ASYMPTOMATIC LV DYSFUNCTION AND HEART FAILURE – NEUROHUMORAL AND METABOLIC ASPECTS OF PHARMACOLOGICAL INTERVENTION.

ASYMPTOMATISCHE LV DYSFUNCTIE – NEUROHUMORALE EN METABOLE ASPECTEN VAN FARMACOLOGISCHE INTERVENTIE.

Proefschrift

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Martin van der Ent
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Promotor:	Prof. dr M.L. Simoons
Overige leden:	Prof. dr P. Verdouw Prof. dr P.W. de Leeuw Prof. dr R. Ferrari
Co-promotor:	dr W.J.Remme

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Voor Anne-Marie en Esther.

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Introduction

Heart failure is a clinical syndrome, which develops as a result of impaired function of the heart. The response of the body to impaired cardiac function includes cardiac and extra cardiac factors, like cardiac remodeling, structural and functional changes in peripheral skeletal muscle and neurohumoral activation. The latter involves increased levels of catecholamines, activation of the renin-angiotensin system, release of ANP, aldosterone and vasopressin and plays a central role in regulating constriction of the peripheral circulation and causes structural and functional changes in the vascular wall, modulation of water and salt retention and sympathetic tone, thus influencing hemodynamic changes. The activated neurohormones have both vasodilating and vasoconstrictive action and growth modulating and promoting actions.

Neurohumoral activation is associated with increased mortality [1], and with the severity of heart failure [2], in particular with clinical parameters like elevated jugular venous pressure, third heart sound and NYHA functional class. The latter symptoms are related to the hemodynamic condition of the heart failure patients. Therefore, careful management of hemodynamic parameters is important in improving myocardial function and relieving symptoms [3] and may be helpful in controlled reduction of neurohumoral activation.

Circulating neurohormone levels have a widespread biological variation. Although changes in hemodynamic parameters may be the result or the cause of changes in circulating neurohumoral levels, there is mostly a poor mathematical correlation [4]. The coexistence of hemodynamic changes and neurohumoral activation suggests that additional compensatory mechanisms may be present [5].

In these days, in the western world, data from untreated heart failure patients are hard to obtain, since treatment is usually started early and sometimes already at an asymptomatic phase. This early start of treatment is based on data from long-term follow-up in the V-HeFT I and II, CONSENSUS, SAVE, SOLVD treatment and prevention trial and the AIRE study [6, 7, 8, 9, 10, 11, 12]. Studies on untreated patients were predominantly done before the publication of these reports. Bayliss et al. observed that in a small group of 12 untreated patients norepinephrine levels were increased, whereas renin and aldosterone levels increased only after start of diuretic treatment, when norepinephrine levels fell to normal values [13]. In another

study 60 untreated heart failure patients were compared to a control group of healthy individuals. Increased levels of norepinephrine, epinephrine, aldosterone and ANP were found, whereas the renin-angiotensin system was not activated [14]. Anand et al. reported a wide variability in renin levels, with on average a substantial increase in renin levels in untreated heart failure in addition to the previously described increase in neurohormones [15].

In addition to the activated neurohormones with both vasoconstrictive and vasodilating action, other vasoactive compounds are activated in heart failure. Plasma endothelin, which is a potent vasoconstrictor and growth stimulating factor, has increased levels in more severe heart failure [16]. In addition, several studies have indicated that insufficient generation of NO by the endothelium may be involved in the impaired vasodilation in heart failure as well [17, 18]. Natriuretic peptides (B and C-type) and prostaglandins, have vasodilating actions, whereas the B-type natriuretic peptide and prostaglandins are elevated in heart failure.

Neurohormones and function in heart failure.

Catecholamines.

Sympathetic activation is an important feature of heart failure. Several studies have suggested that this might be associated with increased norepinephrine levels [19, 20, 21], even when no overt heart failure is present. Norepinephrine has a predominant effect on β_1-receptors. Increased norepinephrine levels in heart failure are caused by increased norepinephrine spillover and decreased uptake. Norepinephrine is an important, but rather insensitive marker of mortality [22, 23]. Patients dying from progressive myocardial pump function failure have shown to have rapid progressively increasing norepinephrine levels [24].

The increase in circulating norepinephrine levels is not only related to the severity of heart failure, but to other factors as well like age and deconditioning [25]. Cardiac norepinephrine release is increased in heart failure and relates with the severity of LV dysfunction [26].

Increased epinephrine levels in heart failure are caused by increased adrenomedullary activity [27]. Epinephrine has vasoconstrictive action through α-receptor activation and has positive chronotropic and inotropic effects in the heart through activation of β_1-receptors. Vasodilation through activation of β_2-receptors is present as well.

Dopamine is a biosynthetic precursor of norepinephrine and has both direct positive effects on myocardial contractility and indirect effects through enhanced release of norepinephrine [28, 29, 30] Dopamine levels may be elevated in heart failure, predominantly in the acute phase [31]. Apart from the effects on β_1 and α-receptors, dopamine activates two different specific dopamine receptors. Activation of DA_1 receptors, located postsynaptically, leads to vasodilation in renal, mesenteric, coronary and cerebral blood vessels, whereas activation of DA_2-receptors, which are located presynaptically, diminish norepinephrine release at the sympathetic nerve endings, resulting in a reduction of vascular resistance and sympathetic drive [32, 33, 34]. Activation of the latter receptor also leads to an inhibition of angiotensin II-induced aldosterone secretion.

In contrast to circulating levels, tissue norepinephrine, epinephrine and dopamine levels are decreased in both idiopathic dilated and ischemic cardiomyopathy [35].

Renin-angiotensin system

In congestive heart failure the renin-angiotensin system is activated. Additional elevation of renin levels occurs during diuretic treatment [19] and enhanced sympathetic activity through stimulation of β receptors. Activation of α_2-receptors inhibits renin secretion. Angiotensin II is elevated as part of the renin-angiotensin system. It consequently increases vasopressin levels and aldosterone and norepinephrine release [36]. The positive effect of angiotensin II on contractility of the heart is a direct effect, since this effect is preserved in the denervated heart [37], and also in the presence of β-receptor blockade [38]. However, an in vitro study by Holubarsch et al. suggested that angiotensin II receptors may be located in the atria only and not in the cardiac ventricles [39]. A significant loss of angiotensin II receptors occurs in end stage heart failure [40] In addition, animal studies have indicated that angiotensin II may influence cardiac function as well by facilitating stimulation-evoked release of norepinephrine [41, 42]. Systemic vasoconstriction by angiotensin II is caused either by a direct effect on angiotensin receptors on the smooth muscle cell or indirectly through releasing endothelin from the endothelium cells [43].

Aldosterone

Increased angiotensin II levels mediate aldosterone secretion from the adrenal cortex. Other factors influencing aldosterone release include endothelins, vasopressin,

catecholamines and prostaglandins [44]. Increased aldosterone levels cause sodium retention, hypokalemia and hypomagnesiemia, vasoconstriction, baroreflex depression, reduced cardiac norepinephrine uptake, arrhythmia and induction of cardiac fibrosis [45]. Activation of the renin-angiotensin system and consequently aldosterone therefore causes vasoconstriction and sodium retention, none of which is beneficial in heart failure.

Vasopressin

Arginine vasopressin is secreted by the posterior pituitary gland and is an antidiuretic hormone, which is elevated in heart failure [19]. Vasopressin is involved in regulation of circulating volume and osmolality and has a suppressive effect on renin release by renal juxtaglomerulary cells. Its secretion is stimulated by β-adrenergic activation and angiotensin II. Vasopressin has direct vasoconstrictive effects and indirect attenuating effects on the sympathetic nervous system in an animal model [46]. Through activation of specific vasopressin (V_2) receptors, vasopressin has some vasodilating action as well [47]. Overall, vasoconstrictive action prevails.

Atrial natriuretic peptide

ANP levels are elevated in heart failure [19], and relate to intracardiac pressures [48, 49, 50]. There are indications that the consequent increased wall stress may be the most important determinator in cardiac ANP release [51]. An increase in ANP levels has direct vasodilating and natriuretic effects, may directly prevent activation of the renin-angiotensin system [52, 53] and consequently or directly reduces catecholamine release [54, 55, 56, 57]. Other natriuretic peptides, BNP and CNP, have similar effects like ANP. CNP however acts through a different guanylate-cyclase receptor then ANP and BNP. CNP levels are not elevated in heart failure, which is in contrast to ANP and BNP [58].

An overview of all previous described effects is given in table 1.

Table 1

Effects of pharmacotherapy on neurohormones, myocardial function and mortality in chronic heart failure.

	Neurohormones			Myocardial function		
	Catecholamines	Renin-angiotensin system	ANP	Exercise tolerance	LV ejection fraction	Mortality
ACE-inhibitors	↓↓	↓[1)]	↓	↑	-	↓
Diuretics	↓ and ↑[2)]	↑	-	-	-	-[3)]
Vasodilators	-	↑	↓	-	↑	↓[4)]
Digoxine	↓	↓	-	-	≈	≈
β-blockers	↓ or ≈	↓	↓	↑	↑	↓
Calcium antagonists	≈ or ↑	≈	-	-	≈	≈ or ↑
PDE-inhibitors	≈	≈	≈	↑	-	↑
Dopaminergic agents	↓↓ and ↑[2)]	↓↓ and ↑[2)]	↓	↑	↑	↑

ACE = angiotensin converting enzyme, PDE = phosphodiesterase , ANP = atrial natriuretic peptide, ↑ = increase, ↓ = decrease, ≈ = no change, - = no known data.

[1)] angiotensin II decreases, renin activity increases.

[2)] both changes have been described.

[3)] there are no data available on diuretic monotherapy in heart failure

[4)] only in combination therapy

Drug treatment

ACE-inhibitors

ACE inhibitors act through direct neurohumoral modulation. Inhibition of the angiotensin converting enzyme prevents the conversion of angiotensin I in angiotensin II, thus reducing angiotensin II levels. This consequently diminishes activation of angiotensin receptors by angiotensin II and directly potentiates bradykinin effects by a reduction of bradykinin degradation [59, 60]. Bradykinin is a potent endothelium dependent vasodilator [61]. The effects of ACE inhibition on adrenergic activation may be secondary to the modulation of the renin-angiotensin system [62]. Data from the CONSENSUS trial indicate that, after treatment with enalapril, there is no longer a positive association between baseline norepinephrine, epinephrine, angiotensin II, aldosterone and ANP levels and increased mortality [63], while such relations persist in the placebo group.

ACE inhibitors have beneficial effects on neurohumoral activation shortly after myocardial infarction. During a 5 day period, administration of ramipril started shortly after myocardial infarction, reduced elevated norepinephrine, epinephrine and vasopressin levels [64], while long-term treatment with ACE inhibitors decreases circulating norepinephrine levels [65]. However, initial reducing effects on aldosterone and norepinephrine release will become less pronounced [66]. During long-term treatment with ACE inhibitors, normalization of angiotensin II levels are observed, probably through increased renin levels, which may require adaptation of the dose regime for proper treatment [67]. Furthermore, induction of angiotensin II formation by cardiac chymases may bypass converting enzyme inhibition [68]. Therefore, monotherapy by ACE inhibitors alone may not be sufficient in overt heart failure.

Diuretics

Treatment with diuretics has beneficial effects on body fluid compartments, but has no attenuating effect on elevated renin and norepinephrine levels in heart failure [69]. Long-term treatment with furosemide monotherapy in heart failure decreases norepinephrine levels and increases plasma renin and ANP levels [70]. The effect on plasma renin levels probably is a result of sodium depletion. The observed reduced effects of sympathetic stimulation on

activation of vasoconstrictor neurohormones during treatment with diuretics may be secondary to the increased renin activity [71]. An increase in renin, angiotensin II, aldosterone and vasopressin levels have been observed after diuretic treatment was started in heart failure [13]. In contrast, both reduction and increase in norepinephrine levels following diuretic treatment have been described [13, 72]. Spironolactone, a potassium sparing diuretic, antagonizes aldosterone at receptor level.

Neurohumoral changes, secondary to diuretic treatment alone may not be beneficial in heart failure at large, however combination therapy with ACE inhibition will enhance beneficial effects [73].

Vasodilators

Pure vasodilators like hydralazine have no direct effect on neurohumoral activation, but secondary effects have been reported. Nitroglycerin, which causes predominantly venodilation, increases renin and aldosterone levels and decreases ANP. Flosequinan, a quinolone-derivate, which is a direct-acting balanced type arteriovenodilator, decreases ANP levels [74, 75]. Clinically, combined vasodilation by hydralazine and nitroglycerine have shown relieve of heart failure symptoms and reduced mortality in the V-HeFT trials and may be used as an alternative to ACE-inhibition when the latter is contraindicated or not tolerated [73].

Digoxin

A recently performed large trial in patients with chronic heart failure treated with diuretics and angiotensin converting enzyme inhibitors showed that additional long-term therapy with digoxin did not reduce overall mortality, but reduced the rate of hospitalization both overall and for worsening of heart failure [76]. In several placebo controlled trials digoxin increases LV ejection fraction and exercise tolerance [77, 78], probably through direct sympatho-inhibitory effects [79, 80]. Several studies indicate a sustained decrease in norepinephrine after chronic treatment with digoxin [81, 82]. Also a decrease in renin levels by digoxin has been reported [77]. Aldosterone levels are decreased by digoxin treatment during 4 weeks [83]. In a small study with 18 patients with heart failure already treated with digoxin, increasing serum digoxin concentrations resulted in a slight increase in aldosterone levels, whereas withdrawal of digoxin had no effect on the neurohumoral profile of these patients over a 12 week period [84].

β-blockers

Since increased sympathetic activation is an important factor in heart failure, β-blockade is potentially useful in heart failure. Etiology, either idiopathic dilating cardiomyopathy or ischemic cardiomyopathy is important in the neurohumoral response in patients with heart failure to β-blockade. Patients with idiopathic dilating cardiomyopathy may have a more pronounced reduction in catecholamine levels then patients with ischemic cardiomyopathy [85]. Treatment with bucindolol, a non-selective β-blocker with primarily vasodilating action, leads to a reduction in renin levels in patients with heart failure [86]. Metoprolol a selective β_1-blocker without intrinsic sympaticomimetic activity improves cardiac function and decreases norepinephrine and epinephrine levels in patients with chronic heart failure [87, 88]. However, metoprolol does not reduce cardiac norepinephrine release, but causes upregulation of beta-receptors which consequently reduces the need for sympathetic stimulation, which may finally result in reduced norepinephrine levels [89]. Carvedilol, a novel third generation non-selective β-blocker does reduce cardiac norepinephrine release [90]. β-Blockers with intrinsic sympaticomimetic activity may counteract the β-blocker-induced reduction in renal renin secretion [91].

Calcium antagonists

Administration of calcium channel blockers causes a higher recurrence of heart failure in patients with LV ejection fraction lower then 40% after myocardial infarction [92]. A more recent study with amlodipine, the PRAISE study, indicated that there was no difference in mortality compared to placebo, however there was no clear benefit in treating heart failure patients with amlodipine compared to placebo [93]. The negative effects observed in the earlier studies may be caused by calcium antagonist-induced norepinephrine release [92]. In contrast, later generation calcium receptor blockers appear to have less effect on norepinephrine release. Acute intravenous administration of nisoldipine decreases systemic vascular resistance and arterial pressure and increases cardiac index, but has no effect on renin and norepinephrine levels [94, 95, 96]. A small, placebo controlled study by Kassis et al showed a reduction in catecholamines by chronic felodipine treatment, probably secondary to improved hemodynamics [97], although impaired baroreceptor function in more advanced heart failure

may diminish a counteractive vasoconstrictive response as well in these patients. So far, there is no clinical use for calcium antagonists in heart failure treatment.

Phosphodiesterase inhibitors and calcium sensitizers

Phosphodiesterase inhibitors have vasodilating and positive inotropic effects. Compounds like vesnarinone, enoximone and milrinone have no direct effect on neurohormones. Changes that may occur are secondary to vasodilation by these compounds. Studies with milrinone showed no favorable effect on neurohumoral activation after one month [98]. So far, all phosphodiesterase inhibitors show an increased mortality during chronic treatment, in spite of improved hemodynamics and relief of symptoms [99, 100]. Especially cAMP induced arrhythmias are frequent lethal adverse events. Agents with additional calcium sensitizing properties like isomazole and pimobendan, also do not affect neurohormones directly or after long-term treatment [101, 102].

Dopaminergic agonists

Studies with levodopa showed an improvement in LV function, with no effect on catecholamine balance [103, 104]. Dopexamine is an agent with dopaminergic and β_2-receptor agonist activity and has potent vasodilating and limited inotropic effects in humans. In addition, norepinephrine and renin levels are increased by dopexamine, through inhibition of norepinephrine uptake by sympathetic nerves [105, 106, 107]. Long-term treatment with ibopamine decreased norepinephrine and aldosterone levels and renin activity [81, 108]. Infusion of dopamine in patients with severe heart failure decreases ANP release [109]. However, long-term treatment with indirect acting β-agonists may become less efficacious due to high adrenergic drive induced receptor changes and neurotransmitter depletion in the failing heart and consequent development of tolerance [110]. Since there is no effective oral dopaminergic treatment available at the present time, the use of these agents is limited to acute heart failure and treatment of endstage heart failure in the clinic.

Discussion

Neurohumoral activation is an important feature in heart failure. Further deterioration of myocardial function through ischemic events, hypertension, aging or other cardiac or non-

cardiac diseases, leads to neurohumoral activation. At early stages, catecholamine levels increase, causing further vasoconstriction, which is amplified by activation of the renin-angiotensin system. This consequently leads to additional vasoconstriction and norepinephrine release. Angiotensin II mediated increase in aldosterone and vasopressin release cause further vasoconstriction and sodium and fluid retention. During this process the hemodynamic situation deteriorates, which places an extra burden on cardiac workload in a situation where cardiac function is already impaired and the clinical symptoms of congestive heart failure worsen. The rate at which this deterioration occurs depends on the factors mediating the worsening of cardiac function. Ischemic events may cause an acute onset of congestive heart failure whereas a factor like aging causes a slow development of symptoms.

Along with the worsening of heart failure several other structural changes occur in the human body. Changes in fiber type composition and energy metabolism in peripheral skeletal muscle have been reported. These changes involve not only the degeneration of muscle due to deconditioning or changes in fiber type composition of the skeletal muscle. These changes may affect the clinical symptoms of the heart failure syndrome like early fatigue and reduced exercise capacity.

Present pharmacological treatment for heart failure as recommended by the European Society of Cardiology, the American College of Cardiology and the American Heart Association [73, 111], mostly aims at attenuation of peripheral vasoconstriction, modulation of increased sympathetic drive, prevention of sodium retention and reduction of circulating volume overload.

ACE-inhibitors are potent neurohumoral modulators and have proven their benefit in large prospective trials like SOLVD, V-HeFT II and CONSENSUS, showing a pronounced reduction in mortality. Acute vasodilation and consequent hypotension may lead to a reflex catecholamine release to compensate, which counteracts the initial beneficial effects of ACE-inhibition. However, in heart failure the baroreceptor reflex is markedly attenuated [112], therefore counteractive vasoconstriction may be of less importance in heart failure patients. Side effects are another limiting factors in ACE-inhibitor administration. Beside contraindications like bilateral renal artery stenosis and angioedema, general symptoms like cough and rash, the possible deterioration of renal function is of some concern. Especially patients with severe heart failure are at risk for developing renal dysfunction due to ACE-inhibitors [113].

The increased sympathetic tone in heart failure, mediated by increased catecholamines may be influenced by β-blockade, which therefore may be beneficial in heart failure. However, negative inotropic and chronotropic effects of β–blockade may jeopardize cardiac function [114, 115]. Studies have indicated that, due to β-blockade, LV ejection fraction, exercise capacity and cardiac function improves, with an overall reduction in circulating norepinephrine levels [116]. Recent trials with carvedilol in heart failure have proven the beneficial effects of β-blockade in heart failure [117, 118].

Vasodilation alone may not be beneficial in heart failure. Although studies with calcium channel entry blockers have shown an increase in exercise capacity, more symptoms of heart failure and increased mortality were seen in patients with reduced cardiac function treated with calcium antagonists compared to those who were not [119]. Secondary changes in neurohumoral activation may occur. Studies with flosequinan, a balanced-type arteriovenodilator did show beneficial effects in heart failure, with secondary changes in neurohormones, but had to be stopped due to toxicity of the compound. The V-HeFT studies showed beneficial effects of combination therapy with hydralazine and isosorbidemononitrate, which may be a useful alternative when treatment with ACE-inhibitors is not possible [73].

Inotropic drugs like dopamine and dobutamine have proven their use in treatment of acute severe heart failure administered intravenously. Oral analogues may be useful in outpatient treatment. However, levodopa, an oral dopamine precursor, has limited use in heart failure due to central side effects, causing nausea, vomiting and dyskinesia. Ibopamine, an oral agent with combined DA_1 and DA_2 agonist with β-adrenergic activity, has shown beneficial effects in short term treatment of heart failure, with improved exercise capacity and a reduction in symptoms of heart failure in the DIMT study. However, in a long-term investigation in heart failure patients, the PRIME study, increased mortality was noted especially in patients with severe heart failure [120]. Nevertheless, DA_2-receptors agonists may be useful in heart failure.

Digitalis glycosides are traditionally widely used in heart failure. Data from the Digitalis Investigator Group showed no clear reduction in mortality in patients with NYHA class II-IV heart failure and sinus rhythm [76]. Other studies indicated that their clinical condition deteriorates when digoxin is withdrawn [121]. Neurohumoral changes seen with these compounds are mainly secondary to inotropic action and improved hemodynamics, although

direct effects of digoxin on norepinephrine have been suggested and sympatho-inhibitory effects precede hemodynamic improvement.

Inotropic agents with vasodilating action, inodilators, like phosphodiesterase inhibitors with or without additional calcium sensitizing properties have shown to improve hemodynamics in heart failure patients. Again long-term studies in heart failure, like the PROMISE trial, indicated an increase in mortality [122]. Arrhythmia's, cAMP induced, may have contributed to this finding. Studies with pimobendan have shown only a very limited effect [123]. Short term studies with isomazole and nanterinone showed improved hemodynamics in heart failure, with secondary changes in neurohormones (chapter 6).

Until now, no neurohumoral data in heart failure are available on novel compounds like AT2 receptor inhibitors, calcium sensitizers, renin inhibitors, vasopressin and endothelin antagonists.

In conclusion, neurohumoral activation in heart failure is an important feature and strongly associated with morbidity and mortality. Modulation of neurohumoral activation can be achieved by pharmacological intervention and altered hemodynamics, to which it is closely associated. Since neurohumoral activation is involved in amplification of hemodynamic deterioration in advanced heart failure, more emphasis should be put on direct attenuation of this mechanism to influence chronic and acute progression of the heart failure syndrome and requires further study.

Aim of the thesis

The aim of the present thesis is to investigate neurohumoral changes in relation hemodynamic parameters at different stages of heart failure and the effects of pharmacological treatment on neurohormones. In chapter 2 the neurohumoral profile of patients at different stages of heart failure are studied and compared to patients without heart failure. In chapter 3 a comparison of the neurohumoral profile is made between patients with and without asymptomatic LV dysfunction, both with and without chronic β-blocker therapy. In chapters 4, 5 and 6 the neurohumoral and hemodynamic effects of a dopamine receptor agonist, an ACE inhibitor and two phosphodiesterase inhibitors are studied. Finally, mitochondrial function in peripheral skeletal muscle is studied in chapter 7, because the

clinical features of the heart failure syndrome can not be fully explained by neurohumoral and hemodynamic parameters.

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Chapter 2

Activation of circulating and cardiac ANP release in absence of additional neurohumoral activation in asymptomatic LV dysfunction in spite of pronounced impaired cardiac function.

M. van der Ent, W.J. Remme

Summary

Several studies indicate early neuroendocrine activation in asymptomatic LV dysfunction. As their temporal relation and correlation with hemodynamic abnormalities are unknown, we studied 107 fasting, supine (≥ 60 min.), untreated patients with coronary artery disease, 25 with symptomatic LV dysfunction (LV ejection fraction 24 ± 2%), 27 with asymptomatic LV dysfunction (LV ejection fraction 34 ± 2%) and 55 with normal LV function (LV ejection fraction 56 ± 1%). Study variables included coronary and systemic hemodynamics and arterial and coronary venous neurohormones. In symptomatic LV dysfunction contractility, relaxation and myocardial pumpfunction were depressed by approximately 25, 65 and 28% respectively and LV filling pressures were elevated by 72% with concomitant activation of catecholamines and the renin-angiotensin-system compared to patients with normal LV function. In contrast, in asymptomatic LV dysfunction, myocardial and pump function were significantly impaired compared to normal subjects by approximately 24%, while RV and LV filling pressures were comparable, the only activated neurohormone was ANP. Therefore, ANP activation precedes other neurohumoral activation. Early ANP activation in asymptomatic LV dysfunction may prevent further deterioration by direct vasodilation and natriuresis and by attenuation of the renin-angiotensin system and catecholamine activation.

Keywords: asymptomatic LV dysfunction, neurohormones, ANP, cardiac function, catecholamines.

Introduction

The early development of the heart failure syndrome, regardless of etiology, is characterised by cardiac remodelling, progressive cardiac dysfunction and early neurohumoral activation. During the subsequent time course of the development of this syndrome, further alterations in the heart and secondary involvement of peripheral systems such as the vascular wall, skeletal muscle and the kidney, occur. These changes, some of them initially reversible, may eventually become irreversible and may amplify the heart failure symptoms [1,2,3,4,5,6]. In the early phase, before development of overt heart failure, neurohormonal mediated vasoconstrictor activity is counteracted by hormones with vasodilating properties such as ANP, prostaglandins and nitrogen oxide. However, the general evolution is one of imbalance where eventually vasoconstriction will prevail. Over time, this imbalance is progressive, leading to increased systemic resistance and further neurohumoral activation. Data from the SOLVD [7] study indicate that plasma norepinephrine, vasopressin and ANP levels are already elevated in patients with asymptomatic LV dysfunction, characterised by an ejection fraction of < 35%. Although, a time related sequence in neurohumoral activation has been suggested, with ANP activation preceding stimulation of the other neurohormones [8, 9], this has not been established yet in humans. In vitro studies in animal CNS tissues have suggested that there might be a direct attenuating effect of ANP on norepinephrine release from the rat adrenal medulla, evoked by angiotensin [10,11] and from rat hypothalamus [12]. The influence of ANP on norepinephrine is supported by the findings both in humans and in isolated rat renal resistance vessels that, in hypertension, ANP acts preferentially on noradrenergic blood pressure control [13,14].

Therefore, the aim of the present study was to investigate ANP levels in asymptomatic LV dysfunction compared to patients with normal LV function and symptomatic LV dysfunction in relation to other neurohormones and their hemodynamic profile.

Methods

Patient selection. Following approval of the Institutional Ethical Review Board and after informed consent, 107 consecutive normotensive patients with ischemic heart disease, indicated by coronary angiography, were studied after an overnight fast without premedication. Patients were divided into three groups, a) patients with a normal LV function

(n=55), b) patients with abnormal LV function (LV ejection fraction < 45%, n=27) without symptoms of heart failure at study entry or history and c) patients with symptomatic LV dysfunction (LV ejection fraction < 45% and NYHA class II-III at study entry). All cardiac medication was withheld 36-72 hours pre study, depending on respective plasma half lives, ACE-inhibitors were withdrawn 3 days pre-study. Myocardial infarction, if present, had to be at least 3 months old. Patient characteristics are shown in table 1.

Instrumentation. Patients were catheterised in the morning. First, Desilet introducer systems were introduced in the right femoral artery and vein, after local anaesthesia with 1% xylocaine. This was followed by routine left and right coronary angiography, after which further study procedures were carried out. These included the introduction of a Desilet introducer system in a brachial vein, through which a 7 Fr Wilton Webster coronary sinus thermodilution flow and pacing catheter was introduced into the midportion of the coronary sinus. The position of the catheter was such that blood could be sampled easily by the catheter, its position stable and no atrial reflux occurred. The latter was assessed by injecting 10 cc glucose 5% at room temperature in the right atrium and observing the coronary flow signal. Next, a 7 Fr Sentron pigtail micromanometer catheter was introduced into the left

Table 1

Patient characteristics

		normal LV function	asymptomatic LV dysfunction	symptomatic LV dysfunction
n		55	27	25
Age (years)		55 ± 1	57 ± 2	61 ± 1
Males/Females		49/6	27/0	24/1
Diseased vessels	3	10	14	7
	2	24	9	8
	1	20	5	7
	0	1	0	3
Previous myocardial infarction		22 (40%)	19 (72%) *	23 (92%) *#
LV ejection fraction (%)		56 ± 1	34 ± 2 *	24 ± 2 *#
LV end diastolic volume	(ml)	61 ± 2	84 ± 5 *	112 ± 7 *#

* $p < 0.05$ versus normal LV function, # $p < 0.05$ versus asymptomatic LV dysfunction.

ventricle through the arterial introducer and a 7 Fr Swan Ganz triple lumen catheter advanced to a pulmonary artery via the femoral vein. The side arm of the arterial introducer system was used to record arterial pressures. After instrumentation, the position of the catheters was recorded on videotape for later check-up during the study.

Hemodynamic measurements and calculations. The fluid-filled catheters were calibrated using Bentley transducers with a zero reference level set at mid chest. The micromanometer was balanced to zero and superimposed on the conventional left ventricular pressure recording. After calibration, all pressures, the first derivative of left ventricular systolic pressure, cardiac output, coronary flow and 3 ECG leads (I, II and V5) were recorded on paper using a Nihon Kohden catheterisation laboratory system. In addition, all hemodynamic parameters, which included mean and phasic systemic arterial, pulmonary arterial and right atrial pressures, LV pressure-derived contractility and relaxation indices [LV peak dP/dt positive and negative, dP/dt/P at 40 mmHg (V_{ce40}) and V_{MAX} total pressure] and coronary flow were determined on-line by a Mennen catheterisation laboratory computer system. This system averages 15-20 consecutive beats to level out respiratory fluctuations. The isovolumetric relaxation parameter Tau was determined off-line in a beat-to-beat program from 15-20 consecutive beats, using a semilogarithmic model [15]. Cardiac output was measured by the thermodilution technique and determined on-line by a Mennen catheterisation laboratory computer system. Coronary flow was determined during a continuous 30-second infusion of 30 ml glucose 5% at room temperature. Although both pulsatile and mean flow curves were recorded, calculations were made from the latter, according to the formula: coronary blood flow (ml/min)= $V_i \times[T_b - T_i)(T_b - T_{cs}) -1] \times 1.08$, where T_b is blood temperature before injection, T_i temperature of injectate, T_{cs} temperature of mixture of coronary sinus blood and injectate and V_i the rate of injection (ml/min). Coronary flow was measured during superficial, shallow breathing. On-line presentation of 1-second measurements allowed for proper estimation of the stability of the flow signal.

