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Acetylcholine stimulated dilatation and stretch induced myogenic constriction in mesenteric artery of rats with chronic heart failure

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Abstract

Rats with chronic heart failure (CHF) develop increased myogenic constriction in mesenteric resistance arteries. Here we investigated increased myogenic constriction in relation to alterations in EDHF- and NO-mediated dilatation in CHF-rats.

Male Spraque–Dawley rats were subjected to myocardial-infarction or sham-surgery. At 9–10 weeks after surgery, isolated mesenteric artery ring preparations were studied in a wire-myograph. Stretch-induced myogenic constriction was obtained by stepwise increase of the internal circumference diameter (0.5–1.2 L100). Cyclooxygenase- and eNOS-inhibitors were employed to study NO- and EDHF-mediated dilatation in response to acetylcholine.

Rats with CHF ($n=8$), but not sham-rats ($n=6$), developed significant myogenic constriction. In addition, the contribution of endothelial dilator mediators was significantly altered in CHF-rats, with increased dependency on NO and decreased EDHF-mediated dilatation. Moreover, EDHF-mediated dilatation was inversely correlated with myogenic constriction in individual CHF-rats ($r=-0.74$, $p=0.04$).

These data demonstrate increased myogenic constriction in mesenteric arteries of rats with CHF post-MI to be correlated to decreased EDHF-mediated dilatation. These findings extend the previous observation that myogenic constriction antagonizes EDHF-mediated dilatation in rat coronary artery under normal conditions, and suggests this relationship also to become functional in mesenteric arteries under pathophysiological conditions of CHF.

Keywords: Myocardial-infarction; Chronic heart failure; Mesenteric artery; Myogenic constriction; NO; EDHF

1. Introduction

Following myocardial-infarction (MI), the gradual progression from compensated cardiac hypertrophy to decompensated heart failure is associated with complex neurohumoral interactions originating from the heart, brain, kidney and peripheral vasculature. These neurohumoral pathways—involving both the renin–angiotensin system and sympathetic nervous system — contribute to increased vasoconstriction and have been primarily held responsible for increased peripheral vascular resistance, a hallmark of chronic heart failure (CHF) [1]. Initially, increased resistance is thought to serve as a compensatory mechanism to help maintain perfusion to the vital organs by sustaining blood pressure in the failing heart. In the long-term, however, increased peripheral resistance renders deleterious effects by augmenting workload on the already impaired cardiac pump [2]. It seems important, therefore, to assess underlying mechanisms of excessive vasoconstriction and increased peripheral resistance in CHF.

In addition to vasoconstriction caused by excessive neurohumoral activation, regulation of vascular diameter also involves adaptations at the level of endothelial and vascular smooth muscle cells, which may act independently from nervous and hormonal mechanisms to sustain a vascular constrictive state [3]. In recent years much attention has been focused on changes at the endothelial level in CHF, which in general comprise reduced synthesis or altered activity of vasodilative mediators and increased effects of

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vasoconstrictors, together referred to as endothelial dysfunction. The rat coronary-ligation MI model is a frequently used animal model of experimental CHF for studying acetylcholine (ACh-) induced relaxation of isolated vascular preparations of MI-rats to investigate mechanisms of endothelial dysfunction. The majority of studies with isolated aortic rings suggest that endothelial dysfunction in this model of post-MI CHF is a time-dependent process with progressive impairment of ACh-induced dilatation due to decreased NO activity that is associated with increased production of vascular superoxide production [4–7]. In contrast to large arteries such as the aorta, in which endothelium-dependent vasorelaxation to a large extent is mediated by NO, in small arteries and arterioles a major amount of the relaxation response seems to be mediated by endothelium-derived hyperpolarizing factor(s) (EDHF) [8]. Although endothelial function has also been investigated in small mesenteric resistance and other small arteries in this model of CHF, these studies are limited in number and have less consistent results [9–13]. Therefore, we set out to characterize endothelial vasodilative mediators in small mesenteric arteries of rats with CHF.

