Clinical research

Putative contribution of prostaglandin and bradykinin to muscle reflex hyperactivity in patients on Ace-inhibitor therapy for chronic heart failure

Adam C. Scotta,1, Roland Wensela,1, Constantinos H. Davos,1, Panagiota Georgiadoua, L. Ceri Daviesa, Andrew J.S. Coatsb, Darrel P. Francisa, Massimo F. Piepolia,c,*

a Imperial College School of Medicine, and Royal Brompton Hospital, London, UK
b University of Sydney, Australia
c Guglielmo da Saliceto Polichirurgico Hospital, Piacenza, Italy

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Aims In patients with chronic heart failure (CHF), an overactive muscle ergoreceptor reflex (chemo-afferents sensitive to the products of muscle work) is thought to play an important role in the origin of dyspnoea. We sought to investigate whether raised intra-muscular prostaglandins (PG) and bradykinin, as estimated by levels within the venous effluent from exercising skeletal muscle may be involved in symptom generation through the stimulation of the ergoreflex.

Methods and results In 19 stable CHF patients and 12 normal controls, cardiopulmonary exercise capacity (peak O2 consumption [peak VO2]) and the ergoreflex contribution to ventilation (post-handgrip regional circulatory occlusion method) were measured. Venous resting and exercise plasma PGE2, PGF1α and bradykinin concentrations were assessed. Eleven patients on angiotensin converting enzyme inhibitors and 10 controls were challenged with ketoprofen infusion (to inhibit PG synthesis and bradykinin activity). Patients vs. controls presented lower exercise tolerance (peak VO2 15.9 ± 0.7 vs. 33.0 ± 1.3 mL/kg/min), an increased ventilatory response to exercise (VE/VCO2 slope 43 ± 2 vs. 27 ± 0.9) (*p < 0.0001 for all comparisons). The overactive ergoreflex of CHF (5.1 ± 1.3 vs. 0.1 ± 0.3 L/min) was significantly related to the increase in PGF1α (adjusted R² = 0.34, *p < 0.005) but not PGE2 (adjusted R² = 0.16, p > 0.05). The increased PG and bradykinin productions both at rest and during exercise in CHF were attenuated after ketoprofen infusion, associated with ergoreflex reduction (–5.1 ± 2.2 L/min, p < 0.05 vs. saline).

KEYWORDS
Exercise; Heart failure; Prostaglandin; Ventilation

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Introduction

Chronic heart failure (CHF) is a multi-organ syndrome with a high morbidity rate. The main symptoms are breathlessness and an early occurrence of fatigue upon exercise. These features are associated with reduced metabolic exercise capacity and abnormally high ventilation on exertion, both are important predictors of mortality, but correlate with the severity of resting left ventricular dysfunction only poorly.

Impairment in skeletal muscle performance and metabolic efficiency, with early acidification and depletion of high-energy phosphate compounds on exercise, seem to play an important role in the generation of exercise-limiting symptoms in CHF. A reduction in peripheral vascular conductance, reflecting an increase in the activity of vasoconstrictive systems, such as the sympathetic nervous system, the renin–angiotensin system and the endothelin system. The activation of pro-inflammatory systems with increased secretion of both bradykinin and vasodilator prostaglandins (PG) (partly mediated by bradykinin) plays a compensatory and homeostatic role in maintaining central and peripheral haemodynamics in this syndrome. Bradykinin is a powerful systemic vasodilator agent by enhancing the activity of constitutive nitric oxide synthase. PGE2 and PGI2 levels are increased locally in the peripheral working skeletal muscle, both at rest and on exercise, contributing to the maintenance of circulatory stability, by counteracting the vasoconstrictive influences.

