Effect of adrenomedullin on the production of endothelin-1 and on its vasoconstrictor action in resistance arteries: evidence for a receptor-specific functional interaction in patients with heart failure

Chris HILLIER*, Mark C. PETRIE†, Michael P. LOVE‡, Fiona JOHNSTON*, Margaret R. MacLEAN‡ and John J. V. McMURRAY†

*Department of Biological Sciences, Glasgow Caledonian University, Glasgow G4 0BA, Scotland, U.K., †Department of Cardiology, Western Infirmary, Glasgow G11 6NT, Scotland, U.K., and ‡Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

ABSTRACT

Endothelin-1 (ET-1) and adrenomedullin (ADM) are both produced in the arterial wall, but have opposing biological actions. Evidence from experimental animals suggests a functional interaction between ET-1 and ADM. We have tested this in humans. Small resistance arteries were obtained from gluteal biopsies taken from patients with chronic heart failure (CHF) due to coronary heart disease (CHD), or with CHD and preserved ventricular function. The contractile responses to big ET-1 and to ET-1 in both sets of vessels were studied in the absence (control) and presence of ADM at 20 pmol/l (low ADM) or 200 pmol/l (high ADM), using wire myography. ADM did not affect the conversion of big ET-1 into ET-1 in vessels from patients with either CHD or CHF. Low ADM did not alter the contractile response to ET-1 in vessels from patients with CHF. Low ADM was not tested in vessels from patients with CHD, but high ADM did not affect this response in arteries from these patients. High ADM did, however, significantly reduce the vasoconstrictor effect of ET-1 in vessels from patients with CHF. The maximum response, as a percentage of the response to high potassium, was 199% (S.E.M. 25%) in the control experiments (n = 14), 205% (27%) in the low-ADM (n = 7) studies and 150% (17%) in the high-ADM (n = 6) experiments (P < 0.001). Furthermore, the Hill coefficient increased from 0.57 ± 0.05 in the absence of ADM to 1.16 ± 0.15 in the high-ADM experiments, indicating that ADM at 200 pmol/l specifically antagonized one receptor type in vessels from patients with CHF. We conclude that there is a one-site receptor interaction between ADM and ET-1 that is specific for vessels from patients with CHF. This functional interaction between ADM and ET-1 in resistance arteries may be of pathophysiological importance in CHF.

Key words: adrenomedullin, endothelin, heart failure, human, resistance arteries.

Abbreviations: ACE, angiotensin-converting enzyme; ADM, adrenomedullin; CHD, coronary heart disease; CHF, chronic heart failure; ECE, endothelin-converting enzyme; ET-1, endothelin-1; pD2, negative log of the concentration of agonist required to effect a 50% response; PSS, physiological salt solution; RAMP, receptor-activity-modifying protein.

Correspondence: Professor John J. V. McMurray, CRI in Heart Failure, Wolfson Building, University of Glasgow, Glasgow G11 6NT, U.K. (e-mail j.mcmurray@bio.gla.ac.uk).

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INTRODUCTION

Endothelin-1 (ET-1), derived from the vascular endothelium, is a potent vasoconstrictor peptide with antinatriuretic and mitogenic properties [1]. Conversely, adrenomedullin (ADM), which is secreted from a wide variety of tissues, including endothelial and vascular smooth muscle cells, is a peptide with vasodilator, natriuretic and anti-mitotic actions [2,3]. Indeed, experimental evidence suggests that these potent vasoactive peptides, produced from a common source, influence each other’s production and activity. The rates of secretion of ADM and ET-1 from vascular smooth muscle cells are similar [4].

Accordingly, ADM and ET-1 appear to act as physiological antagonists. ADM has been found to inhibit the production and actions of ET-1, at least in experimental animals. ADM inhibits the synthesis and release of ET-1 by cultured rat vascular smooth muscle cells [5], cultured rat mesangial cells [6], isolated rat aortic arteries [7] and bovine aortic endothelial cells [8]. These data suggest that ADM may inhibit the activity of endothelin-converting enzyme (ECE). ADM has also been found to inhibit ET-1-stimulated aldosterone secretion [9].