In addition, we have recently obtained evidence that changes may also occur at the level of the vascular smooth muscle cell in CHF. Vascular smooth muscle cells are capable of sensing local mechanical factors including stretch and pressure, which are transmitted into biochemical signals. In this way, small arteries constrict in response to pressure-induced stretch, a reaction known as myogenic response [14]. We found that myogenic constriction is increased in small mesenteric arteries from MI-rats with experimental CHF, as measured in vitro using a pressure-myograph [15]. Furthermore, recent data indicate that there is a relationship between EDHF mediated relaxation and myogenic constriction [16]. Therefore, a second objective of this study was to measure myogenic constriction in mesenteric arteries and relate this to EDHF-mediated relaxation. To this end, we employed an experimental protocol described by Delaey et al [17] for determination of myogenic constriction in isolated choroidal small artery rings mounted in a wire-myograph; this set-up enables several artery preparations to be studied in parallel under various different experimental conditions. With the above in mind, we studied ACh-induced endothelium-dependent dilation in mesenteric artery preparations of rats in which CHF was induced by MI, dissected the contribution of different endothelial mediators and related these to myogenic constriction.

2. Materials and methods

2.1. Rat coronary ligation myocardial-infarction model

Male Sprague–Dawley rats (275–300 g, Harlan, Zeist, The Netherlands) were housed in groups of four to five rats at the animal facility of the University of Groningen with free access to food and drinking water. After a 10-day acclimatization period, rats underwent surgery for induction of experimental MI, as described previously [18,19]. In short, rats were placed on a homeothermic blanket, anaesthetized with 2% isoflurane in 2.5 L oxygen/min, intubated and mechanically ventilated (Amsterdam Infant Ventilator, Hoek/Loos, Schiedam, The Netherlands). A left sided thoracotomy was made, and the proximal portion of the left coronary artery was occluded with a 6-0 silk suture, beneath the left atrial appendage: in sham-operated rats the ligation was placed but not tightened. Subsequently, the thorax was closed and rats were extubated upon spontaneous respiration.

A total of 21 rats underwent either sham-surgery ($n=6$) or surgery for MI ($n=15$). All sham-operated rats survived the surgical procedure and the post-surgical period, whereas total mortality among MI-rats was 40% (6 out of 15). Only MI-rats with infarct-size >20% of the left ventricle (LV) (see below) were included for analysis (8 out of 9), since smaller infarcts do not result in LV dysfunction [18,19]. All animal experimentation was reviewed and approved by the Animal Research Committee at the University of Groningen and conducted in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Determination of cardiac function and morphometry

9 to 10 weeks after surgery, rats were anaesthetized as described above. The right carotid artery was cannulated with a pressure transducer catheter (Micro-tip 3French, Millar Instruments, Germany) connected to a 486-PC equipped with an analog-to-digital converter and appropriate software (Millar Instruments, Germany). A zero-pressure baseline was obtained by placing the pressure sensor in 38 °C saline before the catheter was advanced into the aorta and the LV. After a 3-min period of stabilization, maximal LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), and heart rate were recorded. As indices of global contractility and relaxation, the maximal rates of increase and decrease in LVP (systolic $dP\ dt^{-1}$ and diastolic $dP\ dt^{-1}$) were determined. Hereafter, the catheter was withdrawn to measure systolic and diastolic blood pressure in the aortic root.

After haemodynamic measurements, hearts were rapidly excised and weighed. A transverse slice through the midst of the LV containing the infarcted area was fixed in 4% paraformaldehyde, embedded in paraffin and 10 μm slices were cut and stained for histological analysis. Total epicardial and endocardial circumference of the LV and the epicardial and endocardial scar length of the infarcted areas were determined by means of a computerized planimeter (Quantimet 520, Cambridge Instruments). Infarct size was then calculated by dividing the sum of the scar lengths by the sum of the total circumference and expressed

as percentage of scar to total LV circumference, as described in detail elsewhere [19].

2.3. Preparation and mounting of mesenteric arteries

Intestines were removed and the mesenterium was put in cold Krebs solution. Second to third-order branches of the mesenteric artery were isolated from surrounding perivascular tissue and cut into 2–3 mm long vascular rings. Arterial rings were connected to force transducers in individual organ bath chambers for isometric tension recordings in a wire-myograph (MYO-2, EMKA Technologies, Paris, France). After mounting, the vessel was equilibrated for 30 min in Krebs bicarbonate solution bubbled with 95% O_2/5% CO_2 at 37 °C. Then, the calculated length of the vessel at 100 mm Hg was determined according to Delaey et al [17]. Briefly, the vessel was stretched by a stepwise increase in the distance between two stainless steel wires in steps of 10–20 μm until the calculated transmural pressure exceeded 100 mm Hg. Vessels were held at each length for 1 min and the generated force and internal circumference were used to calculate the wall tension. The internal circumference and corresponding wall tension for each point could thus be fitted on an exponential curve for determination of L100 (i.e. calculated length of the vessel at 100 mm Hg), as previously described [20].

