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Chronic vasopressin V_{1A} but not V_2 receptor antagonism prevents heart failure in chronically infarcted rats

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Abstract

Evidence is increasing that therapeutic modulation of neurohormonal activation with vasopressin receptor antagonists via V_{1A} and V_2 receptors may favourably affect prognosis of heart failure. This study was designed to compare in vivo hemodynamic effects of *early* treatment (1–21 days after infarction) with a V_{1A} (SR-49059 or ((2*S*)1-[(2*R*3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide); 0.3 mg/kg/day) and a V_2 (SR-121463B or (1-[4-(*N*-*tert*-Butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethyoxy)-cyclo-hexane]indol-2one,furmate; 0.5 mg/kg/day) receptor antagonist in myocardial infarcted rats, chronically instrumented for hemodynamic measurements. Left ventricular dysfunction in conscious myocardial infarcted rats, which was evidenced by a significantly decreased cardiac output (myocardial infarction: 70 ± 3 vs. sham: 81 ± 3 ml/min) and stroke volume (myocardial infarction: 190 ± 10 vs. sham: 221 ± 7 μl), was restored by the vasopressin V_{1A} (81 ± 2 ml and 224 ± 5 μl, respectively) but not V_2 receptor antagonist. Improved cardiac output with the vasopressin V_{1A} receptor antagonist resulted from an increased stroke volume at a reduced myocardial infarction induced tachycardia. In addition to the hemodynamic measurements, left ventricular hypertrophy and capillary density were determined, histologically measured as the cross-sectional area of Gomori-stained myocytes and Lectin-stained capillaries per tissue area, respectively. The observed left ventricular concentric hypertrophy (myocardial infarction: 525 ± 38 vs. sham: 347 ± 28 μm^2; $P<0.05$) and reduced capillary density (myocardial infarction: 2068 ± 162 vs. sham: 2800 ± 250 number/mm^2; $P<0.05$) in the spared myocardium of myocardial infarcted rats, remained unaffected by the vasopressin V_{1A} or V_2 receptor antagonist. Thus, chronic vasopressin V_{1A} but not V_2 receptor blockade prevents heart failure in 3-week-old infarcted rats. Moreover, the improved cardiac function could not attributed to changes in left ventricular hypertrophy and/or capillary density.

Keywords: Heart failure; Myocardial infarction; Remodeling; Vasopressin receptor antagonism

1. Introduction

Neurohomonal activation after acute myocardial infarction refers to increased activity of the sympathetic nervous system, renin–angiotensin system, atrial natriuretic peptide and arginine vasopressin (McAlpine et al., 1988; Rouleau et al., 1991). Although initially compensatory in nature, prolonged neurohormonal activation after myocardial infarction has been shown to negatively affect prognosis of heart failure (Packer, 1992b; Francis, 1998). Although angiotensin-converting enzyme inhibitors (Pfeffer et al., 1992) and β-adrenoceptors antagonists (Gu et al., 1998) have been shown to interfere with postinfarction remodeling resulting in improved prognosis of heart failure, current therapy is still not optimal.

Another less investigated approach to achieve inhibition of prolonged neurohormonal activation could be obtained by blockade of the arginine vasopressin system. The antidiuretic hormone vasopressin plays a pivotal role in blood pressure control and salt and water homeostasis through its effects at the vasopressin V_{1A} receptor (Burrell et al., 1994) to cause vasoconstriction and at the renal vasopressin V_2 receptor to mediate antidiuresis (Wong and Verbalis, 2001). The recent development of vasopressin receptor antagonists, such as YM087 or (4′ -[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzazepin-6-yl)carbonyl]-2-phenylbenzanilide monohydrochloride), SR-

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49059 or ((2*S*)1-[(2*R*3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) and OPC-31260 or (5-dimethyl-amino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,4-tetrahydro-1*H*-benzazepine), have allowed reevaluation of the precise role of vasopressin in the development of heart failure (Naitoh et al., 1994). However, currently, there are only a limited number of studies examining the use of vasopressin receptor antagonists as therapy for heart failure. Selective inhibition of the V_1 and V_2 receptor led to immediate improvement in hemodynamic parameters (Creager et al., 1986) and increased diuresis (Wong and Verbalis, 2001).

