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Vascular and renal effects of vasopressin and its antagonists in conscious rats with chronic myocardial infarction; evidence for receptor shift

Inge M. Lankhuizen, Richard van Veghel, Pramod R. Saxena, Regien G. Schoemaker *

Department of Pharmacology, Erasmus University Rotterdam Medical Centre "EMCR", P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

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Abstract

Acute myocardial infarction evokes activation of, among others, the arginine–vasopressin system, resulting in vasoconstriction and fluid retention. In the present study, the vasoconstrictor and antidiuretic effects of vasopressin were examined in vivo in conscious rats with chronic myocardial infarction, in the absence or presence of the V_{1a} receptor antagonist SR-49059 or the V_2 receptor antagonist OPC-31260. In sham rats, vasopressin dose-dependently increased mean arterial pressure (maximum response: 45 ± 3 mm Hg), which was significantly suppressed in infarcted rats (maximum response: 32 ± 3 mm Hg). SR-49059, but not OPC-31260, caused a significant rightward shift of the dose pressure response curve in sham rats, indicating V_{1a} receptor mediation. This rightward shift by SR-49059 was significantly more in infarcted rats. The suppressed response to the agonist and enhanced sensitivity to the antagonist suggest a reduction of V_{1a} receptor number in infarcted rats. In both sham and infarcted rats, the urine production after OPC-31260 (337 ± 14 and 329 ± 30 μl/min, respectively) was about twice of that in vehicle-treated rats (188 ± 25 and 155 ± 24 μl/min, respectively). However, the response in infarcted rats reached its peak quicker and lasted for a shorter period, resulting in a 40% lower area under the curve. Although only measurable during V_2 receptor blockade, the reduction of urine production by vasopressin was significantly more in infarcted compared to sham rats. The enhanced renal response to the agonist and reduced response to the antagonist suggest an increase in V_2 receptor number in infarcted rats. In conclusion, in chronically infarcted rats, vasopressin causes vasoconstriction and fluid retention through the V_{1a} and V_2 receptors, respectively. Altered responses after infarction indicate a shift from V_{1a} to V_2 receptors.

Keywords: Myocardial infarction; Vasopressin; Vasopressin receptor subtype

1. Introduction

Coronary artery disease, often resulting in myocardial infarction, is a common cause of heart failure (Pfeffer et al., 1993). Following an acute myocardial infarction, the sympathetic nervous system, the renin–angiotensin system and arginine–vasopressin system (McAlpine and Cobbe, 1988; McAlpine et al., 1988; Riegger, 1988, 1994; Rouleau et al., 1991) are activated to compensate for the decrease in cardiac output and arterial pressure. Although such compensatory responses may be beneficial initially, epidemiological studies reveal that sustained neurohumoral activation is associated with increased mortality (Schaller et al., 1986). Therefore, it appears that inhibition of these compensatory mechanisms could improve prognosis in patients with heart failure.

The role of the sympathetic nervous system and the renin–angiotensin system in the development of heart failure has been intensively investigated. Indeed, angiotensin-converting enzyme inhibitors, β-adrenoceptor antagonists and diuretics improve the hemodynamic state, quality of life and prognosis (Dargie and Ray, 1989; Pfeffer et al., 1992; Pfeffer, 1993; Vaughan and Pfeffer, 1994). In contrast, the role of the vasopressin system in the development of heart failure as well as the therapeutic possibilities of vasopressin antagonists are less investigated. Vasopressin is produced by the posterior pituitary gland, and its two principal hemodynamic effects, vasoconstriction and fluid retention, are mediated via the V_{1a} and V_2 receptors, respectively (Ostrowski et al., 1992; Burrell et al., 1994a; Carmichael and Kumar, 1994; Nakamura et al., 1994; Liu et al., 1995; Thibonnier, 1998).

* Corresponding author. Tel.: +31-10-408-7530; fax: +31-10-408-9458.

E-mail address: Schoemaker@farma.fgg.eur.nl (R.G. Schoemaker).

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Experimental myocardial infarction in rats, induced by coronary artery ligation, provides a clinically relevant model for the consequences of myocardial infarction (Fishbein et al., 1978; Pfeffer et al., 1979) as a major cause of heart failure. From ex vivo studies in this model, we (Kalkman et al., 1997; Lankhuizen et al., 2000) and others (Stassen et al., 1997) have provided evidence for regionally different vascular responsiveness to vasopressin. However, these data are not necessarily predictive for the whole animal, as a model for a patient. Therefore, in the present study, effects of vasopressin were studied in vivo in conscious rats, in which vascular effects were studied as blood pressure responses, while renal effects were studied by urine production. Both effects were investigated in the absence or presence of the V_{1a} receptor antagonist SR-49059 and the V_2 receptor antagonist OPC-31260.