2.4. Experimental protocol of constriction

To determine myogenic constriction and maximal constriction in response to stimulation with a combination of K^+ plus U46619, the wall tension-internal circumference relationship was obtained in a sequential protocol according to Delaey et al [17], involving a stepwise increase of the internal circumference in 0.1 L100 units over a range from 0.5 L100–1.2 L100. Initially, wall-tension was determined in a Ca^{2+}-free Krebs bicarbonate solution. After 7 min, the solution was changed to a standard Krebs solution with Ca^{2+} present. After 7 min, the vessels were challenged with a combination of K^+ (120 mmol L^{-1}) plus U46619 (1 μmol L^{-1}) for 7 min, after which the solution was changed back to the Ca^{2+}-free Krebs solution. After a final 7 min, the internal circumference was increased by 0.1 L100 and the different steps were repeated.

2.5. Assessment of ACh-induced relaxation

Arteries were allowed to equilibrate for 30 min in a standard Krebs solution at an internal circumference of 0.9 L100 before being preconstricted with 1 μmol L^{-1} U46619. Preconstricted vessels were studied for endothelium-dependent relaxation by applying cumulative doses of ACh (0.1–100 μmol L^{-1}) to the organ bath. To determine the contribution of NO and EDHF in endothelium-dependent relaxation, the response to ACh was additionally studied in the presence of various inhibitors added to the bath 20 min prior to addition of ACh. Indomethacin (10 μmol L^{-1}) was always present in the bath and used to inhibit cyclooxygenase-derived prostanoid production. *N*-monomethyl-L-arginine (L-NMMA, 100 μmol L^{-1}), added to the bath in presence of indomethacin, was additionally used to inhibit NO production when the response of vessels to ACh was tested.

2.6. Drugs

Vascular studies were performed using a Krebs bicarbonate solution with the following composition (mmol L^{-1}): NaCl 120.4, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11.5, and $NaHCO_3$ 25.0, freshly prepared daily. For preparation of the Ca^{2+}-free Krebs bicarbonate solution, $CaCl_2$ was replaced by equimolar concentrations of $MgCl_2$ and supplemented with ethylene glycol-bis-(*b*-amino ethyl ether) tetraacetic acid (EGTA, 2 mmol L^{-1}). A Krebs bicarbonate solution containing K^+ 120 mmol L^{-1} was prepared by equimolar replacement of NaCl with KCl. Compounds for the Krebs bicarbonate solution were purchased from Merck, Darmstadt, Germany. All other compounds were purchased from Sigma (St. Louis, MO, USA). Stock solutions (10 mmol L^{-1}) for indomethacin and U46619 were prepared in 96% ethanol. All other drugs were dissolved in deionized water and diluted with Krebs solution.

2.7. Calculations and statistical analysis

Myogenic constriction was calculated as the difference between the tension measured in normal Krebs solution and that measured in Ca^{2+}-free Krebs solution. Maximal stimulated constriction was taken as the difference between the maximum measured tension in the presence of K^+ plus U46619 and that measured in Ca^{2+}-free Krebs solution.

Table 1
Body weight, heart weight, infarct size, and haemodynamic characteristics of heart failure rats at 9–10 weeks post-MI, and sham-operated control rats

	Sham control rats ($n=6$)	Heart failure rats ($n=8$)
Infarct size (%)	–	42±4*
Body weight (g)	412±8	385±16
Heart-to-body weight ratio (g·kg^{-1})	3.1±0.1	3.8±0.2*
Lung-to-body weight ratio (g·kg^{-1})	3.9±0.1	6.4±1.0*
Heart rate (beats·min^{-1})	316±35	304±22
Systolic pressure (mm Hg)	123±11	110±15
Diastolic pressure (mm Hg)	80±6	76±13
Systolic dP dt^{-1} (mm Hg·s^{-1})	11,269±320	9312±574*
Diastolic dP dt^{-1} (mm Hg·s^{-1})	−10,342±318	−7900±567*
LVESP (mm Hg)	126±9	112±14
LVEDP (mm Hg)	9.6±3.4	14.4±4.4*

LVESP, left ventricular end systolic pressure; LVEDP, left ventricular end diastolic pressure.
Data are expressed as mean±SEM.
* Indicates $p<0.05$ for heart failure vs. sham control.

