Natriuretic peptides maintain sodium homoeostasis during chronic volume loading post-myocardial infarction in sheep

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ABSTRACT

The impaired ability to excrete sodium is a key feature of established congestive heart failure and is also apparent in asymptomatic left ventricular (LV) impairment. However, few studies have examined responses to chronic volume loading immediately post-myocardial infarction (MI). Experimental MI was induced in six sheep by thrombogenic coil coronary artery occlusion, and resulted in significant LV dysfunction with reduced LV ejection fraction (P < 0.001) and subsequent remodelling (increased LV volumes, P = 0.015). Chronic volume loading with 2, 3 and 4 litres/day intravenous saline (each for 7 days) showed no evidence of renal sodium or volume retention in sheep with experimental MI compared with six normal control sheep. Plasma levels of brain natriuretic peptide (BNP), N-terminal pro-BNP and cGMP (all P < 0.05) were higher in the MI group compared with normal control sheep. There were no differences in haemodynamics, body mass or renin–aldosterone levels between groups. This study provides evidence that natriuretic peptides play a pivotal role in preserving volume/electrolyte balance in the early stages of post-MI cardiac dysfunction.

INTRODUCTION

The complete study of coronary ischaemic syndromes entails observations on acute myocardial infarction (MI), asymptomatic left ventricular (LV) dysfunction (LVD) and established heart failure (HF). There is evidence to suggest that available pharmacological therapy can favourably alter the natural history of asymptomatic LVD which commonly precedes HF. Improved understanding of the pathophysiology of this early phase of LV impairment requires an experimental model that reflects the underlying pathology, as well as the haemodynamic and neurohumoral profile, of human disease. We have previously characterized such a model whereby myocardial damage was induced in closed-chest sheep by percutaneous catheter delivery of thrombogenic coils [1]. HF is a complex syndrome caused by a loss of LV function. As ventricular function declines, a hierarchy of neurohumoral systems is progressively stimulated in an attempt to maintain tissue perfusion in vital organs [2]. Although beneficial in the first instance, some of these mechanisms are likely to contribute to further progression of HF through several mechanisms including increases in cardiac load and electrolyte and water imbalance [3]. The impaired ability to excrete ingested sodium is a key feature of patients with established congestive HF [4,5]. Given that a number of symptoms in congestive HF result in large part from this inability to...

Key words: experimental myocardial infarction, haemodynamics, heart, heart failure, natriuretic peptide, sodium homoeostasis.

Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; HF, heart failure; i.v., intravenous; LAD, left anterior descending coronary artery; LV, left ventricular; LVD, LV dysfunction; LVEF, LV ejection fraction; MAP, mean arterial pressure; MI, myocardial infarction; NT-BNP, N-terminal fragment of pro-BNP; PRA, plasma renin activity; RAAS, renin–angiotensin–aldosterone system; RAP, right atrial pressure; TIVCC, thoracic inferior vena caval constriction.

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Excrete sodium and water, it is important to determine what factors regulate fluid and electrolyte balance in the evolution from LVD to HF. Despite this, the pathophysiology of sodium and water retention in HF, particularly with regard to the time of onset of this abnormality during the course of the disease, remains inadequately studied. Volpe and co-workers [6-8] have reported that patients with asymptomatic LVD or mild HF clearly show abnormalities in renal sodium and water handling well before detectable changes in cardiac output. However, few studies have examined the response to chronic volume loading immediately post-MI. Therefore, we have studied the haemodynamic, hormonal and renal effects of chronic volume loading with intravenous (i.v.) normal saline commencing 24 h after experimentally induced MI in sheep.

**METHODS**

**Animals**

Twelve Coopworth ewes (Lincoln University Farm, Christchurch, New Zealand) were housed in an air-conditioned light-controlled room, had free access to water and received a normal laboratory diet of sheep nuts and chaff, providing a daily intake of 75 mmol sodium and 150 mmol potassium. Six sheep underwent experimental MI by catheter delivery of a thrombogenic coil into the left anterior descending coronary artery (LAD) as described previously [5], while the other six sheep served as normal (non-infarcted) controls. Both groups then underwent chronic (3 week) i.v. saline loading, commencing 24 h post-MI/sham. The Animal Ethics Committee of the Christchurch School of Medicine approved the study protocol.