At the end of the study, the arterial pressure curve was compared with a simultaneous recording by the microtip manometer catheter from the aortic root to compensate for differences between proximal and distal arterial pressure measurements.

From the measured hemodynamic variables, the following parameters were calculated: Coronary vascular resistance (mmHg/ml/min) was calculated as the difference between mean arterial pressure (mmHg) and left ventricular mean diastolic pressure (mmHg) divided by

coronary sinus blood flow (ml/min). Systemic vascular resistance (dynes.sec.cm^{-5}) was derived as [mean arterial pressure (mmHg) - mean right atrial pressure (mmHg) / cardiac output (l/min)] x 80. Stroke work index (g.m/m^2) was calculated as stroke index (ml/beat/m^2) x [mean arterial pressure (mmHg) - left ventricular end diastolic pressure (mmHg)] x 0.0136.

Neurohumoral determinations. The levels of catecholamines (norepinephrine, epinephrine, dopamine), active plasma renin concentration, and angiotensin II were assessed by collecting a minimum of 12 ml of blood simultaneously from the left ventricle and coronary sinus in pre-cooled syringes.

For the assay of angiotensin II, 2 ml was immediately transferred into ice cold tubes containing an inhibitor solution (2.4 mg EDTA, 0.3 mg 1,10-O-phenantroline and 0.01 mg of captopril in 50 µl). Blood samples were immediately centrifuged (10 min, 4°C, 3000 g) and the plasma was stored at -70°C. Determination was done by SepPak extraction and HPLC [16]. For catecholamines, 2-3 ml of blood was transferred into chilled heparinized tubes containing 3 mg gluthation and centrifuged within 15 min at 4°C at 3000 g. Plasma was stored at -70°C. Determination of catecholamines (norepinephrine, epinephrine, dopamine) was done by HPLC with fluorimetric detection [17]. For the determination of enzymatically active renin, 2 ml of blood was collected in tubes containing 2.4 mg EDTA. Samples were centrifuged (10

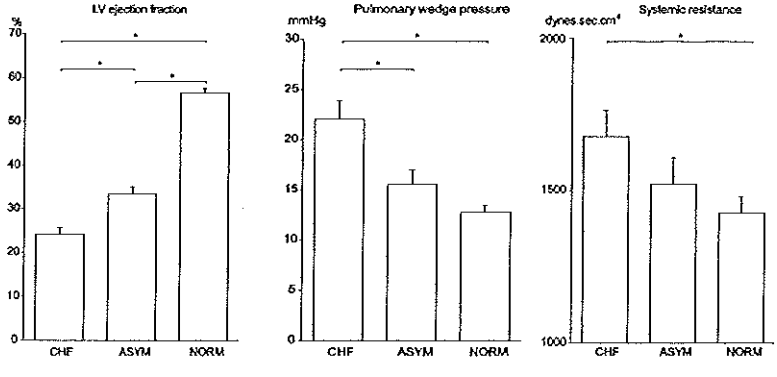

Figure 1

In patients with LV dysfunction (LV ejection fraction ≤ 45%), both symptomatic and asymptomatic LV filling pressures and systemic resistance are increased compared to patients with normal LV function. (CHF= symptomatic LV dysfunction, ASYM= asymptomatic LV dysfunction, NORM= normal LV function).

4°C, 3000 g) and plasma stored at -20°C. Determination was done by radioimmunoassay [18]. Atrial natriuretic peptide (ANP) was determined in plasma prepared from 2 ml of blood collected in a tube containing 2.4 mg EDTA and 500 KIE trasylol and stored at -70°C. ANP was measured by a commercially available radioimmunoassay (ITS, Wijchen, The Netherlands).

Cardiac neurohumoral values were calculated using the formula: (arterial concentration - coronary venous concentration) x coronary blood flow (ml/min), similar to the previously described myocardial oxygen consumption.

Study protocol. Studies were performed in the morning, between 09:30 and 10:30 am. After positioning of the catheters, a stabilisation period of 20 minutes was allowed to reach a minimum interval of 45 minutes between coronary angiography and the study. At the start of the study, patients were in a supine, resting position for at least 1.5 hours. Thereupon, repetitive control measurements were made of systemic and coronary hemodynamic variables to ensure stable baseline values. Systemic hemodynamic variables were assessed both at basal and fixed heart rate. Thereafter, blood was collected for neurohumoral determination, lactate levels and myocardial oxygen utilisation and metabolism.

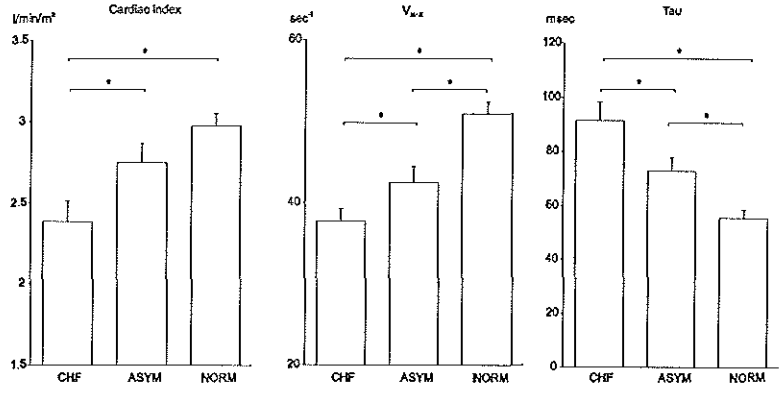

Figure 2

Myocardial pump function is impaired in patients with symptomatic and asymptomatic LV dysfunction. Cardiac index is decreased, contractility and relaxation are impaired. (CHF= symptomatic LV dysfunction, ASYM= asymptomatic LV dysfunction, NORM= normal LV function).

Statistical analysis. Comparisons between group means were performed using a one way ANOVA test. When significant, a post hoc comparison of means was performed using Bonferroni's test. A p value < 0.05 was considered indicative of a significant difference. All tests were performed using SAS/STAT® statistical software. All data are expressed as group means ± standard error of the mean.

Results

Systemic hemodynamics. Cardiac function was depressed in patients with asymptomatic LV dysfunction. Contractility, as indicated by the parameters V_{CE40}, V_{MAX} and dP/dt positive, measured at fixed heart rates, were reduced by 18, 26 and 18% compared to patients with normal LV function. Also, relaxation was impaired in LV dysfunction, indicated by a prolongation of Tau compared to normal patients by 31% and a 25% reduction in dP/dt negative. Cardiac index was not significantly lower in asymptomatic LV dysfunction. In contrast, stroke volume index and stroke work index were reduced in asymptomatic LV dysfunction by 12 and 24% respectively. In contrast, pulmonary wedge pressure and right atrial pressures were comparable in both groups. Despite the reduction in pump function in asymptomatic LV dysfunction, heart rate, mean arterial pressure, systemic resistance and mean pulmonary artery pressure and resistance were not different from patients with normal LV function as well.

In symptomatic LV dysfunction contractility and relaxation were impaired compared to both patients with normal LV function and asymptomatic LV dysfunction. V_{CE40}, V_{MAX} and dP/dt positive were reduced by 31, 26 and 31% compared to normals and by 14, 12 and 17% compared to asymptomatic patients. dP/dt negative was reduced by 44% compared to normals and by 25% compared to asymptomatic patients, Tau was prolonged by 65 and 26% respectively. Cardiac index and stroke volume and work index were reduced by 22, 28 and 43% and by 15, 18 and 26% compared to normal and asymptomatic patients respectively. Heart rate was higher (8%), mean arterial and pulmonary pressure were lower (10 and 36%) and systemic and pulmonary resistances higher (15 and 20%) in symptomatic patients compared to normal subjects. A summary of data is presented in table 2.

Neurohumoral changes. Epinephrine levels were comparable in all patients. Both arterial and coronary venous norepinephrine levels in asymptomatic patients were comparable to normals. The same was observed for the renin-angiotensin system. Both coronary venous and arterial levels of plasma renin and angiotensin II in asymptomatic patients were comparable to patients with normal LV function as well. In contrast to the absence of changes in catecholamines and the renin-angiotensin system in asymptomatic patients, arterial ANP levels and net cardiac ANP release levels were increased by 98 and 83% compared to normal LV function, which is similar to the increase seen in patients with symptomatic LV dysfunction (96 and 73% respectively). In patients with symptomatic LV dysfunction both arterial and coronary venous norepinephrine and renin levels were increased by 59 and 88% and 194 and 177% respectively compared to patients with normal LV function. Net cardiac

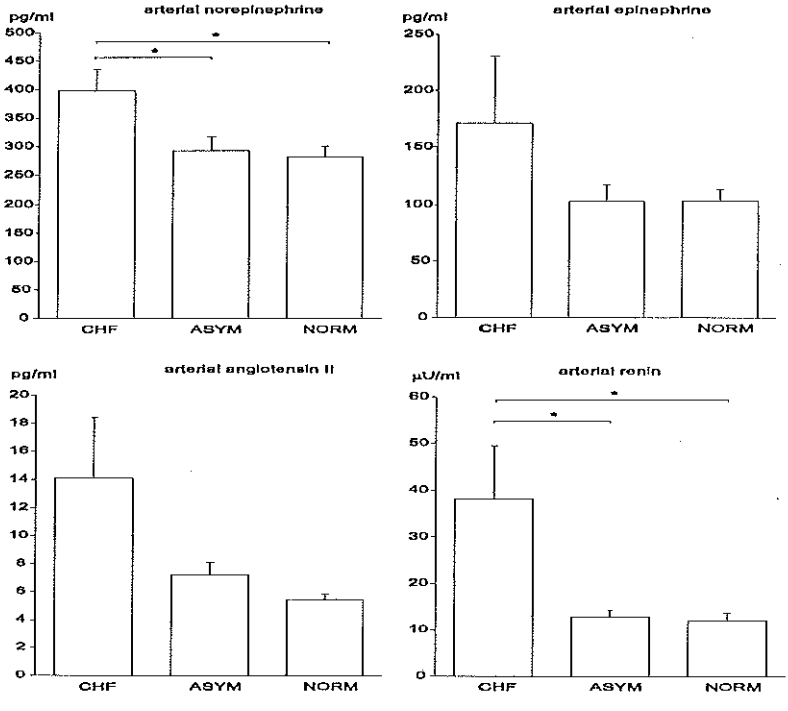

Figure 3

In symptomatic LV dysfunction, neurohumoral activation is present. In contrast, in asymptomatic LV dysfunction, in spite of impaired myocardial pumpfunction and moderately impaired hemodynamics, neurohumoral levels are comparable to normal subjects. (CHF= symptomatic LV dysfunction, ASYM= asymptomatic LV dysfunction, NORM= normal LV function).

norepinephrine release was comparable to normal subjects in asymptomatic patients, but increased by 445% in symptomatic patients. Cardiac epinephrine levels were comparable in all patients. Cardiac angiotensin II uptake was not statistically significantly different in any group. Cardiac renin levels reverted from release in normal subjects to uptake in asymptomatic LV dysfunction and symptomatic LV dysfunction, however these changes were not significant, due to the wide variation in individual values. A summary of data is shown in table 3.

Discussion

Symptomatic heart failure is characterized by overt neurohumoral activation [7]. It is also known that increased catecholamine levels are associated with increased mortality [19]. However, before overt heart failure occurs, there is a variable time course in which there are no symptoms yet, but cardiovascular function becomes progressively impaired. Compensatory mechanisms are still able to maintain stable hemodynamic conditions, until this equilibrium is broken. Pharmacological treatment aims for preservation of this situation.

In the present study, in the resting state, patients with asymptomatic LV dysfunction, indicated by reduced LV ejection fraction and enlarged LV end diastolic volumes, but without signs and symptoms of heart failure, hemodynamics were generally still comparable to normal patients, with the exception of the parameters for myocardial function. In the present study contractility and relaxation, stroke volume and work indexes are impaired in these patients. In contrast, vasotone, left and right ventricular filling pressures and heart rate were still comparable to patients without LV dysfunction. In addition, neurohumoral activation is absent as well, except for increased arterial ANP levels and increased cardiac ANP release. Although in previous studies increased ANP levels related to increased vasotone, we were unable to demonstrate this in our study [20]. Since LV volumes were enlarged, a wall stress factor may be involved.

Investigators from the SOLVD study demonstrated increased catecholamine levels in patients with asymptomatic LV dysfunction, which is in contrast with our findings. In the SOLVD treatment trial however, LV dysfunction was determined by a LV ejection fraction lower than 35%, on average 26% [21]. In our study asymptomatic patients had LV ejection fractions lower than 45% with a mean ejection fraction of 34%, therefore the patients in the SOLVD study may have had more severely affected left ventricles than in our study.

Other data, from a randomly selected subgroup from the SOLVD registry, indicate progressive neurohumoral activation in relation with the severity of heart failure based on clinical parameters [22]. The findings in our study suggest that increased ANP levels are an early sign of heart failure, which occurs before the other neurohormones become activated. Similar findings were done in an asymptomatic LV dysfunction model in dogs [23]. Raised ANP levels in asymptomatic LV dysfunction may directly prevent the activation of the renin-angiotensin system [24, 25] and consequently attenuate catecholamine release [10, 11] or may have a direct effect on catecholamine release [12]. In our study there is no increased sympathetic tone in patients with asymptomatic LV dysfunction, indicated by the fact that there is no difference in heart rate, systemic resistance or mean arterial pressure. In addition, the normal hemodynamics in the asymptomatic LV dysfunction group may be due to sufficient direct vasodilating and natriuretic effects of ANP in this stage of heart failure.

Conclusion. Concerning the above, we conclude from the data from this study that during the development of heart failure due to ischemic heart disease there is a time-related course in hemodynamic impairment, deteriorating cardiac function and neurohumoral activation. In patients with moderately affected LV function by ischemic heart disease, ANP activation occurs early and may be able to maintain the balance between vasoconstriction and

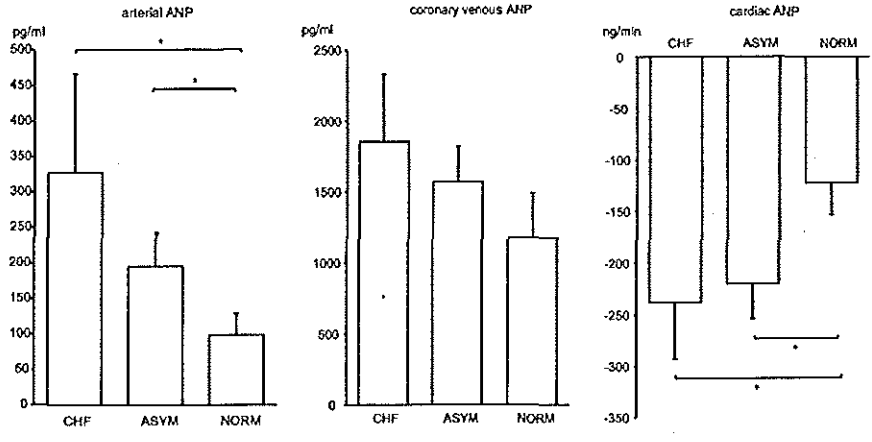

Figure 4

In contrast to the absence of activation of the other neurohormones, measured in the present study, there is an increase in arterial, coronary venous (cv) and cardiac ANP release in patients with asymptomatic LV dysfunction. (CHF= symptomatic LV dysfunction, ASYM= asymptomatic LV dysfunction, NORM= normal LV function)

vasodilation by direct natriuretic and vasodilating effects, effects on the renin-angiotensin system and norepinephrine release. In the development of overt heart failure, further neurohumoral activation occurs. Presently, therapeutic strategies in the treatment of heart failure aim at counteracting the effects of this activation with the use of ACE inhibition and β-blockers. Since both high norepinephrine and ANP levels are associated with increased mortality, it would be interesting to know whether this is true for isolated ANP activation as well.

Table 2

Hemodynamic parameters.

	Normal LV function	Asymptomatic LV dysfunction	Symptomatic LV dysfunction
Heart rate (bpm)	76 ± 2	79 ± 3	82 ± 3*
Mean arterial pressure (mmHg)	107 ± 2	100 ± 3	95 ± 3*
Systemic resistance (dyn.sec.cm^{-5})	1427 ± 53	1521 ± 87	1696 ± 86*
Mean pulmonary artery pressure (mmHg)	16.4 ± 0.59	17.9 ± 1.6	25.8 ± 2.5*#
Pulmonary resistance (dyn.sec.cm^{-5})	129 ± 9	132 ± 15	161 ± 19*#
Coronary blood flow (ml/min)	124 ± 8	161 ± 19	136 ± 14
Coronary vascular resistance (mmHg/ml/min)	1.01 ± 0.06	0.79 ± 0.08	0.97 ± 0.15
V_{CE40} (sec^{-1})	34.7 ± 1.0	28.4 ± 1.2*	24.4 ± 1.1*#
V_{MAX} (sec^{-1})	51.0 ± 1.4	42.5 ± 1.9*	37.5 ± 1.6*#
dPdt positive (mmHg/sec)	1753 ± 49	1445 ± 61*	1204 ± 62*#
Tau (msec)	55.5 ± 3.1	72.8 ± 4.9*	91.8 ± 7.2*#
dPdt negative (mmHg/sec)	1957 ± 43	1464 ± 58*	1091 ± 55*#
Pulmonary wedge capillary pressure (mmHg)	12.8 ± 0.7	15.6 ± 1.6	22 ± 1.8*#
Right atrial pressure (mmHg)	7.9 ± 0.4	5.3 ± 0.7*	6.6 ± 1
Cardiac index (l/min/m^2)	3.0 ± 0.1	2.75 ± 0.12	2.35 ± 0.13*#
Stroke volume index (ml/m^2)	40.6 ± 1.0	35.8 ± 1.1*	29.2 ± 1.6*#
Stroke work index (g.m.m^2)	52.5 ± 1.5	40.0 ± 2.1*	29.7 ± 2.4*#

$p < 0.05$ versus normal LV function, # $p < 0.05$ versus asymptomatic LV dysfunction.

Table 3

Neurohumoral parameters

		Normal LV function	Asymptomatic LV dysfunction	Symptomatic LV dysfunction
Norepinephrine	arterial (pg/ml)	283 ± 19	293 ± 25	449 ± 64*#
	coronary venous (pg/ml)	343 ± 30	321 ± 23	644 ± 105*#
	cardiac (ng/min)	-5.6 ± 2.2	-4.6 ± 3.1	30.5 ± 9.1*#
Epinephrine	arterial (pg/ml)	103 ± 10	102 ± 14	135 ± 43
	coronary venous (pg/ml)	64 ± 7	60 ± 7	93 ± 22
	cardiac (ng/min)	5.0 ± 0.7	5.8 ± 1.0	3.3 ± 1.2
Renin	arterial (μU/ml)	12.0 ± 1.6	12.9 ± 1.4	35.3 ± 9*#
	coronary venous (μU/ml)	12.2 ± 1.5	12.3 ± 1.4	33.8 ± 8.1*#
	cardiac (μU/min)	-44 ± 81	95 ± 78	148 ± 152
Angiotensin II	arterial (pg/ml)	5.4 ± 0.4	7.1 ± 0.9	13.1 ± 3.5
	coronary venous (pg/ml)	5.1 ± 0.4	6.1 ± 0.9	10.7 ± 2.8
	cardiac (pg/min)	45 ± 24	131 ± 111	372 ± 144*
ANP	arterial (pg/ml)	99 ± 31	196 ± 47*	194 ± 39*
	coronary venous (pg/ml)	1173 ± 316	1570 ± 253	1490 ± 327
	cardiac (ng/min)	-122 ± 31	-220 ± 33*	-211 ± 52*

* $p < 0.05$ versus normal LV function, # $p < 0.05$ versus asymptomatic LV dysfunction.

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Chapter 3

Chronic β-blockade does not jeopardize myocardial function in patients with asymptomatic LV dysfunction.

M. van der Ent, W.J. Remme

Summary

Objectives. To evaluate potential deleterious effects in cardiac function of β-blockade in asymptomatic LV dysfunction

Background. In heart failure, chronic β-blockade may provide beneficial clinical and hemodynamic effects. In asymptomatic LV dysfunction, in the absence of significantly increased sympathetic tone, β-blockade could further jeopardize cardiac function.

Methods. We studied 137 consecutive, patients with coronary artery disease during cardiac catheterization. None of these patients ever had signs or symptoms of heart failure. Ninety-three patients had a normal LV function (LV ejection fraction > 45%), 38 of them were on chronic β-blocker therapy. Forty-four patients had LV dysfunction (LV ejection fraction < 45%), 17 of them were on chronic β-blockade. Chronic β-blocker therapy was administered as metoprolol SR 100 mg od.

Results. In subjects with normal LV function and β-blocker therapy cardiac output, contractility and relaxation were impaired by 14%, 19% and 21% respectively compared to those without β-blockade. In contrast, in subjects with LV dysfunction, hemodynamics were not different with β-blockade. Catecholamines were unchanged by β-blockade in any group. The renin-angiotensin system was attenuated by approximately 50% in both normal and LV dysfunction by β-blockade. Circulating ANP was reduced similarly in both groups. However, β-blockade reduced cardiac ANP release by 61% in LV dysfunction and increased it by 163% in normal LV function.

Conclusion. In normal LV function, chronic β-blockade results in a reduction in myocardial muscle and cardiac pump function. In contrast, these effects are much less pronounced or not present in impaired LV function, indicating that chronic β-blockade does not further jeopardize cardiac function in patients with asymptomatic LV dysfunction due to ischemic cardiomyopathy.

Keywords: heart failure, neurohormones, β-blockade, asymptomatic LV dysfunction, hemodynamics.

Introduction

Heart failure is characterized by cardiac remodeling, progressively impaired cardiac function and neurohumoral activation [1, 2]. To compensate for the reduction in tissue perfusion and blood pressure, activation of the sympathetic and renin-angiotensin systems occurs [3, 4], consequently raising systemic vascular resistance and afterload, which puts an extra load on myocardial work. Although the resulting extra load on the heart and the increased myocardial work may initially be counteracted by simultaneous activation of vasodilating and natriuretic peptides such as ANP [5], eventually, the clinical condition of patients with cardiac dysfunction deteriorates towards overt heart failure as vasoconstrictive forces prevail. In addition to increased afterload sympathetic activation stimulates myocardial contractility and heart rate, resulting in increased myocardial oxygen consumption. Also, high levels of catecholamines may induce cardiotoxicity [6]. Several studies have confirmed the potential deleterious effects of sympathetic activation [7, 8]. Since β-blocking agents attenuate the effect of enhanced sympathetic tone, they have been identified as potential useful drugs in the treatment of heart failure [8, 9, 10, 11, 12]. The concept suggested is that long term improvement prevails over the acute negative inotropic and chronotropic effects of these drugs. In asymptomatic cardiac dysfunction, sympathetic activation may be of less importance. Beta-blocking agents may further jeopardize cardiac function in these patients rather than improve it. In the latter event, further neurohumoral activation might ensue. In addition, vasoconstriction as a result of unopposed α-adrenergic response may occur, adding another factor for jeopardizing cardiac function. It is unknown whether a different neurohumoral pattern in relation to a more profound negative hemodynamic effect of chronic β-blockade between asymptomatic LV dysfunction as compared to patients with normal LV function really exists.

The present study evaluates the hemodynamic and neurohumoral profile of patients with ischemic heart disease with asymptomatic LV dysfunction and normal LV function, with or without chronic β-blocker treatment.

Methods

Patient selection.

Following approval of the Institutional Ethical Review Board and after informed consent, 164 consecutive normotensive patients with ischemic heart disease, indicated by coronary angiography, without clinical signs and symptoms of heart failure, were studied after an overnight fast without premedication. Patients were divided into four groups. First, patients were divided in 2 groups, based on LV function (group 1: normal LV function (LV ejection fraction $\geq$ 45%) and group 2: LV dysfunction (LV ejection fraction < 45%)). Each group was subsequently subdivided based on the presence or absence of β-blocker treatment. To minimize heterogeneity in types and dosages of β-blocker treatment, all patients received metoprolol 100 mg once dialy. For at least 5 days prior to the study. Duration of chronic β-blocker therapy had to be at least 3 months. All other cardiac medication was withheld 36-72 hours pre study, depending on their respective plasma half lives, with the exception of the β-blocker groups, who received their last dose of metoprolol 12 hours before the study began. Previous myocardial infarction, if present, had to be at least 3 months old. Patient characteristics are shown in table 1.

Instrumentation.

Patients were catheterized in the morning after an overnight fast, between 9:30 and 10:30 am. First, Desilet introducer systems were introduced in the right femoral artery and vein, after local anesthesia with 1% xylocaine. This was followed by routine left and right coronary angiography, after which further study procedures were carried out. These included the introduction of a Desilet introducer system in a brachial vein, through which a 7 Fr Wilton Webster coronary sinus thermodilution flow and pacing catheter was introduced into the midportion of the coronary sinus. The position of the catheter was such that blood could be sampled easily by the catheter, its position was stable and no atrial reflux occurred. The latter was assessed by injecting 10 cc glucose 5% at room temperature in the right atrium and observing the coronary flow signal. Next, a 7 Fr Sentron pigtail micromanometer catheter was introduced into the left ventricle through the arterial introducer and a 7 Fr Swan Ganz triple lumen catheter advanced to a pulmonary artery via the femoral vein. The side arm of the

arterial introducer system was used to record arterial pressures. After instrumentation, the position of the catheters was recorded on video tape for later check-up during the study.

Hemodynamic measurements and calculations.

The fluid-filled catheters were calibrated using Bentley transducers with a zero reference level set at mid chest. The micromanometer was balanced to zero and superimposed on the conventional left ventricular pressure recording. After calibration, all pressures, the first derivative of left ventricular systolic pressure, cardiac output, coronary flow and 3 ECG leads (I,II and V5) were recorded on paper, using a Nihon Kohden catheterization laboratory system. In addition, all hemodynamic parameters, which included mean and phasic systemic arterial, pulmonary arterial and right atrial pressures, LV pressure-derived contractility and relaxation indices [LV peak dP/dt positive and negative, dP/dt/P at 40 mmHg (Vce_{40}) and V_{max} total pressure] and coronary flow were determined on-line by a Mennen catheterization laboratory computer system. This system averages 15-20 consecutive beats to level out respiratory fluctuations. The isovolumetric relaxation parameter Tau was determined off-line in a beat-to-beat program from 15-20 consecutive beats, using a semilogarithmic model [13]. Cardiac output was measured by the thermodilution technique and determined on-line by a Mennen catheterization laboratory computer system. At the end of the study, the arterial pressure curve was compared with a simultaneous recording by the microtip manometer catheter from the aortic root to compensate for differences between proximal and distal arterial pressure measurements.

From the measured hemodynamic variables, the following parameters were calculated: Systemic vascular resistance (dynes.sec.cm^{-5}) was derived as [mean arterial pressure (mmHg) - mean right atrial pressure (mmHg) / cardiac output (l/min)] x 80. Stroke work index (g.m/m^2) was calculated as stroke index (ml/beat/m^2) x [mean arterial pressure (mmHg) - left ventricular end diastolic pressure (mmHg)] x 0.0136.

Neurohumoral determinations.

The levels of catecholamines (norepinephrine, epinephrine), active plasma renin concentration, angiotensin II and ANP were assessed by collecting a minimum of 12 ml of blood simultaneously from the left ventricle and coronary sinus in pre-cooled syringes.

For determination of catecholamines, 2-3 ml of blood was transferred into chilled heparinized tubes containing 3 mg gluthation and centrifuged within 15 min at 4°C at 3000 g. Plasma was stored at -70°C. Determination of catecholamines (norepinephrine, epinephrine) was done by HPLC with fluorimetric detection [14]. For the assay of angiotensin II, 2 ml was immediately transferred into ice cold tubes containing an inhibitor solution (2.4 mg EDTA, 0.3 mg 1,10-O-phenantroline and 0.01 mg captopril in 50 µl). Blood samples were immediately centrifuged (10 min, 4°C, 3000 g) and the plasma was stored at -70°C. Determination was done by SepPak extraction and HPLC [15]. For the determination of enzymatically active renin, 2 ml of blood was collected in tubes containing 2.4 mg EDTA. Samples were centrifuged (10 min, 4°C, 3000 g) and plasma stored at -20°C. Determination was done by radioimmunoassay [16]. Atrial natriuretic peptide (ANP) was determined in plasma prepared from 2 ml of blood collected in a tube containing 2.4 mg EDTA and 500 KIE trasylol and stored at -70°C. ANP was measured by a commercially available radioimmunoassay (ITS, Wijchen, The Netherlands).

Cardiac neurohumoral values were calculated using the formula: (arterial concentration - coronary venous concentration) x coronary blood flow (ml/min).

Study protocol.

After positioning of the catheters, a stabilization period of 20 minutes was allowed to reach a minimum interval of 45 minutes between coronary angiography and the study. At the start of the study patients were in a supine, resting position for at least 1.5 hours. The actual study took place between 09:30 and 10:30 am. Thereupon, repetitive control measurements were made of systemic and coronary hemodynamic variables to ensure stable baseline values. Systemic hemodynamic variables were assessed both at basal and fixed heart rate. Thereafter, blood was collected for neurohumoral determination.

Statistical analysis.

Comparisons between groups were performed using an one way ANOVA test. For intergroup analysis when appropriate a post hoc test according to Bonferroni was applied. A p value < 0.05 was considered indicative of a significant difference. All tests were performed

using SAS/STAT® statistical software. All data are expressed as group means ± standard error of the mean.