We have recently hypothesised that these humoral factors may also play a causative role in the origin of abnormalities in ventilatory responses to exercise in CHF. The demonstration of a relationship between these humoral factors and the exercise abnormalities may lead to novel treatment for the limiting symptoms in CHF. It is well recognized that sympatho-excitation and cardiovascular and ventilatory responses to exercise are partially regulated by neural feedback from chemosensitive unmyelinated or small myelinated nerve endings located in the interstitium of working muscle: the ergoreceptors. An overactivation of these neural afferents triggered by the metabolic and humoral changes within the muscle in CHF has been described and proposed as a link between peripheral changes and increased sympathetic activity, vasoconstriction and ventilatory drive. A missing element in this chain of reasoning is evidence of the chemical substance(s) involved in eliciting this reflex in a clinical setting. Experiments have demonstrated the role of bradykinin in stimulating muscle reflexes, by acting on kinin B2 receptors, which are also located on the muscle afferents. The effect of bradykinin is critically dependent on the local concentration of PG that enhances the action of this peptide on muscle ergoreceptors. Moreover, synthesis of PG is stimulated by activation of kinin B2 receptors, and there may be a direct effect of PGF1α and PGE2 in sensitising the ergoreceptor afferents. Experimental studies in animal models of CHF have shown that bradykinin can directly stimulate C fibre afferents in the left ventricle.

Local skeletal muscle ischaemia and acidosis (which occur early in exercise in heart failure) are known to release both bradykinin and PG. Research from our laboratory has recently associated the increased acidotic response during exercise, inflammatory mediators such as PG and bradykinin and overactive ergoreflex activation, to the genesis of the abnormal exercise-related symptoms and autonomic responses in the CHF syndrome.

In the present study, we tested the hypothesis that PG and bradykinin play an important role in ergoreflex activation in CHF, by examining the effect of short-term inhibition of these systems on the ergoreflex contribution to ventilation.

Methods

Study population

Our subject population consisted of patients with moderate-to-severe CHF. The patients were all symptomatic on exercise and limited by breathlessness or muscle fatigue. They were consecutively recruited from the outpatient Heart Failure Clinic at our Institution. A healthy group of controls was recruited from "The 316 Club" which is comprised of ex-members of the executive of British Aerospace at Stevenage (England, UK): none had history or clinical signs of heart failure or pulmonary disease.

The study was powered to have 80% power to detect, at the 5% significance level, a 30% change in the ergoreflex value in patients, from an expected baseline value of approximately 8 L/min. The study was not specifically powered to detect differences between patients and controls as the primary aim was to examine the effect of the inhibitors on phenomena in patients. The reproducibility, expressed as standard deviation of difference, was approximately 1.8 L/min from previous work.

The power calculation (which was based on 2-tailed hypothesis testing) recommended a sample size of seven patients.

While most of the patients in our heart failure outpatient clinic are ready to volunteer for studies involving physical manoeuvres and measurements, many are reluctant to have pharmaceuticals infused purely for research (especially when the pharmaceuticals are within groups that the patients have previously been advised to avoid). We therefore invited patients...
to give separate informed consent to the observational study and the ketoprofen infusion study, so that at least the observational study addressed as representative a sample of our clinical patients as possible. In the end, we recruited 10 patients and 11 controls to the full study, with a further nine patients and one control agreeing to undergo only the observational study (making a total of 19 patients and 12 controls). Table 1 presents the clinical characteristics of the whole study population.

The studies were approved by the local ethics committee and conformed to the Declaration of Helsinki. All subjects gave written informed consent.

Protocol

All experimental sessions were performed in a temperature-controlled, air-conditioned room. The subjects were asked to avoid strenuous physical activity for 24 h before each test: a light, caffeine-free breakfast was allowed at least 90 min before each study visit. The tests were preceded by 30 min in a quiet environment.

Cardiopulmonary exercise test

After an initial clinical screening, and a first familiarisation exercise test, a cardiopulmonary exercise test was performed to determine the peak exercise capacity, using a maximal symptom-limited, modified Bruce protocol (commencing at stage 0: 1.0 mph at 5.0% gradient) on a Marquette Case 15 treadmill (Milwaukee, USA). At peak exercise, all subjects were limited by dyspnoea and/or fatigue, and reached a respiratory exchange ratio of 1.1 or greater.