Whereas previous studies have mainly focused on the acute and short-term hemodynamic and renal effects of V_{1A} and V_2 antagonism (Yatsu et al., 1997; Burrell et al., 1998; Lankhuizen et al., 2001), the present study was designed to compare the in vivo hemodynamic effects of *chronic* treatment with a V_{1A} and V_2 receptor antagonist in conscious infarcted rats. These rats have been shown to provide a well-established postinfarction heart failure model (Fishbein et al., 1978). Furthermore, to investigate whether hemodynamic changes could be attributed to effects on postinfarction remodeling, left ventricular hypertrophy and capillary density in the surviving myocardium were determined histologically using image analysis.

2. Materials and methods

2.1. Animals

Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 260–300 g were housed in groups of two or three 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 Laboratoy Animals.

2.2. Myocardial infarction

Rats were subjected to sham surgery or coronary artery ligation. Under pentobarbital anesthesia (60 mg/kg, i.p.), myocardial infarction was induced by ligation of the left anterior descending coronary artery (Fishbein et al., 1978). 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 pushed 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 left ventricular free wall, with small variations in size (Kalkman et al., 1996). Infarct size was determined by planimetry at mid-ventricular levels in transverse slices (Nelissen-Vrancken et al., 1998) as the percentage of left ventricular circumference (Kalkman et al., 1995).

2.3. Treatments and doses

Vasopressin receptor antagonist treatment was administered from day 1 to 21 after myocardial infarction . Therefore, rats were randomized to receive subcutaneous implantation of osmotic minipumps (Alzet® 2001, ALZA Pharmaceuticals, Palo Alto, CA) filled with the vasopressin V_{1A} receptor antagonist SR-49059 or vasopressin V_2 receptor antagonist SR-121463B (generous gifts from Sanofi, Montpellier, France). Minipumps were replaced each week under light ether anesthesia. Sham rats and nontreated myocardial infarcted rats underwent the same anesthesia and surgical procedure without the actual implantation of the minipumps. The V_{1A} receptor antagonist SR-49059 ((2*S*)1-[(2*R*3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) (Serradeil-Le Gal, 1994) and V_2 receptor antagonist SR-121463B or (SR-121463B or (1-[4-(*N*-*tert*-Butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethyoxy)-cyclo-hexane]indol-2one,fumarate were dissolved in dimethyl sulfoxide at 3.75 and 1.25 mg/ml, respectively, to provide a final daily dose of 0.3 and 0.5 mg/kg/day. In pilot experiments, the used dose of the V_{1A} receptor antagonist for 3 weeks caused a rightward shift of the vasopressin pressure–response curve one log unit, while the used dose of the V_2 receptor antagonist chronically increased 24-h urine production by 20% (data not shown).

2.4. In vivo hemodynamics

Six to seven days prior to the actual measurements (at day 21), rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and a flowprobe was implanted. Briefly, after intubation and starting positive pressure respiration, the thorax was opened at the third right intercostal space, and the ascending aorta was dissected from surrounding tissues. A 2.6 mm diameter electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the aorta 1–2 mm above the outlet of the heart using previously described techniques (Schoemaker et al., 1991). The cable was fixed to the ribs, the thorax was closed in layers, and the connector was exteriorized in the neck, where it was sutured to the skin.

At day 18, rats were re-anesthetized and implanted with a J-shaped catheter (PE-10 heat-sealed to PE-50) in the abdominal aorta through the femoral artery to measure arterial blood pressure. Furthermore, through the femoral vein, a catheter (PE-10 heat-sealed to PE-50) was implanted into the abdominal vena cava for infusion and a Silastic (602-175, Dow Corning, Midland, MI, USA) catheter was placed in the thoracic vena cava just before the right atria. All catheters were exteriorized in the neck, filled with heparinized saline, and closed with metal plugs. Animals were allowed to recover 2 days before measurements were done. On the day of measurements (day 21), the conscious rats were placed in plastic cages and connected to the measuring equipment. Signals were fed into a 68B09-based microprocessor and microcomputer, sampling at 500 Hz. Mean values were obtained for arterial and central venous pressure. From the aortic flow signal, besides cardiac output, heart rate and stroke volume were obtained. Total peripheral resistance was calculated as (mean arterial pressure − central venous pressure)/cardiac output. After 60-min stabilization, baseline hemodynamics were recorded.

2.5. Left ventricular hypertrophy

After completion of the functional measurements, the rats were deeply anesthetized, and hearts were excised and weighed after exclusion of the atria and large vessels. Ventricular hypertrophy was macroscopically indicated as the ratio of ventricular weight to body weight. Ventricles were cut into four transversal slices from apex to base and fixated 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 were stained with a Gomori's silver staining (Benjamin et al., 1989) in order to visualize individual myocytes of the viable left ventricular wall. Concentric myocyte hypertrophy in the viable left ventricular 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.

