Pharmacological therapy can increase capillary density in post-infarction remodeled rat hearts

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Abstract

Objective: Postinfarction hypertrophied hearts have been shown to display a lower capillary density and reduced mechanical efficiency amplified by tachycardia. We investigated whether pharmacological reduction of postinfarction tachycardia would induce capillary growth by treating myocardial infarcted (MI) rats with aspirin, methylprednisolone, moxonidine or captopril, during the first 3 weeks after infarction.

Methods and Results: Effects on in vivo heart rate were measured in conscious unrestrained rats, while in vitro heart rate effects were evaluated in isolated perfused hearts. Compared to sham-rats, MI-rats manifested a significant in vivo as well as in vitro tachycardia (increase 9% and 20% vs. sham, respectively). Whereas aspirin, methylprednisolone and moxonidine significantly reduced postinfarction in vivo tachycardia, captopril rather increased in vivo heart rate. In vitro tachycardia of MI-hearts was reduced to sham-values with aspirin and methylprednisolone ($P<0.05$), but not with moxonidine and captopril. Capillary density defined as the number of Lectin stained capillaries per tissue area, which significantly decreased in MI-hearts (decrease 42% vs. sham), was restored by all treatments ($P<0.05$). Concentric left ventricular hypertrophy after MI, defined as increased cross-sectional area of transversally cut Gomori stained myocytes, was indicated by almost double myocyte size ($P<0.05$), while capillary to myocyte ratio remained unchanged. Methylprednisolone, moxonidine and captopril, but not aspirin, prevented hypertrophy ($P<0.05$). However, treatment with aspirin and methylprednisolone, but not moxonidine and captopril, increased capillary to myocyte ratio ($P<0.05$) up to twice the values of non-treated MI hearts, indicating newly formed capillaries.

Conclusions: The above findings confirm that post-MI pharmacological treatment can increase capillary density in the remodeled left ventricle. Whereas prevention of left ventricular hypertrophy normalizes capillary density without actually affecting capillary number, increased capillary to myocyte ratio (at preserved hypertrophic response) indicates actual capillary growth.

Keywords: Angiogenesis; Capillaries; Heart rate; Hypertrophy; Infarction

1. Introduction

One of the major consequences of myocardial infarction (MI) is remodeling of the surviving myocardium including compensatory hypertrophy of cardiomyocytes and interstitial fibrosis, however, without concomitant adaptation of the capillary microvasculature [1]. Accordingly, post-infarction remodeled hearts display a lower capillary density and reduced mechanical efficiency, which may be even further amplified by tachycardia. Normalization of the relation between cardiac muscle growth and capillarization is associated with improved function of non-infarcted myocardium, and may thus beneficially affect clinical outcome [2]. Vascularization in remodeled infarcted hearts can be improved by inhibition of reactive hypertrophy, as has been shown for therapy with ACE-inhibitors [3]. An alternative strategy could be to stimulate capillary growth by long-term reduction of heart rate [4,5]. Bradycardia has been demonstrated to improve tissue perfusion by increasing diastolic time and appears to induce capillary growth irrespective of the cause of heart rate reduction [5–7].
Since MI evokes sympathetic activation [8,9] and tachycardia, we hypothesize that chronic reduction of heart rate after MI would induce capillary growth. During the first 3 weeks post MI, four types of therapy were examined in coronary artery ligated rats [10], with regard to effects on heart rate, left ventricular hypertrophy and capillary density. Captopril and moxonidine both are anticipated to inhibit the hypertrophic response, but with opposite effects on heart rate [11,12]. Methylprednisolone and aspirin did not inhibit heart weight body weight ratio [13], while aspirin is previously reported to reduce heart rate [14].

2. Methods

Male Wistar rats (Harlan, Ziest, The Netherlands) weighing 260–330 g were housed in groups of 2 or 3 on a 12-h light–dark cycle with standard rat chow and water available ad libitum. The experimental protocol was approved by the University ethics committee for the use of experimental animals and conformed with the Guide for Care and Use of Laboratory Animals.