Ergoreceptor test

On separate days, but within a time interval of less than 1 month from the cardiopulmonary exercise test, each subject underwent the ergoreceptor test.

The contribution of the muscle ergoreceptors was evaluated by trapping the metabolites in the exercising muscle after exercise (regional circulatory occlusion method, originally developed by Alam and Smirk in 1937). It has been shown, by inhibiting the washout of the blood from the exercising muscle, to maintain the activation of the ergoreceptors after work and to isolate them from other control mechanisms (i.e., central command, blood-borne factors). This technique is now well accepted and used in different laboratories and its reproducibility has been recently evaluated in our laboratory (co-efficient of variability 23.4%).

The test involved two handgrip exercises performed in a random order by the dominant arm. A five-minute session of rhythmic handgrip was achieved by squeezing the balloon of a sphygmomanometer (30 squeezes/min) at 50% maximal voluntary capacity. The same protocol was followed by three minutes of post-handgrip regional circulatory occlusion on the working limb by inflation of an upper arm biceps tourniquet to 30 mmHg above systolic pressure at the start of the recovery. To this the exercising arm was sealed at the elbow inside a pneumatic chamber in which the air pressure could be manipulated. Thirty minutes separated each bout of exercise.

During the ergoreflex tests, the ventilatory data were computed during the different phases of the handgrip (i.e., the average of the five minutes resting, the last minute of handgrip and the three-min regional circulatory occlusion and control recovery periods). The ergoreflex contribution was computed as the difference of the changes in each ventilatory variable between the mean resting values and the average of the 2nd and 3rd minute recoveries with and without regional circulatory occlusion.

Ergoreflex and inhibition of PG synthesis and bradykinin activity

Ketoprofen was a propionic acid derivate, and a powerful inhibitor of cyclooxygenase (a key enzyme in PG synthesis) and bradykinin action. It was infused in a single-blinded controlled placebo fashion in a subgroup of 11 patients (age 68.1 ± 2.3 years, sex 10 male/1 female, BMI 25.0 ± 1.3, 7 in NYHA class II, 4 in NYHA class III) and 10 healthy controls (age 61.0 ± 4.2, sex 9 male/1 female, BMI 25.5 ± 0.9). The clinical characteristics and concomitant medications of these subgroups were not different from the whole study population (P = NS). In particular, 7 out of 11 patients (63.6%) challenged with ketoprofen were undergoing aspirin therapy.

For 15 min before the ergoreflex tests, ketoprofen (2 mg/kg/h) or equivalent saline (0.9% NaCl, as placebo) were infused, with a time interval between the two infusions of 2 h, in an antecubital vein of the non-dominant arm, contra-lateral to the exercising one. The ketoprofen was infused only after the saline, as the half-life of the drug is approximately 2 h.

Data

Ventilatory data

These were collected at rest, during the cardiopulmonary exercise tests and during the ergoreflex tests. Subjects breathed air through a mouthpiece and wore a nose clip. Ventilation (Vt) and...
respiratory rate were measured continuously online using a calibrated heated pneumotachograph, while oxygen uptake (VO₂) and carbon dioxide production (VCO₂) were measured breath-by-breath using a respiratory mass spectrometer (Amis, Innovid, Odense, Denmark). Peak VO₂ was computed as the average of VO₂ values measured during the last 30 s of the exercise, while the slope of the VE/VCO₂ ratio was calculated over the whole of the exercise period as an index of the ventilatory response.

Blood tests

For blood sampling, a 20-gauge, 1.25 in. catheter (Angiocath) was inserted in a retrograde fashion into an antecubital fossa vein that drained the active muscle of the exercising forearm. During the last minute of each phase of the ergoreceptor test (resting, exercise handgrip, regional circulatory occlusion and control recoveries), deep venous blood was drawn from this catheter into heparinised syringes and analysed for the concentration of PG and bradykinin. Extra care was taken to avoid bleeding or oozing at the site of the cannula during the application of the circulatory occlusion. A local origin of bradykinin and prostaglandin has been experimentally demonstrated in the blood draining from the exercising muscle due to the activation of the kallikrein–kinin–prostaglandin system.