Conversely, ET-1 appears to augment the production of ADM. In cultured rat vascular smooth muscle cells, ET-1 stimulates the production of ADM [10], while increased secretion of ADM is found on activation of the ET1 receptor in canine aortic endothelial cells [11]. Plasma and tissue concentrations of both of these peptides are increased in heart failure [13–22]. ET-1 concentrations are increased in atherosclerotic plaques [23]. ADM can also be identified in macrophages in human atherosclerotic plaques [24]. It has been proposed that ET-1 and ADM may interact to regulate local vascular tone and blood flow in these important conditions.

The present study had two objectives. The first was to determine whether or not ADM inhibits the conversion of big ET-1 into ET-1 by ET-1 ECE in human tissues. The second aim was to examine the effects of ADM on ET-1-induced vasoconstriction. In view of the putative role of both peptides in atherosclerosis and heart failure [2,3,23,24], small resistance arteries from patients with coronary heart disease (CHD) and chronic heart failure (CHF) were used in both sets of experiments.

METHODS

Patients

Written informed consent was obtained from each patient, and all protocols were approved by the local Committee on Medical Ethics.

Patients with CHF

Ambulatory patients with New York Heart Association class II/III CHF were studied. All were receiving long-term (>3 months) treatment with angiotensin-converting enzyme (ACE) inhibitors and diuretics. The aetiology of CHF was CHD in all cases, and each patient had a left ventricular ejection fraction of <40% (Simpson’s biplane method). All patients had suffered a previous myocardial infarction. Patients with renal failure (creatinine >200 μmol/l) or diabetes were excluded.

Patients with CHD

Patients with chronic stable angina attending outpatient clinics were studied. All patients had preserved left ventricular function, determined as an echocardiographic left ventricular ejection fraction of 40% or more (Simpson’s biplane method), and none were treated with an ACE inhibitor.

Materials

ET-1 and big ET-1 were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). ADM was obtained from Nova Calbiochem Pharmaceuticals. All drugs were dissolved in distilled water. Experiments were carried out in physiological salt solution (PSS) with the following composition (mM): NaCl 118.4, KCl 4.7, MgSO4·7H2O 1.2, KH2PO4 1.2, NaHCO3 24.9, CaCl2 2.5, glucose 11.1 and EDTA 0.023, which gives a pH of 7.4 when gassed with a 5% CO2/95% O2 mixture. Studies were performed on a Mulvany–Halpern four-channel wire myograph (JP Trading, Aarhus, Denmark).

Blood sampling, biopsy procedure and artery preparation

After the subject had rested supine for 15 min, blood was drawn from a cannula in an antecubital vein for estimation of blood chemistry and serum cholesterol. Subcutaneous gluteal biopsies were then obtained from each patient under local anaesthesia (1% lidocaine), by the method described previously [25]. Dissected tissue was placed immediately into cold 0.9% NaCl and then transferred to cold PSS. Resistance arteries of approx. 2 mm in length were dissected free of fat and mounted on two 40 μm-diameter stainless steel wires in a four-channel myograph, in which the wires are attached to a force transducer and micrometer respectively. The bath was then gassed and heated for the duration of the experiment.

Experimental protocol

After a rest period of 30 min, each artery was stretched at 1 min intervals to determine the relationship between passive exponential wall tension and internal circumference (L). From the Laplace equation, where P = T/γ
(\(P\) is the effective pressure, \(T\) is the wall tension and \(r\) is the internal radius), the equivalent circumference (\(L_{100}\)) for a transmural pressure of 100 mmHg was calculated for each vessel by an iterative computer method. Each vessel was then set to the normalized internal diameter, \(L_1 = 0.9 \times L_{100}/\pi\), at which contraction is thought to be optimal [26]. Following normalization, the vessels were left for a further 1 h, and were then exposed to a high (123 mM) concentration of potassium (solution identical to PSS, except that sodium was replaced by potassium on an equimolar basis) for a series of 5 min periods until repeatable maximal contractions were achieved.