2.1. Myocardial infarction

Rats were subjected to sham surgery or coronary artery ligation. Under pentobarbital anesthesia (60 mg/kg i.p.), MI was induced by ligation of the left anterior descending coronary artery. Briefly, after intubation of the trachea, an incision was made in the skin overlying the fourth intercostal space with the overlying muscles separated and kept aside. The animals were put on positive pressure ventilation (frequency 65–70/min, tidal volume 3 ml) and the thoracic cavity was opened by cutting the intercostal muscles. The heart was carefully pulled to the left and 6–0 silk suture was looped under the left descending coronary artery near the origin of the pulmonary artery. After returning the heart to its normal position, the suture was tied. Intercostal space was closed by pulling the ribs with 3–0 silk, the muscles were returned to their normal position and the skin incision was sutured. Sham-operated animals underwent the same surgical procedure, without the actual coronary artery ligation. Proper occlusion of the coronary artery resulted in an extensive transmural infarction comprising a major part of the LV free wall, with small variations in size [15]. Infarct size was determined by planimetry at mid-ventricular levels in transverse slices as the percentage of LV circumference [16]. This model is regarded as a well-established model for the consequences of MI, rather than for the causes of MI.

2.2. Treatments

MI-rats were randomized to receive therapy with low-dose aspirin, methylprednisolone, moxonidine or captopril. Low-dose aspirin (25 mg/kg; lysine-acetylsalicylic, Aspegic®, Lorex, Maarssen, The Netherlands) [17] and methylprednisolone (5 mg/kg; methylprednisolone sodiumsuccinate, Solu-Medrol®, Pharmacia & Upjohn, The Netherlands) were dissolved in saline and administered as daily i.p. injections of 1 ml/kg [13]. This dose of aspirin is shown to reduce platelet production of pro-aggregatory and vasoconstrictor thromboxane in favour of anti-aggregatory and vasodilator prostaglandins without exerting anti-inflammatory activity [17,18]. Whereas aspirin treatment was started 2 days before MI surgery and continued until the end of the experiment at 21 days post-MI, methylprednisolone was started at the end of the acute inflammatory phase at 7 days post-MI and also continued to 21 days after surgery [10]. Untreated control rats were receiving once daily saline injections of 1 ml/kg i.p. from 2 days before until 21 days after surgery (control for aspirin treatment) or from 7 to 21 days after surgery (control for methylprednisolone treatment).

The centrally acting sympatholytic moxonidine (Solvay Pharma, Hannover, Germany) was dissolved in acidified saline to provide a final daily dose of 6 mg/kg/day using subcutaneously implanted osmotic minipumps (Alzet® 2001, ALZA Pharmaceuticals, Palo Alto, CA, USA) [11]. Administration of moxonidine was started 24 h following MI and continued until the end of the experiment at 21 days after surgery. Minipumps were replaced each week under light ether anesthesia. Sham-rats and untreated MI-rats underwent the same anesthesia and surgical procedure without the actual implantation of the minipumps.

The ACE-inhibitor, captopril (Squibb, Princeton, NJ, USA), was dissolved in the drinking water (2 g/l), 24 h after MI and continued until the end of the experiment, at 21 days after surgery [19,20]. As shown in a previous paper, 2 g/l captopril accounts for an effective dose of approximately 15 mg per rat per day [21]. Infarction is completed within 24 h in rats. Therefore treatment that was started after 24 h post-MI will not influence infarct size. Consequently, in the present study only aspirin could have had an effect on infarct size, which however was excluded in one of our previous studies on aspirin [17].

2.3. In vivo heart rate and mean arterial pressure

At day 19 after coronary artery ligation, rats were reanesthetized with sodium pentobarbital and a catheter (PE-10 heat-sealed to PE-50) was inserted in the abdominal aorta through the femoral artery. The heparinized saline filled catheter was tunneled under the skin, exteriorized at the back of the neck and closed with a metal plug. The animals were housed separately and allowed to recover 2 days before measurements. On the experimental day, the arterial catheter was connected to a pressure transducer (Viggo-spectramed, DT-XX, Bilthoven, The Netherlands) and signal was fed into a microprocessor and compatible computer, sampling at 500 Hz. After 1 h stabilization, baseline values of mean arterial blood pressure and in vivo heart rate were obtained in the conscious unrestrained animal.
2.4. Isolated heart perfusion