Results

Systemic hemodynamics - group 1 (normal LV function) versus group 2 (asymptomatic LV dysfunction).

By design, LV ejection fraction was lower in group 2 compared to group 1 (35 ± 1 vs 56 ± 1 % respectively). Also LV end diastolic volume indexes were enlarged in group 2 (80 ± 4 vs 60 ± 2 ml, group 1) (Table 1). Mean systemic arterial pressure was slightly higher in normal individuals, but systemic resistance, mean pulmonary pressure and pulmonary resistance, pulmonary wedge pressure and right atrial pressure were comparable in groups 1

Table 1

Patient characteristics.

	Normal LV function		Asymptomatic dysfunction	
	- β-blockade	+ β-blockade	- β-blockade	+ β-blockade
n	55	38	27	17
Age (years)	55.3 ± 1.2	57.3 ± 1.6	56.8 ± 1.6	58.8± 2.6
Range	36-76	41-76	33-70	42-75
Males/Females	49/6	29/9	27/0	14/3
LV ejection fraction %	56.5 ± 1†	55.2 ± 1.1‡	34.0 ± 1.6	36.8 ± 1.4
Range	45-73	45-71	10-44	24-44
LV end diastolic volume (ml/m^2)	61.1 ± 2.2†	61.5 ± 2.6‡	85.7 ± 5.7	70.3± 6.3
Range	30-97	33-101	33-154	27-119
Previous myocardial infarction	23 (41%)	11 (29%)	19 (71%)	10 (59%)

*: $p < 0.05$ -β–blocker vs. + β–blocker within each group.
†: $p < 0.05$ -β -blocker in normal LV function vs. -β-blocker in asymptomatic LV dysfunction.
‡: $p < 0.05$ +β -blocker in normal LV function vs. +β-blocker in asymptomatic LV dysfunction.

and 2. Contractility, indicated by dP/dt positive, V_{CE40} and V_{MAX} were all significantly lower in group 2 (14%, 14% and 13% respectively). Also, relaxation was impaired. In group 2 dP/dt negative was 21% lower and Tau was prolonged by 15%. Although cardiac index was comparable in both groups, stroke volume and stroke work index were reduced in group 2 (37 ± 1 vs 41 ± 1 ml/m^2 and 41 ± 2 vs 51 ± 1 g.m.m^2, group 2 vs group 1 respectively).

Systemic hemodynamics – effects of β-blockade within groups (Table 2).

In group 1 with normal LV function, patients on chronic β-blocker therapy had impaired contractility and relaxation. dP/dt positive, V_{CE40} and V_{MAX} were reduced by 19%, 22% and 22% respectively and dP/dt negative was 12% lower and Tau prolonged by 27% compared to patients without β-blocker. Heart rate and cardiac index were lower (17 and 14% respectively). In contrast, stroke work and volume index were comparable in both subgroups. Also, mean arterial pressure and pulmonary artery pressure were comparable. In contrast systemic and pulmonary resistance were higher in the β-blocker subgroup (15% and 36% respectively). Right atrial pressure and pulmonary wedge pressure were comparable in both subgroups.

In patients with asymptomatic LV dysfunction, group 2, only heart rate and coronary blood flow were lower in the β-blocker treatment subgroup (17% and 35% respectively). Contractility and relaxation parameters, cardiac, stroke volume and work indexes were comparable between patients with and without β-blockade. Also, mean arterial and pulmonary pressures and systemic and pulmonary resistance and right and left ventricular filling pressures were comparable in both subgroups.

Neurohumoral differences - group 1 (normal LV function) versus group 2 (asymptomatic LV dysfunction) and effects of β-blockade within each group (Table 3).

There were no differences in arterial and coronary venous levels nor in the cardiac balance of norepinephrine, epinephrine, angiotensin II, renin or ANP between group 1 and 2. Also, in group 1, norepinephrine and epinephrine levels were comparable in both subgroups. In contrast, arterial and coronary venous levels of renin were significantly lower in the subgroup on β-blocker treatment (45 and 43% for arterial and coronary venous levels). Both arterial and coronary venous levels of ANP were higher after β-blockade (387 and 134% respectively). Cardiac ANP release was increased by 163% in the β-blocker subgroup,

whereas other cardiac neurohormonal levels were comparable. In group 2 norepinephrine and epinephrine levels were also comparable in both subgroups. Although, renin levels were not significantly different in both subgroups, arterial and coronary venous angiotensin II levels were significantly lower in the β-blocker subgroup (3.5 ± 0.4 vs 7.1 ± 0.9 pg/ml (arterial) and 3.6 ± 0.5 vs 6.1 ± 0.9 pg/ml (coronary venous) β-blockade versus non-β-blockade $p < 0.05$). In contrast, cardiac angiotensin II balance was comparable. In both groups, arterial levels of atrial natriuretic peptide (ANP) were higher in the subgroup with β-blockers (99 ± 31 vs 482 ± 49 pg/ml and 196 ± 47 vs 304 ± 9 pg/ml respectively, non-β-blockade versus β-blockade, group 1 and group 2). Coronary venous ANP levels were comparable in both subgroups. Cardiac ANP release increased in group 1 in the β-blockade subgroup, whereas it decreased in the β-blockade subgroup of group 2.

Table 2

Hemodynamic data.

	Normal LV function (group 1)			Asymptomatic LV dysfunction (group 2)		
	all	-β-blocker	+β-blocker	all	-β-blocker	+β-blocker
Heart rate (bpm)	70 ± 1	76 ± 2*	63 ± 2	74 ± 2	79 ± 3*	65 ± 2
Pulmonary wedge pressure (mmHg)	13.8 ± 0.6	12.7 ± 0.7	15.4 ± 1.1	15.7 ± 1	15.5 ± 1.4	15.8 ± 1.6
Right atrial pressure (mmHg)	7.4 ± 0.3	7.9 ± 0.4†	6.9 ± 0.5	6.1 ± 0.6	5.3 ± 0.7	7.2 ± 0.9
Mean arterial pressure (mmHg)	105 ± 2#	107 ± 2	103 ± 2	100 ± 2	100 ± 3	100 ± 3
Systemic resistance (dynes.s.cm^{-5})	1533 ± 43	1426 ± 53	1640 ± 64	1581 ± 73	1520 ± 87	1658 ± 124
Mean pulmonary artery pressure (mmHg)	17.0 ± 0.5	16.4 ± 0.6	17.7 ± 0.9	17.6 ± 1.1	17.8 ± 1.5	17.3 ± 1.5
Pulmonary resistance (dynes.s.cm^{-5})	151 ± 8	129 ± 9*	175 ± 12	145 ± 14	132 ± 15	164 ± 28
Cardiac index (l/min/m^2)	2.77 ± 0.06	2.97 ± 0.07*	2.56 ± 0.08	2.62 ± 0.08	2.74 ± 0.12	2.47 ± 0.11
Stroke volume index (ml/m^2)	41.0 ± 0.8#	40.6 ± 1.1*†	41.3 ± 1.2	36.9 ± 1.0	35.8 ± 1.1	38.3 ± 1.7
Stroke work index (g.m.m^2)	50.9 ± 1.4#	52.5 ± 1.5*†	49.3 ± 2.1	41.4 ± 1.6	40 ± 2.1	43.1 ± 2.4
Coronary blood flow (ml/min)	127 ± 6	124 ± 8	130 ± 10	136 ± 13	161 ± 19*	104 ± 12
Coronary resistance (mmHg/ml/min)	1.03 ± 0.07	1.01 ± 0.06†	1.07 ± 0.14	0.94 ± 0.07	0.79 ± 0.08	1.14 ± 0.12
DP/dt positive (mmHg/s)	1617 ± 37#	1752 ± 49*†	1426 ± 39	1376 ± 46	1444 ± 61	1272 ± 65
V_{CE40} (s^{-1})	31.5 ± 0.8#	34.7 ± 1.0*†	27.1 ± 0.8	27.1 ± 0.9	28.4 ± 1.2	25.1 ± 1.2
V_{MAX} (s^{-1})	46.3 ± 1.2#	51.0 ± 1.4*†	39.8 ± 1.3	40.5 ± 1.4	42.5 ± 1.9	37.5 ± 1.9
DP/dt negative (mmHg/s)	1860 ± 34#	1957 ± 43*†	1721 ± 46‡	1464 ± 48	1464 ± 58	1463 ± 84
Tau (msec)	65 ± 2#	55 ± 3*†	70 ± 2	75 ± 3	73 ± 5	78 ± 5

: $p < 0.05$ normal LV function vs. asymptomatic LV dysfunction.

*: $p < 0.05$ -β-blocker vs. + β-blocker within each group.

†: $p < 0.05$ -β -blocker in normal LV function vs. -β-blocker in asymptomatic LV dysfunction.

‡: $p < 0.05$ +β -blocker in normal LV function vs. +β-blocker in asymptomatic LV dysfunction

Table 3

Neurohumoral data.

		Normal LV function (group 1)			Asymptomatic LV dysfunction (group 2)		
		all	-β-blocker	+β-blocker	all	-β-blocker	+β-blocker
Norepinephrine	arterial (pg/ml)	291 ± 15	283 ± 19	303 ± 26	285 ± 20	293 ± 25	272 ± 35
	coronary venous (pg/ml)	355 ± 26	343 ± 30	372 ± 46	329 ± 28	321 ± 23	340 ± 62
	cardiac (ng/min)	-6.9 ± 1.8	-5.6 ± 2.2	-8.6 ± 3.0	-6.3 ± 2.5	-4.6 ± 3.1	-8.6 ± 4.3
Epinephrine	arterial (pg/ml)	110 ± 8	103 ± 10	120 ± 13	102 ± 10	102 ± 14	99 ± 14
	coronary venous (pg/ml)	68 ± 7	64 ± 7	75 ± 12	56 ± 5	60 ± 7	51 ± 8
	cardiac (ng/min)	5.8 ± 0.8	5.0 ± 0.7	6.9 ± 1.5	5.3 ± 0.7	5.8 ± 1	5.0 ± 1.1
Angiotensin II	arterial (pg/ml)	4.9 ± 0.3	5.4 ± 0.4	4.2 ± 0.5	5.8 ± 0.6	7.1 ± 0.9*	3.5 ± 0.4
	coronary venous (pg/ml)	4.6 ± 0.3	5.1 ± 0.4	3.9 ± 0.5	5.2 ± 0.6	6.1 ± 0.9*	3.6 ± 0.5
	cardiac (pg/min)	36 ± 20	45 ± 24	25 ± 34	83 ± 66	131 ± 111	17 ± 33
Renin	arterial (μU/ml)	8.7 ± 0.8	12.0 ± 1.6*	6.6 ± 0.7	11.2 ± 1.1	12.9 ± 1.4	9.2 ± 1.8
	coronary venous (μU/ml)	8.9 ± 0.9	12.2 ± 1.5*	6.9 ± 1.0	11.5 ± 1.2	12.3 ± 1.4	10.6 ± 1.9
	cardiac (μU/min)	-65 ± 59	-44 ± 81	-78 ± 83	39 ± 64	95 ± 78	-24 ± 103
ANP	arterial (pg/ml)	322 ± 50#	99 ± 31*	482 ± 49	221 ± 37	196 ± 47*	304 ± 9
	coronary venous (pg/ml)	2090 ± 263	1173 ± 316*	2747 ± 285‡	1486 ± 197	1570 ± 253	1215 ± 145
	cardiac (ng/min)	-238 ± 49#	-122 ± 31*	-321 ± 73‡	-189 ± 29	-220 ± 33*	-85 ± 9

: $p < 0.05$ normal LV function vs. asymptomatic LV dysfunction.

*: $p < 0.05$ -β–blocker vs. +β–blocker within each group.

†: $p < 0.05$ -β -blocker in normal LV function vs. -β-blocker in asymptomatic LV dysfunction.

‡: $p < 0.05$ +β -blocker in normal LV function vs. +β-blocker in asymptomatic LV dysfunction

Discussion

In the present study patients with asymptomatic LV dysfunction had more often a myocardial infarction in their history then patients with normal function. Also, LV volumes end diastolic were enlarged in patients with asymptomatic LV dysfunction, irrespective of presence or absence of β-blockade. In contrast to group 1, patients with normal LV function, in whom β-blockade significantly reduced myocardial and cardiac pump function, chronic β-blockade had no such effect in patients with asymptomatic LV dysfunction. β-Blockade clearly depressed contractility and relaxation in patients with normal LV function. Since stroke work and volume index did not change, the concomitant decrease in cardiac index may be caused by the decrease in heart rate. In patients with asymptomatic LV dysfunction who had impaired contractility and relaxation, there was no further effect at all on these parameters in the β-blockade subgroup. The fact that cardiac index did not change in spite of a decrease in heart rate may be due to an actual improvement in cardiac function by β-blockade in this group. Also, Haber et al. found that acute administration of β-blockade in heart failure patients reduced both LV systolic pressure and afterload, whereas in patients with normal LV function LV systolic pressures increased as well but afterload increased [17]. This may explain the reduced myocardial function in normal subjects after β-blockade administration.

In patients with normal LV function chronic β-blockade induced vasoconstriction. Both systemic and pulmonary vascular tone were higher then in patients not on β-blockade. This was not the case in patients asymptomatic LV dysfunction. In the latter group, systemic and pulmonary vascular resistances were comparable between patients with and without β-blockade. Overall, in both subgroups with asymptomatic LV dysfunction contractility and relaxation parameters, with the exception of negative dP/dt, were comparable to those in patients with a normal LV function on β-blockade. Also, selective β_1-blockade in normal cardiac function leads to vascular constriction, of a similar magnitude as observed in asymptomatic LV dysfunction, which does not further progress when β-blockade is added. It may be speculated that this effect on vasotone correlates with an increase in circulating vasoconstrictor neurohormones. In the present study we found no evidence for that.

Vasoconstrictor neurohormones were essentially similar between patients with normal versus asymptomatic LV dysfunction and between subgroups with and without β-blockade.

Moreover, in patients with normal LV function where β-blockade induced vasoconstriction, renin levels were significantly reduced in the β-blockade group. Also, in the asymptomatic LV dysfunction subgroups, arterial renin levels tended to be lower and circulating angiotensin II was significantly reduced by β-blockade. Beta-blockade directly reduces renal renin secretion. This may explain the reduction in renin levels in the normal LV function subgroup on β-blockade. Therefore, the decrease in renin activity is, in addition to the direct effect of β-blockade, probably related to vasoconstriction, which was most pronounced in patients with normal LV function.

Catecholamine levels were essentially unaltered by β-blocking therapy in patients with normal LV function and asymptomatic LV dysfunction. In patients with heart failure metoprolol may decrease circulating norepinephrine levels, with no effect on cardiac nor epinephrine release [18, 19]. This may depend on baseline catecholamine levels and on indicators of enhanced sympathetic tone, such as heart rate. In a substudy of CIBIS, bisoprolol decreased norepinephrine only in patients with baseline norepinephrine levels > 600 pg/ml or a baseline heart rate of more then 90 beats per minute [20]. Obviously, these catecholamine levels were not reached in the present study. Arterial norepinephrine levels in asymptomatic LV dysfunction were within the normal range and comparable to patients with normal LV function. Alternatively, since metoprolol is a β_1-selective blocker this may account for the unchanged circulating norepinephrine in our study. Several studies in heart failure patients have indicated that circulating norepinephrine levels are affected more by non-selective β-blockers [21, 22], suggesting an etiology based response [23]. Patients with idiopathic dilating cardiomyopathy may have more pronounced reduction in norepinephrine levels after β-blockade then patients with ischemic cardiomyopathy. However, the improvement in LV ejection fraction during chronic β-blocker treatment is regardless of etiology [24, 25].

The present study presents the first report on the effects of chronic β-blockade in patients with asymptomatic LV dysfunction, indicating that, compared to patients with normal LV function, there is no difference in circulating catecholamines and cardiac catecholamine balance.

In our study, arterial ANP levels were significantly higher in patients on β-blockade compared to those without β-blockade irrespective of the presence or absence of LV dysfunction. In fact, levels tended to be higher in patients with normal LV function. In heart failure, several studies have reported a significant correlation with LV filling pressures or

pulmonary capillary wedge pressure [26, 27]. Such a correlation was present here as well. Also, a volume increase could lead to higher ANP levels, as its secretion is dependent on atrial wall stretch [28]. However, in the smaller left ventricles in patients with normal LV function similar or even higher ANP levels were found. Also, cardiac ANP release was less in asymptomatic LV dysfunction patients with enlarged LV volumes then in patients with normal LV function. The stimulating effect of β-blockade on ANP per se is not clear and suggests a direct effect on ANP secretion in the absence of a hemodynamic relationship. Activation of ANP is potentially beneficial in early LV dysfunction. The consequences of increased ANP levels in normal LV function is not quite clear. It could be that ANP here also serves to prevent significant hemodynamic deterioration in these patients. Moreover, ANP may protect against neurohumoral activation. It could be speculated that ANP activation in our patient population may have restricted cardiac dysfunction induced increases in norepinephrine and renin-angiotensin.

Limitations. In the present study, we have investigated a selected group of patients, who have shown to tolerate β-blocker therapy, despite a reduced myocardial function. Also, we did not study the patients before and after chronic β-blocker treatment. It is therefore not possible to say to which extent hemodynamic and neurohumoral parameters have changed in these patients since start of the β-blocker treatment. Since none of these patients ever had any sign or symptom of heart failure, it is even possible that some patients, by improving of LV ejection fraction during chronic β-blocker therapy, ended up being in group 1, while initially being in group 2. However, the aim of the present study was to evaluate the hemodynamic and neurohumoral profile of these patients after chronic treatment, and consequently to discriminate whether patients with asymptomatic LV dysfunction needed more compensatory mechanisms to maintain a stable situation. As it appears, in our study, this is not the case.

Conclusion

We conclude that, in patients with a normal LV function, chronic β-blockade results in a significant reduction in myocardial muscle and cardiac pump function, accompanied by systemic and pulmonary vasoconstriction. In contrast, these effects are much less pronounced or not present in patients with impaired LV function, indicating that chronic β-blockade does not further jeopardize cardiac function in patients with asymptomatic LV dysfunction due to ischemic cardiomyopathy.

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Chapter 4

Neurohumoral response to Carmoxirole, a selective dopamine (D_2) receptor agonist, in patients with chronic moderate heart failure.

M. van der Ent, A.F.M. van den Heuvel, W.J. Remme

Summary

Neurohormonal activation and elevated ventricular filling pressures are prominent features in heart failure. Carmoxirole is a DA_2-receptor agonist with limited central activity and modulates sympathetic activation and subsequently reduces pre- and afterload in animals. The effect of carmoxirole on neurohormones and hemodynamics in humans was evaluated in 12 normotensive patients with NYHA class III-IV heart failure on stable ACE-I and diuretic therapy. On 2 consecutive days, carmoxirole (0.25-1.00 mg) was administered, and hemodynamic and neurohormonal measurements carried out. Values given are maximal percent changes from pre-study baseline (significance level $p<0.05$). The lower dose on day 1 (0.25-0.50 mg) reduced circulating norepinephrine, vasopressin and ANP by 40%, 19% and 25%, respectively. In addition, on day 2, at a dose level of 0.75-1.00 mg, plasma renin activity decreased by 30%. Mean arterial pressure and systemic vascular resistance were reduced by 10% and 18%, and pulmonary wedge and right atrial pressure by 38% and 39%, respectively. Cardiac index improved with 20%. Despite a concomitant 12% reduction in heart rate, both stroke volume and stroke work index increased by 32% and 31%, respectively. Mean pulmonary artery pressure decreased by 21%, whereas pulmonary resistance was not affected. Thus, carmoxirole modulates sympathetic activation, accompanied by changes in vasopressin and ANP, and the renin-angiotensin system at higher dosages. These effects lead to a reduction in systemic resistance and heart rate, and an improvement in cardiac pump function and left and right ventricular filling pressures. It is concluded that carmoxirole induces beneficial effects on hemodynamic and neurohumoral parameters in heart failure.

Key words: neurohormones, heart failure, DA_2-receptor, catecholamines, carmoxirole, dopamine agonist.

Introduction

For various reasons, dopaminergic agonists may beneficially affect heart failure [1, 2]. Two distinct dopamine receptor subtypes (DA_1 and DA_2) are involved in the regulation of the cardiovascular system [3, 4, 5]. Stimulation of the post synaptic DA_1-receptor leads to vasodilation, preferentially in renal, mesenteric, coronary and cerebral blood vessels. DA_2-receptor stimulation also leads to a reduction of vascular resistance by diminishing the release of norepinephrine at the sympathetic nerve endings [6]. In addition, DA_2-receptors are present in pre-sympathetic ganglia, where activation also inhibits neurotransmission. Carmoxirole ((3-[4-(1,2,3,6-tetrahydro-4-phenyl-1-pyridinyl)-butyl]-5-indole carboxylic acid hydrochloride (EMD 45 609)) is a new dopamine agonist, selective for presynaptic DA_2-receptors. Data from animal experiments indicate that the effect of carmoxirole on blood pressure results almost exclusively from activity at peripherally located DA_2-receptors [7]. Furthermore, it has been demonstrated in conscious, spontaneously hypertensive rats and in normotensive dogs, that carmoxirole, administered intravenously, increases renal blood flow and reduces renal vascular resistance [8], both direct effects of DA_2-receptor activation.

Available data on the effect of dopamine agonists on hemodynamics and clinical efficacy in heart failure relate to ibopamine, a combined DA_1- and DA_2-receptor agonist, with additional α- and β-adrenergic stimulating activity. In heart failure, ibopamine decreases circulating catecholamines and aldosterone levels, which leads to peripheral vasodilatation. Early, uncontrolled studies did suggest that ibopamine resulted in a beneficial effect on exercise tolerance, central hemodynamic parameters and NYHA functional class [9, 10]. Decreases in circulating catecholamines and actual vasodilating effects have been reported [11, 12]. However, more recent, controlled data failed to show a significant clinical improvement in mild to moderate heart failure [13]. A large survival study, PRIME II, was prematurely stopped because of excess mortality in ibopamine treated patients [14].These negative effects of ibopamine may relate to its additional β-adrenergic activities. In contrast to ibopamine, carmoxirole has no additional adrenergic effects [15], and might therefore be better tolerated in heart failure. As yet, there are no data available of the neurohumoral and hemodynamic effects of carmoxirole in patients with heart failure.

The present study evaluated the acute hemodynamic and neurohumoral response to increasing doses of orally administered carmoxirole in 12 patients with moderate to severe

chronic congestive heart failure (NYHA III-IV), based on ischemic cardiomyopathy or idiopathic dilated cardiomyopathy.

Methods

Patient selection. Following approval of the Institutional Ethical Review Board and after informed consent, 12 normotensive patients with chronic (at least 3 months), stable heart failure (NYHA class III-IV), in spite of optimal therapy, were enrolled in the study. Patients had to be between 18 and 65 years of age, females enrolled had to be of non-child bearing potential. Heart failure of all etiology could be included. The medication should at least consist of digitalis, diuretics and ACE inhibitors and unchanged for two weeks or more. Exclusion criteria included obesity, alcohol or drug abuse, sustained arrhythmias, myocardial infarction within 3 months prior to the study, severe concomitant liver disease, known malignancies or hypotension (systolic pressure $\leq$ 90 mmHg). Dopaminergic medication was not allowed. Irrespective of the etiology of heart failure, LV ejection fraction, measured by radionuclide method (MUGA), had to be $<$ 45%. Patient characteristics are shown in table I. Twelve patients, 10 male, 2 females, average age 67 years (range 60 to 74 years) with heart failure NYHA III and IV in 6 patients each, based on ischemic cardiomyopathy in 11 patients and idiopathic dilated cardiomyopathy in 1 patient, were included. Average LV ejection fraction was 19% (range 5-36%).

Instrumentation. The day before start of the study, a Swan-Ganz catheter was inserted via a brachial or subclavian approach. After insertion, the position of the catheter was confirmed by chest X-ray. Stable baseline conditions were checked by repeated measurements, which had to be within a 5% range.

Hemodynamic measurements and calculations. The Swan-Ganz catheter was calibrated with a zero reference level at midchest. All measurements were processed using Hewlett Packard bedside monitors and computers. Blood pressure was recorder using an automatic blood pressure measurement device, which was integrated in the bedside equipment. Throughout the study, 2 ECG leads (usually II and V5) were monitored continuously on screen. Cardiac output was measured by the thermodilution technique. Heart rate, pulmonary pressures, right atrial pressures and cardiac output were measured in triplicate. Values with less then 10% difference were accepted. From the measured hemodynamic variables, the following parameters were calculated: mean arterial pressure was

calculated as [(2 x diastolic arterial pressure)+systolic arterial pressure] / 3; mean pulmonary pressure was calculated similarly, cardiac index (l/min/m^2) was calculated as cardiac output / body surface area; systemic vascular resistance (dynes.sec.cm^{-5}) was derived as ([mean arterial pressure - mean right atrial pressure] / cardiac output) x 80; stroke volume index (ml/beat/m^2) as 1000 x cardiac index / heart rate; stroke work index (g.m.m^2) as 0.0136 x stroke volume index x (mean arterial pressure - pulmonary capillary wedge pressure); pulmonary resistance (dynes.sec/cm^{-5}) as 80 x (mean pulmonary pressure -pulmonary capillary wedge pressure).

Metabolic determinations. Blood samples were collected from the proximal port of the Swan Ganz catheter, located at the right atrium, for determination of catecholamines, plasma renin activity, angiotensin II, Atrial natriuretic peptide and vasopressin and treated according to previously described methods [16, 17, 18]. Atrial natriuretic peptide was determined by a commercially available radioimmunoassay (ITS, Wijchen, the Netherlands).

Study protocol. Three days before start of the study, patients were admitted to the hospital and put on a 3 gram sodium/24 hour diet. On the morning of the first study day patients received a light breakfast at 7:00 hrs. All cardiac medication was continued until the first study day, with the exception of ACE inhibitors, which had to be stopped 4 days before start of the study. During study days, all medication was withheld until 14:00 hrs. At 9:00 hrs baseline measurements and blood sampling were performed. The administration of the

Table 1

Patient characteristics

Number:	12
Age (years):	66.7 ± 1.2 (60-74 years)
Sex (female/male):	2 / 10
NYHA class:	6 III / 6 IV
Etiology: ischemic/idiopathic	11/1
Duration of heart failure (months):	40.4 ± 9.4 (2-96 months)
LV ejection fraction (%):	19 ± 2 (5-36%)
Initial pulmonary wedge pressure (mmHg):	22.1 ± 1.9 (13-35 mmHg)

studymedication was not blinded to the patient and the investigator. At 10:00 hrs the first dose of carmoxirole was administered orally. At 30, 60, 90 minutes, 2, 2.5, 3, 4 and 24 hours thereafter all hemodynamic measurements were done. At 30 minutes, 1, 2, 3, 4 and 24 hours, blood sampling for neurohormones was performed. After the 4 hour and after the 8 hours timepoint, meals were served and regular medication was administered. On the second day the same protocol was followed, with a higher dose of carmoxirole. Two dosage groups were used: Group A received 0.25 mg on day 1 and 0.50 mg on day 2 and group B received 0.75 mg on day 1 and 1.00 mg on day 2. On the morning of the third day after the 24 hour measurement the Swan-Ganz catheter was removed and the study terminated.

Statistical analysis. Statistical analysis was performed using SAS/STAT® software V6.10. An ANOVA procedure for repeated measurements was applied to the raw data for the analysis of the effects during the first 4 hours of each study day, during which patients only received study medication, and are compared to the baseline values of that day. This method accounts for baseline variation. To identify which timepoints were significantly different from baseline values, post hoc analysis using Dunnet's test was performed. Since changes in both dosage groups A and B were similar in magnitude and direction, all patient data were pooled for the analysis (figure 1). Data are presented as mean ± standard error of the mean.

Results

Carmoxirole was well tolerated by all patients. No side effects were noted. Four patients received medication according to dosage group A, 8 patients according to group B.

Hemodynamic changes. During the first 4 hours of day 1 afterload, indicated by mean arterial pressure and systemic resistance decreased significantly by maximal 10% and 18% from 77.1 ± 2.9 to 68.8 ± 2.6 mm Hg at 3 hrs and from 1976 ± 192 to1563 ± 140 dynes/sec/cm^{-5} respectively at 2.5 hrs (figure 2). In addition, mean pulmonary artery pressure decreased as well by maximal 21%. Despite these vasodilating effects, heart rate decreased by 12%. Right and left ventricular filling pressures, as reflected by right atrial pressure and the pulmonary capillary wedge pressure were elevated at baseline (8.4 ± 1.6 and 22.1 ± 1.9 mm Hg respectively.) and fell by 38% (not significant) and 39% to 6.2 ± 1.5 and 14.1 ± 2.0 both at 2 hrs respectively. Cardiac index and stroke volume index increased from 1.6 ± 0.1 to 1.85 ± 0.17 l/min/m^2 (20%) at 1.5 hrs and from 19.2 ± 1.9 to 24.3 ± 2.4 ml/beat/m^2 (31%) at 3 hrs

respectively (figure 2). After 24 hours heart rate, right atrial pressure, and mean pulmonary pressure were still significantly reduced by 8%, 23% and 15%, whereas stroke volume index was still improved by 27%, all compared to baseline values. Changes from the prestudy baseline values on day 2 were comparable to those on day 1. Statistically significant reductions occurred in heart rate (14%), pulmonary wedge pressure (36%), mean arterial pressure (14%), mean pulmonary artery pressure (23%), right atrial pressure (47%) and systemic resistance (21%), whereas cardiac index, stroke work and stroke volume index increased by 18, 42 and 43% respectively. (table 2).

