Congestive heart failure induces downregulation of P2X₁-receptors in resistance arteries

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Abstract

Objective: Congestive heart failure (CHF) is accompanied by enhanced peripheral sympathetic nerve activity, increased vascular resistance and impaired peripheral blood flow. Besides noradrenaline and neuropeptide Y, the sympathetic nervous system also releases ATP, which has contractile effects mediated by different subtypes of P2-receptors on the vascular smooth muscle cells. The present study was designed to examine postsynaptic changes of the contractile responses to ATP and other extracellular nucleotides in CHF. Methods: CHF was induced by left coronary artery ligation resulting in a reproducible myocardial infarction in Sprague-Dawley rats. Contractile responses were examined in cylindrical segments of aorta and the mesenteric artery after endothelium removal. To determine if an altered response was regulated on the transcriptional level, competitive reverse transcription polymerase chain reaction (RT-PCR) was used to estimate the amount of P2X₁-receptor mRNA. Results: ATP, which is both a P2X₁- and a P2Y-receptor agonist, induced a weaker contraction in the mesenteric artery from CHF as compared to sham operated rats. A decrease in both potency and maximum contraction was shown for the selective P2X₁-receptor agonist, αβ-MeATP, in the mesenteric artery (pEC₅₀=6.04 vs. 5.76, C₅₀=57% vs. 33%, sham vs. CHF operated rats), but not in the aorta. Competitive RT-PCR also revealed decreased P2X₁-receptor mRNA levels in CHF operated rats in the mesenteric artery (9106⁻¹⁰⁻¹⁻¹ molecules/µg, sham vs. CHF operated rats), while it remained unaltered in the aorta. To study the P2Y-receptor induced contractile effects, the P2X₁-receptors were first desensitised with αβ-MeATP (10⁻⁸ M for 8 min). After P2X₁-receptors desensitisation, UTP and UDP induced strong contractions in both the mesenteric artery and in the aorta, while ATP and ADP were much less effective. These contractions were not altered by CHF, indicating that vascular contraction mediated by P2Y-receptors are unaffected by CHF. Conclusion: CHF induces downregulation of P2X₁-receptor stimulated contraction in the mesenteric artery depending on decreased mRNA synthesis for the receptor, while the P2Y-receptor activity remains unchanged. Downregulation of P2X₁-receptors appears to be specific for peripheral resistance arteries. This may represent a compensatory response to enhanced peripheral sympathetic nerve activity and increased vascular resistance in CHF. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Adenosine triphosphate; autonomous nervous system; heart failure; gene expression; receptors

1. Introduction

Congestive heart failure (CHF) is associated with reduced cardiac function resulting in inadequate tissue perfusion. To compensate for this, changes are induced in several cardiovascular regulatory systems, including enhanced peripheral sympathetic nerve activity with increased release of noradrenaline (NA), neuropeptide Y (NPY) and adenosine triphosphate (ATP), followed by increased vascular resistance and impaired peripheral blood flow. Such compensatory responses may initially improve cardiac function and peripheral perfusion, however, in the long term these effects may be deleterious. In
order to design future potential pharmacological treatments of CHF it is important to study these compensatory mechanisms in CHF. It was recently reported that plasma adenosine levels are increased in patients with ischemic and nonischemic chronic heart failure, and that adenosine levels correlate with the severity of the disease [1]. Since circulating ATP is rapidly degraded into adenosine by ectonucleotidases, preferentially present on endothelial cells [2], the elevation of plasma adenosine may in fact reflect increased release of ATP in CHF. The present study was designed to characterise the contractile effects of purines and pyrimidines in the mesenteric artery of the rat, in order to elucidate the role of P2-receptors in CHF.