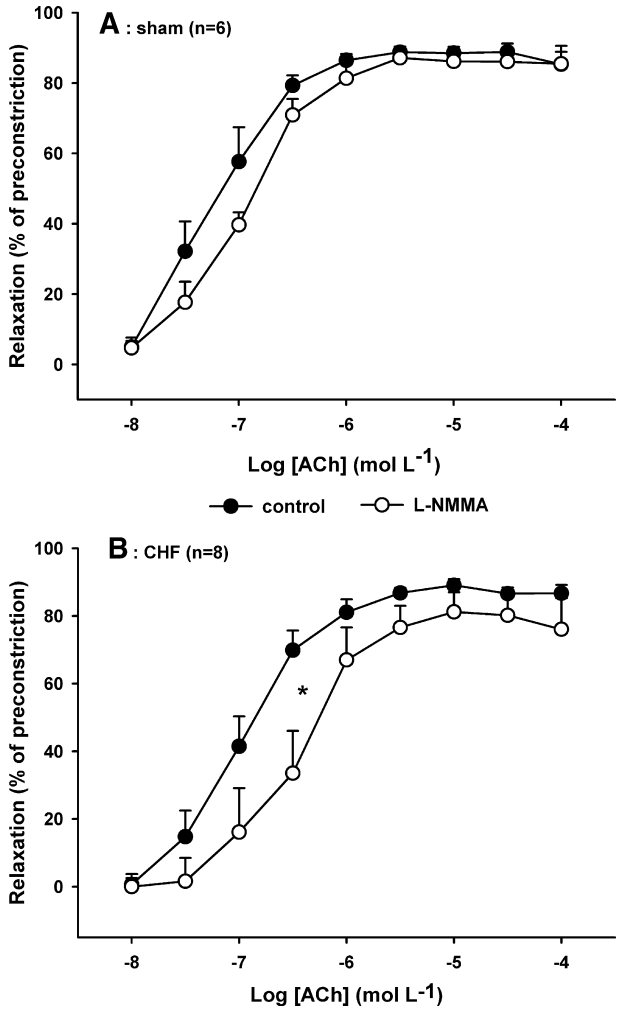

Fig. 1. Acetylcholine (ACh) induced dilatation in isolated mesenteric artery rings of (A, $n=6$) sham-operated rats and (B, $n=8$) myocardial-infarction rats with chronic heart failure (CHF). All responses were studied in presence of 10 μmol·L^{-1} indomethacin to inhibit prostanoid production, representing the control conditions (closed circles). Additional presence of the eNOS-inhibitor L-NMMA (100 μmol·L^{-1}, open circles) significantly shifted the ACh concentration–response curve to the right in rats with CHF, but not in sham-control rats. Data are expressed as mean±SEM. * $p<0.05$ control vs. L-NMMA.

Concentration–response curves and maximal relaxation to ACh were expressed in percentage of preconstriction to U46619, and the concentration of ACh causing half-maximal responses was expressed as negative logarithm of the molar concentration (pEC_{50}). The Area Under each individual Curve (AUC) was determined (Sigma Plot, Jandell Scientific) and expressed in arbitrary units. The AUC was used to present total (individual) ACh dilation (in presence of indomethacin), and for subsequent analysis of differences in ACh dilation with and without L-NMMA, as to estimate the contribution of NO (i.e. response sensitive to L-NMMA) and EDHF (i.e. reminder response to Ach after exclusion of prostanoids and NO in the response [21]), respectively. Data are expressed as mean±SEM. Comparisons were performed using student's t-statistics, or repeated measures ANOVA in case of full concentrations–response curves. Correlation analysis was performed with Pearson's correlation tests. Differences were considered significant at $p<0.05$.

3. Results

3.1. Rat characteristics and cardiac function

At baseline, no differences in body weight were observed (data not shown). After 9 to 10 weeks, body weight was slightly lower in rats with myocardial-infarction (MI-size 42±4%, $n=8$), compared to sham-operated rats, but the difference did not reach statistical significance. Compared to sham-rats, the relative heart weight as well as LVEDP were increased in MI-rats, while systolic and diastolic dP dt^{-1} was decreased (Table 1). MI operated rats displayed fluid in the lungs and increased relative lung weight (Table 1), suggesting congestion of the lungs.