At the end of the protocol, the hearts were rapidly excised under pentobarbital anesthesia and mounted for perfusion with an oxygenated Krebs–Henseleit buffer (composition in mM: NaCl 125, KCl 4.7, CaCl2 1.35, NaHCO3 20, NaH2PO4 0.4, D-glucose 10; pH = 7.4; 37 °C) at a constant pressure of 85 mmHg, using the Langendorff technique. A fluid-filled latex balloon was placed in the left ventricle, connected to a miniature low-volume displacement pressure transducer. LV end-diastolic pressure was set to 5 mm Hg by adjusting the balloon volume. LV function was measured as the isovolumetric developed pressure against the balloon. We imposed a LVEDP of 5 mm Hg for all hearts, although we realize that in vivo LVEDP would have increased because of MI. However, as is known for ACE inhibitors, treatment would influence this parameter as well. Moreover, pilot studies showed similar MI-induced left ventricular dysfunction, when LVEDP was set to 20 mm Hg for both sham and MI heart, though at higher values. Changing LVEDP from 5 to 20 mm Hg did not cause major changes in heart rate (pilot studies). Coronary flow was measured by a flow probe (Transonic Systems, Ithaca, NY, USA) placed in the tubing just before the ostia of the coronary arteries. Ventricular perfusion was defined as the coronary flow corrected for ventricular weight. In vitro heart rate was measured in the spontaneously beating isolated hearts.

After 30-min stabilization, values for left ventricular function, heart rate and coronary flow were recorded for 10 min. At the end of the experiment, coronary capacity, reflected by maximal coronary flow, was obtained with 0.1 ml of 10^{-2} M sodium nitroprusside solution (University Hospital Pharmacy, Rotterdam) injected into the perfusing buffer just before entering the coronary arteries.

2.5. Left ventricular hypertrophy

After completion of the functional measurements, hearts were removed from the Langendorff preparation and weighed after exclusion of the atria and large vessels. Ventricular hypertrophy was indicated as the ratio of ventricular weight to body weight. Ventrices were cut into four transversal slices from apex to base and fixed with 3.6% phosphate-buffered formaldehyde for at least 24 h. After fixation, the slices were dehydrated and paraffin-embedded. Deparaffinized 5-μm thick sections at midventricular level were stained with a Gomori’s silver staining [22] in order to visualize individual myocytes of the viable LV wall, the area with the most pronounced underperfusion [20]. Concentric myocyte hypertrophy in the viable LV free wall, remote from the infarcted area, was measured as the cross-sectional area of transversally cut myocytes showing a nucleus, using image analysis (Zeiss KS 400, Germany). Myocyte density was calculated as the average number of myocytes per tissue area. In each stained slice, measurements were averaged from four different counting fields (± 200 myocytes per heart).

2.6. Capillary density

To visualize capillaries in the myocardium in the same area as used for measurements of myocyte size, endothelial cells were stained with Lectin GSI (Sigma-Aldrich Chemie©, Zwijndrecht, The Netherlands), as previously described by Nelissen-Vrancken et al. [16]. Sections of 5 μm thickness were deparaffinized and rehydrated, and endogenous peroxidase was inhibited by methanol/H2O2 (0.3%) for 15 min. The sections were incubated overnight with the biotinylated Lectin GSI (1:100) at room temperature. Then, in a second step, the signal was intensified with an avidin–biotin (ABC) complex containing peroxidase labeled biotins (1:100) (Lab vision, CA, USA). Finally, the sections were incubated with a Ni–Co amplified 3-3’ diaminobenzidinetetrahydrochloridihydrat (DAB) solution to which a stable peroxide substrate buffer was added (Pierce©, CA, USA). Endothelial cells of capillaries and larger vessels are visualized in the myocardium as a brown precipitate. A background staining was not used in order to avoid interference with the Lectin staining. Since Lectins stain not only capillaries but also other vessels, a size criterion of 10 μm was used to exclude small arterioles and venules. The number of capillaries was counted in the same region of the viable LV free wall (serial sections) in which myocyte size was determined. Image analysis (Zeiss KS 400, Germany) was used to measure capillary density, calculated as the number of capillaries per tissue area in the viable LV wall. The measured total tissue area was corrected for the remaining interstitial space. In transverse, but not longitudinal sections of the viable LV wall, capillary density has been shown to be an adequate measurement for the number of capillaries per tissue area [23,24]. Actual capillary growth was derived from an increased capillary to myocyte ratio, which can be calculated as capillary density divided by myocyte density.