Table 1
Body and ventricular weights measured in the different experimental groups

	Sham	MI	MI + V_{1A}	MI + V_2
N	15	11	7	8
Infarct size (%)	–	40 ± 2	45 ± 4	44 ± 5
Body weight (g)	317 ± 6	304 ± 9	320 ± 6	302 ± 4
Ventricular weight (mg)	891 ± 37	951 ± 22	946 ± 38	937 ± 24
Ventricular/body weight (mg/g)	2.8 ± 0.1	3.1 ± 0.1[a]	3.0 ± 0.1	3.1 ± 0.1[a]

Data are presented as means ± SEM. MI: untreated myocardial infarcted rats; MI + V_{1A}: vasopressin V_{1A} receptor antagonist-treated myocardial infarcted rats; MI + V_2: vasopressin V_2 receptor antagonist-treated myocardial infarcted rats.
[a] $P < 0.05$ vs. sham.

Table 2
In vivo cardiac loading conditions measured in the different experimental groups

	Sham	MI	MI + V_{1A}	MI + V_2
Mean arterial pressure (mm Hg)	105 ± 1	98 ± 2[a]	97 ± 2[a]	94 ± 2[a]
Central venous pressure (mm Hg)	3.4 ± 0.8	3.8 ± 1.1	2.5 ± .1.1	2.0 ± 0.7
Total peripheral resistance (mm Hg min/ml)	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.4 ± 0.1

Data are presented as means ± SEM. MI: untreated myocardial infarcted rats; MI + V_{1A}: vasopressin V_{1A} receptor antagonist-treated myocardial infarcted rats; MI + V_2: vasopressin V_2 receptor antagonist-treated myocardial infarcted rats.
[a] $P < 0.05$ vs. sham.

2.6. Capillary density

To visualize capillaries in the myocardium, endothelial cells were stained with Lectin GSI (Sigma-Aldrich Chemie©, Zwijndrecht, The Netherlands), as previously described by Nelissen-Vrancken et al. (1998). Sections of 5-μm thickness were deparaffinized and dehydrated, and endogenous peroxidase was inhibited by methanol/H_2O_2 (0.3%) for 15 min. The sections were incubated overnight with the biotinylated Lectin GSI (1:100) at room temper-

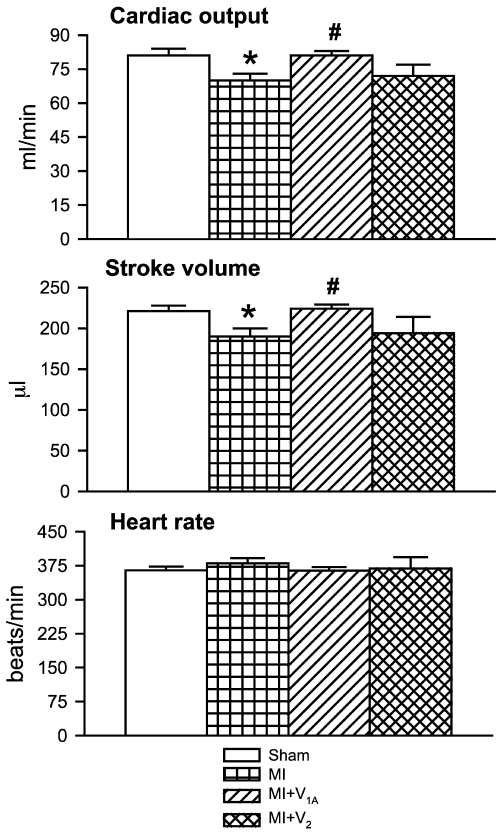

Fig. 1. Cardiac output (upper panel), stroke volume (middle panel) and heart rate (lower panel) measurements obtained from sham, untreated myocardial infarcted and vasopressin receptor antagonist-treated infarcted rats. *: $P < 0.05$ vs. sham; #: $P < 0.05$ vs. MI.

ature. Then, in a second step, the signal was intensified with an ABC (avidin–biotion complex) complex containing peroxidase labeled biotins (1:100) (Lab Vision, CA, USA). Finally, the sections were incubated with a Ni–Co amplified DAB (3-3′ diaminobenzidinetetrahydrochloridedihydrat) solution to which a stable peroxide substrate buffer was added (Pierce©, 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. The number of capillaries were counted in the same region of the viable left ventricular free wall in which myocyte area 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 left ventricular wall. Capillary to myocyte ratio was calculated as capillary density/myocyte density.

