Enhanced acetylcholine and P2Y-receptor stimulated vascular EDHF-dilatation in congestive heart failure

Malin Malmsjö, Anders Bergdahl, Xiao-He Zhao, Xiang-Ying Sun, Thomas Hedner, Lars Edvinsson, David Erlinge

*Division of Experimental Vascular Research, Department of Internal Medicine, Lund University Hospital, Lund, Sweden

bDepartment of Clinical Pharmacology, Gothenburg University, Gothenburg, Sweden

Received 5 October 1998; accepted 12 January 1999

Abstract

Objective: Congestive heart failure (CHF) is accompanied by impaired peripheral blood flow and endothelial dysfunction with decreased release of nitric oxide (NO). Strong evidence supports the existence of another vasodilatory substance, endothelium derived hyperpolarising factor (EDHF), which has not previously been studied in CHF.

Method: CHF was induced by left coronary artery ligation resulting in a reproducible myocardial infarction in Sprague Dawley rats. Vasodilatory responses to acetylcholine and extracellular nucleotides (ATP, ADPβS, ADP and UTP) were examined in cylindrical segments of the mesenteric artery, precontracted with noradrenaline. The combined NO- and EDHF-dilatation (after inhibition of cyclo-oxygenase pathways) was called “total dilatation”, as indomethacin had only minor effects in this system. NO-dilatation was studied in segments pretreated with indomethacin and the potassium channel inhibitors charybdotoxin (10⁻⁷ M) and apamin (10⁻⁶ M), while EDHF-dilatations were studied in the presence of indomethacin (10⁻⁶ M) and L-NOARG (10⁻³ M).

Results: EDHF-dilatations in CHF were strongly up-regulated for ACh (36% vs. 73%; sham vs. CHF operated rats), ADPβS (10% vs. 42%), ADP (0% vs. 21%) and UTP (3% vs. 35%). These dilatations were abolished by a combination of charybdotoxin and apamin, confirming that they were mediated by EDHF. The NO-dilatations on the other hand were down-regulated in CHF as compared to sham operated rats for ACh (93% vs. 76%; sham vs. CHF operated rats), ADPβS (61% vs. 37%), ADP (60% vs. 30%), ATP (49% vs. 34%) and UTP (65% vs. 47%), while a minor decrease was seen in the total dilatation for ACh (87% vs. 75%; sham vs. CHF operated rats), ADPβS (47% vs. 42%), ADP (59% vs. 39%), ATP (52% vs. 39%) and UTP (59% vs. 44%).

Conclusion: In this model of non-atherosclerotic CHF there was a minor decrease in the total dilatation and a marked down-regulation of the NO-mediated dilatation, while the EDHF-dilatation was up-regulated. Increased EDHF-activity in CHF may represent a compensatory response to decreased NO-activity to preserve endothelial function and tissue perfusion.

Keywords: Adenosine; Endothelial function; Heart failure; Nitric oxide; Regional bloodflow

1. Introduction

Congestive heart failure (CHF) is characterised by reduced cardiac function resulting in inadequate tissue perfusion. CHF also induces changes in several cardiovascular regulatory systems, such as vascular smooth muscle cell (VSMC) hypercontractility, endothelial cell dysfunction, activation of the renin-angiotensin and the sympathetic nervous system [1–3].

Endothelial cells induce vasodilatation when stimulated by acetylcholine (ACh) and extracellular nucleotides such as ATP, UTP and ADP. The dilatory mediators, released by ACh, have so far mainly been characterised as nitric oxide (NO), prostaglandins and endothelium-derived hyperpolarising factor (EDHF). We have recently shown that extracellular nucleotides not only stimulate release of NO and prostaglandins [4], but also EDHF by activation of endothelial P2Y-receptors [5,6]. Altered endothelial cell function during CHF may result from an increased activity...
from circulating vasoconstrictors such as catecholamines [7,8], endothelin [9,10], arginine vasopressin [11,12], cytokines such as TNFα [7,13,14], or an altered endothelial shear stress [15,16]. Investigation of endothelial function and stimulated release of NO in CHF has classically been performed by the use of ACh [17]. It has been shown that ACh-induced NO-release is decreased in CHF, and that endothelium-dependent dilatation of resistance vessels is blunted in patients with severe CHF [18–20]. In conductance vessels, flow-dependent dilatation is reduced in CHF, reflecting endothelial dysfunction. Previous reports suggest that decreased levels of NO may be associated with increased activity of EDHF, that in a compensatory manner, maintains endothelial vasodilator function during vascular diseases such as hypertension [21], diabetes [22] and hypercholesterolemia [23].