**Surgical preparation**

Under general anaesthesia (17 mg/kg thiopentone, maintained by a mixture of halothane, nitrous oxide and oxygen), a carotid artery was cannulated with an 8F sheath (Cordis, Miami, FL, U.S.A.) for subsequent measurement of arterial pressure and heart rate and cardiac catheterization procedures, as described below. Two polyethylene catheters were placed in the jugular vein for measurement of right atrial pressure (RAP), blood sampling and i.v. saline administration. A foley catheter was placed per urethra into the urinary bladder to allow complete collection of urine.

**Thrombogenic coil coronary occlusion**

In the experimental MI group ($n = 6$), cannulation of the left coronary circulation was performed via an 8F left ampaltz 1 Softip Guiding Catheter (Schneider, Minneapolis, MN, U.S.A.). A 0.014 inch (0.36 mm) angioplasty wire was advanced down the LAD as far as practicable towards the apex. A 5F straight catheter (Cook Company, Brisbane, Queensland, Australia) was advanced over the angioplasty wire to a position distal to the branching of the first diagonal artery. Under fluoroscopic guidance, impelled by a 0.025 inch (0.64 mm) wire, the thrombogenic coil (Cook Company, Bloomington, IN, U.S.A.) was advanced down the catheter into the artery lumen. Coils (4–5 mm diameter, 3 cm length) were sized larger than the coronary artery lumen diameter. Total occlusion occurred within 2 min and was confirmed by angiography. ECG and arterial pressure were monitored throughout the anaesthetic period. The occurrence of ventricular tachycardia, fibrillation or sustained ectopic activity was treated with a 0.5–1 mg i.v. bolus administration of atenolol. Control animals ($n = 6$) were anaesthetized for the same duration as the experimental MI group.

**I.v. saline loading**

I.v. saline loading was commenced 24 h post-MI/control. Saline (0.9%) was administered at 2 litres/day for 7 days immediately followed by 3 litres/day and 4 litres/day (each for 7 days). Measurements (detailed below) were continued for a further 7 days on cessation of the highest dose of saline. Saline was delivered by means of pressurized i.v. bags connected to the jugular catheter via a metered giving set. Bags were changed daily and accuracy of volume of saline delivery was monitored by weighing the bags.

**Neurohumoral measurements**

Haemodynamic recordings, venous blood samples for hormone assay and live body mass of sheep were recorded immediately prior to commencement of i.v. saline loading and then on days 1, 4 and 7 of each week for the duration of the experiment.

Arterial pressure and RAP were recorded using an online data acquisition system (Dataflow, Crystal Biotech, Hopkinton, MA, U.S.A.). Heart rate and pressures were digitally integrated in 30 s recording periods, and data from four consecutive periods were averaged.

Venous blood drawn for hormone assay was taken into chilled EDTA-coated tubes, centrifuged and the plasma stored at $-80^\circ$C before assay for plasma atrial natriuretic peptide (ANP) [9], brain natriuretic peptide (BNP) [10], N-terminal fragment of pro-BNP (NT-BNP) [11], cGMP [9], endothelin [1], aldosterone [12] and plasma renin activity (PRA) [13].

Complete 24 h urine collections were made throughout the study and measured for volume, sodium, potassium and creatinine excretion by standard methods.

Ventriculography was performed on completion of saline loading protocol (4 weeks post-MI and immediately prior to the sheep being killed) for determination of
LV volumes, LV ejection fraction (LVEF) and regional wall motion. A 7F pigtail catheter was passed through the carotid artery sheath into the left ventricle and a 20 ml bolus of contrast medium (Omnipaque, Nycomed, Birmingham, U.K.) was injected under fluoroscopy with the image captured on video for analysis on an ANCOR workstation (Siemens, Solna, Sweden). Regional wall motion (fractional shortening) was calculated in four quadrants (1–4), these being posterobasal, diaphragmatic, apical anterolateral and anterobasal respectively. Whole hearts were removed and preserved in 10% buffered formalin before examination of gross pathology.

**Statistics**

Results are expressed as the means±S.E.M. ANOVA with time as a repeated measure was used to determine time and group differences between experimental MI and control groups. Significance was assumed when \( P < 0.05 \). Where significant differences were identified by ANOVA, \textit{a priori} Fisher’s protected least-squares difference tests were used to identify time points significantly different from time-matched control.