3.2. ACh-induced relaxation responses in mesenteric rings

Relaxation of mesenteric artery rings to ACh was always studied in the presence of indomethacin (10 μmol L^{-1}) to avoid production and interference of cyclooxygenase-derived vasoactive prostanoids. Vessel preparations were preconstricted with the thromboxane mimetic U46619 and these contractions did not differ between mesenteric arteries from sham and CHF rats (2.45±0.39 N m^{-1} vs. 2.59±0.34 N m^{-1}, respectively; p=ns). Under these conditions, the concentration–relaxation curve to ACh was slightly shifted to the right in mesenteric arteries of CHF rats (representing a 9.4% decrease in terms of AUC) as compared to those of sham-rats, but this shift was

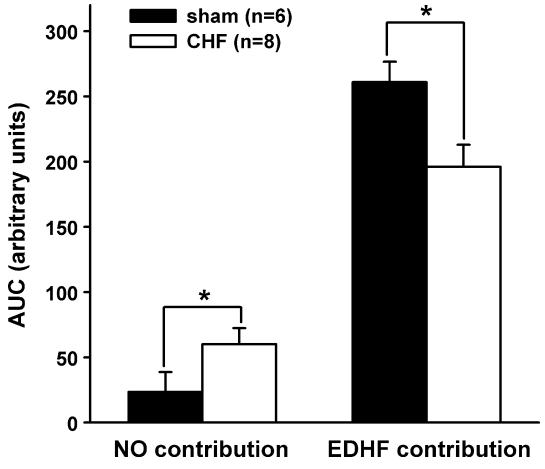

Fig. 2. Comparison of the contribution of NO and EDHF in acetylcholine-induced dilatation in isolated mesenteric artery rings of sham-operated rats (closed bars) and myocardial-infarction rats with chronic heart failure (CHF; open bars). Data are expressed as mean±SEM. * $p<0.05$ sham vs. CHF.

only minimal (pEC_{50} is 7.26±0.12 vs. 6.98±0.11 for sham and CHF, respectively) and statistically not significant (p=ns); moreover, maximal relaxation (87.7±2.4% vs. 87.2±1.5% for sham and CHF, respectively; p=ns) was identical in both groups.

Additional presence of L-NMMA to inhibit NO production significantly shifted the concentration–relaxation curve to ACh to the right in vessel preparations of CHF rats, but not in sham (Fig. 1). Note that this L-NMMA sensitive part of the response to ACh was significantly larger (representing a 156% increase in terms of AUC) in vessel preparations of CHF rats as compared to sham (Fig. 2). Consequently, the EDHF mediated relaxation to ACh that remained in the combined in presence of both indomethacin plus L-NMMA was significantly smaller in vessel preparations of CHF rats (representing a 25% decrease in terms of AUC) as compared to sham (Fig. 2). Collectively,

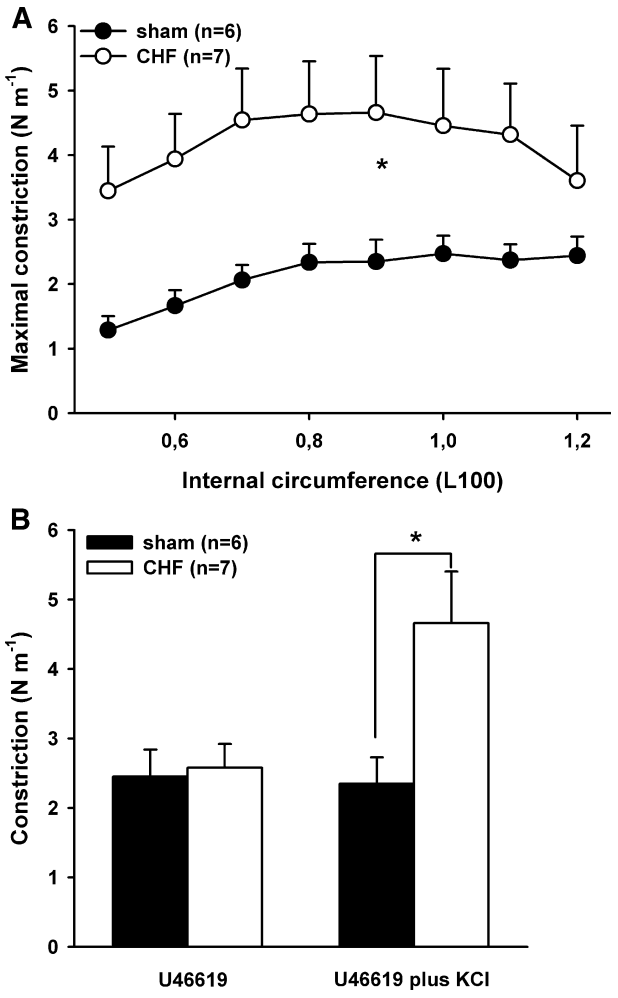

Fig. 3. Comparison of contractile responses of isolated mesenteric artery rings of sham-operated rats (closed circles/bars) and myocardial-infarction rats with chronic heart failure (CHF; open circles/bars) (A; top panel) after stimulation with a combination of K^+ 120 mmol L^{-1} plus 1 μmol L^{-1} U46619 at different lengths of the internal circumference, and (B; lower panel) after stimulation with U46619 only (on the left) or K^+ plus U46619 (on the right) at 0.9L100 of the internal circumference. Data are expressed as mean±SEM. * p<0.05 sham vs. CHF.