Extracellular nucleotides such as ATP, adenosine diphosphate (ADP), uridine triphosphate (UTP) and uridine diphosphate (UDP) induce vasoconstriction by stimulation of P2X- and P2Y-receptors [3]. A number of different P2X-receptors have been cloned, but only P2X1 seems to be present on vascular smooth muscle cells (VSMC’s) [4]. This receptor was first cloned by Valera et al. [5] and is preferentially activated by αβ-methyleneATP (αβ-MeATP), 2-methylthio ATP (2-MeSATP) and ATP. Four different P2Y-receptor subtypes are involved in vascular responses. The P2Y1-receptor is mainly an endothelial receptor, while P2Y2, P2Y4 and P2Y6 are pyrimidine sensitive receptors located both on VSMC’s (contractile) and endothelial cells (dilatory) [6].

It is well known that CHF is accompanied by elevated levels of circulating NA as well as of NPY [7] with blunted vascular responses to these agonists [8,23,26]. To examine if the receptors for the other sympathetic cotransmitter, ATP, are altered we examined the contractile responses mediated by P2-receptors as well as mRNA expression levels in CHF.

2. Methods

2.1. Experimental animals

Experiments were conducted on male Sprague–Dawley rats (ALAB, Sollentuna, Sweden) weighing approximately 500 g. 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 5.00 am to 7.00 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.

2.2. Myocardial infarction

2.2.1. Induction of myocardial infarction

During a short-lasting 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%.

2.2.2. 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 Section 2.3) 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 ×10 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 Pfeffer et al. [9], 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.

2.3. Tissue preparation and contractile response

2.3.1. In vitro experiments

The rats were anaesthetised by inhalation of CO2 (carbon dioxide), and killed by a cardiac cut. The aorta and the mesenteric artery were gently removed and immersed in cold sterile Dulbecco’s modified Eagle’s medium (DMEM) where the arteries were dissected free of adhering tissue under a microscope. The endothelium was removed by perfusion for 5 s with 0.1% Triton X followed by another 5 s of perfusion with a physiologic buffer solution (for composition see below) using a fine needle. 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 [10]. 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.

Endothelium removal was checked by monitoring responses to acetylcholine (ACh). Abolished dilatation indicated a properly removed endothelium. The mesenteric artery segments were allowed to stabilise at a resting tension of 2 mN and the aorta at 4 mN for 1 h before the experiments were started. 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.

2.3.2. Measurement of mechanical responses

Twelve ring segments were studied at the same time in separate tissue baths. As the P2Y-receptors are only very slowly desensitised, the P2Y-receptor agonists could be added cumulatively to determine concentration–response relationships. To avoid desensitisation of P2X₁-receptors, αβ-MeATP was administered in single-dose response (each segment was only exposed to αβ-MeATP once). To study the P2Y-receptor stimulated contractions without interference of simultaneous P2X₁-receptor induced responses, ADP, adenosine 5’O-thiodiphosphate (ADPβS). UTP and UDP were added after desensitisation of P2X₁-receptors using αβ-MeATP (10⁻⁵ M). Both the combined P2X₁- and P2Y-receptor effect and the isolated P2Y-receptor induced contraction (after P2X₁-receptor desensitisation) were studied for ATP.

2.4. Competitive reverse transcription polymerase chain reaction (RT-PCR)

2.4.1. RNA-extraction

The aorta and the mesenteric artery were carefully dissected and the endothelium was removed (see above). The arteries were snap-frozen in liquid nitrogen immediately after acquisition and total cellular RNA was extracted using TRIzol reagent (Gibco, Paisley, Scotland, UK) following the supplier’s instructions. The resulting RNA pellet was finally washed with 70% ice-cold ethanol, air-dried and redissolved in 10 µl diethylpyrocarbonate-treated water. The RNA concentration was determined spectrophotometrically considering a ratio of optical density (OD) 260/280 ≥1.6 as pure.