**RESULTS**

Experiments were carried out without mishap and data collection was complete. In the experimental MI group, coil occlusion induced consistent ECG changes with marked ST wave elevation and raised creatine kinase and troponin T levels (results not shown). Post-mortem macroscopic examination of the hearts revealed well-defined transmural antero-apical infarcts. Ventriculography data (Table 1) showed marked dilation of the left ventricle with an increase in end systolic volume (\( P = 0.015 \)) and a decrease in LVEF (\( P = 0.001 \)) in experimental MI compared with normal controls. There was also significant hypokinesis, as demonstrated by the reduction in fractional shortening indices in quadrants 2–4 (all \( P < 0.05 \)).

**Table 1 Ventriculography data**

<table>
<thead>
<tr>
<th></th>
<th>Normal control</th>
<th>Experimental MI</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end diastolic volume (ml)</td>
<td>104.6 ± 4.9</td>
<td>144.0 ± 18.8</td>
<td>NS</td>
</tr>
<tr>
<td>LV end systolic volume (ml)</td>
<td>54.9 ± 3.0</td>
<td>104.3 ± 11.0</td>
<td>0.015</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>48.5 ± 1.4</td>
<td>26.6 ± 2.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Fractional shortening index (quadrant 1)</td>
<td>2.22 ± 0.19</td>
<td>1.59 ± 0.42</td>
<td>NS</td>
</tr>
<tr>
<td>Fractional shortening index (quadrant 2)</td>
<td>2.05 ± 0.21</td>
<td>1.22 ± 0.24</td>
<td>0.026</td>
</tr>
<tr>
<td>Fractional shortening index (quadrant 3)</td>
<td>1.43 ± 0.23</td>
<td>0.40 ± 0.27</td>
<td>0.011</td>
</tr>
<tr>
<td>Fractional shortening index (quadrant 4)</td>
<td>2.65 ± 0.15</td>
<td>0.87 ± 0.38</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Mean arterial pressure (MAP), heart rate, RAP and body mass were not significantly different between groups, although both heart rate and body mass tended to be higher in the experimental group at most of the time points (Figure 1).

Chronic volume loading induced dose-dependent increases in both urinary volume and sodium excretion, which were not different between the groups (Figure 2). Both urinary volume and sodium excretion were consistently at or just above the input levels (Figure 2, continuous lines), and excretion rates changed immediately (within the 24 h period) in response to increases or decreases in i.v. load. Urinary potassium excretion remained relatively stable across the duration of the experiment, although there was a subtle (but statistically significant, \( P = 0.017 \)) reduction during the first week post-MI compared with normal controls (results not shown).

Plasma ANP levels tended to be higher during and after the higher rates of saline infusion, but differences between the groups were not significant (Figure 3). In contrast, plasma BNP levels were significantly higher early after MI, returning to normal control levels by day 14 post-MI (\( P = 0.002 \), Figure 3). A similar, though more
obvious and sustained, difference between the groups was seen for NT-BNP measurements with the levels remaining higher in the MI group for the duration of the experiment ($P = 0.049$, Figure 3). Similarly, plasma cGMP levels remained elevated for the duration of the experiment in the MI group ($P = 0.017$, Figure 3).

Plasma endothelin levels showed no consistent pattern of response to volume loading and were not different between the two groups (Figure 4). PRA was suppressed during the 3 weeks of volume loading and rebounded immediately upon cessation of i.v. saline, although there was again no difference between the groups (Figure 4). Plasma aldosterone levels, although tending to be higher initially in the MI group, showed no difference between groups in response overall (Figure 4).

**DISCUSSION**

Few studies have examined responses to chronic volume loading immediately post-MI. Therefore, we report the haemodynamic, hormonal and renal effects of chronic volume loading in a sheep model of experimental MI. Thrombogenic coils placed in the LAD induced well-defined transmural antero-apical infarcts associated with significant LVD (reduced LVEF), LV dilation and hypokinesis. There was no evidence of renal volume or sodium retention despite continuous i.v. infusion of normal saline at 2, 3 and 4 litres/day each for 7 days. Plasma BNP, NT-BNP and cGMP levels were higher in the MI group compared with normal controls throughout the volume loading period. In contrast, there were no
Volume loading in post-myocardial infarction left ventricular dysfunction

Figure 3 Plasma natriuretic peptide and cGMP responses to chronic i.v. saline loading in six normal control (∅) and six experimental MI (●) sheep

Results are expressed as the means ± S.E.M. Individual time points significantly different from time-matched data (Fisher’s protected least-squares difference from two-way ANOVA) are indicated as follows: *P < 0.05; †P < 0.01; ‡P < 0.001.

differences in haemodynamics, body mass or renin–aldosterone levels between the two groups.