ATP is stored in and may be released from most cells, e.g. endothelial cells exposed to hypoxia or shear stress as well as trombocytes during aggregation. ATP is also a co-transmitter with noradrenaline (NA) in perivascular sympathetic nervous system [24], which is known to possess increased activity in CHF [25,26]. Recent studies have shown that plasma adenosine levels are increased in patients with ischemic and non-ischemic chronic CHF, and that adenosine-levels are correlated to the severity of the disease [27]. Since circulating (adenosine triphosphate) ATP is rapidly degraded to adenosine by ectonucleotidases, present preferentially on endothelial cells ([28]), the elevation of plasma adenosine may reflect an increased release of ATP in CHF. Thus, it is of considerable interest to study the cardiovascular effects of extracellular ATP in CHF, in order to elucidate possible adaptations to its responses. The present study was designed to examine endothelial function in CHF by investigating the potential alterations in ACh and P2Y-receptor stimulated EDHF and NO-dilatation. To our knowledge there are no previous studies on EDHF-dilatation in CHF.

2. Material and methods

2.1. Experimental animals

Experiments were conducted on male Sprague-Dawley rats (ALAB, Sollentuna, Sweden) weighing approximately 500 g (2–3 months old). All animals were maintained on standard rat pellets and tap water ad libitum and housed in cages in groups of five animals, at +26°C, with 60% humidity and a 05.00 a.m. to 07.00 p.m. light/dark regimen. Animals were caged individually after surgical operation. The protocol was approved by the Ethics Committees for Animal Experiments at the University of Gothenburg and at the Lund University, both in Sweden.

2.2. Induction of myocardial infarction

During a shortlasting methohexital sodium anaesthesia, 60 mg/kg i.p., the rats were intubated and artificially ventilated with a respirator (Carlsson ventilator, No 8908, Mölndal, Sweden). A left thoracotomy was performed, exposing the left ventricular wall. The left coronary artery was ligated by positioning a suture between the pulmonary artery outflow tract and the left atrium. The lungs were thereafter hyperinflated using positive end-expiratory pressure, the thorax was immediately closed and the rats were allowed to recover for 4–8 weeks before the experiments were started. Rats which underwent the same surgical procedure, but without coronary artery ligation, served as controls (sham operated rats). Early mortality (1 day) after surgery was 10% and late mortality (1 month) 2%.

2.3. Evaluation of infarction size

To confirm the induced myocardial infarction in the operated animals a histological follow-up was performed. During the tissue preparation (see below) the hearts from the control and CHF rats were removed and immersed into a 6% formaline solution. The ventricular region of the heart was then cut from the apex to the base in four slices and from each slice 10 μm thin sections were prepared and stained for microscopical evaluation. Photographs were made at X10 magnification and the endocardial circumference of the left ventricle and the extent of the fibrotic area from each slice was measured. Infarct size was evaluated according to [29], i.e. the fibrotic fraction in per cent of the total cross-sectional endocardial circumference of the left ventricle. Rats with a myocardial infarction comprising more than 30% of the left ventricular wall were included in the study.

2.4. In vitro experiments

The rats were anaesthetised by inhalation of CO₂ (carbon dioxide), and killed by a cardiac cut. The mesenteric artery was gently removed and immersed in a cold oxygenated bicarbonate based buffer solution (for composition, see below) where after the superior mesenteric artery before branching was dissected free of adhering tissue under a microscope. The vessels were cut into cylindrical segments (2–3 mm long) which were immediately used in the experiments. Each cylindrical segment was mounted on two L-shaped metal prongs, one of which was connected to a force displacement transducer (FT03C) for continuous recording of the isometric tension, and the other to a displacement device [30]. The position of the holder could be changed by means of a movable unit allowing fine adjustments of the vascular resting tension by varying the distance between the metal prongs. The mounted artery segments were immersed in temperature controlled (37°C) tissue baths containing bicarbonate based buffer solution of the following composition (mM): NaCl 119, NaHCO₃ 15, KCl 4.6, MgCl₂ 1.2, NaH₂PO₄ 1.2, CaCl₂ 1.5 and glucose 5.5. The solution was continuously gassed with 5% CO₂ in O₂ resulting in a pH of 7.4.
Special care was taken to ensure that the endothelium was not damaged. The arterial segments were allowed to stabilise at a resting tension of 2 mN for 1 h, after which the contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60 mM) buffer solution in which NaCl was exchanged for an equimolar concentration of KCl (for composition see above). When two reproducible contractions had been achieved the vessels were used for further studies.