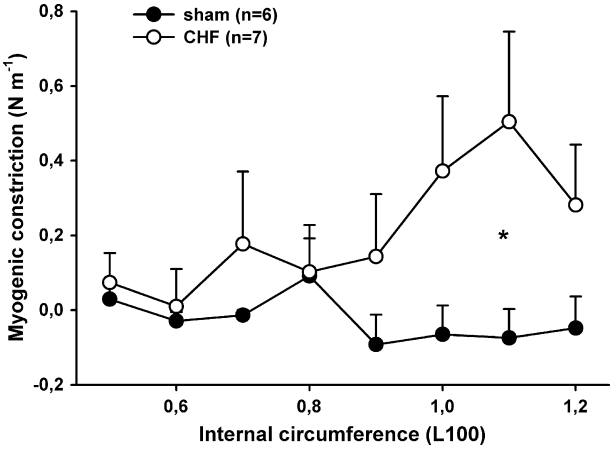

Fig. 4. Comparison of myogenic constriction in response to stretch of isolated mesenteric artery rings of sham-operated rats (closed circles) and myocardial-infarction rats with chronic heart failure (CHF; open circles) at increasing length of the internal circumference. Data are expressed as mean±SEM. * p<0.05 sham vs. CHF.

these data suggest a shift in the relative contribution of endothelial mediators to ACh-induced relaxation in mesenteric artery preparations of CHF rats, with increased dependency on the NO pathway and less contribution of EDHF.

3.3. Contractile responses after stimulation with a combination of K^+ plus U46619

Maximal tension development after challenging the mesenteric arteries with K^+ 120 mmol L^{-1} plus 1 μmol L^{-1} U46619 was studied over a range from 0.5 L100 – 1.2 L100. Although the tension response to K^+ plus U46619 peaked at an internal circumference of 0.9L100 in mesenteric arteries of both sham and CHF rats, absolute tension development was significantly higher in CHF rats as

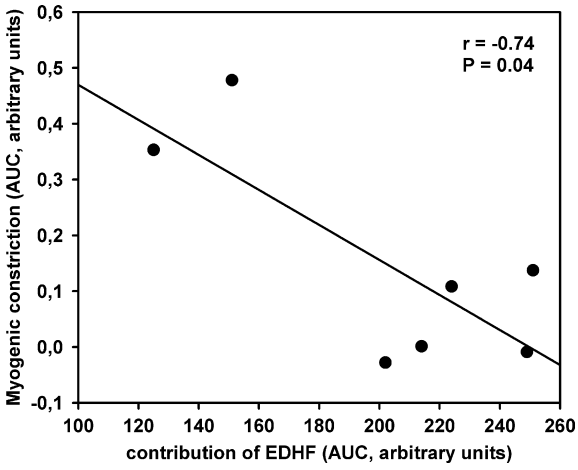

Fig. 5. Scatterplot of the contribution of EDHF to acetylcholine-induced dilatation and myogenic constriction in isolated mesenteric artery rings of individual myocardial-infarction rats with chronic heart failure. The solid line shows the inverse correlation between the both with r=−0.74, p=0.04.

compared to sham over the whole range (Fig. 3A). Interestingly, the increased tension development in mesenteric arteries of CHF rats as seen after stimulation with K^+ plus U46619 at 0.9L100 was not observed after stimulation with U46619 solely at 0.9 L100 (Fig. 3B).

3.4. Myogenic constriction in response to stretch

Wall tension of mesenteric arteries in Ca^{2+}-free Krebs solution increased with increasing internal circumference over a range from 0.5 L100 to 1.2 L100. Importantly, the wall tension in Ca^{2+}-free Krebs solution (i.e. passive tension) did not differ between sham and CHF rats over the whole internal circumference length range studied (data not shown). In presence of Ca^{2+}, however, the developed (hence, active) tension significantly increased in CHF rats, but not in sham-rats. Consequently, myogenic constriction was increased in mesenteric artery ring preparations of CHF rats, but not in sham-rats (Fig. 4). Furthermore, the higher level of myogenic constriction in mesenteric artery ring preparations of CHF rats was significantly correlated with a lower contribution of EDHF (Fig. 5).