2.7. Data analysis

All data are presented as means ± S.E.M. Data of infarcted rats were only included if the infarction comprised the major part of the LV free wall, since small infarctions (<20%; 1–3 per MI group) are found to be hemodynamically fully compensated [12,25]. Estimation of infarct size by macroscopic appearance has proven to be a reliable method to recognize too small infarctions (<20%) [15]. Because of temporal variation and different control administration, each treatment group had its own sham and untreated infarct controls. Statistical analysis of effects of treatment was performed within each group using one-way analysis of variance (ANOVA) (SigmaStat™, Jandel Scientific, Erkrath, Germany) followed by Bonferro-
ni’s post-hoc t-tests for multiple group comparisons [26]. Differences were considered statistically significant if $P < 0.05$.

### 3. Results

#### 3.1. General

Overall mortality following MI was 39% and did not depend on treatment, since death mainly occurred within the first 24 h after coronary artery ligation. No other than surgery related death was observed during the treatment period. Data from four different treatment groups were collected; low-dose aspirin, methylprednisolone, moxonidine and captopril, and compared to their respective sham operated and non-treated infarcted controls. Groups are characterized in Table 1.

Infarct size in untreated and treated MI-hearts was similar for all groups. Except for the lower body weight in untreated MI controls for aspirin, MI did not affect body weight. Methylprednisolone, moxonidine and captopril treated MI-rats manifested a lower body weight compared to their untreated contol MI-rats. Ventricular weight of untreated MI-rats in the methylprednisolone, moxonidine and captopril group was significantly higher than sham-operated control hearts despite replacement of the major part of the left ventricular free wall by lighter scar tissue. Treatment with methylprednisolone, moxonidine and captopril, but not aspirin, normalized ventric-

#### Table 1

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>ASP</th>
<th>MP</th>
<th>MOX</th>
<th>CAP</th>
</tr>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Infarct size (%)</td>
<td>6–12</td>
<td>7–12</td>
<td>7–14</td>
<td>7–10</td>
</tr>
<tr>
<td>Sham</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MI</td>
<td>37 ± 1</td>
<td>43 ± 2</td>
<td>45 ± 3</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>MI+</td>
<td>39 ± 3</td>
<td>41 ± 3</td>
<td>44 ± 3</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>376 ± 6</td>
<td>346 ± 8</td>
<td>333 ± 7</td>
<td>368 ± 5</td>
</tr>
<tr>
<td>MI</td>
<td>340 ± 7*</td>
<td>347 ± 8</td>
<td>320 ± 10</td>
<td>359 ± 4</td>
</tr>
<tr>
<td>MI+</td>
<td>335 ± 8</td>
<td>300 ± 10*</td>
<td>299 ± 9*</td>
<td>334 ± 4*</td>
</tr>
<tr>
<td>Ventricular weight (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>1060 ± 19</td>
<td>1061 ± 52</td>
<td>1174 ± 37</td>
<td>1071 ± 21</td>
</tr>
<tr>
<td>MI</td>
<td>1098 ± 35</td>
<td>1211 ± 54*</td>
<td>1543 ± 75*</td>
<td>1232 ± 56*</td>
</tr>
<tr>
<td>MI+</td>
<td>1147 ± 39</td>
<td>1059 ± 64b</td>
<td>1076 ± 24b</td>
<td>1033 ± 41b</td>
</tr>
<tr>
<td>Ventricular weight/body weight (mg/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>2.8 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>MI</td>
<td>3.3 ± 0.1a</td>
<td>3.5 ± 0.1a</td>
<td>4.7 ± 0.3a</td>
<td>3.5 ± 0.2a</td>
</tr>
<tr>
<td>MI+</td>
<td>3.4 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>3.6 ± 0.2b</td>
<td>3.1 ± 0.1</td>
</tr>
</tbody>
</table>

Data are presented as means ± S.E.M. MI: untreated infarcted rats; MI+: treated infarcted rats; ASP: aspirin; MOX: moxonidine; MP: methylprednisolone; CAP: captopril.