**Effect of ADM on ECE activity**

As conversion of big ET-1 (inactive) into ET-1 (active) is catalysed by ECE, vasoconstriction induced by big ET-1 allows the indirect measurement of ECE activity. Thus, following a further equilibration period, the effect of ADM on ECE activity was examined by constructing concentration–response curves to big ET-1 (3 pM–0.3 \(\mu\)M) in the absence of ADM and following a 30 min incubation with either a low concentration of ADM (20 pmol/l) or a high concentration of ADM (200 pmol/l). Resistance arteries from patients with CHF were incubated with 20 and 200 pmol/l ADM, whereas resistance arteries from patients with CHD were incubated with only the 200 pmol/l concentration of ADM. The low concentration (20 pmol/l) used is similar to the plasma concentration of ADM found in patients with CHF [19]. The high concentration (200 pmol/l) is one estimated to be similar to the local concentration of ADM in the blood vessel wall in patients with CHD and CHF.

**Effect of ADM on ET-1-induced vasoconstriction**

To determine the effect of ADM on ET-1-induced vasoconstriction, the same protocol as above was followed using ET-1 (1 pM–0.1 \(\mu\)M) instead of big ET-1. In brief, arteries taken from patients with CHF were examined in the absence of ADM, and in the presence of 20 and 200 pmol/l ADM. Arteries taken from patients with CHD were examined in the absence of ADM and in the presence of 200 pmol/l ADM.

Responses to big ET-1 and ET-1 are given as the contraction responses relative to the maximal contraction induced by potassium, expressed as a percentage.

**Statistical analysis**

Results are expressed as means±S.E.M. For each ET-1 concentration–response curve, a pD\(_2\) (negative log of the concentration of agonist required to effect a 50% response) was calculated. As this calculation requires a value for the maximum response, pD2 values were not calculated for big ET concentration–response curves (no maximum was obtained with big ET-1 in the concentration range studied). Statistical analysis of maximum responses induced by big ET-1 and ET-1, as well as pD\(_2\) values (ET-1 only), in the presence and absence of ADM and between vessels from patients with CHD and CHF was by one-way ANOVA for repeated measures and Bonferroni’s multiple comparison test. The computer software used was GraphPad Prism. Comparison of values for maximum KCl and for internal diameter was by unpaired Student’s \(t\) test.

**RESULTS**

**Patients**

The clinical characteristics of the patients studied are given in Table 1. Patients with CHF differed from those with CHD in two ways. First, they had impaired left ventricular systolic function. Secondly, all patients with CHF were treated with an ACE inhibitor and a diuretic, while patients with CHD were not; the latter were more often treated with \(\beta\)-blockers (\(P < 0.05\)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHF</th>
<th>CHD</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>16/2</td>
<td>9/3</td>
</tr>
<tr>
<td>Previous MI</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>6 III; 12 II</td>
<td>—</td>
</tr>
<tr>
<td>Coexistent hypertension</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Mean ejection fraction (%)</td>
<td>27 (10)</td>
<td>56 (9)</td>
</tr>
<tr>
<td>Drug therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Diuretic</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Digoxin</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Oral nitrate</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>(\beta)-blocker</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitor</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Aspirin</td>
<td>17</td>
<td>12</td>
</tr>
</tbody>
</table>

| Systolic BP (mmHg) | 121 (23) | 135 (17) |
| Diastolic BP (mmHg) | 69 (10) | 75 (10) |
| Glucose (mmol/l) | 5 (1) | 5 (1) |
| Cholesterol (mmol/l) | 5 (1) | 5 (1) |
| Creatinine (\(\mu\)mol/l) | 110 (46) | 85 (15) |

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Resistance arteries
The mean internal diameter of the arteries was $316 \pm 26 \mu m$. There were no significant differences in normalized artery diameter between the two experimental groups. Similarly, there were no differences in maximal contractile response to high-potassium PSS between the two experimental groups.