2.5. Measurement of mechanical responses

Relaxation was studied in vascular segments precontracted by $10^{-6}$ M NA. Agonists were added cumulatively to determine concentration–response relationships. Twelve ring segments were studied at the same time in separate tissue baths. To avoid desensitisation of receptors, agonists were only added once to each segment. Thus, comparisons were made between the responses in different vessel segments. Antagonists were administered 30 min before application of agonists. All experiments were performed with indomethacin present to abolish prostacyclin-induced dilatory effects. Endothelium-denudation was performed on the dissected intact vessel before mounting by perfusion for 5 s with 0.1% Triton X followed by another 5 s of perfusion with a buffer solution using a fine needle. Endothelium denudation was checked by monitoring responses to ACh. Abolished dilatation indicated a properly removed endothelium. To eliminate the possibility of SMC damage by Triton X, vasodilatory responses to the endothelium-independent vasodilator SIN-1 was assessed (see results).

2.6. Antagonists of the different dilatory mediators

The EDHF-mediated dilatory response was studied after eliminating the prostacyclin- and NO-mediated dilatations by administration of indomethacin and L-NOARG, while the NO-dilatation was studied in artery segments pretreated with indomethacin, charybdotoxin and apamin to abolish all EDHF-effects. Dilatations recorded with only indomethacin present were called `total dilatation' as indomethacin only had minor effects in this system. To block all dilatory mediators, a combination of indomethacin, L-NOARG, charybdotoxin and apamin was used.

2.7. Drugs

Methohexital sodium (Brietal®, Lilly Inc., Indianapolis, IN, USA), adenosine-triphosphate (ATP, $10^{-3}$–$10^{-4}$ M), adenosine-diphosphate (ADP, $10^{-9}$–$10^{-5}$ M), adenosine 5’-diphosphate (ADPβS, $10^{-9}$–$10^{-4}$ M), uridine-triphosphate (UTP, $10^{-9}$–$10^{-5}$ M), acetylcholine (ACh, $10^{-9}$–$10^{-5}$ M), indomethacin ($10^{-5}$ M), noradrenaline (NA, $10^{-6}$–$10^{-5}$ M), Nω-nitro-L-arginine (L-NOARG, $10^{-3}$ M), 3-morpholino-synonimine (SIN-1, $10^{-7}$–$10^{-5}$ M), charybdotoxin ($10^{-7.5}$ M) and apamin ($10^{-6}$ M) (Sigma Co., USA). All drugs were dissolved in 0.9% saline.

2.8. Calculations and statistics

The amplitude of contraction before application of relaxatory agonists was set to 100%. The negative logarithm of the drug concentration eliciting 50% relaxation (EC$_{50}$) was determined by linear regression analysis using the values immediately above and below half-maximum response. R$_{max}$ refers to maximum relaxation. Values are presented as mean±SEM. All experiments were performed on 5–18 segments (animals). Statistical significance was defined as $P<0.05$. ANOVA by repeated measures for the curves (see figures) and by Students t-test for the difference between R$_{max}$ values (see Tables).

3. Results

3.1. CHF status

3.1.1. Myocardial infarction size

Hearts, in which the left coronary artery had been ligated, demonstrated pathological hallmarks of myocardial infarction such as myocyte degeneration, fibrosis in the left ventricular region and cardiac dilatation. The infarction size of the ligated hearts were 38.1±3.5% (mean±SD) of the left ventricular circumference. No myocardial degeneration was seen in the sham operated rats.

3.1.2. Other signs of congestive heart failure

Congestion of the lungs and abdominal organs and of the portal and the lienal veins were seen in the CHF operated rats, supporting the establishment of congestive heart failure. These observations correspond with our findings of increased heart- (0.49±0.03% vs. 0.34±0.02%, CHF vs. sham operated rats) and lung-weights (0.69±0.05% vs. 0.41±0.04%) in CHF rats, when calculated as percentage of total body weight.