* $P < 0.05$ vs. SHAM.

** $P < 0.05$ vs. MI.

#### Table 2

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>ASP</th>
<th>MP</th>
<th>MOX</th>
<th>CAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>In vivo MA (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>106 ± 2</td>
<td>115 ± 2</td>
<td>112 ± 2</td>
<td>133 ± 2</td>
</tr>
<tr>
<td>MI</td>
<td>99 ± 4</td>
<td>108 ± 4</td>
<td>98 ± 3</td>
<td>127 ± 7</td>
</tr>
<tr>
<td>MI+</td>
<td>96 ± 2</td>
<td>99 ± 3</td>
<td>100 ± 5</td>
<td>101 ± 4b</td>
</tr>
<tr>
<td>In vitro LV systolic pressure (mm Hg)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>75 ± 5</td>
<td>89 ± 6</td>
<td>77 ± 6</td>
<td>94 ± 6</td>
</tr>
<tr>
<td>MI</td>
<td>51 ± 4a</td>
<td>58 ± 6a</td>
<td>51 ± 7a</td>
<td>74 ± 7a</td>
</tr>
<tr>
<td>MI+</td>
<td>49 ± 4a</td>
<td>78 ± 7</td>
<td>44 ± 5a</td>
<td>77 ± 14a</td>
</tr>
<tr>
<td>Cardiac perfusion (ml/min/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>10.6 ± 0.6</td>
<td>10.3 ± 0.7</td>
<td>8.6 ± 0.9</td>
<td>9.5 ± 1.4</td>
</tr>
<tr>
<td>MI</td>
<td>8.9 ± 1.0</td>
<td>8.7 ± 1.0</td>
<td>6.6 ± 0.8</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>MI+</td>
<td>9.8 ± 1.4</td>
<td>7.3 ± 0.6</td>
<td>9.4 ± 0.8</td>
<td>8.1 ± 1.1</td>
</tr>
<tr>
<td>Maximal CF nitroprusside (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>19.1 ± 1.1</td>
<td>21.9 ± 1.5</td>
<td>19.9 ± 1.0</td>
<td>20.7 ± 0.7</td>
</tr>
<tr>
<td>MI</td>
<td>17.8 ± 2.2</td>
<td>22.7 ± 1.4</td>
<td>21.8 ± 0.9</td>
<td>19.9 ± 0.9</td>
</tr>
<tr>
<td>MI+</td>
<td>17.8 ± 1.1</td>
<td>19.8 ± 1.1</td>
<td>19.2 ± 1.3</td>
<td>20.1 ± 0.8</td>
</tr>
</tbody>
</table>

Data are presented as means ± S.E.M. MI: untreated infarcted rats; MI+: treated infarcted rats; ASP: aspirin ; MOX: moxonidine; MP: methylprednisolone; CAP: captopril; MAP: mean arterial pressure; LV: left ventricular; CF: coronary flow.

* $P < 0.05$ vs. SHAM.

** $P < 0.05$ vs. MI.
ular weight to sham-values. In addition, ventricular weight to body weight ratio, reflecting total (left and right) ventricular hypertrophy, was increased in all untreated MI-rats, and normalized with moxonidine and captopril (not statistically significant different from sham anymore).

3.2. Functional parameters

Hemodynamics obtained from conscious rats as well as from isolated perfused hearts are summarized in Table 2. Except for the lower value in captopril treated MI-rats, mean arterial pressure was similar for all groups. LV dysfunction of MI-hearts was reflected by a significantly reduced in vitro LV systolic pressure, which was not affected by treatment with aspirin, moxonidine and captopril. Methylprednisolone partially restored in vitro LV systolic pressure, since values were not statistically significant different anymore from sham-values. Coronary flow corrected for ventricular weight (cardiac perfusion), as well as maximal coronary flow obtained with sodium nitroprusside, were similar in all experimental groups.

Values for in vivo (upper panel) and in vitro (lower panel) heart rate are represented in Fig. 1. In general, untreated conscious MI-rats were characterized by a

Fig. 1. In vivo (upper panel) and in vitro heart rate (lower panel) obtained from conscious rats and isolated heart perfusions, respectively. \(* P < 0.05\) vs. Sham; \(* P < 0.05\) vs. MI.