4. Discussion

In the present study, we observed that the contribution of different mediators in endothelium-dependent dilatation in isolated mesenteric artery ring preparations had significantly changed in CHF rats at 9–10 weeks post-MI, showing increased dependency of ACh-induced dilatation on NO and decreased contribution of EDHF. In addition, mesenteric arteries of CHF-rats showed increased stretch-induced myogenic constriction. With the latter, we were able to reproduce in a wire-myograph our previous key finding of increased pressure-induced myogenic constriction in cannulated pressurized mesenteric artery segments of CHF-rats [15], indicating the validity of this technique/protocol for vascular measurements. Moreover, increased myogenic constriction in CHF-rats was inversely correlated to EDHF in the present study. This extends our previous findings of an inverse correlation between myogenic constriction and EDHF in isolated coronary arteries under normal conditions [16] to the occurrence of this relationship in isolated mesenteric arteries in CHF.

Previous studies have reported endothelial dysfunction of coronary arteries as well as large conductance and peripheral arteries in CHF, with considerable implications for myocardial perfusion, cardiac workload, and peripheral vascular resistance [22–24]. In the present study, we investigated small resistance superior mesenteric artery rings of rats with MI-induced experimental CHF for ACh-induced dilation and stretch-induced myogenic constriction. Both are important local mechanisms of vasomotor control and mesenteric resistance arteries are of functional importance as they participate in the regulation of peripheral vascular resistance [25]. Results of previous studies with isolated mesenteric arteries vary from normal ACh-induced dilatation in rats with short- and long-term heart failure [9,10], to minimal and profound impairment of ACh-induced relaxation in heart failure rats relatively soon (4–6 weeks) after MI as reported by Malmsjo et al. [11] and Thuilllez et al. [12], respectively. Some of the observed discrepancies may be related to the different ways ACh-induced dilatation of isolated mesenteric arteries was investigated, particularly whether or not experiments were performed in the presence of indomethacin to exclude the influence of vasoactive prostanoids, as in the latter two studies mentioned above. In this respect, our present study is probably most comparable to that by Malmsjo et al. [11], who also studied ACh-induced dilatations in isolated mesenteric artery preparations in the presence of indomethacin, subsequently referred to as total dilatation to ACh.

Similar to the aforementioned investigators we also found that CHF induced only a minor decrease in total dilatation to ACh, and that the relationship between dilatory mediators was significantly altered. Malmsjo et al [11] reported a marked down-regulation of NO-mediated dilatation while the EDHF-contribution was up-regulated. Such findings seem to be in agreement with the development of an increase in oxidative stress in CHF-as reported by Indik et al [6] in this model at 6 weeks post-MI-causing excessive degradation of NO on the one hand, and suggestions that increased EDHF-activity may function as a compensatory mechanism to preserve endothelial function and tissue perfusion on the other hand [26,27].

In the present study, however, we found the opposite; MI-rats with CHF showed increased contribution of NO to ACh-induced dilatation in mesenteric arteries while EDHF-mediated dilatation was down-regulated. One difference that might account (in part) for the discrepancy with the study by Malmsjo et al [11] could be the later time point post-MI (i.e. 9–10 weeks) at which our study was performed. As suggested by the aorta-studies mentioned in the introduction, the development of endothelial dysfunction in rats with heart failure post-MI seems to be a progressive and time-dependent process. It is possible, therefore, that the observed difference between our studies reflects a time-dependent alteration in endothelial dilatory mediator ratio in the mesenteric artery during progression of CHF post-MI. Interestingly, Bauersachs et al [4] reported upregulation of vascular eNOS expression in CHF-rats at 8 weeks post-MI, and suggested this to presumably act as a counterregulatory mechanism against increased vascular O_2^- generation. In that study, enhanced upregulation of vascular eNOS was not sufficient to restore endothelium-dependent dilatation in the aorta, a vessel type in which vasorelaxation to a large extent is mediated by NO. In the mesenteric artery, however, NO contribution to ACh-induced dilatation is normally much smaller. It is possible, therefore, that upregulation of vascular eNOS — i.e. similar to that in the above study in

the aortas of rats with CHF (in the presence of increased oxidative stress) — is relatively more effective (in increasing NO contribution) in mesenteric arteries, as compared to the aorta. Clearly, this theory is only speculative and has not been specifically tested to our knowledge. Moreover, data on eNOS expression and vascular superoxide production in the mesenteric artery are very limited. Finally, an effect of differences in anaesthetics and rat weight/age — in addition to differences in the time point of sacrifice post-MI — between the present study and the study of Malmsjo et al. [11] cannot be excluded. Nevertheless, the above might reflect development of endothelial dysfunction during progression of CHF post-MI involving time-dependent changes in the relationship between endothelial vasodilator mediators that may be heterogeneous among different vascular beds.