Effects of ADM on ECE activity

Vessels from patients with CHF
Analysis of maximum responses, $pD_2$ values and complete curves showed that neither the low (20 pmol/l) nor the high (200 pmol/l) concentration of ADM affected ECE activity in vessels from CHF patients (Figure 1 and Table 2).

Vessels from patients with CHD
The high concentration of ADM had no effect on ECE activity in arteries from patients with CHD (Figure 2; Table 2).

Effects of ADM on ET-1-induced vasoconstriction

Vessels from patients with CHF
The low concentration of ADM (20 pmol/l) had no effect on ET-1-induced vasoconstriction (Figure 3; Table 2). In contrast, the high ADM concentration (200 pmol/l) significantly reduced the vasoconstrictor effects of ET-1 (comparison of curves: $P < 0.001$) (Figure 3; Table 2). The Hill coefficient (a measure of receptor site activity calculated from the slope of the sigmoidal concentration–response curve) of the ET-1 curve for vessels from patients with CHF in the absence of ADM was $0.57 \pm 0.05$, showing evidence of a two-receptor effect. Incubation with 20 pmol/l ADM had no effect on the Hill coefficient ($0.58 \pm 0.07$). Following incubation with 200 pmol/l ADM, there was an increase in the slope of the curve (Hill co-efficient $1.16 \pm 0.15$), suggesting that this concentration of ADM specifically antagonized one receptor site, leaving the remaining receptor to mediate...

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHF</th>
<th>CHD</th>
<th>CHF</th>
<th>CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 16)</td>
<td>ADM (20 pmol/l) (n = 8)</td>
<td>ADM (200 pmol/l) (n = 8)</td>
<td>Control (n = 11) (n = 8)</td>
</tr>
<tr>
<td>Maximum response to big ET-1 (% of response to KCl)</td>
<td>155 (10) (n = 16)</td>
<td>139 (22) (n = 8)</td>
<td>181 (23) (n = 8)</td>
<td>126 (11) (n = 11)</td>
</tr>
<tr>
<td>Maximum response to ET-1 (% of response to KCl)</td>
<td>199 (25) (n = 14)</td>
<td>205 (27) (n = 7)</td>
<td>150 (17) (n = 6)</td>
<td>170 (34) (n = 11)</td>
</tr>
<tr>
<td>$pD_2$ (mol/l)</td>
<td>$1.12 \times 10^{-9}$ (0.53 $\times 10^{-9}$)</td>
<td>$1.15 \times 10^{-9}$ (0.78 $\times 10^{-9}$)</td>
<td>$9.14 \times 10^{-10}$ (3.53 $\times 10^{-10}$)</td>
<td>$2.10 \times 10^{-9}$ (0.83 $\times 10^{-9}$)</td>
</tr>
</tbody>
</table>
Comparison of ET-1-induced vasoconstriction and ECE activity in vessels from the two patient groups

There was no difference in the concentration–response curves to ET-1 or big ET-1 between vessels from patients with CHD and those from patients with CHF.

DISCUSSION

This is the first study of the functional interaction between ADM and ET-1 in human tissue. It is important to understand these actions, given the putative role of both peptides in atherosclerosis and heart failure [2,3,23,24]. We have described the effect of ADM on the conversion of big ET-1 into ET-1 and on the actions of ET-1 in resistance arteries from patients with CHF and patients with CHD. The concentrations of ADM used were chosen to mimic the range likely to be encountered in the blood and the vascular tissues in subjects with CHF [19].

In our first series of studies, we found that neither the low (20 pmol/l) nor the high (200 pmol/l) concentration of ADM that we used had an effect on the activity of ECE in resistance arteries from patients with either CHF or CHD [16,27]. These findings contrast with those described previously in tissues and cultured cells from experimental animals. Apart from the species difference, one obvious explanation for the lack of an effect of ADM in our present studies is the much lower concentration of ADM that we used. The earlier studies used very high concentrations by comparison. For example, both Kohno et al. [5,6] and Barker and Corder [8] used an ADM concentration of 0.1 μmol/l. The rat ADM used was also more potent than the human form used in our studies [5,6]. It seems probable, therefore, that pharmacological concentrations of ADM are required to inhibit ECE activity, and that ADM is unlikely to have this action at the range of concentrations likely to be encountered physiologically, or pathophysiologically, in the plasma or tissues of humans.