Bradykinin was measured by enzyme immunoassay system using the Bradykinin EIA kit (Peninsula Laboratories Inc., California, USA), with sample extraction using a C18 column. In our laboratory, the reference range is 0.2–7.5 pg/mL. The detection limit is 0.02 pg/mL with an inter-assay variation of <14%. 6-Keto-PGF₁α (PGF₁α) was measured by enzyme immunoassay using the Biotrak 6-keto-PGF₁α enzyme immunoassay system (Amersham Pharmacare Biotech UK Ltd., Buckshire, UK), with sample extraction using Amprep C2 columns: the detection limit was 3.0 pg/mL with an inter-assay variation <15%. In our laboratory, the ranges are from 19.14 to 90.48 pg/mL.

PGE2 was measured by enzyme immunoassay using the Biotrak PGE2 enzyme immunoassay system (Amerham Pharmacare Biotech UK Ltd., Buckshire, UK). The detection limit was 0.5 pg/mL with an inter-assay variation <11.0%. In our laboratory, the reference ranges for healthy normal subjects is 4.4–6.8 pg/mL.

Statistical analysis

The significance of changes in blood metabolite concentrations from rest to exercise was analysed by the paired t-test. For the comparison of ergoreflex contribution to ventilatory variables between controls and patients with CHF an unpaired t test was used. If data did not follow a normal distribution Wilcoxon and Mann–Whitney rank sum tests were performed, respectively. Because the ergoreflex is actually a measure of increment, we computed the relationship between the ergoreflex and the change in PGE2, PGF₁α and bradykinin between rest and exercise. We used adjusted linear regression analysis, which allows for the potential for difference between patients and controls in the mean value of each variable.

In the subgroups of subjects who were challenged with ketoprofen, we compared the effect of the drug vs. saline infusion by computing the differences in the ventilatory response to ergoreflex, and in PG and bradykinin concentration. Changes in these parameters during the interventions were analysed by repeated measures ANOVA and all pairwise comparisons were performed using Dunn’s test. During this interventional phase of the study, the correlations examined were between the ergoreflex measurement and the exercise-induced increment in PGE₂, PGF₁α and bradykinin.

All data are presented as means ± SEM. A two-tailed p-value <0.05 was considered significant.

Results

No adverse event was reported either during the different exercise tests or during the drug infusions.

Cardiopulmonary exercise test

Patients achieved a lower exercise tolerance (peak VO₂) and an increased ventilatory response to exercise (VE/VCO₂ slope) compared to controls (Table 1).

Handgrip exercise and ergoreflex test

Resting respiratory rate was significantly higher, while VO₂ was significantly lower in CHF patients vs. controls. This was associated with higher levels of bradykinin and PGF₁α in CHF patients (Table 2). On exercise, all ventilatory and humoral variables increased in both groups of subjects; however VE, respiratory rate, bradykinin and PG were still significantly higher in CHF patients than in controls (Table 2).

Patients vs. controls showed an increased ergoreflex effect on ventilatory responses, associated with increased concentrations of PG and bradykinin (Table 2). The role of the humoral factors in ergoreflex activation was confirmed by the significant linear correlation between the elevation in concentration of PGF₁α during the ergoreflex test and the ergoreflex-dependent component of ventilation itself (see Fig. 1, adjusted R² = 0.34, p < 0.005; PGF₁α: co-efficient 0.27, standard error (SE) 0.10, p = 0.015; patient vs. control: co-efficient 2.1, SE 1.9, P = 0.28, r = 0.42, p < 0.01, respectively). There was no significant relationship for PGE₂ (adjusted R² = 0.16, p = 0.053; PGE₂: co-efficient 0.46, SE 0.48, p = 0.35; patient vs. control: co-efficient 3.2, SE 2.3, p = 0.17).