2.4.2. RT-PCR

RT-PCR was carried out using the GeneAmp RNA PCR kit (Perkin–Elmer, Foster City, CA, USA) on a GeneAmp PCR system 2400 (Perkin–Elmer). Specific primers for the rat P2X₁-receptor [5], were constructed (forward, 5’-AGAGGCACCTACTACAAGCAGAA-3’; reverse, 5’-GGTAAGGCTGTGGGAAAGA-3’). A synthetic RNA-competitor for P2X₁-receptor, bearing a deletion of 78 base pairs (bp) compared to the wild-type sequence has previously been constructed by Erlinge et al. [11]. For competitive RT-PCR 500 ng total RNA was mixed with decreasing amounts of competitor RNA in five subsequent 1:5 dilution steps. First-strand cDNA synthesis was then performed with the Amplitaq RNA-PCR kit (Perkin–Elmer) in a 20 µl volume using random hexamers. Finally amplification was performed using a modified profile (2 min at 95°C followed by 35 cycles of 1 min 95°C, 1 min 56°C, 30 s 72°C and a final extension step of 7 min at 72°C).

To establish linearity of the assay for the P2X₁-receptor over a wide range of concentrations, 10 pg of wild type plasmid was coamplified with decreasing amounts of competitor plasmid (five step 1:10 dilution series of appropriate competitor plasmid ranging from 100 fg to 1 ng (Fig. 5a, below). The optimal number of amplification cycles was determined by coamplification of duplicate tubes containing 10 pg wild-type plasmid and 5 pg competitor plasmid for 20, 25, 30, 35 and 40 cycles (Fig. 5b, below).

2.4.3. Densitometric analysis

Densitometric analysis of the PCR-products was performed as described previously [11]. Briefly the products were separated on a 2% agarose gel and photographed. The pictures were then digitalised on a flatbed-scanner and analysed densitometrically. The ratio wild-type/competitor was calculated taking the differing lengths of the products into account [30], and plotted on a log scale against competitor plasmid for P2X₁-receptor, bearing a deletion of 78 base pairs (bp) compared to the wild-type sequence has previously been constructed by Erlinge et al. [11].
ing 50% contraction (pEC$_{50}$) was determined by linear regression analysis using the values immediately above and below half-maximum response. C$_{max}$ refers to maximum contraction. Contractile experiments were performed on six segments (animals), and the results were verified by competitive PCR, which was repeated on three animals. Statistical significance was accepted when $P<0.05$, using students t-test. All differences referred to in the text have been statistically verified. Values are presented as mean±standard error of the mean (SEM).

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 37.4±3.9% (mean±SD) of the left ventricular circumference. No myocardial degeneration was seen in the sham operated rats.

3.1.2. Other signs of CHF

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 CHF. These observations correspond with our findings of increased heart- (0.52±0.02% vs. 0.35±0.01%, CHF vs. sham operated rats) and lung-weights (0.67±0.07% vs. 0.43±0.03%) in CHF rats, when calculated as percentage of total body weight.

3.2. Contractile responses

Contractile responses were examined in the rat mesenteric artery and in the aorta. K$^+$-induced contractions were not significantly different in CHF as compared to sham operated rats in the mesenteric artery (5.77±0.11 mN vs. 5.82±0.09 mN; sham vs. CHF operated rats) or in the aorta (7.51±0.07 mN vs. 7.46±0.10 mN). ACh had no dilatory effect indicating that endothelium removal was successful.

3.2.1. ATP

The contractile effect of ATP in the mesenteric artery was reduced in CHF at lower concentrations (where it stimulates P2X$_1$-receptors) but unaltered at the highest concentration (where it also stimulates P2Y-receptors) as compared to sham operated rats (Fig. 1 and Table 1).

3.2.2. P2X$_1$-receptor induced contractions

The P2X$_1$-receptor agonist, αβ-MeATP, was more potent in the mesenteric artery (pEC$_{50}$=6.04) than in the aorta (pEC$_{50}$=4.95). In the mesenteric artery the maximum contraction and the potency was reduced for αβ-MeATP in CHF (C$_{max}$=33±9%, pEC$_{50}$=5.76) as compared to sham operated rats (57±6%, pEC$_{50}$=6.04) (Fig. 2a and Table 1). No reduction was observed in the aorta (88±5% vs. 91±9%; CHF vs. sham operated) (Fig. 2b and Table 2).

3.2.3. P2X$_1$-receptor desensitisation

αβ-MeATP (10$^{-5}$ M) induced a transient contraction. After a period of 8 min the tension was back to normal and if αβ-MeATP was added a second time, no contraction was seen. We thereby concluded that the P2X$_1$-receptors were desensitised after this exposure.

