Small artery structural alterations of patients with microvascular angina (Syndrome X)

S. J. BUND, A. TWEDDEL*, I. HUTTON† and A. M. HEAGERTY
University Department of Medicine, Manchester Royal Infirmary, Manchester, U.K., *Department of Cardiology, University Hospital of Wales, Cardiff, U.K., and †Department of Cardiology, University of Glasgow, Glasgow, U.K.

(Received 12 October 1995/29 July 1996; accepted 1 August 1996)

1. There is increasing evidence that a substantial number of patients who suffer from angina have normal epicardial arteries (Syndrome X), and it has been suggested that these individuals have a generalized disorder of small vessels not confined to the intramyocardial vasculature.

2. Small arteries were therefore obtained from biopsies of skin and subcutaneous fat from nine normotensive patients with Syndrome X and nine matched control subjects. Vessels were dissected and mounted as ring preparations in a myograph for morphological and functional assessment.

3. Morphological measurements revealed a significant increase in media thickness/lumen diameter ratio in arteries from patients with Syndrome X. Contractile responses to U46619 were similar in arteries from patients and control subjects. Endothelium-dependent relaxation induced with acetylcholine and bradykinin was greater in arteries from patients although differences were not statistically significant. Endothelium-independent relaxation induced by forskolin and sodium nitroprusside was not different.

4. In conclusion, these data demonstrate that subcutaneous small arteries from patients with Syndrome X are characterized by increased media thickness/lumen diameter ratio, although contractile responses were normal. Additionally, endothelium-dependent relaxation was not impaired in arteries from these patients. Thus, no significant functional abnormalities were associated with the observed structural differences.

INTRODUCTION

Microvascular angina is a term which is used to describe individuals who often fulfil conventional criteria for angina but who do not have angiographically evident lesions of their epicardial coronary arteries [1, 2]. This may include up to 20% of subjects undergoing diagnostic catheterization for evaluation of chest pain [3]. In the absence of significant atheromatous deposits in medium-sized arteries, it has been suggested that such patients have a disease of small coronary arteries, and this is supported by the finding of reduced coronary reserve in a proportion of such subjects [4–6]. In addition, many patients show intermittent regional abnormalities of myocardial blood flow rather than a continuous uniform fall in reserve [7, 8]. Thus, while a structural change in coronary small arteries might contribute to the pathogenesis of the disease, some form of functional abnormality must also be present. In support of a functional disturbance such patients have been shown to have limited coronary flow responses to the stress of rapid atrial pacing and to vasodilators such as dipyridamole [7, 8]. Furthermore, while blood rheology is normal [9], patients have high circulating levels of insulin [10]. Many studies that have been undertaken to investigate the nature of the disturbance in this disease have focused on the coronary circulation and, of necessity, have been indirect. However, there is accumulating evidence that the condition may be just one manifestation of a more generalized disturbance of arterial function. It has been suggested that there is an increased prevalence of migraine and Raynaud's phenomenon in some patients [11], and it has been shown that patients with microvascular angina have an impaired forearm vasodilator response to ischaemia [12]. Abnormalities may also occur within the pulmonary circulation and be reflected in normal ventilatory responses to exercise [13]. The magnitude of the peripheral vasodilator impairment correlated closely with that seen in the coronary circulation [12]. This finding is of immense importance because it implies that the peripheral vasculature may possess a similar disturbance in function to that found in the heart; therefore a precise examination of the morphological and contractile character-

Key words: acetylcholine, artery, bradykinin, endothelium, morphology, Syndrome X.
Abbreviations: EDRF, endothelium-derived relaxing factor; PSS, physiological salt solution.
Correspondence: Professor A. M. Heagerty, University Department of Medicine, Manchester Royal Infirmary, Oxford Road, Manchester M1 3WL, U.K.
istics of such arteries becomes crucial to our further understanding of this condition.

PATIENTS AND METHODS

Patients diagnosed as having microvascular angina were selected from the outpatient clinic of the Department of Cardiology in Glasgow. The diagnostic criteria included anginal chest pain considered by two cardiologists to be cardiac in nature, completely normal coronary arteriograms (objective evidence of inducible myocardial ischaemia with positive thallium scans with a predictive accuracy for coronary disease of 90% in our population), no other evidence of a cardiac explanation for symptoms such as hypertrophic obstructive cardiomyopathy and left ventricular hypertrophy, and in particular a normal resting ECG and echocardiogram. Patients with left bundle branch block were excluded. No patient had any evidence of any other disease such as essential hypertension, diabetes mellitus, hyperlipidaemia or cardiomyopathy. All were on anti-anginal medication which included nitrates in five patients; this was withdrawn 24 h before any patient was investigated.