3.2. Dilatory responses

Dilatory responses were examined in the rat mesenteric artery. The contractile response to 60 mM K$^+$ did not differ between sham (5.70±0.15 mN), and CHF operated rats (5.85±0.13 mN). Neither did the NA-induced precontraction differ (6.24±0.19 mN vs. 6.32±0.25 mN, CHF vs. sham operated rats).

3.2.1. Endothelium denudation and SIN-1 dilatations

Relaxation to each agonist was totally abolished by
endothelium denudation in both CHF and sham operated rats. SIN-1 induced dilatations did not differ between control vessel segments \( (R_{max} 103\pm5\% \text{ and } pEC_{50} 5.12\pm0.03) \) and denuded vessel segments \( (R_{max} 97\pm4\% \text{ and } pEC_{50} 5.05\pm0.04) \), indicating intact function of the VSMC's after denudation. The SIN-1-induced dilatations did not differ between CHF \( (R_{max} 96\pm3\% \text{ and } pEC_{50} 5.01\pm0.04) \) and sham operated rats \( (R_{max} 101\pm2\% \text{ and } pEC_{50} 4.99\pm0.05) \), indicating that CHF does not affect the dilatory capacity to NO of the smooth muscle cells.

3.2.2. Indomethacin

Indomethacin did not affect the dilatory responses to any of the agonists significantly (reduction of dilatation <5%), indicating a very minor contribution of cyclo-oxygenase products.

3.2.3. Inhibition of NO, EDHF and cyclo-oxygenase pathways

When the artery segments were pretreated with a combination of indomethacin, L-NOARG, charybdotoxin and apamin, all dilatory responses were abolished in both CHF and sham operated rats, indicating that the L-NOARG and indomethacin resistant dilatation was indeed mediated by EDHF.

3.2.4. Total dilatation

Dilatations recorded with only indomethacin present were called “total dilatation” as indomethacin only had minor effects in this system. CHF induced a minor decrease in the total dilatation for ACh, ADPβS, ADP, ATP and UTP (Table 1, Figs. 1a, 2a, 3a, 4a and 5a).

3.2.5. NO-dilatation

The NO-dilatation was studied in artery segments pretreated with indomethacin, charybdotoxin and apamin. Concentration-dependent dilatations, that were significantly lower in CHF as compared to sham operated rats, could be observed for ACh, ADPβS, ADP, ATP and UTP (Table 2, Figs. 1c, 2c, 3c, 4b and 5c).

3.2.6. EDHF-dilatation

EDHF-dilatation was studied in artery segments pretreated with indomethacin and L-NOARG. ACh-, ADP-, ADPβS- and UTP-induced concentration-dependent dilatations that were markedly up-regulated in the CHF rats for ACH, ADPβS, ADP and UTP (Table 3, Figs. 1c, 2c, 3c, and 5c). ATP did not induce EDHF-dilatation either in CHF or in sham operated rats (Table 3 and Fig. 4b).

3.2.7. Relative involvement of EDHF and NO in ach stimulated vasodilatation in sham and CHF operated rats

Based on the calculated area under the curves in Fig. 1a, b and c, the total dilatation for CHF as compared to sham was 78%. The relative shares of the dilatory mediators was in CHF, 57% for NO and 43% for EDHF, and in sham operated rats 77% for NO and 23% for EDHF (Fig. 6).

4. Discussion

The most interesting finding was that this model of non-atherosclerotic CHF induced an up-regulation of the ACh and P2Y-receptor stimulated EDHF-dilatation, while the NO-dilatation was down-regulated.

4.1. The CHF model

A heart failure model was used where the left anterior descending coronary artery was ligated inducing an acute infarction, where after the rats were left to recover for 4–8 weeks. During this period a heart failure state was developed. A myocardial infarction comprising more than 30% of the left ventricular wall has in this heart failure model been shown to decrease cardiac output and increase left ventricular end-diastolic pressure [29]. Furthermore, the infarct size is positively correlated with left ventricular volume and negatively correlated with left ventricular ejection fraction [31]. Other studies of this heart failure model have revealed increased plasma levels of catecholamines and ANP, as well as pulmonary oedema, hepatic congestion and pleural effusion [26,32,33]. The present study shows increased heart- (0.34% of total body weight vs. 0.49%, sham operated vs. CHF) and lung-weights (0.41% vs. 0.69%) in CHF rats. Earlier studies of this heart failure model have shown that the impairment of the left ventricular function is directly related to the loss of