Neurohumoral changes. Norepinephrine levels were elevated at baseline and decreased by 40% from 774 ± 133 to 410 ± 59 pg/ml during the first 4 hours after carmoxirole administration on day 1. Epinephrine and dopamine levels did not change significantly (figure 3). Also, circulating renin and angiotensin II levels were not affected significantly. In contrast, vasopressin decreased by 19% from 1.68 ± 0.25 to 1.35 ± 0.24 pg/ml at 3 hours after carmoxirole administration. Atrial natriuretic peptide (ANP) levels, significantly elevated at baseline, decreased from 1045 ± 105 to 781 ± 111 pg/ml (25%) during the first 4 hours. At 24 hours norepinephrine and ANP levels were still 39% and 18% respectively less compared to prestudy values. Renin activity levels did not change until day 2, on which there was a decrease from 364 ± 78 to 258 ± 63 μU/ml (30%), within the first 4 hours after the second carmoxirole administration. On day 2, compared to prestudy baseline values, norepinephrine decreased by 53% and ANP by 28%, comparable to the changes on day 1. Other neurohormones did not change further on day 2. A summary of data is presented in table 3.

Discussion.

In the present study, patients were suffering from moderate to severe heart failure, NYHA class III-IV. The hemodynamic profile of these patients reflected the severity of their clinical condition. Average cardiac output was low, LV and RV filling pressures raised (pulmonary wedge pressure and right atrial pressure) and neurohumoral activation present (indicated by raised norepinephrine, vasopressin, renin activity and ANP levels). Previous studies by Lokhandwala et al [19] have suggested that DA_2 receptor agonists might be useful in heart failure, since they reduce afterload by decreasing norepinephrine levels and attenuate the

renin-angiotensin-aldosterone system. However, no data are available yet of its hemodynamic and neurohumoral profile in patients with heart failure.

In the present study, carmoxirole reduces pre- and after-load, as indicated by a substantial reduction in systemic resistance and both LV and RV filling pressures and improves myocardial pump function (stroke work, stroke volume and cardiac index). Significant changes in these parameters started at 30 minutes after oral drug intake, reaching the highest levels during the first 4 hours after carmoxirole administration. Maximal changes for all parameters were observed within the first 2 hours after drug intake. The pharmacological profile of carmoxirole has been investigated in a series of in vitro and in vivo studies[7, 15, 20] and suggest a long-lasting effect of at least 8 hours. Therefore, the effects of carmoxirole will be present during the first 4 hours after drug administration, which is the

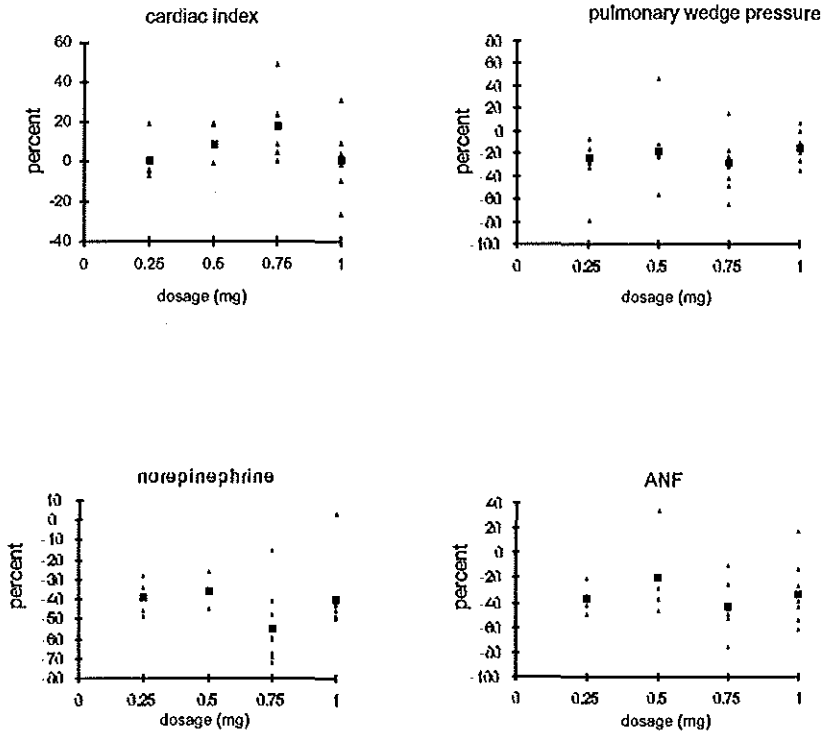

Figure 1

In this figure individual percent changes from baseline are displayed for each dosage group (group A (n=4) 0.25 mg on day 1 and 0.5 mg on day 2 and group B (n=8) 0.75 mg on day 1 and 1 mg on day2). The triangles are individual values; the squares are the average values of that day. As is shown here there is no clear dose related effect.

time interval studied. After 4 hours other medication is given as well, and the effects thereafter are additional to the effects of carmoxirole (figures 2,3,4). Some changes remain present after 24 hours. This may be caused by a lasting effect of improved hemodynamics due to a reduced adrenergic drive through norepinephrine level reduction. Also, the administration of the concomitant medication after 4 hours (ACE-inhibitors) in an improved hemodynamic condition may account for the observation as well. A possible placebo effect will be very limited in the present study, since patients were already kept on a sodium restricted diet for several days, and the Swan Ganz catheter was inserted the day before the study started.

Hemodynamic and neurohumoral changes from prestudy baseline were comparable and consistent on both study days. The most prominent effect was the substantial reduction in norepinephrine levels (up to about 50%). This reflects the reduction of sympathetic tone through activity of carmoxirole at peripherally located DA_2 receptors, since carmoxirole has no capability of passing the blood-brain barrier. As a result, vascular resistance decreased, with a concomitant small reduction in mean arterial pressure. In contrast to vasodilators in general, this did not result in an increase in heart rate. This reflects the direct inhibiting effect of carmoxirole on sympathetic tone through direct inhibition of norepinephrine release. The reduction of the sympathetic activation in heart failure by carmoxirole may have a beneficial effect in treatment and on mortality in these patients [21, 22].

As expected, norepinephrine levels remain reduced substantially on both, consecutive days. Other neurohormones did not change with the exception of ANP and vasopressin. Although the elevated ANP levels decreased significantly, this followed the reduction in left and right ventricular filling pressures and is most likely a secondary effect. Over time, the change in ANP seems to be slower then the change in norepinephrine. Carmoxirole exerts similar neurohumoral effects as ibopamine, but without some negative side effects observed with ibopamine, which are probably related to adrenergic activity. Activation of DA_1-receptors, e.g. by ibopamine, reduces the direct natriuretic effects of ANP [23] in the kidney, whereas activation of DA_2-receptors does not affect this mechanism. Therefore, the natriuretic action of ANP, which is beneficial in heart failure, is preserved by carmoxirole. In addition, activation of DA_1 receptors causes direct renin release from the kidney, whereas DA_2 activation does not [24]. In spite of this, ibopamine reduces plasma renin levels, which is probably secondary to hemodynamic changes [13]. In our study there is no increase in renin activity after drug administration, but a late decrease on day 2. Renin activity is raised at baseline and there is a tendency towards decrease during the study, most pronounced on day 2.

2. This is most likely to be secondary to the substantial decrease in norepinephrine, caused by carmoxirole.

In the present study we could not demonstrate a dose related effect (figure 1). This may be caused by the small number of patients or by the limited duration of the study. Initially, a low dose is as effective as higher doses, however, over time changes in the number

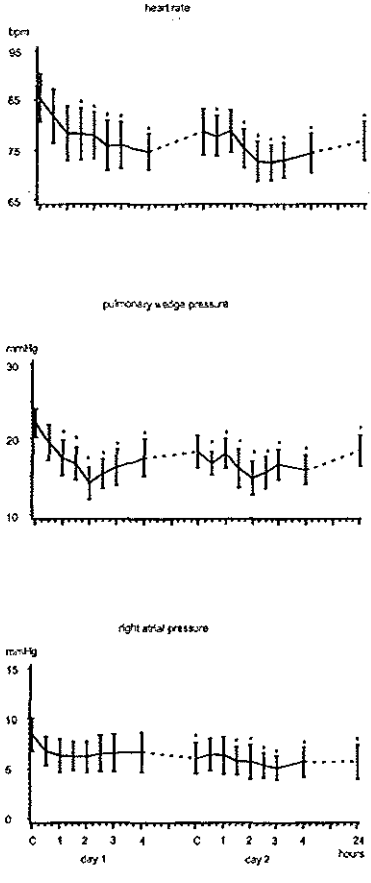

Figure 2

After carmoxirole administration (C) on both consecutive days there is a pronounced reduction in heart rate and LV and RV filling pressures, starting immediately after drug intake and lasting throughout the study.

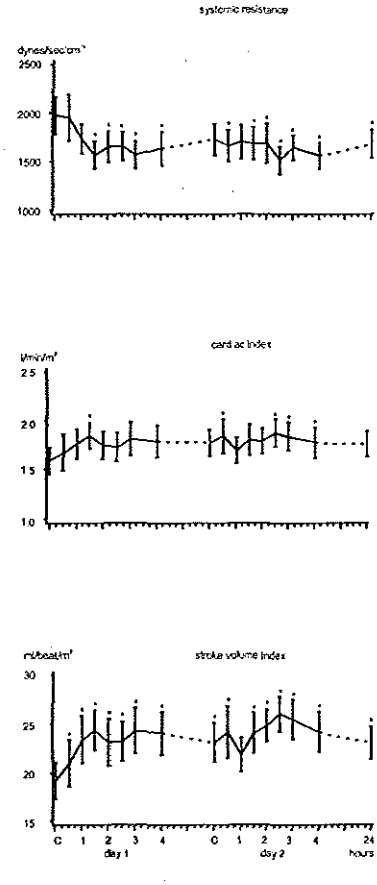

Figure 3

Along with a marked reduction in systemic resistance, cardiac pumpfunction indicated by the cardiac index and stroke volume index improves after carmoxirole administration (C).

receptors or tolerability may occur.

The present study has its limitations. The study was set up as a pilot study. The dosages used, were determined to get a maximum effect which was well tolerated by the patients. A the time of the study, there were no data available of the use of this compound in patients with heart failure, since the compound was developed as an antihypertensive drug. Due to the character of the study, the patient group studied was small (n=12) and there was no control group. Before start of the study, patients were allowed to stabilize, which was checked during baseline measurements. The effect of regression to the mean will be minimized this way. However, the effects noted were very pronounced and clearly related to the carmoxirole administration. Also, only the acute effects of carmoxirole were studied. Therefore,

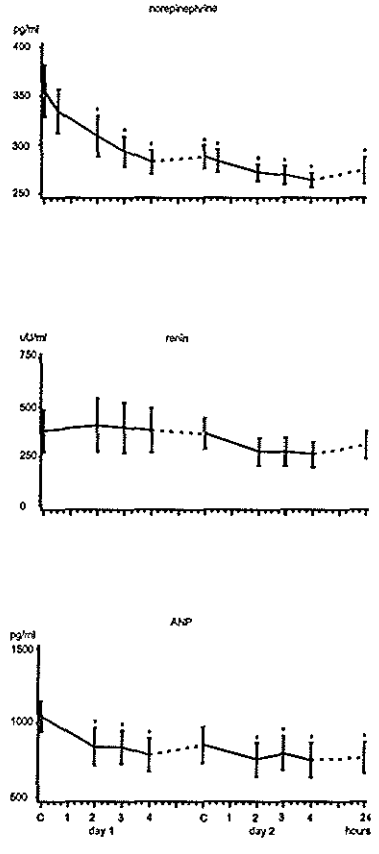

Figure 4

The most important neurohumoral effects are the reduction in norepinephrine and atrial natriuretic peptide (ANP) levels after administration of carmoxirole (C). In spite of reduced vasoconstriction, there are no significant changes in renin activity levels.

extrapolation to larger patient groups and chronic treatment can not be deducted from our data. As all patients remained stable during the study, no additional diuretics or inotropic therapy was required, which could have interfered with our measurements.

In conclusion, as is shown in the present study, the DA_2-receptor agonist carmoxirole is well tolerated by patients with heart failure. Carmoxirole modulates sympathetic activation already at a low dose, accompanied by changes in vasopressin and ANP, and, at the high dose, in the renin-angiotensin system. These effects lead to a reduction in systemic resistance and heart rate, and an improvement in cardiac pump function and left and right ventricular filling pressures. Improvement of hemodynamic and neurohumoral changes induced by carmoxirole may be beneficial heart failure treatment.

Table 2

Hemodynamic parameters.

	Baseline values	Maximum percent changes on day 1 vs. baseline day 1	Maximum percent changes on day 2 vs. baseline day 1
Heart rate (beats/min)	85.5 ± 4.8	-12	-14
Pulmonary wedge pressure (mm Hg)	22.1 ± 1.9	-38	-36
Mean arterial pressure (mm Hg)	77.1 ± 2.9	-10	-14
Mean pulmonary artery pressure (mm Hg)	33.1 ± 2.3	-21	-23
Right atrial pressure (mm Hg)	8.4 ± 1.6	NS	-47
Cardiac index ($l/min/m^2$)	1.59 ± 0.14	20	18
Stroke work index ($g/m/m^2$)	14.3 ± 1.6	31	42
Stroke volume index ($ml/beat/m^2$)	19.1 ± 1.9	32	43
Systemic resistance ($dynes/sec/cm^{-5}$)	1976 ± 192	-18	-21

Hemodynamic parameters. Statistical significant changes ($p < 0.05$) from prestudy baseline values on day 1 and day 2.

Table 3

Neurohormones

	Baseline values	Maximum percent changes on day 1 vs. baseline day 1	Maximum percent changes on day 2 vs. baseline day 1
Norepinephrine (pg/ml)	773 ± 133	-40	-53
Epinephrine (pg/ml)	153 ± 47	NS	NS
Dopamine (pg/ml)	28.3 ± 6.0	NS	NS
Angiotensin II (pg/ml)	13.5 ± 2.9	NS	NS
Renin (μU/ml)	379 ± 109	NS	NS
Vasopressin (pg/ml)	1.68 ± 0.25	-19	NS
ANP (pg/ml)	1045 ± 105	-25	-28

Neurohumoral parameters. Statistical significant changes ($p < 0.05$) from prestudy baseline values on day 1 and day 2.

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Chapter 5

Renal hemodynamic effects in patients with moderate to severe heart failure during chronic treatment with trandolapril.

M. van der Ent, W.J. Remme, P.W. de Leeuw, G.L. Bartels

Summary

Treatment of patients with severe heart failure by ACE inhibition is often limited by worsening of renal function. To evaluate whether trandolapril, a potent lipophilic ACE-inhibitor, affects renal function in severe heart failure, we studied 12 patients with severe heart failure treated with diuretics and digoxin only. Patients received increasing dosages of trandolapril orally (0, 1 and 2 mg) on 3 consecutive days (A). Patients were then discharged on 2 mg trandolapril bid. and re-evaluated 8 weeks later (B). Mean arterial and pulmonary wedge pressures decreased by maximal 14% and 43% and stroke volume and work indexes increased by 24 and 20% at A and similarly at B (11, 45, 25 (ns) and 33% respectively). In contrast, heart rate, systemic resistance, pulmonary artery pressure and cardiac index decreased by 6, 23, 29 and 17% respectively at A only. Renal blood flow improved both at A and B by approximately 40%. In contrast, glomerular filtration rate decreased by 25% at B only, whereas serum creatinine, creatinine clearance and urine osmolality were unaffected during the study. Norepinephrine, angiotensin II and aldosterone levels decreased by approximately 30%, 60% and 65% respectively both at A and B. Renin levels increased by 136% at A and remained elevated at B. Thus, whereas the initial systemic vasodilating and inotropic effects did not persist, long term trandolapril results in sustained neurohormonal modulation, reduced preload and improved organ perfusion, indicated by a persistent increase in renal blood flow and preservation of renal function in severe heart failure.

Keywords: heart failure, neurohormones, ACE-inhibitors, trandolapril, renal function, long-term treatment

Introduction

In heart failure, ACE-inhibition has proven to be beneficial, irrespective of the presence of symptoms and, according to recent clinical guidelines, should be administered as first line therapy to all patients with heart failure [1]. Hemodynamic and symptomatic improvement have been shown in several studies [2,3,4,5] involving both short and long acting ACE inhibitors. Also, controlled trials such as CONSENSUS (severe heart failure) and SOLVD (moderate heart failure) have indicated reduced long-term mortality rates. However, clinical use of ACE-inhibitors in heart failure patients is sometimes limited by the potential deleterious effect of long-acting ACE-inhibitors on renal hemodynamics [6]. Renal insufficiency is one the major adverse effects of ACE-inhibitor treatment. In patients with severe heart failure, the risk on renal insufficiency caused by ACE-inhibition is higher and mostly related to the level reduction of diastolic bloodpressure and concomitant use of diuretics [7].

Trandolapril is a novel, potent, long-acting, third generation ACE-inhibitor, which improves organ perfusion due to pronounced effects on tissue ACE-inhibition and bradykinin degradation [8] with a particular beneficial effect on renal perfusion in hypertensive rats [9]. In an animal model for heart failure, beneficial long-term effects of trandolapril on renal perfusion were observed as well [10]. In addition, the results from the TRACE study showed a reduced mortality in patients with reduced LV function after myocardial infarction [11,12]. However, no data are available on the effect of trandolapril on renal hemodynamics in severe heart failure in man. The present study evaluates the acute and long-term effects of trandolapril in patients with moderate to severe heart failure, with special respect to systemic hemodynamics, neurohormones and renal function and hemodynamics.

Methods

Patient selection. Following approval of the Institutional Ethical Review Board and after informed consent, 12 normotensive patients entered the study. All had NYHA class III or early class IV heart failure for at least 3 months. All patients had heart failure symptoms combined with a cardiothoracic ratio > 0.50 on chest X-ray and/or LV ejection fraction < 0.35

and/or capillary wedge pressure > 15 mmHg. Etiology of heart failure was ischemic cardiomyopathy in 10 patients and idiopathic dilated cardiomyopathy in two. At the start of the study, all patients had normal sodium and potassium levels. As concomitant medication only digoxin, diuretics and short-acting nitrates were allowed. All patients used diuretics, 7 digoxin and 4 short acting nitrates. Previous treatment with ACE-inhibitors had to be stopped for at least one week before start of the study. Patient characteristics are shown in table 1.

Hemodynamic measurements and calculations. The day before start of the study, a multigated blood pool scintigraphy [13] was performed to assess LV ejection fraction, and a Swan-Ganz catheter was inserted via a brachial or subclavian approach. After insertion, the position of the catheter was confirmed by chest X-ray. The Swan-Ganz catheter was calibrated with a zero reference level at midchest. All measurements were processed using Hewlett Packard bedside monitors and computers. Blood pressure was recorded using an automatic blood pressure measurement device, which was integrated in the bedside equipment. During each measurement, 2 ECG leads (usually II and V5) were monitored continuously. Heart rate, pulmonary pressures, right atrial pressures and cardiac output were measured in triplicate. Only successive values within a 10% difference were accepted. Cardiac output was measured by the thermodilution technique. The following parameters were derived from the measured hemodynamic variables: mean arterial pressure was calculated as [(2 x diastolic arterial pressure)+systolic arterial pressure] / 3, mean pulmonary pressure was calculated similarly, cardiac index (l/min/m^2) was calculated as cardiac output / body surface area, systemic vascular resistance (dynes.sec.cm^{-5}) was derived as ([mean arterial pressure - mean right atrial pressure] / cardiac output) x 80, stroke volume index (ml/beat/m^2) as 1000 x cardiac index / heart rate, stroke work index (g.m.m^2) as 0.0136 x stroke volume index x (mean arterial pressure - pulmonary capillary wedge pressure), pulmonary resistance (dynes.sec.cm^{-5}) as 80 x (mean pulmonary pressure -pulmonary capillary wedge pressure). Renal hemodynamics, the renal blood flow and the glomerular filtration rate, were assessed by the clearance of both ^{125}I-hippuran and [^{99}Tc]-DPTA, administered as a simultaneous continuous infusion, which started 2 hours before test medication was given [14, 15]. In addition, urine was collected during 24-hours periods for determination of urea, creatinine, creatinine clearance, proteins, electrolytes and osmolality.

Metabolic determinations. At each timepoint samples of blood were collected from the proximal port of the Swan-Ganz catheter, located at the right atrium, for determination of catecholamines, renin, and angiotensin II, processed and determined according to previously described methods [16,17,18]. Aldosterone was assessed using a commercially available kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA).

Study protocol. Three days before start of the study, patients were admitted at the hospital and put on a 3 gram sodium per 24 hours diet. On the morning of the first studyday patients received a light breakfast at 7:00 AM. All medication was withheld until 2:00 PM. At 9:00 AM baseline measurements and blood sampling were performed. Studymedication administration consisted of placebo on day 1, 1 mg trandolapril on day 2 and 2 mg trandolapril on day 3. At 10:00 AM the first dose of trandolapril (or placebo on day 1) was administered orally. At 30, 60, 90 minutes, 2, 2.5, 3, 4, 8, 12 and 24 hours thereafter hemodynamic measurements were performed. At 30 minutes, 1, 2, 3, 4, 8 and 24 hours, sampling for neurohormones was done. After the 4 hour and after the 8 hours timepoint, meals were served and regular medication was administered. On the second and third day the same protocol was followed, with a higher dose of trandolapril. On the fourth day after the 24 hour measurement the Swan-Ganz catheter was removed and the patients discharged from the hospital, using the highest dose of trandolapril (2 mg bid.). Eight weeks later the patients were readmitted to the hospital, similar to the first time and the day after Swan-Ganz catheter insertion similar measurements were performed as during the first hospitalization, while patients continued trandolapril.

Statistical analysis. Statistical analysis was performed using SAS/STAT® software. Only the 8 patients who completed both hospitalizations were analyzed since evaluation of

Table 1

Patient characteristics

Sex (M/F)		7/1
Age (years)		65.5 ± 2.6
Etiology of cardiomyopathy	Ischemic	7
	Idiopathic	18
Pulmonary wedge pressure at baseline (mmHg)		21.5 ± 2.1
range		15 - 32
LV ejection fraction		23.1 ± 2.3

long-term effects were most important in this study. An ANOVA procedure for repeated measurements with was used for analysis of changes during the first 4 hours of each studyday compared to the baseline values of that day. During this period, no other medication was administered, so changes in this period will be caused by trandolapril only. Afterwards, mean percent changes from prestudy baseline values and percent changes from studyday baseline were calculated for each timepoint on all studydays and evaluated using a Student's t-test. A two-tailed p value < 0.05 was considered statistically significant.

Results

Of the 12 patients who started the study, 8 completed the protocol. Three patients stopped due to an increase in heart failure after the first hospitalization period. One of these died in spite of iv inotropic therapy. Another one of these patients who developed a skin rash, was withdrawn from study medication after the first hospitalization and died one month later due to progressive heart failure. The third one recovered after inotropic therapy. One patient was not evaluated due to a protocol violation.

Hemodynamic changes. On day 1, on which patients received placebo, no major changes in systemic hemodynamics occurred. Heart rate, mean arterial pressure, systemic resistance, mean pulmonary pressure, pulmonary resistance and pulmonary wedge pressure and right atrial pressure did not change during the first 4 hours after placebo administration. In contrast, stroke volume and stroke work index improved by maximal 14% and 17 % respectively. On day 2, with 1 mg of trandolapril there was a decrease in mean arterial pressure by 6% with a concomitant decrease in systemic resistance of 13% and an increase in renal blood flow by 30%. Compared to prestudy baseline values, on day 3, on which the maximal dose was administered, there was a very moderate decrease in heart rate (maximal 6%). Mean arterial pressure decreased by 14% with a concomitant decrease in systemic resistance of 22%, mean pulmonary pressure decreased by maximal 29%, in absence of changes in pulmonary resistance, whereas pulmonary wedge pressure decreased by 43%. Right atrial pressure only showed a trend towards decrease. At rehospitalization 8 weeks later there were baseline differences compared to prestudy values in pulmonary wedge pressure and right atrial pressure (21.5 ± 2.1 vs 13.9 ± 2.1 mmHg and 4.8 ± 1.4 vs 3.1 ± 1.2 mmHg, baseline first vs re-hospitalization respectively). In addition, baseline stroke work index

increased from 24.9 ± 3.0 to 28.5 ± 2.2 g/m/m^2 at the re-hospitalization. During the first 4 hours after the administration of 2 mg trandolapril, the decrease in mean arterial pressure and systemic resistance were still comparable to the decrease on day 3 of the first hospitalization. In contrast, pulmonary pressures showed no significant changes any more, whereas the reduction in LV filling pressure, achieved during chronic treatment, was maintained. Also, stroke volume and cardiac index were not affected, whereas the baseline change in stroke work index was maintained as well. Data are summarized in table 2 and figure 1.

Neurohumoral changes. On day 1, during the first 4 hours after placebo administration norepinephrine, angiotensin II and renin decreased by maximal 18%, 31% and 31% respectively. Epinephrine, dopamine and aldosterone did not change. On day 2 similar changes were seen in norepinephrine and angiotensin II. Renin levels showed no significant change, whereas aldosterone levels decreased by 50%. On day 3, after the highest dose of trandolapril, norepinephrine, angiotensin II and aldosterone decreased by 32%, 62% and 71% respectively, compared to prestudy baseline levels. At baseline measurements at the re-hospitalization period, angiotensin II levels had changed from 14.6 ± 3.2 at prestudy baseline to 10.0 ± 1.4 pg/ml, whereas renin levels were increased from 67 ± 10 to 225 ± 58 μU/ml. After administration of 2 mg of trandolapril, norepinephrine, angiotensin II and aldosterone again decreased by 28%, 57% and 58% respectively, compared to prestudy values. Renin increased by 234 % whereas epinephrine showed no change. A summary of data is displayed in table 3 and figure 2.

Renal hemodynamics and function. During the first 4 hours after placebo administration on day 1, glomerular filtration rates did not change. In contrast, renal blood flow increased by 29% maximally. On day 3 renal blood flow further improved by 44%, again with no change in glomerulary filtration rates. After eight weeks, long-term treatment showed comparable baseline values to prestudy values for both the renal blood flow and the glomerulary filtration rate. After administration of trandolapril there was a decrease in glomerular filtration rate of 25% and an increase in renal blood flow 41% compared to prestudy baseline levels. Creatinine clearance, serum creatinine and urine osmolality, represented here as median and intraquartile range, (34 (49 - 24) ml/min, 121 (134 – 101) μmol/l and 320 (431 – 206) mol/kg resp. at prestudy baseline) did not change throughout the study. A summary of data is displayed in table 2 and figure 3.

Discussion.

In the present study, the participating patients had NYHA class III-IV heart failure. The severity of heart failure in these patients is reflected in the rather high number of patients who did not complete the study, two of whom died. Worsening of heart failure was the major reason for discontinuation, oral therapy being insufficient in these patients. The neurohumoral profile of these patients was not different from the patients who completed the study. The patients who completed the study were stable on diuretics, digoxin and trandolapril. During the time between hospitalizations, these patients had stable NYHA class III heart failure symptoms. No major changes in concomitant therapy were necessary in these patients. The acute effects of trandolapril on hemodynamics and neurohumoral activation are comparable to those seen with other ACE inhibitors. Heart rate, mean arterial pressure, systemic resistance, mean pulmonary pressure and wedge pressure all decreased substantially, right atrial pressure decreased at a slower rate. Myocardial pump function only improved moderately. Most important were the persisting reduction in LV filling pressures. During day 1, after placebo administration, the modulating effects of prolonged supine resting on hemodynamics and neurohormones in these patients are evident and result in less vasoconstriction and increased renal blood flow, and a decrease in norepinephrine and renin-angiotensin levels. The latter is possibly the result of less sympathetic activation, again illustrative of the effect of resting on sympathetic tone and peripheral vasoconstriction. However, further hemodynamic and neurohumoral changes on the subsequent study days relate to trandolapril administration in a dose dependent fashion (figure 1). Neurohumoral changes included a persisting reduction in norepinephrine, angiotensin II and aldosterone and a substantial increase in renin activity after eight weeks of treatment. In particular, no rebound increase in angiotensin II was observed, despite the marked increase in plasma renin concentration, indicating sustained effective ACE-inhibition. These hemodynamic and neurohumoral changes have been described with other ACE inhibitors like lisinopril and ramipril as well [19, 20, 21].

The determination of the creatinine clearance using a 24 hour urine collection method is subject to certain errors. However, since the patients were already in a resting state before start of the study, the endogenous production of creatinine can be considered constant, dietary changes concerning meat consumption are minor as well and urine collection at the coronary

care unit was carefully done. Renal perfusion was increased already after the first day when placebo was administered. In addition, after trandolapril a further and persisting increase was observed. After long term treatment, glomerulary filtration rates were mildly reduced. This was not accompanied by a deterioration of renal function, since serum creatinine did not rise, and creatinine clearance and urine osmolality values did not fall. We conclude that renal function was not affected by trandolapril treatment.

Although our patients with severe heart failure were at risk of deterioration, this did not occur, most likely due to the increase in renal blood flow. Improved renal perfusion in patients with heart failure has been described with other ACE inhibitors also [22], but is usually associated with a decline in renal function [19, 20] In contrast, this did not happen with trandolapril in our study.

Limitations of the study. The group of patients studied is small and severely affected by heart failure. It is unclear whether our data can be extrapolated towards a larger group. Also, the absence of a placebo group makes it hard to determine to which extent regression to the mean contributes to our results. However, in this patient group it will be difficult to withhold ACE inhibitor treatment. Still, our data are comparable to those observed in other ACE inhibitors [19, 20, 21, 22]. Overt hemodynamic and neurohumoral changes were seen after trandolapril administration, some in a dose dependent way. In clinical terms, an 8 week period to determine long-term effects of ACE-inhibition may be rather short, but, in view of the study objectives, of sufficient duration. Determination of cardiac output by thermodilution method may be difficult in these patients due to low cardiac output, and common existence of some degree of tricupidalis and mitralis valve regurgitation. However, the values obtained were reproducible within the limits described in the methods section and within the range that could be expected in these patients.

Drop out patients. Four out of twelve patients did not complete the study, which is a rather high number. The patient that was withdrawn due to a protocol violation was not replaced. The two patients that suffered from worsening of heart failure were withdrawn from studymedication. After hospitalization and treatment with intravenous inotropic medication one of them died. The other one recovered, resuming ACE inhibitor treatment. The last patient was withdrawn from the study due to development of a rash. After withdrawal of ACE-inhibitor treatment, heart failure worsened and the patient died in spite of enhanced therapy.

