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Expression, localisation and function of ACE and chymase in normal and atherosclerotic human coronary arteries

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Abstract

The expression, localisation and function of enzymes responsible for the local formation of angiotensin II in atherosclerotic and non-atherosclerotic human coronary arteries were studied. Human epicardial coronary arteries expressed mRNA for both ACE and chymase. Immunohistochemical studies revealed that ACE was localised to the vascular endothelium, and to a lesser extent the medial smooth muscle cells, in both large and small arteries. Chymase was detected in both types of vessel but was shown to be associated with mast cells. The contractions to angiotensin I in large arteries were inhibited only by a combination of quinaprilat and soyabean trypsin inhibitor. In the intramyocardial arteries the response to angiotensin I was markedly inhibited in the presence of chymostatin. These findings demonstrate that a dual pathway for the synthesis of angiotensin II is active in diseased and non-diseased coronary arteries.

Keywords: Experimental; Vasculature; Multicellular; Pharmacology; ACE inhibitors; Coronary circulation; Coronary disease; Arteries; Vasoactive agents

1. Introduction

Angiotensin II is believed to play a key role in the onset and progression of the atherosclerotic process. In the early stages of the disease, angiotensin, via stimulation of adhesion molecules, assists in the recruitment of monocytes/macrophages into the vessel wall (Pastore et al., 1999). Angiotensin may play a role in the development of these cells into foam cells following activation of NADP/NADPH oxidase, and oxidation of LDL (Warnholtz et al., 1999). Increases in oxidant stress will also down-regulate the protective effects of endothelium-derived nitric oxide. Superimposed on these detrimental effects of angiotensin II is the direct action on the vascular smooth muscle cells.

Considering this repertoire of potentially damaging effects on the vessel wall, it is not surprising that pharmacological modulation of the renin–angiotensin pathway has been shown to be of benefit in a number of animal models of atherosclerotic disease, as well as in the treatment of patients with cardiovascular diseases (The SOLVD Investigators, 1991; Osterrieder et al., 1991). The mainstay of this therapeutic strategy has been to block the formation of the effector peptide by inhibition of angiotensin converting enzyme (ACE). However, this strategy may be limited by the presence of alternative pathways for angiotensin II. This concept is supported by a number of clinical and experimental observations. For example, increases in plasma angiotensin II levels following exercise are not prevented by an ACE inhibitor (Miura et al., 1994). ACE inhibitors alone have been shown to be unable to completely block angiotensin II generation in the human myocardium and detrusor muscle, and have no effect on the contractile effect of angiotensin I in isolated blood vessels (Urata et al., 1990a; Lindberg et al., 1994; Borland et al., 1998).

The capacity to generate angiotensin II via an ACE-independent mechanism has been demonstrated in human myocardium, internal mammary arteries, saphenous veins, radial arteries and gastroepiploic arteries (Borland et al., 1998; Okunishi et al., 1987; Lindberg et al., 1997; Voors et al., 1998; Wolny et al., 1997; Chester and Borland, 2000).

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The evidence for dual or multiple pathways for angiotensin II formation is supported by the efficiency of angiotensin type I (AT_1) receptor antagonists in animal models of atherosclerotic disease, as well as in normalising vascular structure in patients with essential hypertension. Additional reductions in blood pressure can be obtained by co-administration of angiotensin receptor antagonists with an ACE inhibitor (Azizi et al., 1995).

Studies in coronary vessels have identified the action of a serine protease enzyme that mediates contractions to angiotensin I in isolated epicardial vessels (Wolny et al., 1997). However, no assessment has been made of either regional variations of chymase within the coronary circulation, or the impact of arteriosclerosis on the expression or function of this alternative angiotensin II-forming enzyme. Animal studies that have attempted to address this question are of limited value, due to the known species differences with respect to the action of chymase on angiotensins. There is little information as to how the expression of angiotensin II-forming enzymes are affected by the presence of atherosclerotic disease. Expanding our knowledge in this area will help in the development of more efficient pharmacological control of the renin–angiotensin system.