Table 1

Relaxation maximum \( (R_{max}) \) and \( pEC_{50} \) values for the total dilatation for different agonists in sham operated and CHF rats. There were no statistical difference between \( R_{max} \) (sham vs. CHF, Student’s t-test). \( N=\text{number of different animals} \)

<table>
<thead>
<tr>
<th></th>
<th>Sham:</th>
<th>CHF:</th>
</tr>
</thead>
<tbody>
<tr>
<td>R_{max} (%)</td>
<td>pEC_{50} (-\log M)</td>
<td>R_{max} (%)</td>
</tr>
<tr>
<td>ACh</td>
<td>87±4</td>
<td>8.1±0.13</td>
</tr>
<tr>
<td>ADPβS</td>
<td>47±9</td>
<td>6.18±0.15</td>
</tr>
<tr>
<td>ADP</td>
<td>59±7</td>
<td>5.64±0.11</td>
</tr>
<tr>
<td>ATP</td>
<td>52±10</td>
<td>5.34±0.27</td>
</tr>
<tr>
<td>UTP</td>
<td>59±6</td>
<td>5.66±0.22</td>
</tr>
</tbody>
</table>
myocardium [29], with the infarction-sizes being divided into small (5–19%), moderate (20–39%) and large (>40%) [34]. Loss of more then 30% of the myocardium of the left ventricle (referring to the method described above) results in a pump-dysfunction with reduced values of peak flow, systolic and mean arterial pressure. The
damage of the left ventricular wall was in the present study histologically proven, and showed a myocardial infarction comprising 38±4% of the endocardial circumference. Also, we noted signs of organic congestion as described above. Our model can therefore be considered successful according to CHF criteria denoted by the literature. We used this model to examine the endothelial function in CHF by investigating potential alterations in ACh- and P2Y-receptor-stimulated EDHF- and NO-dilatation.

### 4.2. Total dilatation and NO-mediated dilatation

Investigation of endothelial function and stimulated release of NO in CHF have classically been performed by the use of ACh [17]. Endothelium-dependent dilatation of resistance arteries is blunted in patients with severe CHF [18]. In conductance vessels, flow-dependent dilatation is reduced. These findings reflect endothelial dysfunction in CHF. The ACh-stimulated release of NO is impaired while the bloodflow response to nitroglycerin or sodium nitroprusside, both endothelium-independent vasodilators, is preserved indicating a normal response of the VSMC’s to
ADPβS, ADP, ATP and UTP, indicating a CHF induced endothelial dysfunction (Table 2, Figs. 1b, 2b, 3b, 4b and 5b). The NO-donor SIN-1 induced relaxations that were unaffected by CHF, indicating preserved VSMC response to exogenous NO.

The total dilatation of the vessel, induced by ACh and P2Y-receptor stimulation, was slightly decreased, indicating general endothelial dysfunction (Table 1, Figs. 1a, 2a, 3a, 4a and 5a). The decrease in total dilatation was not as pronounced as for the NO-dilatation, indicating a mechanism that compensates for the decreased NO-effect.

4.3. EDHF-dilatation

The vasodilatory effects of ACh have so far mainly been ascribed to NO and prostaglandins. Since Taylor and Weston [35] demonstrated endothelium-dependent relaxation by a factor that could cause relaxation by increasing the membrane potential of the VSMC’s, it has been generally accepted that ACh also induce release of EDHF. Although the identity of EDHF remains unknown it has been proposed that EDHF represents epoxyeicosanoic acids [36]. According to the criteria denoted by the literature identification of EDHF requires evidence that it is released by the endothelium, not inhibited by antagonists of NO-synthetase or cyclo-oxygenase pathways and that it induces hyperpolarisation and relaxation of VSMC’s that can be blocked by the potassium channel inhibitors charybdotoxin and apamin [37–40]. In the mesenteric artery activation of P2Y-receptors induce endothelium-dependent vasodilatation mediated by a factor distinct from NO and prostacyclin that is blocked by charybdotoxin and apamin indicating that it is EDHF [5]. This was confirmed by electrophysiological experiments in which both ACh and P2Y-receptor agonists induce endothelium dependent hyperpolarisation of the VSMC’s [6]. The hyperpolarisations were antagonised by charybdotoxin and apamin, confirming the use of this combination to inhibit EDHF.