Parameters of baseline hemodynamics and renal function of the drop out patients at study entry and at the end of the first hospitalization period are displayed in table 4. It can be noticed that their renal function was rather impaired. Their values were at the outer limits of the intraquartile ranges. Their renal function did not deteriorate during treatment. None of these patients was withdrawn from the study because of deterioration of renal function. It is therefore unlikely that treatment with an ACE inhibitor, trandolapril, as a single factor, is implicated in the bad outcome of these patients. It must be kept in mind that the patients in our study belong to a group with a very poor prognosis. Data from the CONSENSUS trial have shown a 1-year mortality rate in patients with NYHA class IV heart failure of 52%, with an even higher incidence of hospitalizations. The bad outcome of the drop out patients is not worse then can be expected in this patient group.

Conclusion.

Trandolapril improves hemodynamics and reduces neurohumoral activation in patients with severe heart failure, with preservation of renal function.

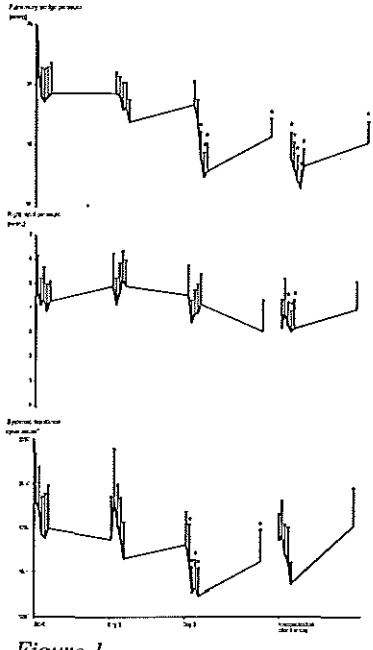

Figure 1

Left and right ventricular filling pressures are decreased after long-term treatment. Systemic vascular resistance is significantly lower at the third day and remains reduced, although not statistically significant after long-term treatment with trandolapril 2 mg bid. (*: $p < 0.05$ change from baseline).

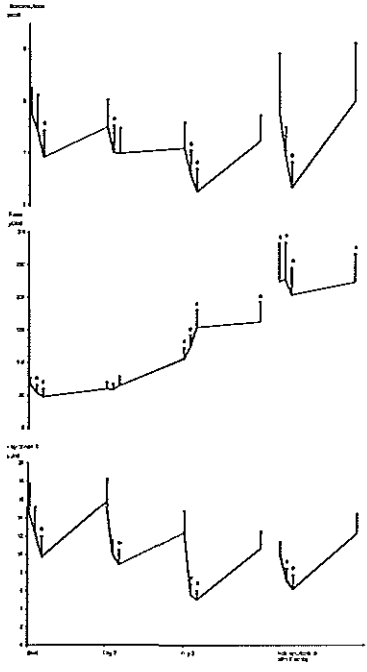

Figure 2

A dose dependent and persisting modulation in neurohumoral activation is noted after trandolapril treatment. Norepinephrine levels decrease, angiotensin II levels decrease as well, no rebound increase in angiotensin II levels is observed in this study. Renin levels progressively increase during treatment. (*: $p < 0.05$ change from baseline).

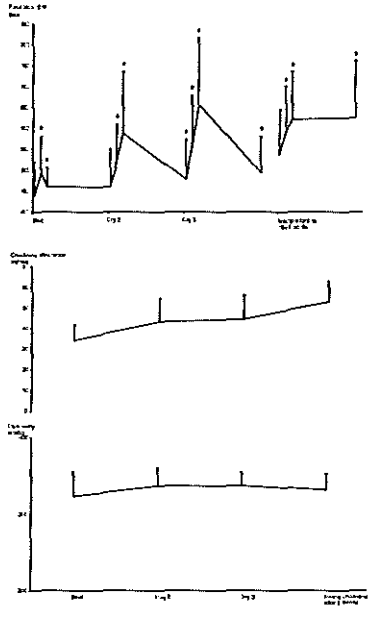

Figure 3

Renal function is preserved during long-term treatment. Renal blood flow gradually increases, whereas creatinine clearance and osmolality are not affected by trandolapril treatment. There is even a trend towards improvement, although not significant, of creatinine clearance over time. (*: $p < 0.05$ change from baseline).

Table 2

Baseline systemic and renal hemodynamic values and maximum percent changes from baseline during the first 4 hours after drug administration.

	First hospitalization				Re-hospitalization	
n=8	Baseline	Day 1 Placebo % change	Day 2 1 mg % change	Day 3 2 mg % change	Baseline	Day 1 2 mg % change
Heart rate (bpm)	75 ± 4	2.2	2.3	-5.8 *	71 ± 4	-6.9
Pulmonary wedge pressure (mmHg)	22 ± 2	-11.7	-16.7	-43.2 *	14 ± 2#	-44.5 *
Mean arterial pressure (mmHg)	85 ± 4	-1.7	-5.7 *	-14.1 *	82 ± 4	-11.0 *
Mean pulmonary artery pressure (mmHg)	29 ± 2	-8.5	-9.3	-29.1 *	25 ± 3	-20.3
Right atrial pressure (mmHg)	5 ± 1	-20.1	32.0	-23.8	3 ± 1	-47.0 *
Cardiac index (l/min/m^2)	2.13 ± 0.31	14.0	12.1	16.6 *	2.18 ± 0.16	21.6
Pulmonary resistance (dyn.s.cm^{-5})	191 ± 26	-13.8	21.7	15.5	227 ± 39	104.7
Stroke volume index (ml/beat/m^2)	29.3 ± 5.0	14.2 *	13.5	23.7 *	31.0 ± 2.3	24.9
Stroke work index (g/m/m^2)	24.9 ± 3.9	17.4 *	14.7	19.5 *	28.6 ± 2.2	-33.2 *
Systemic resistance (dyn.s.cm^{-5})	1900 ± 773	-9.9	-13.0 *	-22.5 *	1676 ± 151	-17.0
Glomerulary filtration rate (ml/min)	86.8 ± 9.6	-10.9	-11.1	5.0	69.4 ± 5.3	-24.8 *
Renal blood flow (ml/min)	438 ± 80	28.7 *	29.4 *	44.1 *	536 ± 108	40.6 *

Baseline values are expressed as mean ± standard error of the mean, * $p < 0.05$ compared to baseline values, # $p < 0.05$ baseline first hospitalization compared to baseline rehospitalization. Dosage: day 1:

Table 3

Baseline neurohumoral values and maximum percent changes from baseline during the first 4 hours after drug administration.

		First hospitalization			Re-hospitalization	
n=8	Baseline	Day 1 Placebo % change	Day 2 1 mg % change	Day 3 2 mg % change	Baseline	Day 1 2 mg % change
Norepinephrine (pmol/l)	4.8 ± 0.5	-17.7 *	-16.9 *	-31.6 *	4.8 ± 1.1	-27.5 *
Epinephrine (pmol/l)	0.53 ± 0.14	-11.4	-24.7	-32.1 *	0.42 ± 0.12	-18.7
Angiotensin II (pg/ml)	14.6 ± 3.2	-31.2 *	-32.9 *	-62.3 *-	10.0 ± 1.4	-57.1 *
Renin (µU/ml)	67 ± 10	-31.1 *	-10	135.6 *	225 ± 58#	233.5 *
Aldosterone pg/ml)	723 ± 177	-29.2	-49.9 *	-71.1 *	399 ± 104	-58.3 *

Baseline values are expressed as mean ± standard error of the mean, * $p < 0.05$ compared to baseline values, # $p < 0.05$ baseline first hospitalization compared to baseline rehospitalization. Dosage: day 1: placebo, day 2: 1 mg, day 3: 2 mg, rehospitalization: 2 mg.

Table 4

Data from drop out patients.

		Drop out #1	Drop out #2	Drop out #3
Reason for drop out		Skin rash	Worsening of heart failure	Worsening of heart failure
Outcome		Death	Death	Recovered
Baseline pulmonary wedge pressure (mmHg)		27	27	29
Baseline right atrial pressure (mmHg)		16	20	15
Baseline serum creatinine		150	130	128
Creatinine clearance (ml/min)	at baseline	6	28	16
	at end of study	8	15	34
Osmolality (mol/kg)	At baseline	357	631	323
	At end of study	375	592	386
Renal blood flow (ml/min)	At baseline	188	625	310
	At end of study	171	297	326
Glomerulary filtration rate (ml/min)	At baseline	58	123	Missing
	At end of study	65	63	Missing

Data from drop out patients. All patients have elevated LV and RV filling pressures, indicators of severe heart failure. Renal function parameters indicate a pronounced impaired renal function. Treatment with trandolapril has variable effects in these patients. Deterioration of renal function due to trandolapril treatment was not the reason for discontinuation of the study in any of these patients.

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Chapter 6.1

Acute hemodynamic effects and preload-dependent cardiovascular profile of the partial phosphodiesterase inhibitor nanterinone in patients with mild to moderate heart failure.

W.J. Remme, M. van der Ent, G.L. Bartels, D. van Schelven,

D.C.A. van Hoogenhuyze, and C.J. Storm.

Acute Hemodynamic Effects and Preload-Dependent Cardiovascular Profile of the Partial Phosphodiesterase Inhibitor Nanterinone in Patients with Mild to Moderate Heart Failure

W.J. Remme, M. van der Ent, G.L. Bartels, D. van Schelven, D.C.A. van Hoogenhuyze, X.H. Krauss, H.A.C.M. Kruijssen, and C.J. Storm

Sticares Cardiovascular Research Foundation, Rotterdam, The Netherlands

Summary. Nanterinone (UK-61,260) is a novel positive inotropic and balanced-type vasodilating drug, only partially based on phosphodiesterase III inhibition. Preliminary data from controlled studies suggest satisfactory long-term efficacy and safety. As its acute hemodynamic effects in humans are unknown, an oral dose of 2 mg nanterinone was studied in 14 patients with heart failure (NYHA class II-III) on chronic diuretic and angiotensin-converting enzyme (ACE) inhibitor treatment. Before the study, patients were on a 2 g salt-balanced diet, and they received their last medication 16 hours before each study day. Hemodynamic measurements were carried out before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, 12, and 24 hours after administration of the study drug. All patients received placebo and nanterinone on 2 consecutive days. Following nanterinone, systemic vascular resistance decreased immediately from 1699 ± 82 (mean ± SEM) at baseline to 1368 ± 80 at 1 hour. Changes persisted for 12 hours. Concomitantly, there was an immediate and significant fall in pulmonary wedge pressure to 38% of baseline at 1.5 hours, together with a 20% reduction in pulmonary artery pressure. Heart rate remained unchanged and arterial pressures showed only a short, significant decrease. Cardiac index rose significantly from 2.28 ± 0.15 at baseline to a highest value of 2.65 ± 0.14 l/min/m^2 at 1 hour. Changes persisted for 3 hours. Placebo had no effect on these variables. As, in view of its potential venodilating properties, hemodynamic improvement by nanterinone may depend on pre-existing left ventricular filling pressure, patients were subsequently grouped according to baseline pulmonary wedge pressure of >12 mmHg (H-PCWP) and ≤12 mmHg (L-PCWP). Cardiac index improved by 26% in H-PCWP and by 17% in L-PCWP (NS). In contrast, PCWP fell more markedly in H-PWCP than in L-PCWP (40% and 23%, respectively, $p < 0.05$). Thus, oral nanterinone results in a significant acute hemodynamic improvement and is well tolerated. Although changes in left ventricular filling pressure are more pronounced in patients with elevated pre-existing PCWP, cardiac pump function improves equally in patients with normal or low left ventricular filling pressure at baseline.

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Key Words. heart failure, positive inotrope, phosphodiesterase inhibition, hemodynamics, humans, calcium sensitization

The conventional therapy for heart failure, that is, diuretics and digitalis, has recently been complemented and, to a certain degree, improved by vasodilator therapy, in particular by converting enzyme inhibitor treatment. Nevertheless, as the syndrome remains characterized by an ever increasing incidence and a considerable morbidity and hospitalization rate, additional forms of pharmacological therapy are mandatory. One approach that has been pursued for many years aims at improving myocardial contractile force. However, with the exception of digitalis glycosides, orally active positive inotropes, which act mainly through cyclic AMP–dependent mechanisms, that is, beta-adrenergic compounds or cyclic AMP phosphodiesterase inhibitors, have not been successful where clinical efficacy is concerned. Instead, such agents have been shown to be arrhythmogenic and to increase mortality in patients with moderate to severe or with severe heart failure. In contrast, agents with partial rather than predominant phosphodiesterase-inhibiting properties may be useful and possibly safe, when given in addition to conventional therapy in patients with mild to moderate, rather than severe, heart failure [1].

Nanterinone (UK 61,260), a quinolone derivative, induces positive inotropic and arterial vasodilating effects, without affecting heart rate in conscious animals [2,3]. Moreover, it improves relaxation and contractility in cardiac preparations of patients with heart failure [4]. Preliminary data indicate that long-term treatment with nanterinone improves exercise capac-

Address for correspondence: W.J. Remme, MD, PhD, Sticares Cardiovascular Research Foundation, P.O. Box 52006, 3007 LA Rotterdam, The Netherlands.

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ity in patients with moderate heart failure without significant side effects [5]. Although its mode of action is as yet undefined, the positive inotropic effect does not relate to an effect on receptors, adenylate cyclase, or cellular membrane activity; neither does the drug affect calcium homeostasis [6]. Although nanterinone has phosphodiesterase III-inhibiting properties, its relative inotropic potency in anesthetized dogs is approximately five times that of milrinone and is longer lasting [2]. One study investigating the effect of nanterinone on cytoplasmic calcium fluxes and developed force suggested similar properties as pimobendan, a partial phosphodiesterase inhibitor and calcium sensitizer [7]. Whether nanterinone has identical calcium-sensitizing properties is as yet unknown. Nanterinone acutely improves systemic hemodynamics in a canine heart failure model [3]. However, its hemodynamic profile in heart failure patients is unknown. The present study investigates the acute systemic hemodynamic effects of nanterinone in patients with mild to moderate heart failure on background therapy of diuretics and converting enzyme inhibition in a single-blind, crossover comparison with placebo. In addition, its hemodynamic profile was studied in patients with normal as compared with elevated left ventricular filling pressures at baseline, as previous studies indicate that the hemodynamic effects of phosphodiesterase inhibitors may depend on preload [8]. Thirdly, the study evaluated the safety and tolerability of nanterinone.

Methods

Patients

After the study protocol had been accepted by the institutional review board and informed consent obtained, 14 patients (11 males and 3 females; mean age 63 ± 2 years, range 45–77 years), with mild to moderate heart failure due to ischemic or idiopathic dilated cardiomyopathy, were enrolled in the study. To be eligible, signs and symptoms of symptomatic heart failure had to be present for at least 2 months, despite background therapy of diuretics and a converting enzyme inhibitor (enalapril). Only patients with a left ventricular ejection fraction ≤45%, as indicated by radionuclide or angiographic evaluation, were included. Patients were either males, aged between 21 and 80 years, or postmenopausal females. Patients with both minimal and severe heart failure, defined as NYHA classification I or IV, respectively, or with signs of unstable heart failure, were excluded. Moreover, patients with unstable angina, a recent (<2 months) myocardial infarction, coronary artery bypass graft or angioplasty, ventricular dysrhythmias requiring drug treatment, clinically significant stenotic valvular disease, supine systemic arterial hypotension or hypertension (<90 or >200 mmHg, respectively), or significant hepatic, renal, or metabolic disease were excluded. Concomitant cardiovascular therapy other than diuretics and/or enalapril was not allowed, with the exception of digitalis for the treatment of atrial fibrillation. In the latter case, digitalis therapy had to be stable for the last 4 weeks before entry into the study.

Individual demographic and clinical patient characteristics are given in Table 1. Of the 14 patients included, 4 were in NYHA class II and 10 were in NYHA class III. The duration of heart failure symptoms averaged 5.4 ± 1 years (range 2 months to 11 years). The average left ventricular ejection fraction at baseline was 21 ± 2% (range 6–44%). The underlying etiology was ischemic cardiomyopathy in nine and idiopathic dilated cardiomyopathy in five patients. Only two patients were on digitalis therapy for atrial fibrillation.

Study design

The study was designed as a single-blind comparison of a single oral dose of placebo and a single oral dose of nanterinone over 2 successive days. The study was preceded by a 5-day prestudy period, during which patients were hospitalized and kept on a 2 g salt balanced diet. At the beginning of the prestudy hospitalization phase, all cardiovascular medication was withdrawn, with the exception of diuretics, angiotensin-converting enzyme (ACE) inhibitors, and digitalis, which were continued at an unchanged dose and were administered at night, approximately 15 hours before baseline measurements on the 2 consecutive study days. On the day preceding the study, patients were admitted to the coronary care unit.

Instrumentation

Instrumentation for the study was carried out approximately 16–18 hours before the first study day and comprised a Swan Ganz triple-lumen thermodilution catheter positioned in a pulmonary artery for measurements of pulmonary artery, pulmonary wedge, and right atrial pressures, and an arterial line in a radial artery for the measurement of arterial pressures. Cardiac output was assessed by the thermodilution technique, using a bedside cardiac output computer (Edwards Laboratories). Cardiac output measurements were made at least in triplicate. Only successive values with a variation of <10% were ac-

Table 1. Patient characteristics

Sex	11 male/3 female
Age (x̄, range)	62.5 years (45–77)
CHF classification	4 NYHA II/10 NYHA III
Duration CHF symptoms (x̄, range)	5.4 years (2 months to 11 years)
Etiology	9 ischemic CMP/5 idiopathic CMP
LV ejection fraction (x̄, range)	21% (6–44%)

LV = left ventricular; CMP = cardiomyopathy.

cepted. Heart rate was recorded from a continuous two-lead electrocardiogram (ECG).

Hemodynamic measurements and calculated variables

Measured hemodynamic parameters included heart rate (HR, min^{-1}); systolic, diastolic, and mean arterial pressures (SAP, DAP, and MAP, respectively); and systolic, diastolic, and mean pulmonary artery pressures (SPAP, DPAP, and MPAP, respectively), mean pulmonary wedge and right atrial pressure (PCWP and RAP, respectively), and cardiac output (CO, l/min). The pulmonary artery and right atrial pressures were measured at midrespiration, to ensure stable values. From the above-mentioned parameters the following variables were calculated: cardiac index (CI, $l/min/m^2$) = [CO/BSA, where BSA = body surface area (m^{-2})], stroke volume index (SVI, ml/m^2) = CI/HR, stroke work index (SWI, $g.m.m^{-2}$) = [(MAP − PCWP) × SVI × 0.0136], systemic vascular resistance (SVR, $dynes.sec.cm^{-5}$) = [(MAP − RAP) × 80/CO], and pulmonary vascular resistance (PVR, $dynes.sec.cm^{-5}$) = [(MPAP − PWCP) × 80/CO].

Study protocol

On each study day, repetitive control hemodynamic measurements were carried out 2.5 hours after a light liquid breakfast to ensure stable baseline values. Following control evaluations, patients received placebo on day 1 and 2 mg nanterinone on day 2. On each study day, all hemodynamic variables were reassessed at 30, 60, 90, and 120 minutes after drug administration, followed by measurements at 3, 4, 8, 12, and 24 hours. Patients were allowed to eat immediately after the 4-hour hemodynamic assessment and received an evening meal as well as background medication immediately following the 8-hour assessment period. After completion of the 24-hour measurement of the second study day, patients were discharged.

Statistical analysis

Baseline hemodynamic variables were compared between control and nanterinone treatment days, using a paired t-test, whereas changes from baseline during each study day were analyzed using an analysis of variance for repeated measurements, followed by a multiple comparison procedure according to Dunnett. In a separate analysis, the acute hemodynamic effects of nanterinone were compared between patients with a baseline left ventricular filling pressure (pulmonary wedge pressure) ≤12 mmHg and >12 mmHg. Baseline hemodynamic variables of these two groups were compared using an unpaired t-test, whereas changes from baseline within each group were analyzed using analysis of variance for repeated measurements, followed by a multiple comparison procedure according to Dunnett. Furthermore, maximal percent changes in hemodynamic variables were compared between the two groups using a paired t-test. For all analyses, a two-tailed p value of $<.05$ was considered indicative of a significant difference. All values are given as mean ± SEM.

Results

Baseline values on each treatment day were comparable. Placebo did not affect systemic hemodynamics, except for a moderate 3–5% reduction in heart rate during the first 2 hours after administration, accompanied by a small, but significant increase in stroke volume (Fig. 1). In contrast, following nanterinone heart rate did not change. Still, cardiac and stroke index increased by 16% and 18%, respectively, together with a 19% reduction in systemic vascular resistance. Changes were already apparent at 30 minutes after drug administration and persisted for at least 12 hours. In contrast, whereas the compound decreased left ventricular filling and mean pulmonary arterial pressures by 36% and 19%, respectively, these effects, although occurring early, between 30 and 60 minutes, were relatively short lasting, being present for only 2½ hours (Fig. 2). Despite the systemic vasodilating effects, arterial pressures did not decrease, except for a short-lasting 7% reduction at 60 minutes. Nanterinone did not affect right atrial pressure or pulmonary vascular resistance.

Preload-dependent effect of nanterinone

To assess the influence of the baseline left ventricular filling pressure on the effects of nanterinone, patients were grouped as those with a baseline pulmonary wedge pressure >12 mmHg (group I, n = 7) or <12 mmHg (group II, n = 7) on day 2, before the administration of nanterinone. All patients in group I were in NYHA class III, whereas four patients in group II were in NYHA class II and 3 were in class III. In addition to wedge pressure, right atrial and mean pulmonary artery pressures were lower in group II, whereas arterial mean pressures and stroke work index were less in group I. Otherwise, hemodynamic parameters did not differ between groups (Table 2).

In Figure 3 maximal percent changes in both groups during the first 4 hours after drug administration are displayed. Nanterinone reduced systemic vascular resistance to a greater extent in group II. Only in this group was a significant decrease observed. In contrast, although nanterinone improved cardiac pump function in both groups, these effects were clearly more marked in patients with increased left ventricular filling pressures at baseline (group I; Fig. 3). In the latter group, cardiac index increased by 26%, but by only 17% in group II. Moreover, stroke volume index increased significantly in group I, but not in group II. In group I, nanterinone reduced pulmonary artery mean and pulmonary wedge pressure by 24% and 40%, respectively. Although it did not significantly affect these variables in group II, in the

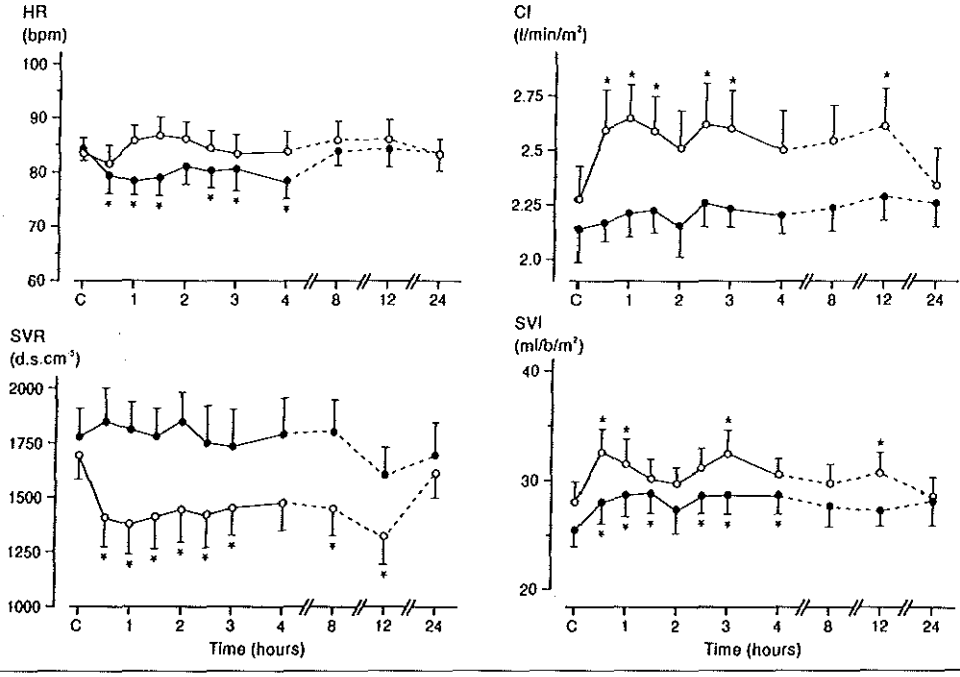

Fig. 1. Sequential effects of nanterinone (open circles) and placebo (closed circles) on heart rate (HR), systemic vascular resistance (SVR), and cardiac index (CI), and stroke index (SWI). Whereas placebo had a small, temporary effect on HR and stroke volume, nanterinone significantly improved CI and SWI, and reduced SVR starting at 30 minutes after administration and continuing to at least 12 hours thereafter. C = baseline.

latter changes in left ventricular filling pressure were identical to those in group I, ranging between 30% and 40%, and following the same time course as in group I (Fig. 4).

Long-term hemodynamic effects of nanterinone

In addition to the above-mentioned protocol, 11 patients continued with the double-blind study medication for 8 weeks. Six patients received 2 mg nanterinone and five received placebo. After this outpatient phase, patients were hospitalized and a repeat hemodynamic study was performed. During the second hospitalization, patients continued with oral study medication until after hemodynamic re-evaluation. As a limited number of patients was studied per group, only baseline values were compared between long-term treatment and day 1 of the study.

Nanterinone was well tolerated. No side effects occurred in either group. Although in the patients treated with nanterinone heart rate and mean arterial pressure tended to decrease by 4% and 5%, respectively, compared with baseline before treatment (Table 3), these changes were not significant due to small sample size. The other hemodynamic variables remained essentially unchanged during long-term treatment. There were no changes in the placebo group either.

Discussion

The present study indicates that nanterinone acutely improves cardiac pump function as compared with placebo in patients with stable moderate heart failure, despite the concomitant use of converting enzyme inhibition and diuretic therapy. Also, the compound induces systemic vasodilating effects and significantly decreases left ventricular filling and pulmonary artery pressures without changes in heart rate or clinically important reductions in systemic arterial pressures. Although nanterinone improved cardiac output in all patients irrespective of whether baseline left ventricular filling pressure was abnormally increased or low to normal, this effect was clearly more pronounced when preload was elevated. Finally, the oral administration of 2 mg nanterinone did not lead to side effects and was well tolerated, both acutely and during long-term treatment.

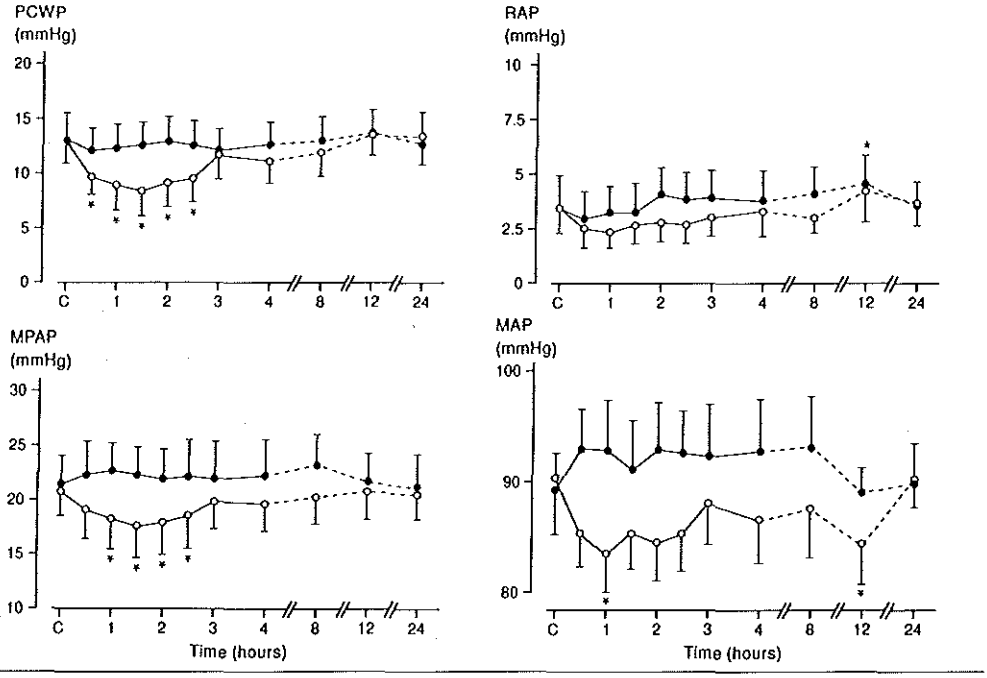

Fig. 2. Significant, but short-lasting reductions in pulmonary wedge pressure (PCWP) and mean pulmonary artery pressure (MPAP) immediately following nanterinone administration (open circles). In contrast, right atrial pressure (RAP) and mean arterial pressure (MAP) remained unchanged. Placebo (closed circles) had no effect on these variables (C = baseline).

Table 2. Patient characteristics: High versus low left ventricular filling pressures

	Group I (PCWP >12 mmHg) (n = 7)		Group II (PCWP ≤12 mmHg) (n = 7)
Age (years)	63.7 ± 2.3	ns	61.3 ± 3.7
NYHA class	III		II (4 patients) III (3 patients)
LV ejection fraction (%)	18.0 ± 2.8	ns	23.7 ± 3.7
PCWP (mmHg)	20.1 ± 1.9	*	6.1 ± 1.2
MAP (mmHg)	84 ± 2.8	*	96 ± 3.3
RAP (mmHg)	6.1 ± 2.7	*	1 ± 0.4
PAM (mmHg)	30.1 ± 2.3	*	12.1 ± 2.7
HR (beat/min)	80 ± 5.1		86 ± 4.0
CI (l/min/m^2)	2.07 ± 0.14	ns	2.49 ± 0.26
SVI (ml/beat/m^2)	26.1 ± 3.2		29 ± 2.8
SWI (g.m.m^2)	22.8 ± 3.2	*	35 ± 3.6
SVR (dynes.sec.cm^{-5})	1705 ± 110		1693 ± 129
PVR (dynes.sec.cm^{-5})	207 ± 43		135 ± 37

CI = cardiac index; HR = heart rate; LV = left ventricular; MAP = mean arterial pressure; PAM = mean pulmonary pressure; PCWP = pulmonary wedge pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure; SVI = stroke volume index; SVR = systemic vascular resistance; SWI = stroke work index. *$p < 0.05$ Group I vs II.