3.2.4. P2Y-receptor induced contractions

To study the contractile effect of P2Y-receptors, the P2X$_1$-receptors were first desensitised (as previously described). UTP and UDP were by far the most effective and potent stimulators of P2Y-induced contraction in both aorta and mesenteric artery, while ATP and ADP were much less effective, and ADPβS was inactive (Fig. 3, Tables 1 and 2). The ATP-, ADP-, UTP- and UDP-induced contractions were not significantly different in CHF as compared to sham operated rats, either in the mesenteric artery or in the aorta, indicating that the P2Y-receptor induced contractions were unaltered (Fig. 4a, 4b, Tables 1 and 2).

3.3. Competitive RT-PCR

Densitometric analysis of P2X$_1$-receptor PCR-products produced a linear coamplification for the wild type and competitor plasmid, which was established over the entire dilution range as shown in Fig. 5a. Optimal cycle number,
Table 1
Contractile responses to \( \alpha\beta\text{-MeATP}, \text{ATP}, \text{ADP}, \text{UTP}, \text{UDP} \) and \( \text{ADP}\beta\text{S} \) in mesenteric artery from CHF and sham operated rats

<table>
<thead>
<tr>
<th></th>
<th>Sham C_{max} (%)</th>
<th>pEC_{50} (\text{log M})</th>
<th>CHF C_{max} (%)</th>
<th>pEC_{50} (\text{log M})</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha\beta\text{-MeATP} )</td>
<td>57±6</td>
<td>6.04±0.06</td>
<td>33±9</td>
<td>5.76±0.06</td>
</tr>
<tr>
<td>ATP</td>
<td>36±6</td>
<td>(3.36±0.12)</td>
<td>36±6</td>
<td>(2.77±0.19)</td>
</tr>
<tr>
<td>P2X-desensitised</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTP</td>
<td>171±16</td>
<td>3.01±0.12</td>
<td>193±23</td>
<td>2.99±0.11</td>
</tr>
<tr>
<td>UDP</td>
<td>140±15</td>
<td>(2.71±0.08)</td>
<td>162±8</td>
<td>(2.66±0.06)</td>
</tr>
<tr>
<td>ATP</td>
<td>28±7</td>
<td>(2.32±0.02)</td>
<td>25±7</td>
<td>(2.38±0.08)</td>
</tr>
<tr>
<td>ADP</td>
<td>28±9</td>
<td>(2.30±0.02)</td>
<td>20±5</td>
<td>(2.33±0.02)</td>
</tr>
<tr>
<td>ADP\betaS</td>
<td>0±0</td>
<td></td>
<td>0±0</td>
<td></td>
</tr>
</tbody>
</table>

\( \alpha\beta\text{-MeATP} \) and \( \text{ADP}, \text{UTP}, \text{UDP} \) and \( \text{ADP}\beta\text{S} \) were added after desensitisation of P2X-receptors with \( \alpha\beta\text{-MeATP} \). ATP is presented both in the absence and presence of prior P2X-receptor desensitisation. Contractions are expressed as percentage of an initial contraction induced by 60 mM K\(^+\). Data are shown as \( C_{max}\pm\text{SEM} \) and pEC_{50}-values that are within brackets (for ATP, ADP and UDP) are calculated as 50% of the contraction reached at the highest concentration of the agonist, since the dose–response curves did not reach a plateau-phase that could define the maximum concentration.

i.e., those which resulted in a measurable signal within the exponential phase, was between 25–35 cycles. Both the competitor and the wild type sequences were amplified in parallel (Fig. 5b). Thus, the established linearity of the assay enabled an exact measurement of the absolute concentration of the target fragments in the sample.