2.7. Data analysis

All data are presented as means ± SEM. Data of infarcted rats were only included if the infarction comprised the major part of the left ventricular free wall, since small infarctions are found to be hemodynamically fully compensated (Pfeffer et al., 1979; Schoemaker et al., 1991). Estimation of infarct size by macroscopic appearance has proven to be a reliable method to recognize too small infarctions (< 20%) (Kalkman et al., 1996). Differences between groups were analyzed (SigmaStat™, Jandel Scientific, Erkrath, Germany) using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc *t*-tests for multiple group comparisons (Wallenstein et al., 1980). Differences were considered statistically significant if $P < 0.05$.

3. Results

Results comprize data from sham rats, untreated as well as vasopressin V_{1A} and V_2 receptor antagonist-treated myocardial infarcted rats ($N = 7–15$). Overall mortality following myocardial infarction was 35% and did not depend on the treatment used, since death mainly occurred within the first 24 h after coronary artery ligation, before starting treatment. No other than surgery related death were observed during the treatment period.

3.1. Group characterization

The experimental groups used for hemodynamic studies are characterized in Table 1. Infarct size was similar in untreated as well as vasopressin receptor antagonist-treated myocardial infarcted rats. Whereas significant changes were observed in neither body nor in ventricular weight, the ratio of these two parameters (ventricular/body weight) was increased in myocardial infarcted rats and not affected by treatment. The lack of effect of myocardial infarction on

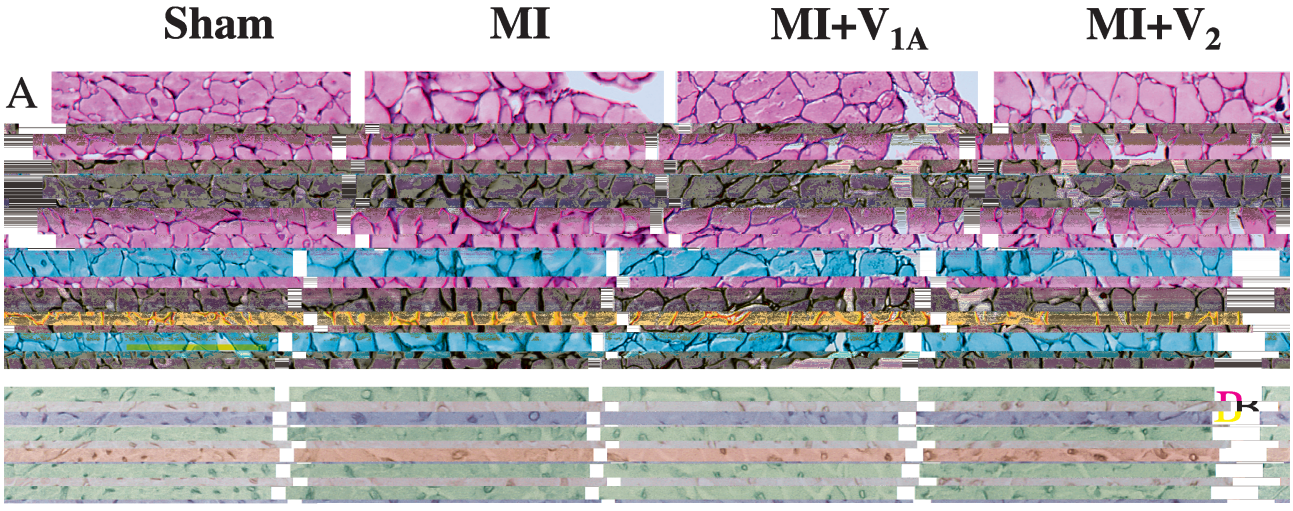

Fig. 2. Representative micrographs of Gomori as well as Lectin-stained sections in the left ventricular viable wall of the different experimental groups, showing individual myocytes (Panel A) and individual capillaries (small dark brown circles) (Panel B), respectively. The bar in left upper photomicrograph indicates 100 μm and accounts for all micrographs. MI: untreated myocardial infarcted rats; MI + V_{1A}: vasopressin V_{1A} receptor antagonist-treated myocardial infarcted rats; MI + V_2: vasopressin V_2 receptor antagonist-treated myocardial infarcted rats.

ventricular weight, despite replacement of a substantial part of the left ventricle by much lighter scar tissue implies hypertrophy of the spared myocardium.

