication April 7, 2005; revisions received May 10, 2005; accepted for publication May 25, 2005.

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J Thorac Cardiovasc Surg 2005;130:1130-6

0022-5223/$30.00

doi:10.1016/j.jtcvs.2005.05.028

In addition to energy storage, adipose tissue secretes a significant number of biologically active substances, such as leptin, adiponectin, angiotensinogen, resistin, and steroid hormones.[1,2] Most of the systemic blood vessels are surrounded by a certain amount of perivascular adipose tissue (PVAT) situated outside the adventitial layer. In rat aorta recent studies, including ours,[3-5] have reported that PVAT releases a relaxing factor that attenuates vascular contractile responses to agonists, including serotonin, angiotensin II, and phenylephrine, through the activation of potassium channels in vascular smooth muscle cells. Hyperpolarization of smooth muscle cell membrane caused by potassium channel activation, as in the case of vascular response to endothelium-derived hyperpolarizing factor (EDHF), is an important mechanism for vasodilation.[6] However, the fact that rat aortic PVAT can release a relaxation factor does not necessarily mean that PVAT in a human subject would function similarly because rodent aorta is surrounded by brown adipose tissue that is absent in adult human subjects.[7] Therefore the clinical relevance of the finding in rats needs to be examined in human vessels. The purpose of this study is to examine whether this relaxation factor is also present in the PVAT of human arteries.

Methods

Patients with coronary artery disease who underwent elective coronary artery bypass grafting (CABG) were included in this study. The study conforms to the principles outlined in the Declaration of Helsinki, and institutional approval was obtained.

Vessel Preparation and Contractility Study

Terminal segments of human internal thoracic arteries (ITAs) that were usually discarded during CABG were collected in oxygenized (95% O_2 and 5% CO_2) physiologic salt solution at 4°C with the following composition: NaCl, 119 mmol/L; KCl, 4.7 mmol/L; KH_2PO_4, 1.2 mmol/L; $MgSO_4$, 1.2 mmol/L; $NaHCO_3$, 25 mmol/L; $CaCl_2$, 1.6 mmol/L; glucose, 11 mmol/L. Care was taken to ensure minimal handling of the ITAs during operation to minimize damage. The ITAs were transported to our laboratory within 60 minutes in physiologic salt solution on ice.

Paired ITA rings, one with intact PVAT ($PVAT^+$) and one with PVAT removed ($PVAT^-$), were prepared from each segment. A wired myograph system was used to study the contraction and relaxation responses of the ITAs, as described in our previous report.[8] ITAs with or without PVAT were studied at the same basal tension. After equilibration for at least 60 minutes, the ITA was challenged with 60 mmol/L KCl twice at an interval of 30 minutes to check the viability of the tissue. Concentration-contraction response curve for U 46619 (0.3-300 nmol/L) was constructed and expressed as a percentage of KCl contraction. The concentration that causes 50% of the maximal contraction was estimated by fitting each concentration-response curve. Relaxation response to sodium nitroprusside was tested in ITAs precontracted with phenylephrine (1-3 μmol/L) and expressed as a percentage of the precontraction value.

Bioassay Experiments and Morphologic Study

The effects of the relaxing factor released by PVAT were studied in the following way. The donor aliquot was incubated either with ITAs with intact PVAT or with PVAT alone for 20 to 25 minutes and then transferred to the recipient ITAs with PVAT removed. The donor and the recipient ITAs were simultaneously contracted with the same contracting agent (1 μmol/L phenylephrine or 60 mmol/L KCl), and the transfer of the aliquot was carried out when the contraction of the donor and recipient arteries had reached a plateau. The capacity of the organ bath tube was 4 mL, and the amount of aliquot transferred was 3 mL. The relaxation of the recipient ITAs was expressed as a percentage of the precontraction value. Ion channel blockers were introduced when the relaxation response had reached a plateau. Some ITAs were fixed in 10% formalin for morphologic studies. Cross-sections of ITAs were stained with hematoxylin and eosin or Gomori trichrome for light microscopy.

TABLE 1. Patient characteristics, including risk factors and medications

Physical factors	
Number	52
Men, n (%)	44 (85)
Women, n (%)	8 (15)
Age (y)	62.8 ± 1.3
Height (cm)	172.2 ± 1.1
Weight (kg)	87.4 ± 2.1
Overweight, n (%), BMI 25-29.9	24 (46)
Obese, n (%), BMI ≥30	20 (38)
Health factors, n (%)	
Hypertension	32 (62)
Diabetes mellitus	24 (46)
Hypercholesterolemia	26 (50)
Smoker	42 (81)
Myocardial infarction	39 (75)
Medications, n (%)	
Aspirin	33 (63)
Clopidogrel	14 (27)
β-Blockers	39 (75)
ACE inhibitors	37 (71)
Calcium channel blockers	21 (40)
Statin	35 (67)
Nitrates	43 (83)

BMI, Body mass index; *ACE*, angiotensin-converting enzyme.

Statistical Analyses

Results are presented as means ± standard error of the mean. Statistical analyses were carried out with SigmaStat (SPSS, Inc, Chicago, Ill). Before statistical testing, normality was examined for all the data, and the minimum P value was .072. The Student t test was used to identify the contractile difference between vessels with or without PVAT in response to KCl and to phenylephrine. The paired t test was used to assess the relaxation response of the ITAs ($PVAT^-$) to transfer of aliquot from ITAs ($PVAT^+$) and from PVAT alone. Two-way repeated-measures analysis of variance (ANOVA) was used to determine any significant difference between the concentration responses to U 46619 in ITAs with or without PVAT. Post hoc t tests were used to identify the concentration at which statistically significant differences occurred, when ANOVA indicated significant differences between $PVAT^+$ and $PVAT^-$ ITAs. One-way repeated-measures ANOVA was used to assess the effects of K^+ channel blocker on the relaxation caused by aliquot transfer.

Results

Patient Characteristics

ITAs from a group of 52 patients consisting mostly of male subjects were studied (Table 1). The average age was 62.8 years, with a range of 43 to 82 years. Forty-six percent of them were overweight, and 38% were obese. Eighty-one percent of them were smokers. Of the patients, 92.3% had 1 or more risk factors for coronary heart disease, and 96.2% were receiving 1 or more type of medication for hypertension, hypercholesterolemia, antianginal therapy, and anti-

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