Fig. 2. Gomori stained sections in the LV viable wall of sham-hearts, untreated and treated MI-hearts, showing individual myocytes. The bar in left upper photomicrograph indicates 100 μm, and accounts for all micrographs. ASP: aspirin; MOX: moxonidine; MP: methylprednisolone; CAP: captopril.
marked in vivo tachycardia, which was normalized to sham-values with aspirin, moxonidine, and methylprednisolone. With captopril a further increase rather than normalization of in vivo heart rate was observed. Moreover, in vitro heart rate measured in isolated perfused hearts, was significant higher in all untreated MI vs. their sham control hearts. Treatment with aspirin and methylprednisolone, but not moxonidine or captopril, resulted in normalization of the in vitro tachycardia.

3.3. LV hypertrophy

As ventricular weight body weight ratio (Table 1) represents total ventricular hypertrophy, which includes right ventricle and interventricular septum as well, left ventricular hypertrophy was studied separately in more detail by histological analysis. Representative photomicrographs of Gomori stained sections in the viable LV free wall are shown in Fig. 2. MI-induced LV hypertrophy at cellular level was confirmed by a significantly increased myocyte cross-sectional area and was prevented in moxonidine, captopril, methylprednisolone, but not aspirin treated rats. These observations were substantiated by the actual measurements as presented in Fig. 3.

3.4. Capillary density

Representative photomicrographs of Lectin stained sections in the LV viable wall showing individual capillaries are represented in Fig. 4. A significantly reduced capillary density observed in hypertrophied MI hearts, was normalized to sham-values with moxonidine, methylprednisolone and captopril and even significantly increased above sham-values with aspirin. These observations were substantiated by the actual measurements as presented in Fig. 5 (upper
4. Discussion

4.1. Heart rate

Chronically infarcted rats were characterized by a marked in vivo tachycardia, which is generally considered to result from sympathetic nervous system activation to compensate for the loss of function after MI. Whereas treatment with aspirin, methylprednisolone and moxonidine normalized MI-induced in vivo tachycardia, captopril further increased in vivo heart rate of MI-hearts. These observed effects of treatment are in general accordance with previous observations. A lower heart rate due to aspirin treatment is also reported in a clinical study in which heart failure patients were treated with low-dose aspirin [27]. The tachycardic effect of captopril is in accordance with previous functional observations in early captopril treated rats, and could probably be attributed to compensation of a reduced stroke volume in order to maintain cardiac output [12]. With regard to moxonidine, a clear relation between sympathetic suppression and reduced in vivo heart rate has been previously demonstrated in experimental [11] and clinical studies [28,29]. However, the reduction of the in vivo tachycardia by aspirin and methylprednisolone treatment cannot as easily be explained by reduced sympathetic activity, which is further supported by unchanged plasma catecholamine concentrations in aspirin [14] and methylprednisolone treated rats (data not shown). Alternative mechanisms should be evaluated.

Interestingly, when the hearts of untreated MI-rats were isolated and perfused, thereby circumventing influence of sympathetic nerve activity and circulating catecholamines as the major determinants of in vivo tachycardia, heart rate was still found to be higher than in sham-operated hearts. The presence of local sympathetic activity that could have remained after isolation of the heart was excluded by the absence of heart rate response to $10^{-6}$ M propranolol (data not shown).

Intrinsic heart rate is normally defined as the in vivo heart rate found after autonomic nervous system blockade by simultaneous inhibition of muscarinic and β-adrenergic receptors [30]. The heart rate measured in our Langendorff preparations was considerably lower than in vivo, and independent of autonomic receptor inhibition (pilot studies). Moreover, in vitro values closely match with values obtained in our previous in vivo studies with autonomic blockade using hexamethonium [31]. Therefore it seems feasible to assume that the in vitro heart rates in the present study represent intrinsic heart rates, as has been suggested before [14].

Nevertheless, chronic treatment with aspirin and methylprednisolone, but not moxonidine and captopril, prevented the in vitro tachycardia of isolated MI-hearts. Hence, the lower in vivo heart rate observed with aspirin and methylprednisolone might be due to a reduced intrinsic heart rate rather than to a suppressed sympathetic nervous system activity.