3.2. In vivo hemodynamics

Table 2 summarizes in vivo cardiac loading conditions. Myocardial infarction was associated with a reduced mean arterial pressure, which was not affected by vasopressin receptor antagonists. In all experimental groups, hearts operated at comparable loading conditions: a similar preload (central venous pressure) and afterload (total peripheral resistance). Differences in preload were undetectable. Left ventricular dysfunction in conscious myocardial infarcted rats, which was substantiated by a decreased cardiac output and stroke volume, was significantly restored by the vasopressin V_{1A} but not V_2 receptor antagonist (Fig. 1, upper and middle panel). Improved cardiac output with the vasopressin V_{1A} receptor antagonist resulted from an increased stroke volume, rather than increased heart rate (Fig. 1, lower panel).

3.3. Cardiac remodeling

Representative photomicrographs of Gomori-stained sections in the left ventricular viable wall are shown in Fig. 2 (panel *A*). Myocardial infarction induced left ventricular hypertrophy at cellular level, which was confirmed by a significantly increased myocyte cross-sectional area, was not affected by vasopressin V_{1A} or V_2 receptor antagonist treatment. These observations were substantiated by the actual measurements as presented in Fig. 3 (upper panel). Fig. 2 (panel *B*) shows representative photomicrographs of Lectin-stained sections in the left ventricular viable wall showing individual capillaries. Reduced capillary density, which was observed in myocardial infarction induced hypertrophied hearts, remained unaffected by treatment with vasopressin receptor antagonsists. This was confirmed by the actual measurements in Fig. 3 (lower panel). In addition, capillary to myocyte ratio was similar in all groups (sham: 1.31 ± 0.12; myocardial infarction: 1.37 ± 0.14; myocardial infarction + V_{1A}: 1.27 ± 0.08; myocardial infarction + V_2: 1.41 ± 0.19).

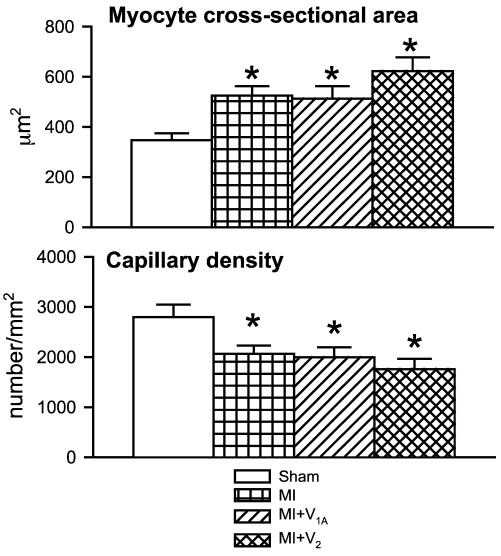

Fig. 3. Actual measurements for myocyte cross-sectional area (upper panel) and capillary density (lower panel) in the different experimental groups. *: $P < 0.05$ vs. sham.

4. Discussion

4.1. In vivo hemodynamics

The important role of neurohormonal activation in the progression of heart failure and left ventricular dysfunction is well established (Packer, 1992a). That vasopressin may contribute importantly to this process, is supported by prior studies demonstrating elevated arginin vasopressin levels in acute as well as chronic heart failure (Szatalowicz et al., 1981; Goldsmith et al., 1983; Rouleau et al., 1994). When antivasopressor V_1 receptor antagonists became available for intravenous use in humans, it was shown to produce a hemodynamic improvement with transient decrease in systemic vascular resistance and increased cardiac output (Nicod et al., 1985). Moreover, understanding of the functional significance of vasopressin in heart failure have also been achieved with V_2 receptor antagonists, which were shown to improve diuresis and increase free water clearance in experimental models (Yamamura et al., 1998; Yatsu et al., 1999) as well as humans (Kalra et al., 2001).