The competitive RT-PCR assay was used to measure P2X-receptor mRNA levels in rat mesenteric artery and aorta VSMC’s. Higher levels of mRNA (molecules/\( \mu \text{g} \) total mRNA) were detected in the mesenteric artery (9106\( \pm \)33\( \pm \)3\( \times \)10\(^6\)) than in the aorta (582\( \pm \)10\(^3\)\( \pm \)211\( \pm \)10\(^3\)). In CHF rats a 13-fold reduction in the amount of P2X-receptor mRNA was observed in the mesenteric artery (9106\( \pm \)33\( \pm \)3\( \times \)10\(^6\) vs. 714\( \pm \)10\(^6\)) than in the aorta (582\( \pm \)10\(^3\)\( \pm \)267\( \pm \)10\(^3\); sham vs. CHF operated rats) (Fig. 6).

4. Discussion

4.1. The CHF model

A heart failure model was used in which the left anterior descending coronary artery was ligated inducing an acute infarction, after which the rats were left to recover for 4–8 weeks. During this period a heart failure state developed. Other studies with this heart failure model have revealed increased plasma levels of catecholamines and atrial natriuretic peptide, as well as pulmonary oedema, hepatic congestion and pleural effusion [12,26,29]. It has also been demonstrated that the impairment of the left ventricular function is directly related to the loss of myocardium and that the infarct size is positively correlated with left ventricular volume and negatively correlated with left ventricular ejection fraction [9,13,14]. Loss of more than 30% of the myocardium of the left ventricle 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
Table 2
Contractile responses to αβ-MeATP, ATP, ADP, UTP, UDP and ADPβS in aorta from CHF and sham operated rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th></th>
<th>CHF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax (%)</td>
<td>pEC (−log M)</td>
<td>Cmax (%)</td>
<td>pEC (−log M)</td>
</tr>
<tr>
<td>αβ-MeATP</td>
<td>91±9</td>
<td>4.95±0.03</td>
<td>88±5</td>
<td>4.98±0.05</td>
</tr>
<tr>
<td>P2X-desensitised:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTP</td>
<td>170±21</td>
<td>3.41±0.18</td>
<td>178±25</td>
<td>3.25±0.22</td>
</tr>
<tr>
<td>UDP</td>
<td>179±16</td>
<td>(3.13±0.30)</td>
<td>161±9</td>
<td>(3.03±0.27)</td>
</tr>
<tr>
<td>ATP</td>
<td>38±10</td>
<td>(2.99±0.21)</td>
<td>46±15</td>
<td>(3.07±0.13)</td>
</tr>
<tr>
<td>ADP</td>
<td>25±10</td>
<td>(3.14±0.09)</td>
<td>30±4</td>
<td>(3.11±0.14)</td>
</tr>
<tr>
<td>ADPβS</td>
<td>0±0</td>
<td>-</td>
<td>0±0</td>
<td>-</td>
</tr>
</tbody>
</table>

* ATP, ADP, UTP, UDP and ADPβS were added after desensitisation of P2X$_1$-receptors with αβ-MeATP. Contractions are expressed as percentage of an initial contraction induced by 60 mM K$. Data are shown as Cmax±SEM and pEC$_{50}±SEM$. pEC$_{50}$ values that are within brackets (for ATP, ADP and UDP) are calculated as 50% of the contraction reached at the highest concentration of the agonist, since the dose–response curves did not reach a plateau-phase that could define the maximum concentration.

37±4% of the endocardial circumference. Furthermore, the heart and lung weights were increased in CHF rats.

4.2. Contractile responses mediated by P2-receptors

Ralevic and Burnstock [3] have demonstrated that ATP is more potent at inducing vascular contraction than UTP in the mesenteric artery, and that the ATP response is abolished after desensitisation of P2X$_1$-receptors with αβ-MeATP, while the UTP response is unaffected. This suggests that the contractile effect to extracellular ATP in blood vessels is mediated mainly by P2X$_1$-receptors with a smaller contribution of P2Y-receptors. Similar findings for ATP- and UTP-elicited contractions have been reported for the rat aorta [15]. In the present study the P2X$_1$-receptor induced contractions was studied with the specific agonist αβ-MeATP, while the P2Y-receptor induced contractile responses were studied after desensitisation of P2X$_1$-receptors using longer preincubation with αβ-MeATP.