2. Materials and methods

2.1. Animals

Male Wistar rats (260–300 g; Harlan Zeist, The Netherlands) were used. Rats were housed in groups of two or three, on a 12/12-h light/dark cycle, with standard rat chow and water ad libitum. The experimental procedures were approved by the Ethical Committee for the use of experimental animals within the Erasmus University Medical Centre, Rotterdam.

2.2. Surgical preparations

Myocardial infarction was induced by coronary artery ligation (Johns and Olson, 1954; Pfeffer et al., 1979). Briefly, animals were anaesthetised with pentobarbital sodium (60 mg/kg; i.p.) and the trachea was intubated for positive pressure ventilation (3 ml; ±70 strokes/min). The thorax was opened between the 3rd and 4th rib to localise the left anterior descending coronary artery near the origin of the pulmonary artery. A 6-0 silk thread was tied around the coronary vessel and the thorax was carefully closed (Lankhuizen et al., 2000). Sham animals (control) underwent the same surgical procedures except for the actual ligation. Infarct size was judged after the completion of experimental procedures. Proper occlusion of the coronary artery results in a transmural infarction covering a major part of the left ventricle free wall (about 40% of left ventricular circumference), with small variations in size (Pfeffer et al., 1979; Schoemaker et al., 1990). Accidental occlusion of a side branch results in infarcts of substantially smaller sizes. Estimation of infarct size by macroscopic appearance has proven a reliable method to recognise too small infarctions ($< 20\%$) (Kalkman et al., 1996). Data of animals with such small infarcts were excluded from analysis, since these infarcts are known to be hemodynamically fully compensated (Pfeffer et al., 1979; Schoemaker et al., 1991; Kalkman et al., 1996).

On the 18th day following myocardial infarction, rats were anaesthetised with pentobarbital sodium (60 mg/kg; i.p.) and J-shaped catheters were inserted in the abdominal aorta (vascular experiments only) as well as the vena cava through the femoral artery or vein, respectively. Catheters were subcutaneously guided to the neck of the animal, where they were fixed and extruded to facilitate measurement of arterial blood pressure and intravenous infusion, respectively. The animals were housed individually, and allowed 2 days for recovery. Then, vascular or renal experiments were performed in conscious, freely moving rats.

2.3. Vascular experiments

A pilot study was performed to investigate the overall hemodynamic effects of vasopressin in vivo. For that, six myocardial infarcted and four sham rats were instrumented for full hemoynamic measurements in vivo in the conscious rats, including cardiac output, heart rate, stroke volume, central venous pressure, mean arterial pressure and total peripheral resistance (Schoemaker et al., 1991). Animals were placed in perspex experimental cages ($30 \times 24 \times 30$ cm) and connected to the measuring equipment. Following a stabilisation period, dose–response curves for vasopressin were obtained through cumulative injections (10^{-13}–10^{-8} mol in 100 μl; i.v.). Based on the results of these experiments, we chose for blood pressure responses to vasopressin to represent the in vivo vascular response in the present study.

Animals were placed in perspex experimental cages ($30 \times 24 \times 30$ cm) and connected to the measuring equipment. Heart rate and arterial blood pressure were continuously registered by a computer. Following a stabilisation period of 45–60 min, a bolus injection of vehicle (dimethyl sulfoxide, DMSO; 1 ml/kg; i.v.) was administered, and 15 min later, a dose–response curve for vasopressin was obtained through cumulative injections (10^{-13}–10^{-9} mol in 100 μl; i.v.). Pilot studies have shown that in this preparation, the vasopressin dose–response curve can be repeated with the same result when at least 60 min recovery is allowed in between the curves. Therefore, 60 min following the first dose–response curve, a bolus injection (i.v.) of either the V_2 receptor antagonist OPC-31260 (2 mg/ml · kg) (Nakamura et al., 1994; Wang et al., 1996) or the V_{1a} receptor antagonist SR-49059 (1 mg/ml · kg) (Serradeil-Le Gal et al., 1993) was administered (i.v.), and 15 min later, a second vasopressin dose–response curve was obtained. Two days later, the animals were subjected to the same protocol, receiving the other receptor antagonist.

Subsequently, the animals were anaesthetised with pentobarbital (i.v.) and hearts were excised and infarct size was judged macroscopically (see Section 2.2).