The mechanism by which the in vitro heart rate in aspirin and methylprednisolone treated MI-hearts was reduced is largely unknown. One may speculate it to be attributed to an effect of prostaglandins on the pacemaker activity. Indeed, prostaglandins have been shown to increase the sinus node pacemaker activity in isolated preparations independent of adrenergic response, and anti-inflammatory treatment with indomethacin could reduce this activity [32]. Moreover, positive chronotropic effects of prostaglandins occur in nanomolar doses, suggesting a strong connection with sinus node activity [33]. In the present study, the lower heart rate observed with aspirin and methylprednisolone could be explained by even a minor effect on prostaglandin levels. Although the low-dose aspirin may not be expected to have substantial anti-inflammatory actions, low concentrations have been shown to attenuate prostaglandin levels and some local interference with the inflammatory response to infarction has been suggested [17,34].

A second possible mechanism by which heart rate could have been reduced, may come from an interaction between sinus node activity and collagen, modulated by stretch [35]. Since aspirin and methylprednisolone have been shown to interfere with collagen deposition in the surviving myocardium of infarcted rats [13], it leaves the possibility that collagen in the sinus node, which accounts for a major part
of its volume, was also affected. However, moxonidine [11] and ACE-inhibitors [36] have also been shown to inhibit collagen deposition in the spared myocardium, but without reducing in vitro heart rate.

4.2. Left ventricular hypertrophy and capillary adaptation

The heart responds to overload following MI with compensatory hypertrophy, which is aimed to restore the myocardial function and normalize increased wall stress. In the present study ventricular hypertrophy was macroscopically reflected by an increased ventricular weight to body weight ratio, including changes in left and right ventricle as well as interventricular septum. Previous studies have shown that cardiac underperfusion at 3 weeks post-MI is limited to the spared LV myocardium, which displayed the most pronounced hypertrophy [20]. At cellular level, hypertrophy of the viable left ventricular free wall was microscopically demonstrated by an almost doubled myocyte thickness. Moreover, these hypertrophied cardiomyocytes in the spared myocardium are associated with decreased capillary density. Post-MI hypertrophy includes eccentric as well as concentric components, as the myocytes grow in length as well as in thickness [1]. Concentric rather than eccentric hypertrophy has been associated with a reduced ischemic tolerance [37]. In accordance with previous observations [2,38], the morphometric indices from the present study clearly demonstrated a concentric hypertrophic response of the spared myocardium, which was associated with a decreased number of capillaries per tissue area. It appeared that, despite an increased cardiomyocyte cell volume, capillary number per myocyte remains unaltered, and hence makes the myocardium prone for insufficient oxygenation [39]. Micro-vascularization in postinfarction remodeled hearts can be improved by inhibition of reactive hypertrophy, thereby restoring capillary density, which can be associated with improved ischemic tolerance. Indeed, in the present study, prevention of compensatory hypertrophy was associated with a restored capillary density in the spared myocardium as was shown in methylprednisolone, moxonidine and captopril treated MI-hearts. However, whether prevention of compensatory hypertrophy in the early phase after MI would be beneficial for cardiac function is questionable [12].

Surprisingly, although aspirin did not prevent compensatory hypertrophy, capillary density was even increased above sham-values suggesting actual capillary growth. These findings were fully supported by the values of capillary to myocyte ratio, which were doubled when compared to sham-hearts. Capillary growth indicated by a significantly increased capillary to myocyte ratio could also be observed in methylprednisolone treated MI-hearts, although to a lesser extent. Theoretically, an increased capillary to myocyte ratio could be obtained by either increased number of capillaries or by decreased number of myocytes. Although we cannot exclude the latter possibility, the former seems more feasible regarding results on heart weight/body weight ratios; that does not imply substantial loss of tissue.

A final remark concerns the fact that although capillary density may represent the capacity of oxygen supply to the myocyte, coronary flow as such is determined at the level of resistance sized arteries. In the present study, maximal coronary flow obtained with nitroprusside was similar in all groups, indicating that flow capacity in all hearts was similar, supporting that indeed capillary density represents oxygen supply capacity.