Ketoprofen challenge

The subgroups challenged with ketoprofen (11 CHF patients and 10 normals) did not differ from the whole group of study population in terms of exercise tolerance, ventilatory responses to exercise and ergoreflex responses. Patients had a significantly lower peak VO₂ (16.2 ± 1.1 vs. 33.7 ± 1.1 mL/kg/min), higher VE/VCO₂ slope (43.1 ± 3.2 vs. 26.5 ± 0.7) and ergoreflex contribution to the ventilatory responses (6.5 ± 2.1 vs. −0.2 ± 0.5 l/min) (p < 0.05 vs. normals for all comparisons).

The drug infusions did not affect the ventilatory and heart rate results during the handgrip tests in either group of subjects (Table 3). Instead, after ketoprofen
infusion, reductions in resting PG, exercise PG and bradykinin concentrations vs. respective saline values for both subject groups were observed. The resting concentrations of both PGF1α and PGE2 were no longer increased in patients compared to controls, whilst the concentrations of resting and exercise bradykinin and exercise PGs were elevated in CHF (Table 3).

During the ergoreflex test in CHF patients, reduced metabolite blood concentrations (only PGE2 was still elevated with respect to normals) were present after ketoprofen infusion. This change was associated with a decrease in the ventilatory and respiratory rate components of the reflex, which were no longer different to those in the control group (Table 3).

**Discussion**

The results of this study confirms and extends previous observations on the role of humoral vasodilator substances in triggering increased ergoreflex activity in CHF: ergoreflex activity correlates with the concentrations of PGF1α and PGE2 in the venous effluent of the exercising skeletal muscle. The reduction in PG and bradykinin plasma concentration observed after ketoprofen infusion was associated with a reduction in ergoreflex contribution to ventilation. This suggests a causative relationship between muscular effluent concentrations of bradykinin and PG, and symptoms of exercise tolerance and dyspnoea, throughout the activation of the muscle reflex.

This is in-keeping with previous human investigations showing a role for PG in the regulation of the ventilatory responses to effort, through stimulatory effects on muscle receptors. These vasodilator factors may aggravate the symptoms of exercise intolerance in CHF patients by inducing muscle ergoreflex stimulation.

We found that administration of the cyclooxygenase inhibitor ketoprofen abolished the ergoreceptor response, and this abolishment was associated with a marked reduction in PG and bradykinin levels. It must be kept in mind that all these patients were taking angiotensin converting enzyme inhibitors and 7 out of 11 were on aspirin, so we cannot be certain whether the observed effects result from the disease process or its treatment.
### Table 3

<table>
<thead>
<tr>
<th>Ventilatory</th>
<th>Hormonal</th>
</tr>
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<tbody>
<tr>
<td>VE</td>
<td>VO₂</td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Saline</strong></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>12.70 ± 1.00</td>
</tr>
<tr>
<td>Exercise</td>
<td>16.90 ± 1.00#</td>
</tr>
<tr>
<td>Ergoreflex (**)</td>
<td>8.00 ± 2.20</td>
</tr>
<tr>
<td><strong>Ketoprofen</strong></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>13.10 ± 0.70#</td>
</tr>
<tr>
<td>Exercise</td>
<td>17.20 ± 0.90#</td>
</tr>
<tr>
<td>Ergoreflex (**)</td>
<td>1.37 ± 0.60</td>
</tr>
<tr>
<td><strong>Normals</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Saline</strong></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>11.40 ± 0.60</td>
</tr>
<tr>
<td>Exercise</td>
<td>15.00 ± 0.50#</td>
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<tr>
<td>Ergoreflex (**)</td>
<td>0.10 ± 0.30</td>
</tr>
<tr>
<td><strong>Ketoprofen</strong></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>11.60 ± 0.60</td>
</tr>
<tr>
<td>Exercise</td>
<td>15.00 ± 0.90#</td>
</tr>
<tr>
<td>Ergoreflex (**)</td>
<td>0.10 ± 0.30</td>
</tr>
</tbody>
</table>

*p value <0.05 vs. respective saline. For other abbreviations, refer to Table 1.*
complicates the interpretation of our findings insofar as the increased ergoreflex activity may reflect relative muscle ischaemia (or anaerobic metabolism) or an over-activation of the bradykinin and PG systems.