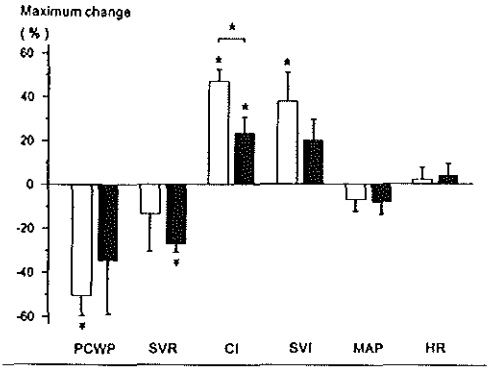

Fig. 3. Differential effects of nanterinone in patients with elevated (>12 mmHg, open bars) or low to normal (≤12 mmHg, closed bars) pulmonary wedge pressure (PCWP). In patients with elevated LV filling pressure, nanterinone significantly increased stroke volume index (SVI) and improved cardiac index (CI) to a greater extent than in the other group, despite the fact that the systemic vascular effects and reductions in left ventricular (LV) filling pressure were comparable.

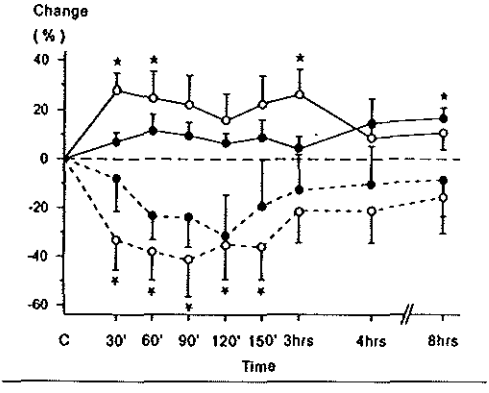

Fig. 4. Sequential effects of nanterinone on cardiac index (CI, solid lines) and pulmonary wedge pressure (PWCP, broken lines) in patients with increased (open circles) or normal to low (closed circles) PCWP at baseline. Although in patients with increased LV ventricular pressures nanterinone significantly improved CI and decreased PCWP, in patients with normal to low left ventricular (LV) filling pressures temporal changes were comparable in both groups.

Systemic hemodynamic profile of nanterinone

The systemic hemodynamic profile of nanterinone in patients with heart failure reflects its phosphodiesterase-inhibiting properties, at least to such an extent that systemic arterial vasodilating effects were present, cardiac output improved, and left ventricular filling pressure decreased, together with a reduction of pulmonary artery pressures. As such, the compound's cardiovascular effects compare well with those of predominant phosphodiesterase inhibitors, such as amrinone, milrinone, and enoximone [9–14]. These agents are generally considered to be positive inotropic compounds and are devoid of digitalis-like properties. However, in view of these arterial or balanced-type vasodilating effects, the result of cAMP phosphodiesterase inhibition, they are better categorized as inodilator drugs [15]. Indeed, the vasodilating effects of these agents may be significant in the treatment of severe heart failure [16] and, reportedly, may predominate in normal individuals [17]. Also, in mild to moderate heart failure, the vasodilating effects of milrinone may prevail [1]. Our data suggest that vasodilation also contributed to the overall hemodynamic profile of nanterione in a similar patient group as in the latter study.

Although animal data suggest that the positive inotropic effects of nanterinone predominate and occur at lower dose levels than its vasodilating activity [3], the present study shows that, at the dose level used, in heart failure patients systemic arterial vasodilating properties may significantly contribute to its positive effects on cardiac pump function. In fact, systemic unloading of the left ventricle could entirely explain the overall hemodynamic improvement by nanterinone in our patients. In particular, the temporal relation between systemic vasodilating effects and the increase in cardiac output suggests a functional correlation. In contrast to the systemic arterial effects, changes in pulmonary arterial pressures and in pulmonary wedge pressures were clearly of shorter duration.

Whether positive inotropic properties played an additional role in the improved cardiac pump performance in our patients is impossible to state. The presence of positive inotropic properties cannot be deduced from our study in the absence of appropriate methods to examine the latter. Certainly, positive chronotropic effects, also a direct result of phosphodiesterase inhibition, did not play a role. Significant additional venodilating properties appear less likely, as no changes occurred in right atrial pressures. Also, the effect of nanterinone on pulmonary artery pressures cannot be explained by a drug effect on the pulmonary arterial vascular system, because pulmonary resistance remained unchanged.

Thus, nanterinone has beneficial hemodynamic effects, as indicated by a significant increase in cardiac

Table 3. Long-term hemodynamic effects of nanterinone

	Nanterinone (n = 6)		Placebo (n = 5)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Heart rate (beats/min)	88.2 ± 3.2	84.3 ± 4.8	85.2 ± 2.9	82.2 ± 6.6
Mean arterial pressure (mmHg)	89.5 ± 4.8	85.2 ± 6.0	87 ± 6.0	86.4 ± 5.0
Pulmonary wedge pressure (mmHg)	16.5 ± 3.2	14.2 ± 3.7	8.8 ± 4.0	10.4 ± 1.4
Cardiac index (l/min/m^2)	2.1 ± 0.2	2 ± 0.3	2.3 ± 0.3	2.4 ± 0.2
Stroke work index (g/m/m^2)	24.2 ± 4.5	26.5 ± 7.4	29.5 ± 6.0	30.4 ± 4.0
Systemic vascular resistance (dynes/sec/cm^{-5})	1846 ± 131	1901 ± 220	1614 ± 119	1505 ± 140

and stroke index and a lowering of left ventricular filling pressure, due at least to systemic unloading of the left ventricle and, possibly, to positive inotropic properties without apparent positive chronotropic effects, both during acute administration and during long-term treatment. The absence of any increase in heart rate conforms with previous observations with this compound in both in vitro experiments and studies in both anesthetized and unanesthetized dogs [2]. Of importance, in the present study changes in heart rate also did not occur in patients with low to normal left ventricular filling pressures. As such, nanterinone clearly differs from the phosphodiesterase inhibitor milrinone. The latter increases heart rate in patients with mild to moderate heart failure and low to normal left ventricular filling pressures [8]. This increase, in the absence of a significant fall in blood pressure and/or cardiac output, most likely reflects a direct, cAMP-related effect on the heart. The absence of heart rate–stimulating properties suggests that nanterinone does not resemble predominant phosphodiesterase inhibitors, such as milrinone.

Preload-dependent hemodynamic effects of nanterinone

As the cardiovascular actions of nanterinone depend at least in part on cAMP phosphodiesterase-inhibiting properties, a combination of positive inotropic, arterial dilating, and venodilating effects is to be expected. Drug-induced improvement of left ventricular pump function in patients with heart failure will depend entirely on the instantaneous contribution of these separate effects, and on baseline cardiac function, in particular on the level of left ventricular filling pressure. Whereas an increase in contractility will lead to an increase in cardiac output at any given preload, the effect of afterload reduction may vary depending on the magnitude of pre-existing left ventricular filling pressure. More importantly, when the latter is normalized or low as a result of diuretic and ACE inhibitor therapy in mild heart failure, venodilatation may in fact be counteractive and offset the effect of arterial vasodilation and positive inotropism, and thereby lead to a deterioration of cardiac pump function, instead of the expected improvement. Indeed, such a reduction in cardiac output has been observed with both predominant and partial phosphodiesterase-inhibiting agents in patients with mild heart failure and a left ventricular filling pressure <15 mmHg [8,18].

In the present study, nanterinone had no such negative effects on cardiac pump function. The compound improved cardiac output in both patients with elevated wedge pressures and in those with normal to low left ventricular filling pressures. However, the improvement in pump function was more pronounced in those with a high preload. Also, stroke volume or work did not increase in patients with normal to low left ventricular filling pressures, despite comparable reductions in systemic vascular resistance and, hence, unloading effects on the left ventricle. Thus, both the absence of heart rate effects and different preload-dependent hemodynamic properties suggest that, at least as far as cardiovascular properties are concerned, nanterinone may differ from milrinone, a prototype of the predominant phosphodiesterase inhibitors. This could be important where long-term treatment is concerned. Possibly as a result of preload-dependent reductions in cardiac output in mild to moderate heart failure patients, milrinone loses its systemic arterial vasodilator effect, perhaps as a result of secondary neurohormonal stimulation [1,19,20]. Although too few patients wanted to participate in the long-term study, there was no evidence of a hemodynamic deterioration in the six patients who continued with nanterinone.

The present study suggests that nanterinone induces beneficial hemodynamic effects in patients with mild to moderate heart failure. Further evaluation of this compound in heart failure is necessary to investigate whether these acute hemodynamic effects translate into long-term clinical improvement. Preliminary data from a long-term, multicenter, placebo-controlled study indeed indicate that nanterinone, in dosages ranging between 1.5 and 3 mg daily, improves exercise capacity in mild heart failure [5].

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Chapter 6.2

Contrasting preload-dependent hemodynamic and neurohumoral effects of isomazole, a partial phosphodiesterase inhibitor and calcium sensitizer.

M. van der Ent, Willem J. Remme, G. Louis Bartels, Peter de Leeuw,

Diederik C.A. van Hoogenhuyze, Dick A.C.M. Kruijssen

Contrasting Preload-Dependent Hemodynamic and Neurohumoral Effects of Isomazole, a Partial Phosphodiesterase Inhibitor and Calcium Sensitizer

MARTIN VAN DER ENT, MD, WILLEM J. REMME, MD, PhD, G. LOUIS BARTELS, MD, PETER W. DE LEEUW, MD, PhD,* DIEDERIK C.A. VAN HOOGENHUYZE, MD, DICK A.C.M. KRUIJSSEN, MD

Zuiderziekenhuis, Rotterdam; Maastricht, The Netherlands

ABSTRACT

Background: Currently evaluated positive inotropic agents that act predominantly through phosphodiesterase III-inhibiting properties, have been disappointing in the treatment of heart failure. Lack of efficacy as a result of diminished cellular cyclic adenosine monophosphate and vasodilating tolerance and side effects are prevalent. In contrast, calcium sensitization is preserved in heart failure and agents that combine phosphodiesterase-inhibiting and calcium-sensitizing properties may be more efficacious. Isomazole is such a novel agent with combined properties. This study investigated the acute hemodynamic and neurohormonal effects of intravenous isomazole (3 μg/kg/min for 30 minutes).

Methods and Results: The effects of preexisting preload were evaluated in 18 patients with heart failure, New York Heart Association class II/III, and elevated (>15 mmHg, n = 11, group I) and normal (n = 7, group II) pulmonary wedge pressure at baseline. In the overall group, isomazole increased myocardial contractility and relaxation and decreased systemic resistance by 20%. Left and right filling pressures fell by 35–45%, accompanied by a 69% reduction in cardiac atrial natriuretic peptide release. In contrast, levels of arterial norepinephrine and renin both increased by 27%. Cardiac output increased in group I (23%), but fell in group II (18%), accompanied by a 51% increase in arterial norepinephrine. Cardiac atrial natriuretic peptide decreased in group I, but not in group II.

Conclusions: Isomazole induced positive inotropic and lusitropic effects and arterial vasodilation in all patients. Cardiac pump function improved only in group I, accompanied by a reduction in sympathetic activity and renin-angiotensin and aldosterone levels and a more pronounced decrease in cardiac atrial natriuretic peptide release. In contrast, in patients with normal to low preload, the further reduction in preload led to a deterioration of pump function and increased sympathetic tone.

Key words: phosphodiesterase inhibitors, calcium sensitization, heart failure, humans, neurohormones, myocardial function.

Despite recent developments, currently available heart failure therapy is not universally efficacious and is often limited by side effects. One approach that has been pursued for many years aims at improving the impaired cardiovascular function, the primary event in heart failure. Agents used in this context include drugs that combine both positive inotropic effects and vasodilating properties, that is, inodilator compounds.

*From the Sticares Cardiovascular Research Foundation and Zuiderziekenhuis, Rotterdam, and *University Hospital of Maastricht, Maastricht, The Netherlands.*

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Reprint requests: W. J. Remme, MD, PhD, Sticares Foundation, PO box 52006, 3007 La Rotterdam, The Netherlands.

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Orally active inodilator agents that have been evaluated in humans all share phosphodiesterase (PDE) III-inhibiting actions, which lead to an increase in cellular cyclic adenosine monophosphate (cAMP) levels and subsequently to enhanced inotropy and cardiac relaxation and to vasodilation. Agents with predominant PDE-inhibiting properties, such as milrinone and enoximone, acutely improve cardiovascular pump function in heart failure, in part the result of arterial vasodilation; however, late tolerance has been reported (1). Moreover, cellular cAMP levels are reduced in failing hearts, possibly as a result of beta-receptor down-regulation, which may also explain the lack of long-term efficacy of these cAMP-dependent inodilators. Side effects are of concern, including ventricular arrhythmias and the likelihood of myocardial oxygen consumption increasing, which is unfavorable in patients with ischemic heart disease. Conversely, agents that share partial PDE-inhibiting properties and additional inotropic properties such as calcium-sensitizing effects may be more efficacious. Through the additional inotropic properties they are less dependent on the contribution of PDE-inhibition with fewer side effects. Moreover, the process of calcium sensitization remains intact in failing hearts. On the downside, calcium sensitization slows relaxation (2), which is already impaired in failing hearts. Whether the latter becomes clinically relevant, however, depends on the relative contribution of PDE-inhibition, which in turn enhances relaxation, even in heart failure. In this study, we investigated the acute hemodynamic and myocardial energetic effects of isomazole, an agent with calcium-sensitizing properties and, in addition, partial PDE-inhibiting effects, administered intravenously to patients with myocardial dysfunction as a result of ischemic cardiomyopathy or idiopathic dilated cardiomyopathy. As vasodilator agents may cause reflex neurohormonal responses, which may counteract their direct vascular effect, we also evaluated neurohormonal changes. Furthermore, we compared the effect of isomazole in patients with normal versus elevated left ventricular end-diastolic pressures, as previous studies have shown that the level of left ventricular filling pressure may determine the acute hemodynamic effects of PDE III inhibition (3).

Materials and Methods

Patient Selection

After informed consent and after approval by the local Ethical Review Committee, 18 patients, 1 female and 17 males, mean age 59.7 ± 2.0 years (range, 43–74 years), with mild to moderate heart failure, New York Heart Association classification II/III, participated in the study. Left ventricular ejection fraction, measured angiographically, had to be ≤45%. Patients with renal and hepatic insufficiency, insulin-dependent diabetes mellitus, body weight <40 kg or >100 kg, obstructive valvular disease, a myocardial infarction <1 month old, or a supine systolic blood pressure <100 mmHg were excluded. The etiology of heart failure was ischemic cardiomyopathy in 16 patients and idiopathic dilated cardiomyopathy in 2 patients. All patients were normotensive at entry. Two patients were on digitalis treatment, 12 received diuretics, 5 converting enzyme inhibitors, 5 calcium antagonists, 8 beta-1-blocking agents, and 9 long-acting nitrates. Digitalis and diuretic treatment were continued until 24 hours before the study. Other cardiac therapy was withdrawn 3 to 5 days prestudy. At entry, 13 patients were New York Heart Association class II and 5 class III. Average ejection fraction was 22.1 ± 1.6% (range, 10–35%) and average left ventricular end-diastolic volume measured 111 ± 7 mL/m² (range, 59–164 mL/m²). Clinical and angiographic criteria are given in Table 1.

Catheterization Procedures and Instrumentation

Studies were carried out during left and right heart catheterization, performed in the morning, after an overnight fast and without premedication. After routine coronary arteriography, carried out according to the Seldinger technique, a No. 7-F Wilton Webster coronary sinus thermodilution pacing catheter was positioned via a brachial vein in the midportion of the coronary sinus, such that the proximal thermistor was at least 2 cm beyond the orifice of the coronary sinus and its position stable. The absence of atrial reflux was assessed by a bolus injection of 10 mL 5% glucose at room temperature in the right atrium. Next, a No. 8-F Sentron pigtail microtip manometer was positioned in the left ventricle through a No. 9-F arterial Desilet introducer system in the femoral artery. The side arm of this system was used to record arterial pressures. Finally, a No. 7-F balloon-tipped triple-lumen thermodilution catheter was advanced into the pulmonary artery through a femoral vein. Care was taken that the catheter tip was stable without baseline drift on the thermodilution signal.

The position of the catheters was recorded on video disk and regularly checked throughout the study.

Hemodynamic Measurements and Calculations

After calibration of the fluid-filled catheters with a zero reference level set at midchest, the micromanometer pressure was balanced to zero and superimposed on the conventional pressure tracings. Pressures in the right

Table 1. Patient Characteristics

	All Patients	Baseline LVEDP > 15 mmHg (Group I)	Baseline LVEDP ≤15 mmHg (Group II)
No.	18	11	7
Male/female	17/1	10/1	7/0
Age (range)	60 ± 2 (43–74)	60 ± 3 (43–74)	59 ± 3 (50–68)
NYHA class II/III	14/4	9/2	5/2
Previous infarction	16	9	7
Number of diseased vessels			
None	2	1	1
1 vessel	3	1	2
2 vessels	7	6	1
3 vessels	6	3	3
LV ejection fraction (%)	22 ± 2	21 ± 2	24 ± 3
LV end-diastolic volume (ml/m²)	111 ± 7	121 ± 8	96 ± 10*
Medication			
Angiotensin-converting enzyme inhibitors	5	4	1
Digoxin	2	2	—
Diuretics	12	8	4
Nitrates	9	4	5
Calcium antagonists	5	3	2
Beta-blockers	8	3	5

LV, left ventricular; LVEDP, left ventricular end-diastolic pressure; NYHA, New York Heart Association.
*$P < 0.05$, group I versus group II.

atrium and femoral and pulmonary arteries were measured using Bentley transducers (Bentley–Baxter, Uden, The Netherlands). Pressure and flow signals and the first derivative of left ventricular pressure (LV dP/dt) were recorded on paper at different paper speeds (ie, 10–25 mm/s), using an RMC 1100 Cath Lab System (Nihon Kohden). Cardiac output was measured on-line by a Mennen Cath Lab Computer System (Mennen Medical, Rekovot, Israel). All pressures and pressure-derived contractility indices were determined on-line throughout the study. In a beat-to-beat analysis, this system averages 15–20 consecutive beats to level out respiratory variations. Isovolumetric pressure-derived contractility indices (peak positive dP/dt, dP/dt/P at 40 mmHg [V_{cc40}], and V_{max} total pressure) and peak negative dP/dt were calculated and displayed on-line. In contrast, the isovolumetric relaxation index Tau was measured off-line (4). Throughout the study contractility and relaxation parameters were measured at a fixed heart rate set at 15 beats above baseline heart rate. All other hemodynamic measurements were carried out at basal, unfixed heart rates. Thermodilution cardiac output was determined in triplicate with a variation <5%. Coronary sinus blood flow was measured during a continuous 30-second infusion of 30 to 35 mL 5% glucose at room temperature. Although both pulsatile and mean flow curves were recorded, calculations were made from the latter, according to the formula coronary blood flow (mL/min) = $V_i \times [T_b - T_i)(T_b - T_c) - 1] \times 1.08$, where T_b is blood temperature before injection, T_i temperature of injectate, T_c, temperature of mixture of coronary sinus blood and injectate, and V_i rate of injection (mL/min). On-line presentation of 1-second measurements allow for proper estimation of the stability of the flow signal.

At the end of the study, the pressure curves from the femoral artery were compared with a simultaneous recording from the aortic root to compensate for any difference between proximal and distal arterial pressures.

Left Ventricular Angiography

Left ventricular angiography was performed in the 30 RAO projection. Nonionic contrast material (0.65 mL/kg body weight), was injected by a Medrad infusion system at a rate of 14 mL/s. All angiograms were performed in midrespiration, at a fixed pacing heart rate, 15 beats above basal heart rate. Left ventricular volumes were determined semiautomatically, using the Mennen Angio Cath Lab Program and calculated by the area–length method, corrected by Heitzens' formula (5).

Metabolic Measurements

Sampling of 1 mL of blood was carried out from the left ventricle and the coronary sinus for instantaneous determination of oxygen saturation values on an OSM-80 oximeter (Waters Associates). For lactate, exactly 1 mL of blood was collected simultaneously from the left ventricle and the coronary sinus and transferred quickly into precooled glass tubes containing 2 mL of ice-cold 0.6 M $HClO_4$, mixed thoroughly, and kept on ice. Imme-

diately after the study, the samples were weighed and centrifuged for 20 minutes at a speed of 2000g. Thereupon, the supernatant was frozen for subsequent assay of lactate. The latter was carried out in triplicate by an enzymatic technique as previously described (6).

Neurohormonal Measurements

The levels of catecholamines (norepinephrine, epinephrine, dopamine), active plasma renin concentration, angiotensin II, and aldosterone were assessed by collecting a minimum of 12 mL of blood simultaneously from the left ventricle and coronary sinus in precooled syringes.

For the assay of angiotensin II, 2 mL was immediately transferred into ice-cold tubes containing an inhibitor solution (2.4 mg EDTA, 0.3 mg 1,10-*O*-phenanthroline, and 0.01 mg of captopril in 50 μL). Blood samples were immediately centrifuged (10 min, 4°C, 3000g) and the plasma was stored at −70°C. Determination was done by SepPak extraction and high-performance liquid chromatography (7). For catecholamines, 2 to 3 mL of blood was transferred into chilled heparinized tubes containing 3 mg glutathione and centrifuged within 15 minutes at 4°C at 3000g. Plasma was stored at −70°C. Determination of catecholamines (norepinephrine, epinephrine, dopamine) was done by high-performance liquid chromatography with fluorometric detection (8). For the determination of enzymatically active renin, 2 mL of blood was collected in tubes containing 2.4 mg EDTA. Samples were centrifuged (10 min, 4°C, 3000g) and plasma stored at −20°C. Determination was done by radioimmunoassay (9). Aldosterone was measured with a commercially available radioimmunoassy kit (Coat-A-Count, Diagnostic Products, Los Angeles, CA) in plasma obtained from 2 mL of blood collected in 2.4 mg of EDTA. Angiotensin I-converting enzyme (ACE) was measured in plasma prepared from heparinized blood by a colorimetric method (Fujirebio ACE Color Kit, Bipharma Diagnostics, Amsterdam, The Netherlands). Atrial natriuretic peptide (ANP) was determined in plasma prepared from 2 mL of blood collected in a tube containing 2.4 mg EDTA and 500 KIE Trasylol and stored at −70°C. Atrial natriuretic peptide was measured with a commercially available radioimmunoassay (ITS, Wijchen, The Netherlands).

Calculations

From the measured variables, the following parameters were derived: Coronary vascular resistance (mmHg/ mL/ min) was calculated as the difference between mean arterial pressure (mmHg) and left ventricular mean diastolic pressure (mmHg) divided by coronary sinus blood flow (mL/min). Systemic vascular resistance (dyn·s cm^{-5}) was derived as (mean arterial pressure [mmHg] − mean right atrial pressure [mmHg]/cardiac output [L/min]) × 80. Stroke work index (g·m/m^2) was calculated as stroke index (mL/beat/m^2) × (mean arterial pressure [mmHg] − left ventricular end-diastolic pressure [mmHg]) × 0.0136. Myocardial oxygen extraction (mL O_2/mL) was calculated as arterial − coronary venous oxygen content (mL/O_2/mL) and myocardial oxygen consumption (mL/min) as the product of myocardial oxygen extraction (mL/O_2/mL) and coronary blood flow (mL/min). Percentage myocardial lactate extraction was calculated as 100 × (arterial lactate content [mmol/L]) − coronary venous lactate content [mmol/L]/ arterial lactate content [mmol/L]). Myocardial lactate (mmol/L) uptake was determined by multiplying the difference in arterial and coronary venous lactate content (mmol/L) by coronary blood flow (mL/min). Myocardial catecholamine (nmol/min), angiotensin II (pmol/min), renin (μmol/L/h), and aldosterone (pmol/min) balances were calculated by multiplying the respective differences in arterial and coronary levels by the instantaneous coronary sinus blood flow (mL/min).

Study Protocol

Patients were studied 45 to 60 minutes after the last coronary angiogram and 25 to 30 minutes after instrumentation. Consequently, all studies were performed between 10:30 and 11:30 a.m. with the patient resting and supine for approximately 1.5 to 2 hours. First, multiple control measurements of all hemodynamic variables were performed to ensure stable baseline values. Next, arterial and coronary venous blood samples for metabolic and neurohormonal parameters were collected, and, subsequently, left ventricular angiography was carried out. After a 10-minute stabilization period, isomazole was administered in a dose of 3 μg/kg/min during 30 minutes. Repeat hemodynamic, metabolic, and electrocardiographic measurements were carried out every 10 minutes for 1 hour following drug administration at both basal and fixed heart rates. Neurohormones were determined 5, 30, and 60 minutes after isomazole administration. At the end of the study, 60 minutes after isomazole administration, a second left ventricular angiogram was made in a fashion identical to the control angiogram.

Statistical Analysis

All data are presented as group means ± SEM. As several studies with PDE inhibitors have shown that patients with high LV filling pressures have a different response than patients with lower filling pressures, patients were divided based on LV end-diastolic pressure >15 mmHg or ≤15 mmHg for subgroup analysis

(group I, n = 11, high LVEDP, and group II, n = 7, low LVEDP, respectively). Changes per assessment period from control values were evaluated using an analysis of variance for repeated measurements with missing values; baseline differences and differences during treatment between subgroups were analyzed using an unpaired Student *t*-test. All software was provided by SAS/STAT statistical software (SAS, Cary, NC). A two-tailed *P* value < .05 was indicative of a significant difference.

Results

Isomazole was well tolerated. No adverse events occurred. Overall study group and subgroup characteristics are given in Table 1.

Systemic Hemodynamic Effects

Overall Patient Group Analysis. Isomazole resulted in an improvement of myocardial function. The contractility parameters $V_{cf/P}$, V_{max}, and dP/dt positive increased significantly by 19, 18, and 15%, respectively, with a maximal effect 20 minutes after onset of infusion. Simultaneously, Tau and Tau_1 improved with maximum reductions of 27% and 18% ($P < .001$), respectively. Systemic resistance decreased by 20% at 20 minutes. Heart rate increased temporarily by only 5% at 30 minutes ($P < .05$) and mean arterial and left ventricular systolic pressures gradually decreased with a significant 12% reduction at 30 minutes after onset of infusion. In contrast, cardiac pump function remained unaltered during the study, as neither cardiac output nor stroke volume or stroke work index changed during the investigation. Left ventricular end-diastolic pressure fell from 21 mmHg (baseline) to 11 mmHg (40 minutes, $P < .0001$), and right atrial pressure decreased by 35% ($P < .0001$). Left ventricular angiography showed no change in LV ejection fraction after isomazole. Left ventricular end-diastolic and systolic volumes had decreased significantly but comparably 60 minutes after isomazole, from 111 to 99 mL/m² and 87 to 76 mL/m², respectively (both $P < .05$ versus control values) (Table 2).

Subgroup Analysis. At baseline, cardiac pump function and contractility and relaxation indices were significantly lower and heart rate higher in patients with a high LVEDP (Table 3). Isomazole improved all contractility and relaxation indices to a similar extent in both groups. In contrast, cardiac output increased in group I (23%), but decreased by 18% in group II (both $P < .001$). Similar changes were noted for stroke volume and stroke work index. Concomitantly, in group I systemic vascular resistance fell by 24% ($P < .0001$) after 20 minutes, but did not change in group II (Figs. 1, 2). Left ventricular filling pressures and right atrial pressure declined significantly but comparably in both groups. Left ventricular ejection fraction did not change in either group. Left ventricular end-diastolic volumes decreased by 13 and 6% and end-systolic volumes by 13 and 10%

Table 2. Sequential Systemic Hemodynamic Effects of Isomazole (Overall Patient Group)

		Minutes After Isomazole Administration					
Parameter	Control	10	20	30	40	50	60
Basal heart rate							
LVSP (mmHg)	128 ± 3	124*	120*	113*	116*	116*	117*
MAP (mmHg)	95 ± 3	91	88*	84*	87*	87*	88*
LVEDP (mmHg)	20 ± 2	15*	12*	11*	10*	13*	12*
RAP (mmHg)	8 ± 1	5*	6*	5*	6*	5*	6*
PAMP (mmHg)	22 ± 2	18*	17*	16*	17*	17*	18*
HR (beats/min)	86 ± 3	87	90*	92*	89*	89	88
CO (l/min)	4.7 ± 0.3	4.9	5.1	4.7	4.8	4.6	4.6
SVI (mL/beat/m²)	29.1 ± 1.8	29.6	30.1	27.1	28.4	27.7	27.4
SWI (g/m/m²)	30.1 ± 2.4	30	31.3	27.1	29.6	28.1	28.7
SVR (dyn·s cm⁻⁵)	1605 ± 120	1460*	1316*	1366*	1374*	1431*	1454*
PVR (dyn·s cm⁻⁵)	132 ± 24	121	136	143	153	150	166
Fixed heart rate							
dP/dt positive (mmHg/s)	1261 ± 61	1468*	1497*	1484*	1416*	1364*	1432*
dP/dt negative (mmHg/s)	1149 ± 57	1228	1203	1147	1141	1212	1174
$V_{cf/P}$ (second⁻¹)	26 ± 1	31*	32*	31*	30*	29*	29*
V_{max} (second⁻¹)	40 ± 2	48*	49*	48*	47*	45*	45*
Tau (milliseconds)	76 ± 6	63*	60*	57*	62*	60*	60*

CO, cardiac output; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; MAP, mean arterial pressure; PAMP, pulmonary artery mean presure; PVR, pulmonary vascular resistance; RAP, right arterial pressure; SVI, stroke volume index; SVR, systemic vascular resistance; SWI, stroke work index; $V_{cf/P}$, dP/dT/P at 40 mmHg; V_{max}, total pressure. Data are means ± SEM (control) and means (isomazole)
*$P < .05$ versus control.