4.3. Interaction between heart rate and capillary growth

Independent of its origin, bradycardia is associated with capillary growth [5], which could provide high clinical benefit. Capillary growth has been shown by either bradycardic pacing [6,40] or infusion of bradycardic drugs [4,41]. Lower in vivo heart rates were shown to improve the balance between oxygen demand and supply by enhanced tissue perfusion through longer diastolic time [42] in addition to increased myocardial capillarization [5,6]. Indeed, the lower in vivo heart rate after moxonidine treatment was associated with increased capillary density. For captopril treated rats, however, even an increased heart rate coincides with increased capillary density. Therefore, a distinction should be made between increased capillary density as an indirect result of prevention of LV hypertrophy, and actual capillary growth. Reduction of myocyte size by itself increases capillary density without changing capillary number. Moreover, the reduced myocyte size may inhibit the stimulation of capillary growth, since there may be less metabolic demand from the reduced amount of contractile tissue. Restored capillary density resulting from prevented hypertrophy seems to be the case in both moxonidine and captopril treated MI-hearts, since capillary to myocyte ratio remained unchanged. Similar findings were observed in spontaneously hypertensive rats with the ACE-inhibitor temocapril [43].

An interaction between lower heart rate and cardiac capillarization has been suggested from studies with exercise training induced bradycardia [44,45]. While heart rate after autonomic blockade (intrinsic heart rate) was shown to be reduced as a part of adaptation to training [46], morphometric measurements in exercise induced hypertrophied pig hearts have revealed compensated capillary proliferation [47]. This suggests that in exercise training-induced lower heart rates, growth of new capillaries occurs in proportion to myocyte hypertrophy. This phenomenon was observed in aspirin- and less pronounced in methylprednisolone treated MI-hearts, although in aspirin hearts capillary density even exceeded values of control hearts. Aspirin treated hearts display lower heart rates concomitant with capillary growth, as indicated by an increased capillary to myocyte ratio. Significant capillary growth in aspirin treated MI-hearts may offer an attractive explanation for the improved in vivo hemodynamics observed in conscious aspirin-treated MI-
rats [14]. The exact mechanism, however, by which the lower heart rate may have stimulated capillary growth remains yet unclear. Even the other way around, it may also be feasible that capillary growth itself could have affected the heart rate by improving perfusion to the myocardium. Alternatively, both heart rate and capillary density changes may be independent manifestations of drug action. On the other hand, mechanical factors, such as stretch of the capillary wall, or release of growth factors stored in the capillary basement membrane [5] could also have played a role. Indeed, vascular endothelial growth factor (VEGF) was shown to play a key role in bradycardia induced angiogenesis [4]. Interestingly, possible synergism between anti-inflammatory therapy and basic fibroblast growth factor has been shown to improve capillary density in rats [48].

Finally, a critical remark should be made, that despite the observation that all therapies in the present study improved capillary density in the spared part of the left ventricle, left ventricular dysfunction of MI-hearts, as reflected by a decreased in vitro LV systolic pressure, was not improved by aspirin, moxonidine and captopril, and only partially restored with methylprednisolone. However, in vitro LV function does not always predict in vivo cardiac function; in vivo hemodynamics were indeed improved with aspirin. LV systolic pressure, was not improved by aspirin, moxonidine and captopril, and only partially restored with methylprednisolone. Alternatively, both heart rate and capillary density changes may be independent manifestations of drug action. On the other hand, mechanical factors, such as stretch of the capillary wall, or release of growth factors stored in the capillary basement membrane [5] could also have played a role. Indeed, vascular endothelial growth factor (VEGF) was shown to play a key role in bradycardia induced angiogenesis [4]. Interestingly, possible synergism between anti-inflammatory therapy and basic fibroblast growth factor has been shown to improve capillary density in rats [48].

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4.4. Conclusions

So far, the results of the present study indicate that pharmacological therapy post-MI indeed can increase capillary density. Enhanced capillary density could be obtained by prevention of reactive hypertrophy without changing capillary number. Chronic in vivo reduction of heart rate, however, could not be associated with increased capillary density, whereas reduction of in vitro heart rate coincided with capillary growth in chronically infarcted hearts. Furthermore, in the present study, therapy with a low-dose aspirin in the early phase after MI seems to benefit most, in terms of inducing capillary growth.

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