2.4. Renal experiments

Under normal conditions, urine excretion is about 12–18 ml per 24 h, most of which is excreted during the dark/active period. To investigate the effect of vasopressin and vasopressin antagonists on urine excretion, a higher constant output is required. In conscious animals, this can be achieved by a constant i.v. fluid infusion so at least every 30 min, one spontaneous urination occurs. Pilot studies showed that with infusion rates of NaCl (0.077 M) at 150 and 300 μl/min, urine excretion stabilised after 2 h, and remained constant over the next 5 h (the duration of the protocol). In the present study, the infusion rate of 150 μl/min was used.

After prior habituation, animals were placed in metabolic cages and venous catheters were connected for infusion. NaCl (0.077 M) infusion was started at a rate of 150 μl/min. Since pilot studies showed a stabile urine production after 2 h at this infusion rate, after an equilibration period of 2 1/2 h, a bolus injection of vehicle (DMSO; 1 ml/kg), OPC-31260 (2 mg/kg; 1 ml/kg) or SR-49059 (1 mg/kg; 1 ml/kg) was administered (i.v.), and 30 min later, the infusion was switched to NaCl containing vasopressin (24 pmol/min; 150 μl/min) for 2 h (Windle et al., 1995). During the whole experiment, urine samples were collected every 30 min. Two days later, the animals were subjected to the same procedure, receiving one of the other compounds, resulting in each animal being subjected to two of the three protocols (vehicle, OPC-31260 or SR-49059). Not all three protocols could be carried out in one animal, since the protocol was rather aggravating for the rats. Subsequently, the animals were anaesthetised with pentobarbital (i.v.), hearts were excised and infarct size was judged macroscopically.

To confirm the efficacy of the used dose of vasopressin, mean arterial pressure was measured in four separate animals during a 4-h stabilisation period (0.077 M NaCl; 150 μl/min), followed by a 2 h infusion of NaCl containing vasopressin (24 pmol/min; 150 μl/min).

The diuretic response to the V_2 receptor antagonist OPC-31260 and the interference with exogenous vasopressin was studied in more detail. Therefore, two groups (infarct $n = 4$; sham $n = 6$) received a bolus OPC-31260 (2 mg/kg; 1 ml/kg) 30 min before NaCl infusion was randomly switched to either NaCl containing vasopressin or fresh NaCl solution. After 2 h, the infusion was switched again to a fresh NaCl solution for 90 min. Two days later, the animals were subjected to the other procedure, resulting in paired observations.

2.5. Drugs

[Arg^8]-vasopressin (acetate salt) (Sigma, USA) was dissolved in distilled water at a concentration of 10^{-5} M and on the day of experiment further diluted in 0.077 M NaCl. 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 et al., 1994) and OPC-31260 (5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,4-tetrahydro-1*H*-benzazepine) (Yamamura et al., 1992) were gifts from Sanofi Recherche, Montpellier, France (courtesy: Dr. D. Nisato) and dissolved in dimethyl sulfoxide at 2 and 1 mg/ml, respectively. All solutions were stored at −80 °C.

2.6. Data analysis

2.6.1. Vascular experiments

All data are expressed as means ± S.E.M. Curves were analysed with GraphPad Prism® to obtain $-\log EC_{50}$ (pD_2) and maximal responses. We concede that maximal vasoconstriction was not reached with the highest dose of vasopressin (10^{-9} mol in 100 μl) in the presence of SR-49059, but the curve was fitted assuming the maximal response to be the same as in the animals without the antagonists. Data were subjected to statistical analysis using paired *t*-tests (two dose–response curves at the same day) for differences between vehicle- and antagonist-curves. For differences between EC_{50} values of the curves from sham and infarcted rats, unpaired *t*-tests were used. The level of significance was taken as $P < 0.05$.

2.6.2. Renal experiments

All data are expressed as means ± S.E.M. Differences in responses of all six groups were tested for statistical significance with one-way Analysis of variance, followed by post hoc Bonferroni's multiple comparison test. The level of significance was taken as $P < 0.05$.

3. Results

3.1. General

Ligation of the left descending coronary artery resulted in transmural infarctions in the left ventricle free wall. Overall mortality was less than 20% and occurred within the first 24 h after surgery.

In the vascular study, 26 animals were used. Five of them died within 24 h after ligation of the coronary artery. No animals were excluded for too small (< 20%) infarct sizes, while two animals were excluded because of obstructed cannula.