A higher potency to the selective P2X$_1$-receptor agonist, αβ-MeATP, was observed in the mesenteric artery as compared in the aorta (Table 1 and 2). Furthermore, CHF

Fig. 3. Concentration-dependent contractions to αβ-MeATP, ATP, ADP, UTP, UDP and ADPβS in segments of mesenteric artery from sham operated rats. ADP, UTP, UDP and ADPβS were added after desensitisation of P2X$_1$-receptors with αβ-MeATP. ATP is shown both in the absence (open symbols) and presence (filled symbols) of prior P2X$_1$-receptor desensitisation. Contractions are expressed as percentage of an initial contraction induced by 60 mM K$. Data are shown as means±SEM of six experiments (animals).

Fig. 4. Concentration-dependent vasoconstriction to UTP and UDP in segments of mesenteric artery (a) and aorta (b) from sham (filled symbols) and CHF operated rats (open symbols) after P2X$_1$-desensitisation. Contractions are expressed as percentage of an initial contraction induced by 60 mM K$. Data are shown as means±SEM of six experiments (animals).
induced a decrease in potency and maximum contraction for αβ-MeATP in the mesenteric artery but not in the aorta. The mesenteric artery is in many ways a representative model of a peripheral resistance vessel [3], while the aorta is a large conducting artery. Thereby, our results suggest a more important physiological role for P2X<sub>1</sub>-receptors in peripheral resistance than in large conducting arteries.

The biphasic shape of the contraction curves for ATP is dependent on the activation of P2X<sub>1</sub>-receptors at low concentrations and of P2Y-receptors at high concentrations. The ATP-stimulated contractions at lower concentrations were reduced in the mesenteric artery from CHF, in agreement with reduced P2X<sub>1</sub>-receptor mediated contractions (Fig. 1 and Table 1). The unchanged contraction for ATP at the highest concentration supports the conclusion that CHF does not affect P2Y-mediated contractions. After P2X<sub>1</sub>-receptor desensitisation, no significant difference between CHF and sham operated rats could be observed for the weak remaining ATP- or ADP-induced contractions in the mesenteric artery or in the aorta. These results indicate that ATP mediates most of its contractile effect via P2X<sub>1</sub>-receptors with only a minor contribution of P2Y-receptors, and that the P2X<sub>1</sub>-induced contractile response is downregulated in the mesenteric artery from CHF operated rats. The responses to UTP and UDP were prominent despite desensitisation with αβ-MeATP, which is in accordance with the lack of desensitising effect of αβ-MeATP on P2Y-receptors [3]. The contractile responses to UTP and UDP were not altered in CHF in the mesenteric artery or in the aorta, indicating that the pyrimidine sensitive P2Y-receptors (P2Y<sub>2</sub>, P2Y<sub>4</sub> and P2Y<sub>6</sub>) are not significantly altered in CHF. The P2Y<sub>1</sub>-receptor selective agonist, ADPβS, was ineffective in our system, which suggests that P2Y<sub>1</sub>-receptors do not mediate contraction. This is consistent with recent data showing that P2Y<sub>1</sub>-receptor mRNA is only present in minute amounts in VSMC’s in the blood vessel wall [11].