Table 3. Baseline Systemic Hemodynamic Variables

	All Patients	Group I	Group II
LV end-diastolic pressure (mmHg)	16.5 (14–30)	30 (19–31)	14 (11–15)*
Mean pulmonary artery pressure (mmHg)	21.5 (14–30.5)	24 (22–32)	13 (12–20)*
Right atrial pressure (mmHg)	6 (5–11)	10 (6–14)	5 (4–6)*
Cardiac output (mL/min)	4.37 (3.88–5.65)	3.98 (3.44–4.43)	5.92 (4.38–6.28)
Stroke volume index (ml/m²)	28.7 (23–34)	25.1 (21.6–31.6)	30.4 (28.9–37.4)*
Systemic resistance (dyn·s cm⁻⁵)	1487 (1362–1790)	1683 (1493–2116)	1334 (1165–1465)*
Stroke work index (g/m/m²)	29.3 (22.1–36.2)	22.6 (19–28.5)	37.4 (33.1–45.6)*
V_{ce40} (second⁻¹)	24.2 (20.6–29.2)	23.3 (19.5–24.6)	27.9 (26.1–31.1)*
V_{max} (second⁻¹)	37.7 (33.2–44.2)	35.2 (30.9–37.8)	45.2 (39.2–45.4)*
Tau (milliseconds)	84 (65–100)	99 (94–117)	65 (61–71)*

Group I: LV end-diastolic pressure > 15 mm Hg, n = 11, group II: LV end-diastolic pressure ≤ 15 mmHg, n = 7. Baseline values and statistically significant differences between patients with elevated LV end-diastolic pressure (group I) and patients with normal LV end-diastolic pressure (group II). Data are presented as medians (intraquartile range). LV, left ventricular; V_{ce40}, dP/dT/P at 40 mmHg; V_{max}, total pressure.
*$P < .05$, group I versus group II.

in groups I and II, respectively (all $P < .001$ vs baseline, no group difference).

Coronary Hemodynamic Effects and Myocardial Energetics

Overall Patient Group and Subgroup Analysis. Neither coronary flow or resistance nor myocardial oxygen demand was affected by isomazole. In contrast, myocardial oxygen consumption decreased significantly, by 19% at 30 minutes after isomazole in the overall group (Table 4). Subgroup analysis did not indicate any change in coronary hemodynamics and myocardial energetics in either group. Myocardial lactate extraction did not change throughout the study.

Effects of Isomazole on Circulating Neurohormones and Cardiac Neurohormonal Balance

Overall Patient Group Analysis. Arterial norepinephrine levels increased after 30 minutes by 27% from 329 ± 50 to 420 ± 59 pg/mL ($P < .01$) and remained elevated throughout the study. Also, arterial renin activity increased by 27% from 30 ± 13 to 38 ± 14 μU/mL ($P < .05$) at 60 minutes. Arterial ANP levels did not change, but coronary venous ANP levels fell progressively with a 59% decrease ($P < .001$) at 60 minutes. As arterial ANP did not change, cardiac ANP release was significantly reduced by 54% from 214 ± 42 to 106 ± 29 ng/min at 30 minutes and by 69% (to 68 ± 15 ng/min) at 60 minutes after onset of isomazole infusion. Other circulating neurohormones and cardiac neurohormonal balances remained unaltered (Table 5).

Subgroup Analysis. There were no baseline differences in neurohormonal levels between the groups. Arterial norepinephrine increased in group II by 54% from 242 ± 61 to 371 ± 80 pg/mL ($P < .001$). No other neurohormonal changes occurred in this group. In group I, arterial norepinephrine remained unaltered, but arterial angiotensin II levels fell significantly by 22%, whereas coronary venous aldosterone and ANP levels and cardiac ANP release decreased by 34, 57, and 67% after 60 minutes, respectively (Fig. 3).

Discussion

Isomazole is a novel compound with calcium-sensitizing properties and partial cAMP–PDE III-inhibiting effects (10). Studies in the *in vivo* model as well as in isolated organs have shown a profound increase in contractility, together with a modest increase in heart rate (11–13). In an experimental model of LV dysfunction, isomazole resulted in a marked hemodynamic improvement, with an increase in contractility and cardiac out-

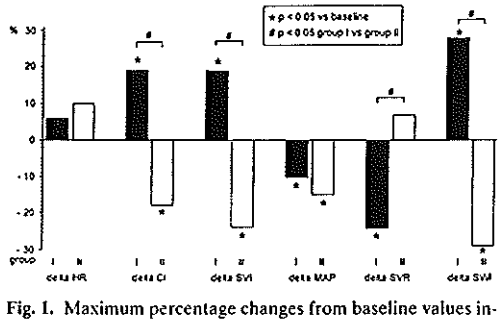

Fig. 1. Maximum percentage changes from baseline values indicate an improvement of cardiac index (CI), stroke volume index (SVI), stroke work index (SWI), and systemic resistance (SVR) in group I (LV end-diastolic pressure > 15 mmHg) but not in group II (left ventricular [LV] end-diastolic pressure < 15 mmHg). Heart rate (HR) is not affected in either group, whereas mean arterial pressure (MAP) is affected similarly in both groups.

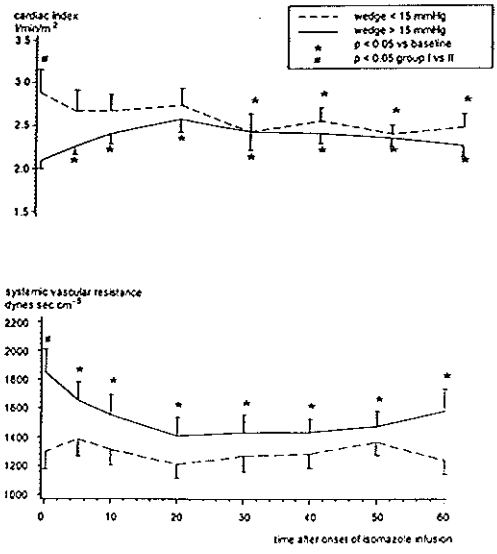

Fig. 2. Isomazole improved cardiac function in patients with elevated preload only. Concomitantly, systemic vascular resistance was reduced in these patients as well. Baseline cardiac index and systemic resistance differed significantly between both groups.

put and a reduction in LV filling pressure and systemic resistance. Thus far, only limited data are available regarding the hemodynamic profile and neurohormonal response of intravenous isomazole in patients with LV dysfunction (12,14).

In this study, isomazole increased contractility, improved relaxation, induced systemic vasodilation, and resulted in a significant reduction in LV filling pressure. Cardiac output, however, was not affected; neither was stroke volume or stroke work. Our observations in patients with LV dysfunction and mild to moderate heart failure differ from studies in more severe heart failure with agents with combined PDE-inhibiting and calcium-sensitizing properties, such as pimobendan, in which cardiac output generally improves (15). Subgroup analysis in our study revealed that the lack of improvement in cardiac output with isomazole is preload dependent. Cardiac output increases in patients with elevated LV filling pressures, but falls in patients with a normal preload (Fig. 4). Taking into account the more pronounced reduction in right atrial and LV systolic pressure and systemic resistance in group I against the background of a comparable inotropic effect in both patient groups, it would seem that the balanced vasodilating effect of the drug is more effective when LV filling pressures are elevated. The fall in cardiac output in conditions with low LV filling pressures reflects previous observations with nitrates, at least at the lower dose levels, where venodilating properties prevail. In our study, isomazole clearly reduced right ventricular filling pressures, suggestive of a venodilating effect. A similar, preload-dependent effect on cardiac pump function has been reported for milrinone (3). Another explanation for the better effect or isomazole in group I relates to wall stress. By decreasing abnormal wall stress through after- and preload reduction, cardiac function may improve more in these patients, with enlarged ventricles, than in those in whom changes in wall stress are less important, that is, in patients with normal ventricular size and normal to low filling pressures. This may be particularly true when the underlying etiology is ischemic cardiomyopathy.

Effects of Isomazole on Relaxation

For a number of reasons, calcium sensitization constitutes a more optimal approach to the positive inotropic support in heart failure than PDE inhibition (16). Its major drawback concerns slowing of relaxation, which may be considerable when agents with exclusive calcium sensitizing properties are used (17). This negative effect on relaxation may also counteract an improvement in

Table 4. Sequential Coronary Hemodymanic Effects of Isomazole (Overall Patient Group)

Parameter	Control	Minutes After Isomazole Administration					
		10	20	30	40	50	60
CSBF (mL/min)	150 ± 16	150	136	133	129	135	136
CVR (mmHg/mL/min)	0.77 ± 0.08	0.76	0.75	0.73	0.78	0.72	0.74
MVO_2 (mL/min)	19.2 ± 2.3	18.5	16.5	15.7*	15.9*	16.9	16.2
DP	10.9 ± 0.5	10.7	10.7	10.4	10.3	10.2	10.3
Lactate extraction (%)	22.5 ± 3.3	19.8	9	16.2	13.6	16.7	15.5
Lactate uptake (mol/min)	20.5 ± 3.6	15.4	7	11.1	10.4	14.4	16.9

CSBF, coronary sinus blood flow; CVR, coronary vascular resistance; DP, double (rate–pressure) product; MVO_2, myocardial oxygen consumption. Data are means ± SEM (control) and means (isomazole).
*$P < .05$ versus control.

Table 5. Effects of Isomazole on Circulating Neurohormones and Cardiac Neurohormonal Balance (Overall Patient Group)

Neurohormone	Control	30 Minutes	60 Minutes
Norepinephrine			
Arterial (pg/mL)	349 ± 53	444 ± 61*	436 ± 58*
Coronary venous (pg/mL)	461 ± 89	532 ± 76*	556 ± 86*
Cardiac balance (pg/min)	−24.8 ± 12	−13 ± 5.3	−16.7 ± 6.4
Epinephrine			
Arterial (pg/mL)	144 ± 61	123 ± 50	128 ± 44
Coronary venous (pg/mL)	101 ± 42	85 ± 40	89 ± 35
Cardiac balance (pg/min)	6 ± 2.9	5.8 ± 2.2	6.1 ± 2.1
Dopamine			
Arterial (pg/mL)	47 ± 7	67 ± 13	45 ± 8
Coronary venous (pg/mL)	46 ± 8	43 ± 5	47 ± 7
Cardiac balance (μU/min)	0.45 ± 0.83	4.6 ± 2.09	−0.1 ± 0.89
Renin			
Arterial (μU/mL)	31 ± 14	34 ± 15	40 ± 16*
Coronary venous (μU/mL)	30 ± 13	33 ± 13	35 ± 15
Cardiac balance (μU/min)	93 ± 203	267 ± 426	494 ± 445
Angiotensin II			
Arterial (pg/mL)	15 ± 6	15 ± 6	21 ± 7
Coronary venous (pg/mL)	11 ± 5	11 ± 7	13 ± 7
Cardiac balance (pg/min)	0.51 ± 0.2	0.31 ± 0.14	0.76 ± 0.3
Aldosterone			
Arterial (pg/mL)	173 ± 54	125 ± 51	152 ± 52
Coronary venous (pg/mL)	169 ± 54	121 ± 50	134 ± 47
Cardiac balance (pg/min)	0.12 ± 0.67	0.52 ± 0.92	0.92 ± 2.56
Atrial natriuretic peptide			
Arterial (pg/mL)	321 ± 116	223 ± 83	304 ± 164
Coronary venous (pg/mL)	1850 ± 393	988 ± 234*	768 ± 164*
Cardiac balance (ng/min)	−227 ± 46	−103 ± 31*	−71 ± 15*

Data are means ± SEM.
*$P < .05$ versus control.

contractility (18). Additional cAMP-dependent properties, for instance by way of partial PDE inhibition, may oppose these negative effects on relaxation. In this study, the isovolumetric relaxation parameters Tau, Tau_1, and Tau_2 improved rather than deteriorated, indicating the existence of PDE-inhibiting properties of isomazole. Similar lusitropic effects have been observed in studies with pimobendan, a comparable-type agent (15,19).

Coronary Hemodynamics, Myocardial Energetics, and Metabolism

Coronary flow and resistance were not affected by isomazole. The reduction in myocardial oxygen consumption in the absence of alterations in coronary flow and resistance is consistent with previous findings with pimobendan (15), but differs from observations with predominant PDE inhibitors such as milrinone and enoximone under similar conditions, in which coronary flow and myocardial oxygen consumption increase secondary to increased myocardial oxygen demand (20,21). The absence of this nonfavorable effect on myocardial energy expenditure with isomazole possibly relates to its calcium-sensitizing properties as the acute vasodilating properties in this study were comparable to those in the latter studies. In several *in vitro* and *in vivo* experiments calcium sensitization increased cardiac contractile force, without additional energy cost.

Circulating Neurohormones and Cardiac Neurohormonal Balance

Enhanced sympathetic and/or renin–angiotensin activity often results from acute vasodilating maneuvers and may lead to reflex vasoconstriction and tachyphylaxis (22,23). In this study, arterial norepinephrine and renin levels increased in the overall group, most likely related to the concomitant reduction in arterial pressure. In contrast, coronary venous ANP levels and cardiac ANP release decreased, most likely reflecting the reduction in LV and right ventricular filling pressures. Subgroup analysis in this study indicated that the arterial norepinephrine levels did not change in patients with elevated LV filling pressures, but, in contrast, rose significantly in patients with low to normal filling pressures, in whom cardiac output fell during isomazole administration. Secondary to this sympathetic activation in the latter, systemic vascular resistance increased significantly, counteracting the vasodilating effects of isomazole. In patients with increased preload the renin–angio-

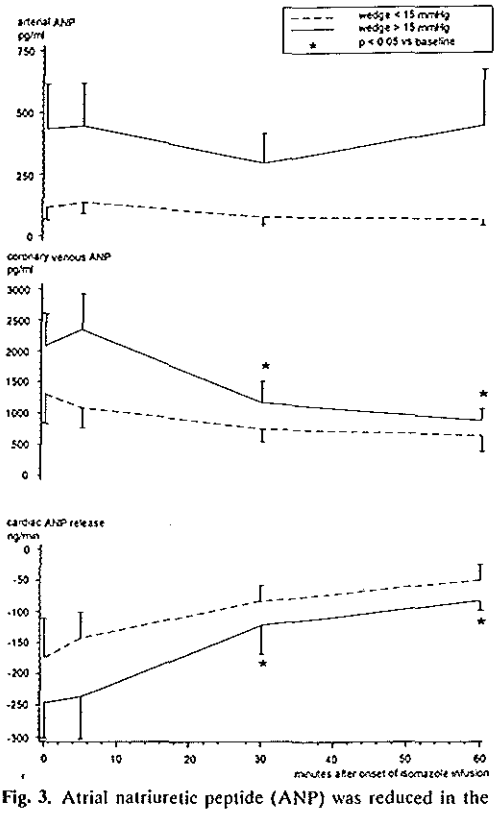

Fig. 3. Atrial natriuretic peptide (ANP) was reduced in the coronary sinus of patients with elevated wedge pressure only. Cardiac ANP release was reduced significantly more in these patients than in patients with normal wedge pressures at baseline.

tensin system became less activated, most likely reflecting the improvement in cardiac output without sympathetic activation. This improvement in cardiac pump function and the numerically greater reduction in right ventricular and LV filling pressures in these patients translated into a significantly greater reduction in coronary venous ANP levels and in cardiac ANP release. Previous investigators have indicated a direct relation between circulatory ANP levels and changes in right atrial pressure (24,25).

Limitations of the Study

Although patient characteristics are quite similar in both groups, considerable differences in hemodynamic parameters, such as LV and right ventricular filling pressures, cardiac output, and systemic resistance, are observed. This may be related to the withdrawal of pre-

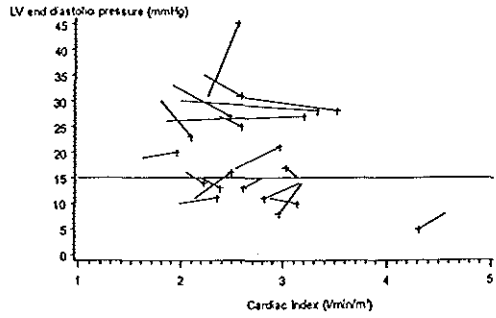

Fig. 4. Individual changes from baseline. The final values of the changes from baseline are marked with plus signs. The majority of patients in group I (baseline [LV] left ventricular end-diastolic pressure > 15 mmHg) have an increasing cardiac index, whereas patients in group II generally have a slight decrease in cardiac index.

study medication, which was quite different in both groups. In group I, there were more patients on ACE inhibitors, digoxin, and diuretics.

As there was no placebo group in this study there is no indication to what extent regression to the mean and random variation contributes to the observed effects; however, the effect of regression to the mean was minimized by waiting until a stable baseline situation was reached, which was checked by multiple control measurements. The acute effects observed afterward were pronounced and clearly related to the substance under investigation. The small number of patients, short duration of the study, and use of a single dose of isomazole allow extrapolation to larger groups and longer duration only with great caution. Also, this study does not allow differentiation between PDE-inhibiting effects and calcium-sensitizing effects. Their relative contribution to the overall hemodynamic profile of isomazole is not possible to establish; however, the positive lusitropic and the vasodilating effects clearly point to the presence of PDE-inhibiting properties, whereas the positive inotropic effects elaborate the presence of calcium-sensitizing properties.

Clinical Implications

Although during long-term oral use the hemodynamic and neurohormonal effects may be quite different from those during acute, intravenous administration, our study suggests a favorable effect of intravenous isomazole in conditions in which cardiac filling pressures are elevated, that is, advanced or acute exacerbations of heart failure. On the other hand, its usefulness is questionable in stable mild heart failure, particularly when LV filling pressures are likely to be normalized or even

low due to concomitant ACE inhibition and diuretic therapy.

Isomazole, as a partial PDE inhibitor with calcium-sensitizing properties, has a more favorable profile than compounds with predominantly PDE-inhibiting properties, at least where myocardial oxygen consumption and neurohormonal activation are concerned. As isomazole was tolerated well in our study, further investigations for evaluation of long-term effects, efficacy, and safety will have to be performed.

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Chapter 7

A non-invasive selective assessment of type I fibre mitochondrial function using ^{31}P NMR spectroscopy. Evidence for impaired oxidative phosphorylation rate in skeletal muscle in patients with chronic heart failure.

M. van der Ent, J.A.L. Jeneson, W.J. Remme, R. Berger, R. Ciampricotti and F. Visser.

A non-invasive selective assessment of type I fibre mitochondrial function using ^{31}P NMR spectroscopy

Evidence for impaired oxidative phosphorylation rate in skeletal muscle in patients with chronic heart failure

M. van der Ent*, J. A. L. Jeneson†, W. J. Remme*, R. Berger†, R. Ciamprlcotti‡ and F. Visser§

**Sticares Foundation, Rotterdam, The Netherlands; †Laboratory of Metabolic Diseases, University Children's Hospital, Utrecht, The Netherlands; ‡Streekziekenhuis De Honte, Terneuzen, The Netherlands; §Academisch Ziekenhuis Vrije, Amsterdam, The Netherlands*

Background Skeletal muscle abnormalities contribute considerably to the clinical expression of heart failure. Deconditioning, underperfusion and an increased number of type IIb glycolytical fibres lead to early lactate production and muscle fatigue at low exercise levels. Aerobic muscle metabolism may also be impaired, as suggested by biopsy studies. Thus far, no data are available from non-invasive studies to indicate the extent of aerobic muscle dysfunction during low-grade exercise which does not induce acidosis.

Methods and results Mitochondrial function of skeletal muscle during fibre type I activation was studied in 22 patients with chronic heart failure [NYHA class III, left ventricular ejection fraction 28 ± 2%, (patients)] on ACE inhibitors, diuretics and digoxin, and in 20 normal subjects, using ^{31}P NMR spectroscopy of a single right forearm flexor muscle during three mild intermittent exercise levels (0–40% of maximum voluntary contraction) and recovery time. At rest, the inorganic phosphate/phosphocreatine ratio was different [0·13 ± 0·005 (patients) vs 0·09 ± 0·002 (normal subjects), P=0·0001]. However, intracellular pH was comparable. Local acidosis (tissue pH <6·9) was avoided to prevent fibre type IIb activation. Calculated resting phosphate potential levels were comparable, but the slope and intercept of the linear relationship of phosphate potential and workload were significantly lower in patients than in normal subjects (11·7 ± 0·7 vs 15·8 ± 0·6 and 139 ± 7 vs 196 ± 7, patients vs normal subjects, indicating early exhaustion of intracellular energy at lower exercise levels. Also, maximum calculated workload at which tissue ADP stabilized was lower in patients than in normal subjects (88 ± 7% vs 120 ± 4% of maximum voluntary workload, patients vs normal subjects. P<0·05). Time to recovery to pre-test phosphocreatine levels was prolonged by 46% in patients compared to normal subjects (P<0·05).

Conclusions In heart failure, oxidative fibre mitochondrial function in skeletal muscle is impaired, as reflected by the reduced phosphate potential and oxidative phosphorylation rate, early exhaustion and slowed recovery of intracellular energy reserve at workloads, which do not affect intracellular pH.
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Key Words: Heart failure, mitochondrial function, ^{31}P NMR spectroscopy, skeletal muscle, phosphorylation rate, muscle fibre type.

Introduction

Reduced exercise tolerance is a typical clinical feature of the heart failure syndrome. Several studies have suggested that besides deconditioning and underperfusion, secondary to the reduction in cardiac pump function, intrinsic changes in peripheral skeletal muscle contribute to this phenomenon[1]. Changes in the fibre type composition of skeletal muscle, and abnormalities in the energy metabolism in skeletal muscle cells have been described in 'in vitro' studies[2]. In vivo investigations in muscle biopsy specimens have also suggested that oxidative ATP synthesis is impaired in heart failure patients. Moreover, mitochondrial crista volumes are reduced, which suggests mitochondrial dysfunction[3].

^{31}P NMR studies during exercise in patients with heart failure have demonstrated abnormal metabolic changes, such as early acidification, increased

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Correspondence: W. J. Remme, MD, PhD, Sticares Foundation, P.O. Box 52006, 3007 LA Rotterdam, The Netherlands.

phosphocreatine utilization and concomitantly increased inorganic phosphate levels. Moreover, recovery to pre-exercise phosphocreatine levels is slowed in some patients[4–7]. As yet, the extent to which this mitochondrial dysfunction contributes to the symptomatology of heart failure is unclear. To address this issue 'in vivo', using ^{31}P NMR spectroscopy techniques, it is required that the oxidative capacity of a homogeneous population of fibre types and, consequently, of comparable mitochondrial content is studied. Results from ^{31}P NMR studies investigating mitochondrial function reported so far have been ambiguous as they did not meet this requirement since both oxidative and glycolytic fibres were recruited in the voluntary exercise regimens used[6]. In studies conducted on forearm flexor muscles and employing voluntary handgrip exercise, this could be attributed to poor control over the mechanical workload on individual fibres within the population of fibres under investigation[8–11]. Recently, an experimental design for ^{31}P NMR measurement of the work–cost relationship in single human finger flexor muscle was described in which the mechanical workload could be carefully controlled, thus allowing selective recruitment of only oxidative fibres within the sampled muscle mass[11].

In the present study, we applied this experimental design to measure the variation in phosphate metabolite concentrations and pH at different levels of muscle power output in oxidative fibres of forearm muscle of patients with stable, moderate heart failure. Our results indicate that mitochondrial dysfunction does contribute to the reduced exercise tolerance of skeletal muscle in heart failure.

Methods

Patients

After informed consent and after approval of the protocol by the local Ethical Review Committee, 20 male and two female patients with heart failure NYHA class III and a left ventricular ejection fraction $\leq$45% participated in the study. All patients had been on stable ACE inhibitor and diuretic therapy for at least 4 weeks before nitrates were withdrawn the day before the study; other vaso-active medication was stopped 3–5 days pre-study, depending on plasma half-lives. Control data of forearm skeletal muscle bioenergetics were obtained from 20 healthy untrained volunteers. Fifteen subjects were male, five female, mean age 22 years (range 8–40 years). The ^{31}P NMR data of this group are reported elsewhere[12]. Patient characteristics are shown in Table 1.

Table 1 Patient characteristics

Number of patients	22
Sex	20 males, 2 females
Age	63 ± 2 years (range 43–77 years)
NYHA class	III
Previous infarction	15
Idiopathic cardiomyopathy	7
LV ejection fraction	26 ± 2% (range 10–44%)

Controls: 20 healthy, untrained volunteers, aged 8–40 years.

NMR measurements

^{1}H MR images and ^{31}P NMR spectra were obtained using a Philips S15 HP whole body NMR spectrometer (Eindhoven, The Netherlands) operating at 1·5 T (clear bore diameter after removal of the body coil: 70 cm). Positioning of the patients and localization of the flexor digitorum profundus muscle of the right forearm was performed using magnetic resonance imaging. ^{31}P NMR spectra were obtained by collecting data from 50 exercise cycles at each workload, using a 400 ms window during exercise. Data from the spectrum before and after the contracted state of the muscle were discarded. Data processing was performed off-line.

Exercise protocol

The exercise protocol and equipment have been described elsewhere[10]. Briefly, exercise involved bulb-squeezing with visual feedback of power-output on an audio-signal using only the tips of the fourth and fifth digit at three steady-state workloads normalized to maximum voluntary contraction in a ramp protocol. Rather than using fixed workloads for all, the load of the second and third work level for each individual were set by the supervising physician by monitoring on-line the metabolic response to the first, lowest workload to meet each subject's individual oxidative capacity. The frequency of bulb-squeezing was four times per minute. Power output was measured and recorded for calculation of actual power output at each workload. At the end of each protocol, τ, the time to recovery to pre-study phosphocreatine levels, was measured.

Calculation of metabolite concentrations

The ATP and total creatine concentrations were assumed to be identical for normal subjects and patients. The mean values of these metabolites reported for human upper leg muscle (8·2 mM and 42·7 mM, respectively)[13] were used in our calculations. The phosphate (Pi) and phosphocreatine (PCr) concentrations in the resting state were calculated from the measured ratios over ATP, using saturation correction factors of 1·2 and 1·4, respectively, determined from fully relaxed spectra. The average free ADP concentration in fibres within the sampled muscle mass was calculated from the creatine kinase equilibrium according to the equation:

$$[ADP]=[ATP][Cr]/(1{\cdot}66\times10^{9})(10^{-pH})[PCr] \quad (1)$$

where $1{\cdot}66 \times 10^9$ is the equilibrium constant for creatine kinase reported by Veech[14] and [Cr] is the creatine concentration. From these values, the concentration-dependent term ln([ATP]/[ADP][Pi]) in M^{-1} of the free energy of ATP hydrolysis (ΔG_p) or phosphate potential was calculated.

Determination of oxidative phosphorylation capacity

Two measures of the muscle's capacity for oxidative phosphorylation were obtained based on the steady-state equations relating to cellular ATP consumption rate (dominated by ATPase rate) and ATP synthesis rate (dominated by mitochondrial ATPase rate at constant pH 7·0). The derivation of the steady-state equations that were used has been described previously[12]. The first equation concerns a linear function of the phosphate potential vs steady-state power output data of the form:

$$P = m \times \ln([ATP]/[ADP][Pi]) + b \qquad (2)$$

where m and b are parameters containing kinetic proportionality and proton-coupling stoichiometry constants of oxidative phosphorylation (dimensions: % maximum voluntary contraction × $mol.l^{-1}$ and % maximum voluntary contraction, respectively). The absolute value of m proportionally reflects the cellular oxidative phosphorylation capacity and was used as the first 'in vivo' measure of the latter[15]. Secondly, an estimate of the maximal steady-state power output (which is oxidative ATP fuelled) was obtained for each subject from the fit of a hyperbolic function to the [ADP] vs steady-state power output data of the form:

$$P = C1 \times [ADP]/[ADP] + Km) - C2 \qquad (3)$$

where P is the steady-state power generated by muscle fibres (maximum voluntary $contraction^{-1}$), C1 and C2 are parameters reflecting maximal and basal oxidative phosphorylation rate (dimension: both in % maximum voluntary contraction) and Km is the [ADP] at half-maximal respiration rate (in μM) for the given boundary condition of the total adenine nucleotide and creatine content of the flexor digitorum profundus muscle. At high [ADP] values, the function given by the equation approaches a value of (C1–C2), the maximal steady-state power output (% maximum voluntary contraction) which was used as the second measure of the cellular capacity for oxidative phosphorylation.

Statistical calculations

ANOVA linear and non-linear curve fitting were performed using SAS/STAT® software (version 6·10). All data are presented as mean ± standard error of the mean. Unpaired Student's t-tests were performed on measurement comparisons between groups where a *P*-value $\leq 0{\cdot}05$ was considered statistically significant.