Our second series of studies examined the effects of ADM on the vasoconstrictor response to ET-1 in human small resistance arteries. While the lower concentration of ADM had no effect on ET-1-induced vasoconstriction in arteries from patients with CHF, was more than a non-specific vasodilator effect (Figure 4). No receptor-specific inhibition similar to that observed in arteries from CHF patients was seen in arteries from patients with CHD. The Hill coefficient in these arteries was 0.77 ± 0.38 before ADM and 0.56 ± 0.38 following incubation with 200 pmol/l ADM.

Vessels from patients with CHD

The high concentration of ADM (200 pmol/l) had no effect on ET-1-induced vasoconstriction in vessels from patients with CHD, suggesting that the effect seen in arteries from patients with CHF was more than a non-specific vasodilator effect (Figure 4). No receptor-specific inhibition similar to that observed in arteries from CHF patients was seen in arteries from patients with CHD. The Hill coefficient in these arteries was 0.77 ± 0.38 before ADM and 0.56 ± 0.38 following incubation with 200 pmol/l ADM.

The inhibition was, therefore, receptor-specific. The effect of this antagonism is to leave the maximum response and pD₂ relatively unchanged, but to alter significantly the appearance of the curve at the lower concentrations (Figure 3; Table 2).

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(0.58 ± 0.07), indicating a two-receptor effect. In the presence of 200 pmol/l ADM, however, the Hill coefficient increased to 1.16 ± 0.15, suggesting that 200 pmol/l ADM specifically antagonized one receptor site, leaving the remaining receptor to mediate the response. These findings are intriguing in the light of evidence of altered vascular ET receptor regulation in CHF. Fu et al. [28] reported an approx. 60% reduction in ET receptor density in the mesenteric arteries of rats with CHF induced by coronary ligation (although the dissociation constant was increased by 2.8-fold compared with controls). ET receptor subtyping was not performed, although the authors suggested, on the basis of functional responses to ET-1 infusion, that ET₂ receptor down-regulation may have occurred. Other studies have shown arterial ET₂ receptor up-regulation, as well as ET₁ receptor down-regulation, in experimental and human CHF [29,30]. Altered ADM receptor regulation has also been reported in CHF, and this too may have contributed to the different functional interaction between ADM and ET-1 in vessels from CHF compared with CHD patients. Óie et al. [31] described significant increases in myocardial ADM receptor and receptor-activity-modifying protein-2 (RAMP-2) mRNA levels in rats with CHF induced by coronary ligation. Radioligand studies confirmed a 1.6-fold increase in specific ADM binding sites in the failing left ventricle, despite increased tissue and plasma concentrations of ADM. Interestingly, treatment with the mixed ET₁/ET₂ receptor antagonist bosentan prevented the increase in the levels of RAMP-2 mRNA, but not ADM mRNA, in non-infarcted myocardium. This is further evidence for an ET₁–ADM interaction. The possible receptor alterations and receptor cross-talk involved in the specific functional interaction that we have identified in CHF is worthy of further investigation.

In conclusion, we have found evidence of a functional interaction between ADM and ET-1 in resistance arteries from patients with CHF. No such interaction was found in vessels from patients with CHD. Our findings suggest that a specific interaction between ADM and ET-1 occurs in CHF, and that this may be of pathophysiological significance in this syndrome. Certainly, ADM has potentially beneficial haemodynamic, renal and hormonal actions in CHF [32].

ACKNOWLEDGMENTS

This work was supported by a grant from the National Heart Research Fund and the Scottish Office Home and Health Department. M.C.P. was funded by British Heart Foundation Junior Research Fellowship No. FS/97031:1997.

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12. Reference deleted


Received 4 January 2001/14 February 2001; accepted 28 March 2001