In contrast to previous studies, which mainly examined the acute effects of vasopressin receptor antagonism, the present results demonstrate that *chronic* treatment with the V_{1A} receptor antagonist SR-49059, but not the V_2 receptor antagonist SR-121463B, could improve in vivo hemodynamics in a postinfarction rat model of heart failure. Whereas left ventricular dysfunction in untreated conscious myocardial infarcted rats was reflected by a significantly decreased stroke volume and cardiac output, chronic V_{1A} receptor blockade restored these functional parameters. Furthermore, whereas acute administration of V_{1A} and V_2 receptor antagonists in myocardial infarcted rats significantly reduced mean arterial blood pressure and more than doubled urine production (Lankhuizen et al., 2001), *chronic* treatment did not alter cardiac loading conditions as reflected by mean arterial pressure, central venous pressure and total peripheral resistance. Thus, an improved stroke volume and cardiac output with the V_{1A} receptor antagonist, but not V_2 receptor antagonist, could not be attributed to changes in pre- or afterload. In addition, a lowered in vivo tachycardia as observed with the vasopressin V_{1A} receptor antagonist may be advantageous in terms of improving myocardial oxygen delivery by enhanced tissue

perfusion through longer diastolic time (Stewart et al., 1993).

The hemodynamic results of this study are fully supported by observations in a rat model of postinfarction heart failure in which short-term therapy with a combined V_{1A}/V_2 receptor antagonist resulted in an increased cardiac output; however, at substantial increased urine output (Mulinari et al., 1990). Hemodynamic improvement with this combined V_{1A}/V_2 receptor antagonist could, based on the findings of the present study, be mainly attributed to the effects of V_{1A} antagonism.

4.2. Cardiac remodeling

Alterations in the cardiac structure may be responsible for improved cardiac function in vasopressin V_{1A} receptor antagonist-treated myocardial infarcted rats. Therefore, its effects on left ventricular hypertrophy and capillary density were investigated. Postinfarction remodeling is associated with alterations in shape and size of the injured left ventricle and compensatory hypertrophy of the spared myocardium with reduced capillary density (Anversa et al., 1986). Although angiotensin-converting enzyme inhibitors (Sanbe et al., 1995) and β-adrenoceptor antagonists (Fowler, 1998) are now extensively used to treat functional and structural consequences following large myocardial infarction, little is known about the effects of vasopressin antagonists on cardiac remodeling.

In the present study, left ventricular hypertrophy associated with a lower capillary density was observed in the spared myocardium of untreated myocardial infarcted rats, which was not affected by treatment with the vasopressin V_{1A} or V_2 receptor antagonist. The lower capillary density directly results from left ventricular hypertrophy since capillary to myocyte ratio remained unchanged. Hemodynamic improvement at a preserved hypertrophic response during early postmyocardial infarction treatment supports our hypothesis that complete prevention of myocardial infarction induced compensatory hypertrophy, as was observed with *early* angiotensin-converting enzyme inhibitor therapy, deteriorates rather than improves left ventricular hemodynamics in myocardial infarcted rats (Schoemaker et al., 1991). Recent studies have demonstrated that in addition to other neurohormones, vasopressin increases the rate of protein synthesis in the myocardium, leading to myocyte hypertrophy, a direct effect mediated by the V_{1A} receptor (Fukuzawa et al., 1999; Nakamura et al., 2000). However, in the present study, antagonizing the V_{1A} or V_2 vasopressin receptors did not affect myocyte hypertrophy and, without a direct effect on capillary growth, capillary density remained reduced. A minor role of vasopressin compared to the renin–angiotensin system in promoting left ventricular hypertrophy might be one explanation. From these results, we may assume that improved cardiac function observed with the vasopressin V_{1A} receptor antagonist is not due to changes in hypertrophic response and myocardial capillarity.

4.3. Conclusions

It is conceivable that in the setting of neurohormonal blockade with angiotensin-converting enzyme inhibitors, β-adrenoceptor antagonists, and aldosterone antagonists, other neurohormones, such as vasopressin, begin to play a more prominent role in the subsequent progression of heart failure. Currently, there are only a limited number of studies examining the use of vasopressin receptor antagonists in human heart failure. In general, vasopressin receptor antagonism has been shown to improve hemodynamics and diuresis in several models of heart failure. The results of the present study showed that chronic vasopressin V_{1A} but not V_2 receptor blockade prevented heart failure in chronically infarcted rats. Interestingly, in our hands, this is the very first treatment administered during the first 3 weeks that actually improved cardiac output. Previous work resulted at the most in a sustained depressed cardiac output, but at a lower heart rate and improved stroke volume (Schoemaker et al., 1998). The observed improvement of cardiac function in these rats could not be attributed to changes in left ventricular hypertrophy and capillary density. However, in line with the observations with angiotensin-converting enzyme inhibitor therapy (Schoemaker et al., 1991), effect of *early* treatment with vasopressin receptor antagonists may not predict effects of more *delayed* treatment. These aspects need further investigation.

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