Established congestive HF is characterized by impaired LV function, which results in compensatory activation of several neurohumoral systems including the renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system. In association with activation of these systems, renal perfusion pressure and glomerular filtration rate decrease and tubular sodium reabsorption increases [14]. Indeed, the impaired ability to excrete ingested sodium is a key feature of patients with congestive HF [4,5]. Recent studies have demonstrated that renal sodium retention can occur in patients with asymptomatic LVD and mild HF [6–8], and in experimental HF, without increased activation of the RAAS [14]. It is difficult to elucidate mechanisms by which sodium and water retention occur in very early heart disease as most patients immediately receive (or have already received) treatment that affects the mechanisms being studied. Therefore, primary data on the development of the oedematous syndrome are few. Nonetheless, it has been proposed that in the first few days after acute MI in a hitherto healthy and medically untreated person, sodium and water retention develops in a phase with increased efferent sympathetic nerve activity and increased activity of the RAAS [15]. In a small (n = 8) group of untreated patients Abildgaard et al. [16] showed there is indeed sodium and water retention early (2 days) after acute MI without LV failure, which was mediated by an increased tubular reabsorption rate in the distal nephron and associated with an increase in extracellular volume and mass indicating water retention. Although hormone levels were not measured in their study [16], the authors postulated that the observed sodium and water retention is likely to be mediated by increased activity of the RAAS.

Despite the inherent difficulties in obtaining human data, there appear to be few reports of animal studies examining renal sodium and water handling immediately post-MI. Rats with experimental MI show reduced sodium excretion of an acute saline load, with the degree of impairment related to size of infarct [17]. However, these experiments were performed at a single time-point (3 weeks post-MI) and neurohormonal activation (or lack of it) was not assessed in this study. The neurohumoral responses to congestive HF are typically presented as
either ‘on’ or ‘off’, with the implication that these responses persist unabated throughout the course of the disease. However, Francis et al. [18] have examined, at sequential time-points, the progression of HF after MI in the rat. Their findings demonstrate a temporal divergence among humoral changes in congestive HF as LV remodelling progresses, thus challenging the standing paradigm that neurohumoral activation in HF is a monolithic and monophasic response. Amongst their findings, they demonstrated that post-MI rats exhibit significant decreases in urine sodium and volume from baseline levels at all time-points measured (weekly for 4 weeks). However, these changes occurred in the setting of marked activation of PRA, arginine-vasopressin and ANP. It is important to note here that in established asymptomatic LVD in humans (without activation of systemic RAAS) sodium retention can be ameliorated by administration of angiotensin-converting-enzyme inhibitors [7,19]. In the absence of systemic RAAS activation, augmented ‘local’ RAAS (in tissues such as heart or kidneys) presumably contributes, at least in part, to impaired renal excretion of sodium and volume in these patients. Thus it remains unclear whether renal sodium retention occurs immediately post-MI and, if not, what factors serve to maintain circulatory hoemoostasis in the setting of early cardiac damage.

The present study was performed in a model of thrombogenic coil-induced MI which leads to significant LVD (LVEF 26.6% versus 48.5% in normal controls at 4 weeks) and subsequent remodelling of the left ventricle as judged by significantly increased LV volumes. Comparison of ventriculography data, obtained at 4 weeks post-MI in the present study, with the very early 1-week data published in a previous series of sheep [1], confirms the dynamic remodelling of the left ventricle in response to thrombogenic coil-induced MI in sheep. LVEF was similar (21–26%) in both studies, but there was no significant LV dilation observed 1 week post-MI in the previous study [1]. Degree of dilation measured at 4–5 weeks post-MI in the present study was similar to that previously observed in our laboratory measured 5 weeks following open-chest coronary artery ligation [20]. Thus, although remodelling is shown to be progressive with maintained low LVEF but ongoing dilation of the left ventricle, it appears that the chronic volume loading regime employed in the present study did not exacerbate LV function/dilation. Despite LV impairment and remodelling, there is no apparent activation of the circulating RAAS in this model.