Although several studies have attempted to explain altered NO contribution to ACh-induced dilatation in CHF by studying associated changes in eNOS expression and vascular superoxide production, we are not aware of any studies addressing potential mechanisms underlying altered EDHF contribution in CHF. One important limitation for such studies is the fact that the nature of EDHF and its pathway for synthesis has not uniformly and unequivocally been established, hence, making it difficult to explain decreased EDHF, as found in CHF in the present study, in these terms.

We previously studied EDHF in relation to myogenic constriction. Normal rat coronary arteries show a relatively high level of myogenic constriction and lower EDHF contribution to ACh-induced dilatation, as compared to mesenteric arteries of similar size, and we recently showed that the degree of myogenic constriction in coronary arteries is inversely related to EDHF contribution in individual rats [16]. This functional relationship probably exists because both processes share common mechanisms through specific potassium channels. Hence, reduction of myogenic constriction by NS1619 (a specific opener of K_{ca}) but not cromakalim (an opener of K_{ATP}), profoundly enhanced EDHF contribution to ACh-induced dilatation in coronary arteries, which then show features similar to the normal mesenteric artery — i.e. low myogenic constriction and high EDHF [16]. We previously found that in rats with CHF, however, myogenic constriction in mesenteric arteries is markedly increased [15], and we were therefore interested whether increased myogenic constriction may go hand-in-hand with decreased EDHF.

The results of the present study in the mesenteric arteries of rats with CHF at 9–10 weeks post-MI confirm our previous finding of increased myogenic constriction, and additionally suggest that EDHF is impaired and inversely related to individual rats with CHF. It thus appears that an inverse functional relationship between myogenic constriction and EDHF as observed in normal coronary artery can also be demonstrated in mesenteric arteries under pathophysiological conditions of CHF.

Our results are quantitatively limited in the number of observations, and qualitatively in that EDHF was determined by exclusion only and that the results do not identify whether myogenic constriction and EDHF are causally related. Nor do we know the time frame in CHF development post-MI during which alterations occur. Therefore, we can only speculate about initial changes in the one to cause subsequent (or associated) changes in the other, and what is underlying these initial changes. One hint with respect to the latter may come from our present finding that the contractile response to a fixed dose of U46619 did not differ between sham control rats and those with CHF, whereas the contractile response to a combination of same dose of U46619 plus a fixed concentration of K^+ was significantly increased only in rats with CHF. Such findings may be suggestive of a more selective alteration at the level of ion channels in CHF rather than general changes at the level of the contractile apparatus (hence, contraction with K^+ is due to opening of voltage-gated calcium-channels). Heart failure is associated with changes in (myocardial) ion channel expression [28], although little is known about ion channel expression in relation to vascular function in CHF [29]. Since EDHF and myogenic constriction act via specific K^+ channels, it might be interesting to assess temporal changes in vascular K^+ channels in rats with CHF post-MI in relation to both processes in future studies [27,30]. Another direction to pursue may be the possibility that changes in cytochrome P450 arachidonate metabolites, such as EETs and 20-HETE, could play a role in CHF. Cytochrome P450 metabolites have been implicated in EDHF-mediated dilatation as well as myogenic constriction, and their potential involvement in an inverse relationship between both may be argued [31,32], including the possibility of modulation of the heme moiety of P450 by altered NO production [33] as observed in the present study.

In conclusion, the present study provides evidence for increased myogenic constriction and significant alterations in the relationship between endothelial mediators in the mesenteric arteries of rats with CHF at 9–10 weeks post-MI, in which decreased EDHF contribution to ACh-induced dilatation is inversely related to increased myogenic constriction. Future studies investigating causal relationships between these changes may be directed at alterations in vascular K^+ channels and/or cytochrome P450 arachidonate metabolites in CHF. Ultimately, our findings give insight into local mechanisms contributing to increased peripheral vascular resistance in CHF and may provide a rationale for therapeutic interventions.

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