The result of our study is in contrast with the concept of the compensatory role of these hormones in CHF. A different pathophysiological mechanism of action should be advocated when a relationship between vasodilatory hormones, such as PG and bradykinin, and ergoreflex stimulation is considered. The development of desensitization to PG infusion has been shown to characterise those CHF patients with a more advanced status. We hypothesise that long-lasting elevated blood concentrations of these hormones may be detrimental and ultimately responsible for the symptoms of exercise intolerance. A continuous triggering of the muscle reflex operated by these humoral factors may exaggerate the ventilatory response to exercise and increase the vasoconstrictive and sympathetic drives, thereby contributing to a vicious circle in the heart failure syndrome.

Our results may at least partially explain the relative lack of effect of angiotensin converting enzyme (ACE) inhibitors on peak oxygen uptake and exercise capacity and/or symptoms in heart failure. Interestingly, it has recently been reported that the highest dosage of ACE-inhibitors, while being undoubtedly beneficial for survival, may exert an unfavourable effect on aerobic exercise tolerance, possibly by increasing the blood concentration of vasodilatory humoral factors and thus enhancing their adverse secondary effects on muscle afferents. Furthermore, our observations may provide a mechanism for harm from interventions for heart failure that not only act as ACE-inhibitors but also are specifically intended to augment bradykinin and prostaglandins (for example Omapatrilat, whose recent trials OVERTURE and OCTAVE were disappointing).

No direct evidence of a beneficial effect for long-term therapy of an inhibition of local vasodilation is available. This report may stimulate future investigation in a large prospective setting.

**Limitations**

The correlations between PG, bradykinin blood levels and the ergoreflex component of the ventilatory responses show that these measurements only partially explain the variability in responses. These humoral mediators are therefore clearly not the sole triggers of the muscle reflex and contributors to the limiting symptoms. A number of vasoactive agents, including angiotensin II, noradrenaline and bradykinin can directly stimulate PG synthesis and alterations in metabolic clearance may also contribute to the increase in circulating levels. Regardless of their source, all these humoral changes limit the magnitude of the regional and systemic vasoconstriction that is observed in CHF.

The lack of a decrease in the ventilatory response during exercise in parallel with the decrease in PG levels is grounds for making our conclusions tentative. During exercise, multiple inputs contribute to ventilation. Some of them have been shown to be overactive in CHF patients (such as peripheral and central chemoreceptors) and may be responsible for the abnormal hyperventilation. Thus modulation of one input may increase another, particularly if this has been obtained by an intervention which has modified the adequacy of blood flow or had an impact on central processing of ventilation.

The fact that in our patient population all individuals were under chronic ACE-inhibitor therapy (known to inhibit the breakdown of bradykinin and raise the level of bradykinin and to enhance prostaglandin formation), and twelve were on aspirin (an inhibitor of cyclooxygenase) (Table 1) may have affected our findings.

**Conclusion**

Local vasodilatory mediators such as PG or bradykinin play an important role in the genesis of the symptoms of exercise intolerance in heart failure. In this study, we have identified the potential for relief of abnormal reflex overresponsiveness by blockade of these mediator pathways. However, while symptomatic relief may be a sufficient achievement for some groups of patients with heart failure, for the majority it would be important to establish long-term safety and mortality efficacy. We hope that this study might encourage the development of safe agents specifically targeted against exercise-limiting symptoms, since permitting regular physical exertion is now itself a potential avenue of long-term prognostic benefit.

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