4.3. Transcriptional regulation of the P2X<sub>1</sub>-receptor

Competitive RT-PCR has previously been used to show decreased levels of angiotensin-receptor mRNA in CHF [16], which corresponds well with findings of decreased angiotensin-receptor levels [17]. To examine if the presently observed decrease in P2X<sub>1</sub>-receptor induced contrac-
tile responses was dependent on transcriptional regulation, we quantified the level of P2X$_r$-receptor mRNA by competitive RT-PCR, recently developed in our laboratory [11]. Synthetic mRNA-competitors bearing a deletion of 78 bp compared to the wild type sequence were added in known amounts to RNA prior to RT-PCR, thus allowing quantification of the number of P2X$_r$-receptor mRNA copies in CHF and sham operated rats. Our experiments demonstrate that P2X$_r$-receptor mRNA and its competitor were amplified in parallel and that the cycle number used to give a measurable signal (35 cycles) lay within the exponential phase. Thus, the established linearity of the assay enabled exact measurement of the concentration of P2X$_r$-receptor mRNA. P2X$_r$-receptor mRNA levels were decreased in CHF ($714 \times 10^5$ molecules/$\mu$g) as compared to sham operated rats ($9106 \times 10^5$ molecules/$\mu$g) in the mesenteric artery, whereas it was unaltered in the aorta. The decreased P2X$_r$-receptor mediated contractions to ATP and the potency of $\alpha\beta$-MeATP in CHF suggest that the P2X$_r$-receptors are downregulated in CHF, which is supported by the decreased P2X$_r$-receptor mRNA levels. The more potent response to $\alpha\beta$-MeATP in the mesenteric artery as compared to the aorta also correlates with the higher amount of P2X$_r$-receptor mRNA ($9106 \times 10^5$ molecules/$\mu$g) as compared to aorta ($582 \times 10^5$). The high levels of P2X$_r$-receptor mRNA and the observed P2X$_r$-receptor downregulation during CHF in the mesenteric artery may in fact indicate an important pathophysiological role for P2X$_r$-receptors in peripheral resistance arteries.

4.4. Previous evidence of P2X$_r$-receptor regulation

Recent reports indicate that P2-receptors are selectively regulated in the VSMC’s. Contractile P2X$_r$-receptors are downregulated both at the functional and mRNA level, in the shift from the contractile to the synthetic phenotype [18,19,11], which is a central pathophysiological process in the development of atherosclerosis and in restenosis after angioplasty [20]. An increased sympathetic cotransmitter role for ATP has been demonstrated in spontaneous hypertensive rats as compared to normotensive rats [21], which is consistent with findings of decreased P2X-receptor mediated contraction in patients with hypertension [22]. Thus downregulation of P2X$_r$-receptors may be a general response to increased release of ATP.

4.5. Receptor regulation in CHF

In CHF a number of compensatory mechanisms are activated to increase tissue perfusion and to maintain circulation. In patients with CHF increased activity of the sympathetic nervous system and elevated levels of circulating catecholamines [8], NPY [7,27] and adenosine, possibly reflecting ATP [1], can be detected. The responses to NA on systemic blood pressure and on vascular resistance are blunted in patients with heart failure [8]. $\beta$-adrenoceptors located on circulating lymphocytes are downregulated, presumably as a consequence of prolonged elevation of circulating catecholamines in CHF [23]. Treatment with isoproterenol, a $\beta_1$-receptor agonist, reduces ventricular wall $\beta_1$-adrenoceptor density and myocardial contractility [24], which can be reversed by the $\beta_1$-receptor antagonist metoprolol [25]. Similar receptor downregulation has been demonstrated for the sympathetic cotransmitter NPY, where a decreased responsiveness to vascular postjunctional NPY receptors [26] may be due to the increased plasma levels of NPY in patients with CHF [7,27]. The cause of the decrease in P2X$_r$-receptor induced contraction and reduced mRNA levels can tentatively be ascribed to elevated levels of ATP in CHF, acting as a negative regulator on the P2X$_r$-receptors. This hypothesis is supported by a previous study by Edwards et al. [28] showing that ATP-induced VSMC depolarisation is much lower in innervated than in denervated rat cerebral arteries.

5. Conclusion

The present study demonstrates that CHF induce downregulation of P2X$_r$-receptor stimulated contraction in the mesenteric artery depending on decreased mRNA synthesis for the receptor, while the P2Y-receptor activity remains unchanged. Downregulation of P2X$_r$-receptors appears to be specific for peripheral resistance arteries. This suggests a compensatory response to enhanced peripheral sympathetic nerve activity and increased vascular resistance in CHF.

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[3] Ralevic V, Burnstock G. Effects of purines and pyrimidines on the sympathetic nervous system and elevated levels of circulating catecholamines [8], NPY [7,27] and adenosine, possibly reflecting ATP [1], can be detected. The responses to NA on systemic blood pressure and on vascular resistance are blunted in patients with heart failure [8].