The study was approved by the local ethics committees in both centres, although in Glasgow a limit of 10 patients was stipulated initially and permission to study healthy control subjects was denied. All patients gave full informed consent. The results of the study were compared with those from a group of control subjects recruited in Leicester and matched for age, sex and body weight. Small arteries (internal diameter <300 μm) were obtained from a skin biopsy taken under local anaesthesia, as described previously [14]. All patients and control subjects gave informed consent to the biopsy procedure. The samples were then transported to the study centre in Leicester. Biopsies were kept in ice-cold physiological salt solution (PSS) before use. The PSS had the following composition (mmol/l): NaCl, 119; NaHCO3, 25; KCl, 4.7; KH2PO4, 1.18; CaCl2, 2.4; EDTA, 0.026; glucose, 5.5 (pH 7.4 when gassed at 37°C and bubbled with 5% CO2/95% O2 for 60 min before measurement of vessel morphology, with the vessels just held under tension, by water immersion microscopy at ×320 magnification. After morphological measurement arteries were set to an internal circumference, L0, as determined previously [16]. Briefly, the relation of passive tension to internal circumference was measured. The internal circumference that vessels would have when relaxed and subject to a transmural pressure of 100 mmHg (L100) was calculated from the law of Laplace. Vessels were then set to a normalized internal circumference, L0, where L0 = 0.9 × L100. Arterial contractile responses are maximal at L0 [16]. Internal diameters, Io, were calculated as L0/π. Assuming constant media volume, the normalized media thickness (mo) a vessel would have at L0 was calculated. Arteries were then stimulated by means of the following protocol: first three times for 2 min with KPSS (PSS with KCl substituted for NaCl), and once with KPSS containing noradrenaline (5 μmol/l) (Sigma Chemical Co, Poole, Dorset, U.K.). Complete relaxation was allowed between each stimulation. Previous work on human intramyocardial resistance vessels had demonstrated that few conventional agonists produce consistent vasodilation [17]. However, good responses are seen with the thromboxane mimetic U46619 [17]. Cumulative concentration–response curves were therefore constructed with this agonist to examine contractile function in microvascular angina. Contractile responses are presented in three ways: (i) ΔT, defined as active tension and calculated as force divided by twice the segment length; (ii) ΔP, defined as effective active pressure and calculated as ΔT/r (r = radius) from the law of Laplace; (iii) ΔS, defined as media stress and calculated as ΔT/mo.

Relaxation responses to bradykinin and acetylcholine (endothelium-dependent) and forskolin (endothelium-independent) were determined in random order. The lowest concentration of U46619 required to induce maximal tone was applied before cumulative addition of a dilator agonist. Finally, U46619-preconstricted arteries were challenged with a single concentration of sodium nitroprusside (0.1 mmol/l).

STATISTICS

The measurements of each vessel from a single biopsy were averaged to a single value. Data are presented as means±SEM. Values were compared using Student’s t-test. Scheffe’s test was performed to compare data points in the concentration–response curves.

RESULTS

A total of 10 patients with microvascular angina volunteered to undergo skin biopsy, but a technical failure led to arteries only being harvested from nine subjects (three males and six females). The results were compared with those obtained from arteries from nine well-matched control subjects. The demographic details of the two groups are shown in Table 1. In particular, the blood pressure was normal and not significantly different from that obtained in control subjects. There were significant differences in vascular morphology between the two groups (Table 2). There was a significant increase in media thickness in vessels from patients with microvascular angina, while mean lumen diameter was
Table 1. Demographic details of patients with microvascular angina and matched control subjects. NS, not significant.

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<tr>
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<th>Patients</th>
<th>Control subjects</th>
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<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/3</td>
<td>6/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.3 ± 3.7</td>
<td>49.4 ± 4.0</td>
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<tr>
<td>Height (m)</td>
<td>1.62 ± 0.02</td>
<td>1.64 ± 0.04</td>
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<tr>
<td>Weight (kg)</td>
<td>71.6 ± 6.8</td>
<td>70.0 ± 6.0</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128.0 ± 4.5</td>
<td>128.0 ± 8.5</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82.0 ± 2.7</td>
<td>77.0 ± 3.8</td>
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 decreased in arteries from patients but this did not attain statistical significance. The haemodynamically important parameter, media thickness/lumen diameter ratio, was increased in vessels from patients and was significantly different from that seen in control subjects (Table 2).