2. Methods

2.1. Tissue collection

Lengths of left anterior descending coronary artery (LAD CA) with underlying myocardium were obtained from either unused donor hearts (aged 15–51 years) or from patients who were undergoing heart and heart–lung transplantation for the conditions of dilated cardiomyopathy (DCM) (aged 12–55 years) and ischaemic heart disease (IHD) (aged 29–55 years). On harvesting, tissues were placed directly into sterile Hank's balanced salt solution (HBSS), pH 7.4 and transported to the laboratory. Epicardial coronary vessels were dissected free of underlying myocardium and surrounding connective tissues. On macroscopic examination, all vessels removed form IHD patients showed significant atherosclerotic changes, while those from the other two groups showed no or only mild disease. Short 3–4 mm lengths of artery were snap frozen in liquid N_2 for mRNA extraction, immunohistochemical and autoradiographic studies. Individuals from whom LAD CAs were obtained had been receiving a variety of medications depending on the underlying disease. Patients with IHD and DCM were prescribed combination therapies, including calcium channel antagonists, β-blockers, anti-arrhythmic drugs, diuretics, potassium channel blockers, lipid lowering agents, ACE inhibitors, nitrates, anticoagulants and analgesics. No patient was prescribed an AT_1 receptor antagonist. Responses from those patients whom had been receiving ACE inhibitors prior to surgery did not affect the responses observed with ANG I or ANG II. Individuals from whom donor heart tissue was obtained had no previous history of cardiovascular disease, and in all cases were not exposed to inotropes prior to donation.

2.2. RNA extraction

Blood vessel samples were homogenised in liquid N_2 and total RNA extracted following the RNAzol B protocol, in accordance with the manufacturers instructions (ams Biotechnology, Whitney, UK). A 1 μg sample of total RNA from each blood vessel preparation was reverse-transcribed into first-strand cDNA using oligo dT primer and Superscript II reverse transcriptase (Life Technologies Inc, Paisley, UK).

2.3. Reverse transcriptase-polymerase chain reaction amplification

Oligonucleotide primers were designed according to the nucleotide sequences encoding human endothelial ACE (Hubert et al., 1991), human heart chymase (Urata et al., 1991) and human β-actin (Ponte et al., 1984) using a "primer 3" computer program (Whitehead Institute for Biomedical Research/MTT Center for Genomic Research, USA) (Urata et al., 1991). Primer sequences for ACE, 5′ CACCAATGACACGGAAAGTG 3′ (sense), and 5′ TCAGCCTCATCAGTCACCAG 3′ (antisense), and chymase, 5′ CCTACATGGCCTACCTGGAA 3′ (sense), and 5′ TCCAGGATTAATTTGCCTGC 3′ (antisense), were synthesised by Pharmacia Biotech, Cambridge, UK. The anticipated product sizes were 615 base pairs (bp) for ACE and 651 bp for chymase. The primers were specifically designed to span introns, to ensure that the polymerase chain reaction (PCR) product amplified was from the mRNA, and not genomic DNA. PCR was carried out using 100 ng cDNA, for 35 cycles (30 s denaturing at 94 °C; 30 s annealing at 56 °C for ACE and 60 °C for chymase and 30 s extension at 72 °C), followed by a 10-min extension step at 72 °C, in 10× PCR buffer containing, 4 mM each dNTP, 0.1 mol/L DTT, 1.5 mmol/L $MgCl_2$, 10 pM each gene specific primer, and 2.5 units (5 U/μl) of Platinum Taq DNA polymerase (all, GibcoBRL, Paisley, UK). Control samples were human umbilical vein endothelial cells (HUVECs) for ACE, and human left ventricle (LV) tissue for chymase. Negative controls for each tissue sample consisted of a cDNA reaction mixture minus reverse transcriptase enzyme. To assess the quality of cDNA used as a template, β-actin cDNA was amplified in a separate PCR reaction using primers with an annealing temperature of 60 °C and sequences of 5′ CCTCGCCTTTGCCGATCC 3′ (sense), and 5′ AAGCTGTAGCCGCGCTCG 3′ (antisense). The expected product size was 647 bp. PCR products were visualised on a polyacrylamide gel (6%), following electrophoresis and silver staining.