Large volumes of normal saline (2–4 litres/day or 300–600 mmol of Na+/day) were continuously infused intravenously commencing 24 h post-MI. Under these experimental conditions, there was no evidence of renal sodium retention and body mass did not increase. In fact, close inspection of daily figures of volume and sodium excretion (Figure 2) showed that, as with normal control sheep, sheep with experimental MI showed a remarkable ability to modulate output levels to either match or be slightly above (i.v. load) input levels. In addition, there was no delay in responding to increased (or decreased) volume/salt load with excellent ‘dose-dependent’ congruency. We postulate that the augmented natriuretic peptide system (as indicated by significant increases in plasma BNP and cGMP) is pivotal in preserving volume/ electrolyte balance in spite of the clear substantial cardiac injury involved. Furthermore, we speculate that the augmented natriuretic peptide system acts to suppress the RAAS in this early phase of cardiac dysfunction. Indeed, a number of previous studies have proposed such a role for ANP in other models of cardiac disease. When Lee et al. [21] studied a canine HF model without activation of ANP, thoracic inferior vena caval constriction (TIVCC), they showed marked activation of RAAS and sodium retention compared with an acute pacing model in which plasma ANP was activated. Furthermore, administration of exogenous ANP in the TIVCC dogs, to mimic levels achieved with pacing, normalized both the RAAS and urinary sodium excretion. In addition, results from another group show that reduction of plasma ANP levels by prior atrial appendectomy in dogs with pacing-induced HF resulted in increased activation of the RAAS and marked sodium and water retention compared with pacing in dogs with intact atria [22]. The Mayo Clinic group has also studied the role of ANP in a canine model of early or asymptomatic LVD. Dogs paced at the modest rate of 180 beats per min for 10 days exhibited increased plasma ANP levels, but no RAAS activation or sodium retention [23]. The authors concluded that significant LVD with peripheral vasoconstriction can be associated with normal renal function, and thus suggests an important functional role for the neurohumoral profile in asymptomatic LVD in preserving sodium balance. Further studies employing the same model of asymptomatic LVD showed that either atrial appendectomy (which reduced circulating levels of ANP) or antagonism of ANP with HS-142-1 was associated with significant activation of RAAS and decreased sodium excretion both before and during acute volume expansion [24]. Taken together, natriuretic peptides appear pivotal in preserving renal function across a wide spectrum of cardiac dysfunction, including immediately post-MI (present study). While a pivotal role for natriuretic peptides is proposed, these studies do not preclude other neurohumoral factors, such as adrenomedullin, playing a similar role. It is likely that the renoprotective effects of the natriuretic peptides are eventually overcome with progressing cardiac disease as LV function worsens associated with, among other factors, reduction in renal perfusion pressures and activation of RAAS, despite continued augmentation of the natriuretic peptide system.

Plasma NT-BNP levels were more discriminatory than plasma ANP or BNP between the MI and normal control
groups, with levels remaining significantly higher in the MI group for the duration of the experimental period. This finding is consistent with previous studies in humans which showed that, for a given degree of cardiac injury, absolute and proportional increments in NT-BNP exceed those of BNP [25,26], suggesting that it may be a more sensitive marker of cardiac damage and LVD. It is likely that plasma cGMP levels, which also remained significantly elevated in the MI group for the duration of the study, serve to ‘integrate’ more subtle changes occurring in plasma ANP and BNP levels.

In conclusion, this is one of the first reports of renal sodium/volume handling immediately post-MI, performed in a sheep model with significant LVD, activation of natriuretic peptides, but no activation of the RAAS occurred. It shows that, despite chronic volume loading commenced 24 h after experimental MI, there was no evidence of impaired renal excretion of sodium or volume. Furthermore, there was no exacerbation of LV function/remodelling or neurohormonal indicators of LVD/HF. This study provides further evidence that natriuretic peptides play a pivotal role in promoting sodium excretion in the early stages of cardiac dysfunction.

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