Results

Accurate, MRI-guided, surface coil placement on the superficial region of the flexor digitorum profundus, corresponding to fibres involved in flexing of the fourth and fifth digit, was achieved in all patients. Also, exercise was completed by all patients according to protocol. In the resting state, phosphocreatine levels were lower in patients than in normal individuals ($33{\cdot}4 \pm 0{\cdot}5$ vs $36{\cdot}6 \pm 0{\cdot}6$ mM, respectively) and phosphate levels were higher ($4\,3 \pm 0{\cdot}2$ vs $3{\cdot}2 \pm 0{\cdot}1$ mM, respectively). Consequently, the phosphate/phosphocreatine ratio was different ($0{\cdot}13 \pm 0{\cdot}005$ in patients vs $0{\cdot}09 \pm 0{\cdot}002$ in normals). Intracellular pH in patients was similar to that in normal individuals ($7{\cdot}05 \pm 0{\cdot}03$ vs $7{\cdot}06 \pm 0{\cdot}02$, respectively). During exercise, the magnitude of changes in phosphate and phosphocreatine was greater in patients, as illustrated in Fig. 1, which shows a typical series of ^{31}P NMR spectra obtained from forearm muscle of a control and a patient at identical power output levels. Importantly, intracellular pH decreased only to $6{\cdot}96 \pm 0{\cdot}11$ at the highest workload both in normal individuals and in patients. This is also illustrated in Fig. 1, by the observation that the line widths of phosphate and phosphocreatine were similar at all power output levels. In seven patients, intracellular pH intermittently dropped to low values (6·95–6·71) during the first workload, but recovered to the normal range of values at subsequent workloads. Data from these workloads were excluded from the analysis. Figure 2 shows the linear correlation of the concentration-dependent term of the phosphate potential (ln(ATP/ADP × Pi), and power output predicted by equation (2) for the pooled data of all 22 patients ($r^2 = 0{\cdot}72$). At zero workload, i.e. the resting state, the phosphate potential was comparable in both groups. Individual line fitting used to calculate slope m, had corrected r-square values ranging from 0·86 to 0·99. Compared to normal individuals, both the average slope m and intercept b were significantly lower, $11{\cdot}7 \pm 3{\cdot}0$ % maximum voluntary contraction × M and 139 ± 8 % maximum voluntary contraction in patients than in normal individuals ($15{\cdot}8 \pm 2{\cdot}5$ % maximum voluntary contraction × $mol.l^{-1}$ and 196 ± 7 % maximum voluntary contraction). Average values for m and b in patients were approximately 70% of normal phosphorylation rates. Figure 3 shows the variation of the average intracellular [ADP] in fibres with power output for the pooled data of all 22 patients. In patients, higher intracellular [ADP] levels are associated with lower power output levels compared to normal individuals. In addition to the phosphorylation rates, the average C1 value for maximal O_2 consumption, estimated for each individual separately, differed significantly, 57 ± 20 % maximum voluntary contraction in

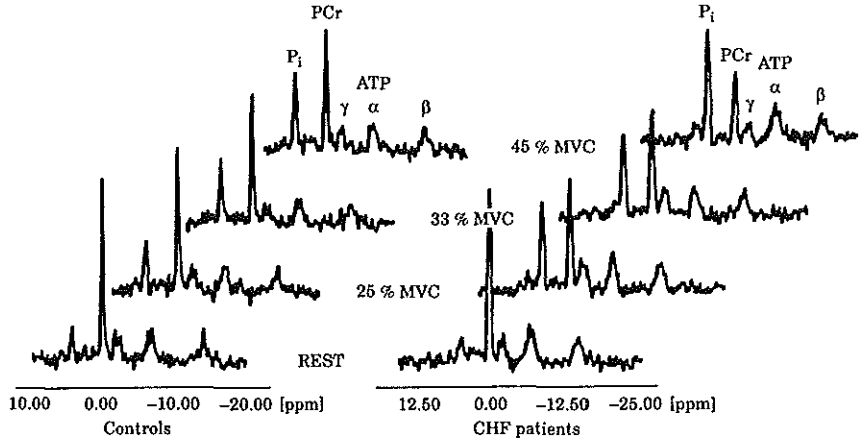

Figure 1 Presentation of ^{31}P NMR spectra taken at rest and during exercise from the flexor digitorum profundus muscle in patients with chronic heart failure and normal untrained individuals. Note the rapid decline in the phosphocreatine (PCr) peak and the rapid incline in the inorganic phosphate peak (Pi) in heart failure patients compared to normals, already at very low exercise levels. Also, at the highest exercise level, the peak widths are still narrow, which is indicative of stable intracellular pH.

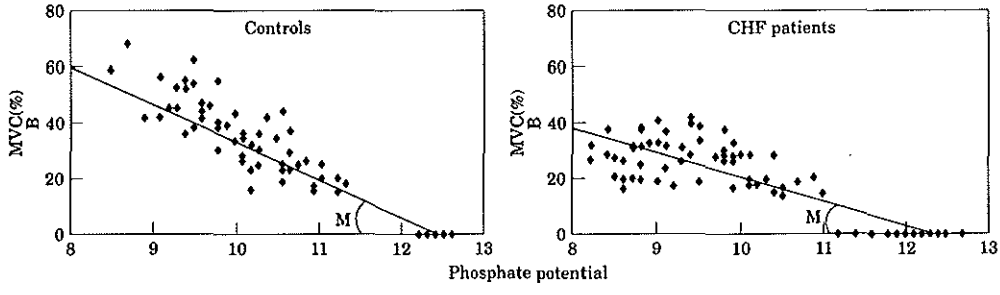

Figure 2 Graph showing the linear relationship between the phosphate potential and maximum voluntary contraction (MVC). Note that the slope m and intercept b are significantly lower in heart failure patients (CHF) compared to normal individuals (controls), which is indicative of lower phosphorylation rates. At zero workload the average values for the phosphate potential are comparable in both groups.

patients vs 120 ± 17 % maximum voluntary contraction in normal individuals, a decrease of 53%. However, values at zero workload were comparable in both groups. The C2 value, indicative of basal O_2 consumption, was comparable in both groups (29 ± 1 in normals vs 25 ± 4 % maximum voluntary contraction in patients). In contrast, maximal steady-state power output (C1–C2), calculated for each individual from the fit of the equation (3) to the [ADP] vs power output data, was lower in patients (63 ± 3 vs 91 ± 3 % maximum voluntary contraction in normals). As Fig. 4 shows, there is a positive linear correlation between slope m and (C1–C2). Adjusted r^2 for this fitted line is 0·74. Along this line, a group-wise distribution of normal subjects and heart failure patients can be noticed, where heart failure patients are associated with the domain corresponding to lower mitochondrial function. Individual data are displayed in Table 2. The calculated time constant τ of phosphocreatine resynthesis after exercise was found to be significantly prolonged in the group of heart failure patients, 65 ± 19 s vs 42 ± 11 s in normal subjects, a prolongation of 55%.

Discussion

In the present study of skeletal muscle bioenergetics in heart failure we observed a significantly altered work–energy cost relationship of forearm finger flexor muscle of patients with stable, moderate heart failure. Importantly, the magnitude of changes in phosphocreatine and phosphate levels and in intracellular pH to

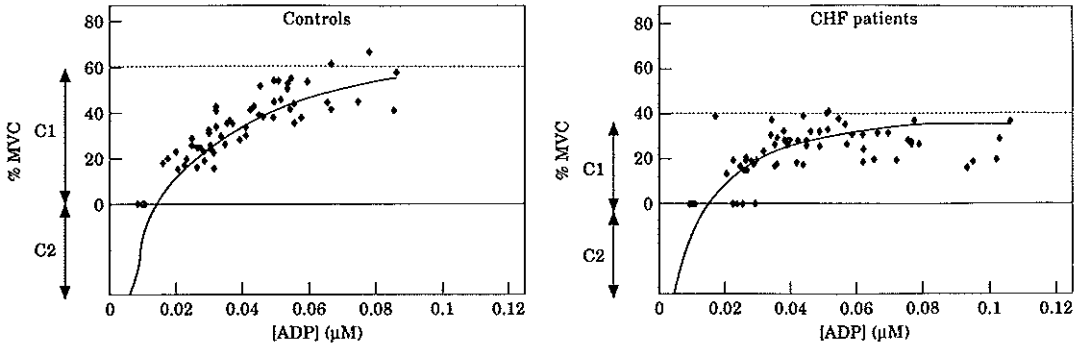

Figure 3 Graph showing the hyperbolic relationship between intracellular ADP concentrations and maximum voluntary contraction (MVC). In CHF patients the C1 value, indicating maximal O_2 consumption, is significantly lower than in normals. The basal O_2 consumption however, is comparable in both groups. C1–C2, which represents the maximal ATP-fuelled steady-state power output, is significantly lower in CHF patients than in normals.

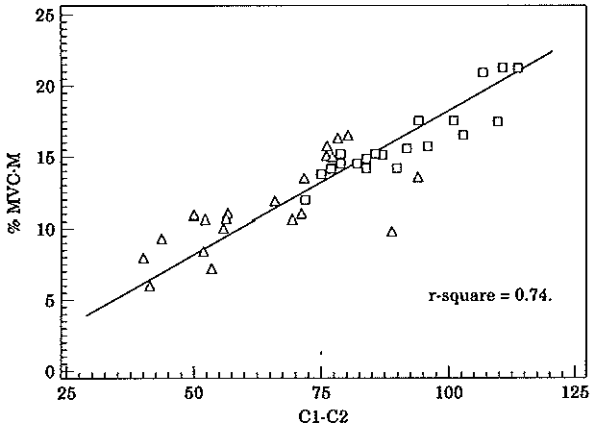

Figure 4 Graph showing the linear correlation of phosphorylation rate m against the maximal steady-state power output C1-C2. Correlation between these parameters is highly significant. Along this line, there is a difference in distribution between heart failure patients and normal individuals. △=chronic heart failure patients; □=controls.

exercise at normalized, submaximal workloads was strikingly homogeneous for the 22 patients. No patients exceeded the range of mean ± 2 standard deviations for these parameters. This observation is in sharp contrast with the findings in previously reported studies of forearm flexor muscle bioenergetics in heart failure patients, where a highly heterogeneous metabolic response to identical exercise regimens was observed amongst patients[4]. We attribute this homogeneity in our data to the adequate control of the population of fibres that was metabolically and mechanically sampled in the present study. This was also illustrated by the observation that in both patients and normal individuals at the highest workload the lowest-measured intracellular pH was 6·90, only 0·15 units lower than the resting state value (7·05) and homogeneous amongst fibres within the sampled muscle mass, as indicated by the narrow line width of the phosphate peak (Fig. 1). According to studies on the transition of aerobic to anaerobic metabolism in human forearm muscle employing both ^{31}P NMR and electromyography, this indicates that oxidative (type I and IIa), and not glycolytic (IIb) fibres were recruited during the voluntary exercise programme used[16,17]. In addition, the maximum workload in this study was at a very modest level (no more than 40% of maximum voluntary contraction). Thus, the maximum work demanded by the protocol was not limited by the number and oxidative capacity of mitochondria present in the recruited fibres. Therefore, the present protocol was not used to determine the exercise limit in these

Table 2 Individual values of metabolic parameters assessed by ^{31}P NMR spectroscopy of human forearm muscle

Heart failure patients				Normal individuals			
Patient number	Baseline Pi/PCr ratio	C1–C2	m	Patient number	Baseline Pi/PCr ratio	C1–C2	m
1	0·13	76	16	1	0·08	114	21
2	0·12	52	8	2	0·08	90	14
3	0·12	44	9	3	0·10	84	14
4	0·11	57	11	4	0·09	110	17
5	0·13	48	18	5	0·09	111	21
6	0·14	78	16	6	0·08	79	14
7	0·12	40	8	7	0·09	103	16
8	0·09	56	11	8	0·09	101	17
9	0·13	66	12	9	0·10	92	15
10	0·13	70	11	10	0·08	107	21
11	0·11	56	10	11	0·08	75	14
12	0·17	77	15	12	0·09	79	15
13	0·08	71	11	13	0·08	72	12
14	0·15	54	11	14	0·10	82	15
15	0·15	72	13	15	0·10	94	17
16	0·15	94	13	16	0·09	84	15
17	0·14	80	16	17	0·07	87	15
18	0·13	76	15	18	0·11	86	15
19	0·15	50	11	19	0·07	77	14
20	0·17	89	10	20	0·10	96	16
21	0·08	41	6				
22	0·10	54	7				
$\bar{x}$	0·13	63	11·7	$\bar{x}$	0·09	91	15·8
± SEM	± 0·005	± 3	± 3·0	± SEM	± 0·002	± 3	± 2·5

Average Pi/PCr ratios at rest differ significantly between patients and normal individuals. However, these values do not relate to the metabolic parameters of activated mitochondria during low-grade exercise. Impaired maximum phosphorylation rates (m) and maximum ATP-fuelled steady-state power output (C1–C2) can be associated with normal baseline Pi/PCr ratios and vice versa.

patients. In this protocol, work was only used to get the mitochondria out of their resting state. The prerequisite condition for valid interpretation of our results with respect to mitochondrial functionality, i.e. that the measurements have to be performed in a population of fibres that is relatively constant in its mitochondrial content in both patients and normal subjects, was therefore adequately met. This contrasts with previous studies and is likely to contribute to the reproducibility of the data obtained in our study. The use of a control group that was not age matched in this study is justified. An 'in vitro' study by Trounce *et al.* suggested that mitochondrial function declines with age[18], however this was most pronounced in the age group over 75, which had skeletal muscle respiration rates of about half the rate in younger subjects. In our study, respiration rates were reduced by about 50% independent of age, whereas all our patients were younger than 75 years. Moreover, previous studies failed to observe age-related differences in skeletal muscle bioenergetics, using nuclear magnetic resonance[19]. This suggests that there were skeletal muscle abnormalities other than functional changes induced by age alone. Even so, the protocol used in our study does not depend on changes in the number of mitochondria or secondary muscular atrophy. This is accounted for by the use of a relatively low power output during exercise[10].

In a small subgroup of patients, a transient rapid decline of pH was observed during exercise at the first workload only, recovering to the range of normal values during exercise at subsequent workloads. Although local blood flow was not measured in this study, this may be interpreted as reflecting a temporal flow impairment causing rapid deoxygenation and increased anaerobic glycolytic flux. Apparently, spontaneous flow adaption occurred at subsequent workloads in view of the transient nature of the decline in pH. This phenomenon has also been observed in normal subjects, albeit less commonly[20]. Since this was only observed in a small subgroup of patients, the results of the present study can be considered supportive of the hypothesis that flow impairment is not a major contributor to the abnormalities of skeletal muscle bioenergetics in heart failure[4,6,21].

In patients, abnormal baseline phosphate/phosphocreatine ratios were observed in the presence of normal baseline phosphorylation rates. In addition, basal phosphorylation rates and oxygen consumption were comparable in patients and normal subjects. However, the lower phosphorylation rates during exercise, indicated by a lower value for slope m and intercept b (Fig. 2), demonstrate that the intracellular energy buffer is depleted more rapidly in patients than in normal individuals. This is confirmed by the lower maximal O_2

consumption (C1) and lower maximal steady-state power output (C1–C2), reflected by more ADP production at lower power output levels. Under conditions of adequate flow and homogeneous, muscle fibre activation. this observation is in agreement with previous studies of skeletal muscle energy metabolism[2,3,7]. Also, the prolonged recovery after exercise is in line with a reduced capacity for oxidative phosphorylation.

In the present study, skeletal muscle mitochondrial dysfunction was present and is most likely involved in the clinical status of our heart failure patients. 'In vivo' oxidative phosphorylation capacity parameters were all reduced to the same extent, i.e. approximately by 30%. In a plotted representation of maximal phosphorylation rates and maximum steady-state power output, reflecting mitochondrial function (Fig. 4), heart failure patients can be distinguished from normal subjects. The protocol used provides us with a non-invasive means of collecting 'in vivo' data, which are highly reproducible. Whether these values are related to the severity or duration of heart failure cannot be deduced from our study results.

In conclusion, in patients with chronic heart failure, aerobic metabolism in peripheral muscle becomes already impaired at very modest exercise levels under conditions where acidosis is avoided and predominantly oxidative phosphorylation pathways are activated. Therefore, the cellular capacity for oxidative phosphorylation is substantially reduced in these patients compared to normal individuals.

In addition to the established lower mitochondrial density of skeletal muscle in heart failure, mitochondrial dysfunction may contribute to the clinical feature of reduced exercise tolerance. The underlying mechanisms are not entirely clear. Abnormal skeletal muscle flow may be one cause, although in our study abnormalities in aerobic metabolism already occurred under conditions in which flow may be presumed to be adequate. Deconditioning could be another reason. Several studies suggest that exercise training may improve skeletal muscle metabolism in heart failure patients, although these studies do not focus on aerobic metabolism, as was done in the present study[22,23].

The observation of intrinsic abnormalities in oxidative phosphorylation in the present study supports the potential usefulness of metabolic therapy aimed at improving aerobic muscle metabolism. This novel form of therapy is rapidly gaining interest in the management of heart failure.

Note added in proof

It has since been learned that equation (3) that was used to estimate the metabolic parameter C1-C2 is second, not first order in [ADP] (Jeneson JAL, Wiseman RW, Westerhoff HV, Kushmerick MJ: The signal transduction function for oxidative phosphorylation is at least second order in ADP. J Biol Chem, 1996, 271: 27995–27998). While this affects the *absolute* value of C1-C2 that is obtained from fitting eqn (3) to the ([ADP], power-output) data, this would not alter the *relative* change in C1-C2 in patients versus controls that was studied here (Fig. 4).

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Chapter 8

Summary

Summary

Several studies have indicated that neurohumoral activation, involving catecholamines, the renin-angiotensin system, ANP, aldosterone and vasopressin, is closely related to mortality and morbidity in chronic heart failure. Hemodynamic effects of different neurohormones are either vasodilating or vasoconstrictive. In addition growth modulating and promoting actions have been described.

The aim of the present thesis was to investigate neurohumoral changes in relation hemodynamic parameters at different stages of heart failure and the effects of pharmacological treatment on neurohormones.

Chapter 1 provides an overview of the known hemodynamic effects of the different neurohormones and the effects on hemodynamics and neurohormones of different medications like ACE-inhibitors, diuretics, vasodilator agents, digoxine, β-blockers, calcium antagonists, phosphodiesterase inhibitors and dopaminergic agents.

Chapter 2 presents a study on 107 patients with either normal LV function, asymptomatic LV dysfunction or symptomatic LV dysfunction and compares these groups, with respect to hemodynamic parameters and neurohumoral levels. Markedly impaired myocardial function and neurohumoral activation (catecholamines, renin, angiotensin II and ANP) were present in patients with symptomatic LV dysfunction. In contrast, in patients with asymptomatic LV dysfunction only ANP levels were increased, in spite of significant impaired myocardial and pump function. This suggests that ANP activation precedes activation of other neurohormones and prevents or delays further deterioration towards symptomatic LV dysfunction by direct vasodilation and natriuresis and by attenuation of the renin-angiotensin system and catecholamine activation.

Chapter 3 addresses a study on 137 patients with normal LV function or asymptomatic LV dysfunction, both with and without chronic β-blocker therapy, with respect to hemodynamic parameters and neurohumoral levels. Cardiac output, myocardial contractility and left ventricular relaxation were impaired in patients with normal LV function treated with β-blockade, whereas these effects were not seen in the asymptomatic LV dysfunction group.

In contrast, β-blockade reduced cardiac ANP release in patients with asymptomatic LV dysfunction and increased cardiac ANP release in normal LV function. Thus, in patients with normal LV function, chronic β-blockade results in a reduction in myocardial contractility and cardiac pump function. Since these effects are much less pronounced or not present in impaired LV function, chronic β-blockade does not further jeopardize cardiac function in patients with asymptomatic LV dysfunction.

In chapter 4 data on 12 patients with severe heart failure, undergoing treatment with carmoxirole, a selective dopamine (D_2) receptor agonist, are reported. Carmoxirole modulates sympathetic activation, accompanied by decreases in vasopressin and ANP, and activation of the renin-angiotensin system at higher dosages. These effects lead to a reduction in systemic vascular resistance, lowering of heart rate, resulting in an improvement in cardiac pump function and left and right filling pressures. Therefore, carmoxirole produces beneficial effects on hemodynamic and neurohumoral parameters in heart failure.

Chapter 5 reports data from 12 patients with severe heart failure during chronic treatment with trandolapril, a potent lipophilic ACE inhibitor. Neurohormones, systemic and renal hemodynamics were studied. Long-term treatment with trandolapril resulted in sustained reduction in norepinephrine, angiotensin II and aldosterone levels, reduced preload and improved renal perfusion, indicated by a persistent increase in renal blood flow and preservation of renal function in severe heart failure.

In chapter 6 the acute effects of two phosphodiesterase inhibitors, nanterinone and isomazole, both with calcium sensitizing properties, are presented. Both improve myocardial function, however this effect is preload dependent. Markedly reduced neurohumoral activation was observed. In patients with normal or low preload, determined as a pulmonary capillary wedge pressure lower then 15 mmHg, treatment with phosphodiesterase inhibitors in these studies led to a deterioration of pump function and increased sympathetic tone. This implies that treatment with these agents may be beneficial only in patients with markedly increased preload at the dosages used in these studies.

In chapter 7 the mitochondrial function of peripheral skeletal muscle in 22 patients with heart failure NYHA class II-III, and 20 patients with normal LV function was

investigated. We applied a novel non-invasive method, using ^{31}P NMR spectroscopy, and found that in heart failure, oxidative fiber mitochondrial function in skeletal muscle is impaired. This may contribute to the clinical features of the heart failure syndrome.

Conclusion

Increased sympathetic drive and norepinephrine levels may be the most prominent neurohumoral feature in the clinical syndrome of heart failure and are closely related to the severity of heart failure and mortality. Activation of other neurohormones is a predominantly compensatory mechanism to deal with this situation. Activation of ANP in early LV dysfunction may prevent norepinephrine levels to become elevated and cause further deterioration towards overt heart failure. In more advanced stages of heart failure the renin-angiotensin system, aldosterone, vasopressin and other catecholamine become activated, resulting in more increased sympathetic drive and vasoconstriction. Therefore, pharmacological intervention to break the negative spiral of cascading neurohumoral activation and deteriorating hemodynamic situation should be started early and adequately. However, the use of phosphodiesterase inhibitiors should be restricted to the more severe cases of heart failure. Careful hemodynamic monitoring and additional monitoring of neurohumoral status contributes to adequate pharmacological treatment of heart failure, and should be performed in patients with progressive heart failure. It should be appreciated that, in chronic heart failure, changes in energy metabolism occur in peripheral muscle, which may lead to persisting symptoms in adequately treated heart failure.

Therefore, future research on heart failure should focus on modulation of neurohumoral activation and improving quality of life and improvement of muscle function, if possible. Improvement in quality of life may outweigh improved survival per se, from a patient's point of view.

Samenvatting

Verschillende studies hebben laten zien dat activatie van neurohormonen, (catecholamines, het renine-angiotensine systeem, ANP, aldosterone en vasopressine) nauw gerelateerd is aan mortaliteit en morbiditeit bij hart falen. Hemodynamische effecten van verschillende neurohormonen zijn zowel vasoconstrictief als vasodilaterend van aard. Daarnaast zijn groei beïnvloedende en bevorderende eigenschappen beschreven.

Het doel van dit proefschrift was de neurohumorale veranderingen te onderzoeken in relatie tot hemodynamische parameters bij verschillende stadia van hart falen en de effecten van farmacologische behandeling op deze neurohormonen.

Hoofdstuk 1 geeft een overzicht van de bekende hemodynamische effecten van de verschillende neurohormonen en de effecten op hemodynamiek en neurohormonen van verschillende medicamenten, zoals ACE-remmers, diuretica, vasodilaterende stoffen, digoxine, β-blokkers, calcium antagonisten, phosphodiesterase remmers en dopaminerge stoffen.

In hoofdstuk 2 wordt een studie besproken waarbij 107 patiënten met een normale LV functie, asymptomatische LV disfunctie of symptomatische LV dysfunctie groepsgewijs met elkaar worden vergeleken, met betrekking tot hemodynamische parameters en neurohormoon spiegels. Patiënten met symptomatische LV disfunctie hadden een duidelijk verminderde myocard functie en neurohumorale activatie (catecholamines, renine, angiotensine II en ANP). Bij patiënten met asymptomatische LV disfunctie daarentegen, waren alleen ANP spiegels verhoogd, terwijl er wel een duidelijk verminderde myocard en pompfunctie was. Dit suggereert dat activatie van ANP optreedt voordat andere neurohormonen geactiveerd worden, en dat dit verdere verslechtering naar symptomatisch hart falen voorkomt of vertraagd. Dit door directe vasodilatatie en natriurese of indirect door vermindering van activatie van het renine-angiotensine systeem en catecholamines.

Hoofdstuk 3 gaat over een studie met 137 patiënten met normale LV functie of asymptomatische LV disfunctie, al dan niet behandeld met ß-blokkade, met betrekking tot hemodynamische parameters en neurohormoon spiegels. Onder behandeling met ß-blokkade waren, bij patiënten met normale LV functie, de cardiac output, linker ventrikel contractiliteit en relaxatie verminderd, terwijl dit bij patienten met asymptomatische LV disfunctie behandeld met ß-blokkade niet het geval was. In de laatste groep was de cardiale ANP

productie verminderd, terwijl deze toegenomen was bij patiënten met normale LV functie, behandeld met ß-blokkade. De conclusie was dat chronisch ß-blokkade myocard contractiliteit en de pompfunctie verminderde. Aangezien deze effecten niet of nauwelijks gezien werden in de groep met asymptomatisch LV disfunctie, wordt gesteld dat behandeling met chronische ß-blokkade in deze groep geen risico geeft op verdere verslechtering.

In hoofdstuk 4 worden 12 patiënten met ernstig hart falen beschreven, die behandeld worden met carmoxirole, een selectieve dopamine (D_2) receptor agonist. Carmoxirole verminderd sympatische activatie. Dit gaat gepaard met vermindering in vasopressine en ANP spiegels en vermindering in activatie van het renine-angiotensine systeem, dit laatste met hogere doseringen. Dit leidt tot een vermindering in systemische vaatweerstand en hart frequentie. Dit leidt vervolgens tot een verbetering in cardiale pompfunctie en linker en rechter ventrikel vullingsdrukken. De conclusie is dat behandeling met carmoxirole bij patiënten met ernstig hart falen een gunstig effect heeft op hemodynamische en neurohumorale parameters.

Hoofdstuk 5 gaat over 12 patiënten met ernstig hart falen die langdurig behandeld worden met trandolapril, een krachtige lipofiele ACE-remmer. Er werden neurohumorale en systemische en renale hemodynamische parameters bestudeerd. Langdurige behandeling met trandolapril geeft een blijvende vermindering van norepinephrine, angiotensine II en aldosterone spiegels. Tevens wordt de preload en renale perfusie verbeterd, hetgeen wordt aangegeven door een blijvende toename in renale perfusie en behoud van renale functie bij patiënten met ernstig hart falen.

In hoofdstuk 6 worden de acute effecten van twee phosphodiesterase remmers bestudeerd, te weten isomazole en nanterinone. Beiden hebben deels een calcium sensitizerende werking. Beiden verbeteren de myocard functie en verminderen neurohumorale activatie. Dit effect blijkt afhankelijk van de preload. Bij patiënten met een normale of lage preload (gedefinieerd als een pulmonale wiggedruk van lager dan 15 mmHg) verslechterde de pompfunctie en nam de sympatische tonus toe. Dit impliceert dat behandeling met deze middelen, met de doseringen die in deze studie zijn gebruikt, mogelijk alleen gunstig is bij patiënten met een duidelijk verhoogde preload.

In hoofdstuk 7 wordt de mitochondriale functie bestudeerd in perifere skeletspier bij 22 patiënten met hart falen (NYHA klasse II-III) en bij 20 patiënten met normale LV functie. Hierbij werd een nieuwe niet-invasieve methode toegepast, die gebruik maakt van ^{31}P NMR spectroscopie. We vonden dat bij patiënten met hart falen de mitochondriale functie van

oxidatieve vezels in skeletspier verminderd is. Dit kan bijdragen tot de klinische symptomen van het hart falen.

Conclusie

Toegenomen sympatische activiteit en norepinephrine spiegels lijkt de belangrijkste neurohormonale parameter te zijn bij hart falen en is nauw gerelateerd aan de ernst van het hart falen en mortaliteit. Activatie van andere neurohormonen treedt hierbij voornamelijk als compensatoir mechanisme op. Activatie van ANP bij vroege LV disfunctie kan een stijging in norepinephrine spiegels, en vervolgens verdere verslechtering naar hart falen, voorkomen. Bij verder gevorderde stadia van hart falen ontstaat activatie van het renine-angiotensine systeem, aldosterone, vasopressine en andere catecholamines. Dit resulteert in een toename in sympatische activatie en vasoconstrictie. Om deze reden is het van belang dat farmacologische behandeling vroeg en adequaat gebeurd. Het gebruik van phosphodiesterase remmers dient beperkt te worden tot de ernstige gevallen van hart falen. Bij iedere patiënt met progressief hart falen dient de hemodynamische en neurohumorale status gemeten te worden om adequate farmacologische behandeling in te kunnen stellen. Er dient rekening gehouden te worden met het feit dat, bij chronisch hartfalen, er veranderingen optreden in het energie metabolisme in perifere skelet spieren, waardoor bepaalde symptomen, ook bij adequaat behandeld hart falen blijven bestaan.

Het is daarom van belang dat toekomstig onderzoek zich richt op vermindering van neurohumorale activatie en verbetering van de kwaliteit van het leven en op verbetering van de perifere spier functie, waar mogelijk. Van uit het oogpunt van de patient kan verbetering van kwaliteit van het leven kan belangrijker zijn dan het verlengen van de levensduur.

Curriculum Vitae

Martin van der Ent werd op 25 augustus 1961 te Rotterdam geboren. Van 1973 tot 1980 bezocht hij de Comenius Scholen Gemeenschap te Capelle aan den IJssel, alwaar hij het diploma Atheneum B behaalde. Van 1980 tot 1983 studeerde hij aan het Van 't Hoff Instituut te Rotterdam voor de HBO-B opleiding tot klinisch chemisch analist. Aansluitend studeerde hij van 1983 tot 1990 geneeskunde aan de Erasmus Universiteit Rotterdam. Van 1990 tot 1991 werkte hij als AGNIO interne geneeskunde en poortarts in het Drechtsteden ziekenhuis, locatie Jacobus te Zwijndrecht. Van 1991 tot 1995 was hij werkzaam bij Sticares, stichting voor cardiovasculair onderzoek te Rotterdam. Hier werd het in dit proefschrift beschreven onderzoek verricht. Van 1995 tot 1998 was hij werkzaam als AGNIO cardiologie in achtereenvolgens het Zuiderziekenhuis en het Academisch Ziekenhuis Dijkzigt beiden te Rotterdam. In 1998 is hij begonnen aan de opleiding tot cardioloog aan het Hartcentrum Rotterdam (opleider Prof. dr. J.R.T.C. Roelandt). Hiertoe volgt hij op dit moment de vooropleiding interne geneeskunde in het Zuiderziekenhuis te Rotterdam (opleider dr A. Berghout).

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