The present study shows endothelium-dependent dilatations, distinct from NO and prostacyclin, to ACh, ADPβS, ADP and UTP since they were not blocked by L-NOARG and indomethacin. A combination of charybdotoxin and apamin totally abolished these dilatations indicating that they were mediated by EDHF. The high concentration of L-NOARG (10^{-3.5} M) used in the present experiments has in previous studies been shown to abolish the release of NO [41]. Furthermore, addition of the NO-scavenger PTIO to L-NOARG (10^{-3.5}) did not reduce the dilatation further, indicating that all the NO-production was blocked (data not shown). We have previously examined the specificity for charybdotoxin and apamin as EDHF-inhibitors on other vasodilators acting independently of the endothelium. They did not modify the relaxation to the NO-donor SIN-1 and thus we conclude that they do not affect the direct NO-mediated activation of the VSMC’s [5].

ACh, ADPβS-, ADP- and UTP-induced EDHF-dilata-
Table 2
Relaxation maximum (R_{max}) and pEC_{50} values for the NO dilatation for different agonists in sham operated and CHF rats. Statistical differences between R_{max} (sham vs. CHF, Student’s t-test) are indicated (*, P<0.05). N=number of different animals

<table>
<thead>
<tr>
<th></th>
<th>Sham:</th>
<th>CHF:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R_{max} (%)</td>
<td>pEC_{50} (-log M)</td>
</tr>
<tr>
<td>ACh</td>
<td>91±5</td>
<td>7.49±0.15</td>
</tr>
<tr>
<td>ADP\betaS</td>
<td>61±6</td>
<td>5.96±0.12</td>
</tr>
<tr>
<td>ADP</td>
<td>60±8</td>
<td>4.71±0.23</td>
</tr>
<tr>
<td>ATP</td>
<td>49±3</td>
<td>5.36±0.26</td>
</tr>
<tr>
<td>UTP</td>
<td>65±11</td>
<td>5.58±0.09</td>
</tr>
</tbody>
</table>

Table 3
Relaxation maximum (R_{max}) and pEC_{50} values for the EDHF dilatation for different agonists in sham operated and CHF rats. Statistical differences between R_{max} (sham vs. CHF, Student’s t-test) are indicated (**, P<0.01; *** , P<0.001). N=number of different animals

<table>
<thead>
<tr>
<th></th>
<th>Sham:</th>
<th>CHF:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R_{max} (%)</td>
<td>pEC_{50} (-log M)</td>
</tr>
<tr>
<td>ACh</td>
<td>36±8</td>
<td>6.19±0.24</td>
</tr>
<tr>
<td>ADP\betaS</td>
<td>10±3</td>
<td>5.15±0.28</td>
</tr>
<tr>
<td>ADP</td>
<td>0±0</td>
<td>–</td>
</tr>
<tr>
<td>ATP</td>
<td>0±0</td>
<td>–</td>
</tr>
<tr>
<td>UTP</td>
<td>3±1</td>
<td>4.18±0.04</td>
</tr>
</tbody>
</table>

Previous reports suggest that decreased levels of NO may be associated with increased activity of EDHF [21]. During physiological conditions, the production of EDHF is damped by NO. Impaired NO-synthesis, alleviates this intrinsic inhibition of EDHF and endothelial vasodilator function is maintained [43]. Up-regulated EDHF-activity may compensate for an increased vascular tonus elicited by decreased NO-synthesis in hypercholesterolemia [23] and hypertension [21], or by increased vasoconstriction in diabetes [22]. Our experiments showed EDHF-mediated dilatations that were several-fold stronger in the CHF rats for ACh, ADP\betaS, ADP and UTP (Table 3, Figs. 1c, 2c, 3c and 5c). We therefore speculate that a compensatory up-regulation of EDHF-mediated dilatation may represent a balancing adaptation to endothelial dysfunction and decreased NO-release and tissue perfusion in CHF (Fig. 6). The mechanism underlying the increased EDHF-dilatation is not known. It could depend on an enhanced synthesis of EDHF triggered by reduced levels of NO or by some of the many humoral factors that are increased in CHF. On the other hand the up-regulated EDHF-dilatation might depend on a greater responsiveness of the VSMC’s to EDHF.

5. Conclusions
The present study demonstrates that CHF induces a slight decrease in the total dilatation of the blood vessel while the relationship between the dilatory mediators is altered. EDHF-dilatations stimulated by ACh and P2Y-receptor agonists are up-regulated, while the NO-dilatations are down-regulated (Fig. 6). The increased EDHF-
References