There was no difference in vascular sensitivity to U46619 in vessels from patients with microvascular angina when compared with control subjects (Fig. 1). The concentration–response curves for acetylcholine and bradykinin are shown in Figs 2 and 3. The maximum relaxation was greater with both drugs in vessels from patients with microvascular angina (Table 3), but the difference was not statistically significant. When compared by Scheffé’s test the concentration–response curves were not found to be significantly different, as was also the case for forskolin (data not shown). The maximum relaxation responses to forskolin and sodium nitroprusside were normal in microvascular angina (Table 3).

DISCUSSION

These results demonstrate that there is a structural alteration in the architecture of the vascular wall in peripheral small arteries of patients with microvascular angina. Indeed, the magnitude of the increase in media thickness/lumen diameter ratio is similar to that previously reported in established essential hypertension [16, 18]. Such changes are not known to antedate human hypertension [19] and yet have been found here in a normotensive population, and as such would explain, to a degree, the previously reported finding of abnormal forearm blood flow in such patients [12]. It is possible that the patients we studied were already hypertensive when recruited, but that the medication they regularly received for angina was controlling this. This seems unlikely because searching the previous notes did not reveal raised blood pressure readings in any subject. Also, there was no evidence of left ventricular hypertrophy on echocardiography in any subject, and finally antihypertensive medication would be unlikely to lower pressure to such normal
vascular morphology. Second, this finding might be attributed to previous use of nitrate therapy which was withdrawn 24 h before biopsies were obtained from the patients. However, comparisons between the responses in vessels from those taking nitrates and those patients not on such drugs showed similar results. Third, our findings are in contrast to what is found in hypertension and hypercholesterolaemia, where endothelium-dependent dilatation may be depressed. The lipid profiles of the patient population in this study were normal (data not shown). Enhanced endothelium-dependent relaxation could explain why the study population was normotensive despite having substantial changes in vascular morphology. Second, this finding might be attributed to previous use of nitrate therapy which was withdrawn 24 h before biopsies were obtained from the patients. However, comparisons between the responses in vessels from those taking nitrates and those patients not on such drugs showed similar results. Third, our findings are in contrast to levels in previously established hypertensive patients [18]. Furthermore, follow-up 3 years later has only revealed one subject to have developed mild hypertension (probably associated with gross obesity). In small arteries from patients with microvascular angina, functional investigations revealed that endothelium-dependent relaxation was not impaired. Direct smooth-muscle relaxation provoked by sodium nitroprusside or forskolin was normal. Three points should be made. First, this finding is contrary to what is found in hypertension and hypercholesterolaemia, where endothelium-dependent dilatation may be depressed. The lipid profiles of the patient population in this study were normal (data not shown). Enhanced endothelium-dependent relaxation could explain why the study population was normotensive despite having substantial changes in vascular morphology. Second, this finding might be attributed to previous use of nitrate therapy which was withdrawn 24 h before biopsies were obtained from the patients. However, comparisons between the responses in vessels from those taking nitrates and those patients not on such drugs showed similar results. Third, our findings are in contrast to the impaired endothelium-dependent relaxation observed in the coronary vasculature in Syndrome X [20]. While the cause of the morphological changes remains uncertain, these results confirm that there is a generalized abnormality in the vascular tree in microvascular angina rather than one confined to the heart. Furthermore, while indirect evidence would suggest that such changes may well also be present in the myocardium, a functional change is also potentially exciting in providing an explanation of why such patients develop regional abnormalities on thallium cintigraphy during episodes of pain. It is possible that transient local disturbances in endothelium-dependent dilator function occur in such regions which leads to a reduction in either the production of endothelium-derived relaxing factor (EDRF), or increased degradation of EDRF and consequently enhanced vascular tone, leading to areas of underperfusion due to unopposed vasoconstriction. If this is correct, there is the possibility of exploring new therapeutic options in such patients, such as the administration of substrates for the production of EDRF.

**ACKNOWLEDGMENT**

We are grateful to the British Heart Foundation which supported this study.

**REFERENCES**