In the renal study, 48 animals were used. Nine of them died within 24 h after ligation of the coronary artery. Two animals were excluded for too small (< 20%) infarct sizes, and four animals were excluded because of obstructed cannula.

3.2. Vascular experiments

The full hemodynamic pilot experiment showed dose-dependent effects in the dose range of 10^{-13}–10^{-10} mol

for heart rate (sham 360 ± 10 to 235 ± 33 beats/min; MI 384 ± 18 to 294 ± 31 beats/min), mean arterial pressure (sham 100 ± 1 to 156 ± 7 mm Hg; MI 96 ± 5 to 131 ± 7 mm Hg) and cardiac output (sham 79 ± 8 to 40 ± 10 ml/min; MI 62 ± 3 to 35 ± 4 ml/min). Central venous pressure did not change, whereas stroke volume and total peripheral resistance were not influenced by vasopressin, except for the highest dose. Moreover, for the latter parameter, no differences were observed between sham and infarcted rats.

In the present study, the dose pressure response curves to vasopressin with or without the antagonists SR-49059 and OPC-31260 are shown in Fig. 1, and the pD_2 values are presented in Table 1. Vasopressin induced a substantial, concentration-dependent increase in mean arterial pressure and a considerable decrease in heart rate. In sham animals, mean arterial pressure was increased maximally 45 ± 3 mm Hg from baseline (123 ± 3 mm Hg). In animals with myocardial infarction heart failure, mean arterial pressure was increased maximally 32 ± 3 mm Hg from baseline (103 ± 3 mm Hg). Baseline ($P < 0.0001$) as well as maximal increase in mean arterial pressure ($P < 0.005$) was significantly lower in infarcted rats compared to sham rats. The pD_2 values for the pressure response were similar in sham and infarcted rats, as were the hill slopes of the dose–response curves. Although SR-49059 caused a significant rightward shift ($P < 0.0001$) in both sham and infarcted rats, this shift was significantly more in infarct than in sham rats. OPC-31260 did not antagonise the vasopressin-induced increase in mean arterial pressure in sham animals, but caused a significant rightward shift ($P < 0.05$) in infarct animals.

Although slightly higher in infarcted rats, baseline heart rate was not significantly different between sham and infarcted rats, but vasopressin-induced decreases in heart rate were significantly smaller in infarcted compared to sham rats. At the highest vasopressin dose, heart rate was decreased by 96 ± 10 beats/min from 345 ± 7 beats/min baseline in sham animals ($n = 10$) and 51 ± 8 beats/min from 359 ± 8 beats/min baseline in animals with myocardial infarction ($n = 9$). Since heart rate decreases as a result of increasing blood pressure because of baroreflex activation, baroreflex activity in infarcted and sham rats was compared using the slope of the change in heart rate per change in blood pressure. The baroreflex activity was not significantly different for sham and infarcted rats (-1.79 ± 0.18 and -1.62 ± 0.20, respectively). Results were similar after pretreatment with either antagonist with respect to heart rate changes following blood pressure changes (data not shown).

Table 1
pD_2 values (in mol) of the pressure response to vasopressin in conscious rats

Treatment	Sham	Infarct
Vehicle	11.68 ± 0.15	11.58 ± 0.20
SR-49059 (1 mg/ml kg)	9.78 ± 0.10[a]	9.19 ± 0.25[a,b]
OPC-31260 (2 mg/ml kg)	11.35 ± 0.14	11.02 ± 0.18[c]

[a] $P < 0.0001$ vs. vehicle.
[b] $P < 0.05$ vs. sham.
[c] $P < 0.05$ vs. vehicle.

3.3. Renal experiments

About 2 h after starting the NaCl infusion, urine flow had stabilised at 103 ± 7 μl/min (Fig. 2). In both sham ($n = 9$–10 per group) and infarcted ($n = 10$–12 per group) rats, urine flow increased after administration of vehicle (188 ± 25 and 155 ± 24 μl/min, respectively). Similar increases were seen with the V_{1a} receptor antagonist SR-49059 (157 ± 34 and 170 ± 16 μl/min, respectively), but neither reached statistical significance. However, urine flow was significantly ($P < 0.001$) increased after administration of the V_2 receptor antagonist OPC-31260 (337 ± 14 and 329 ± 30 μl/min, in sham and infarct rats, respectively) and was approximately twice the values in vehicle-treated rats. In infarcted rats, maximal urine flow immediately returned to baseline after the vasopressin infusion was started, whereas in sham rats, the increase of urine

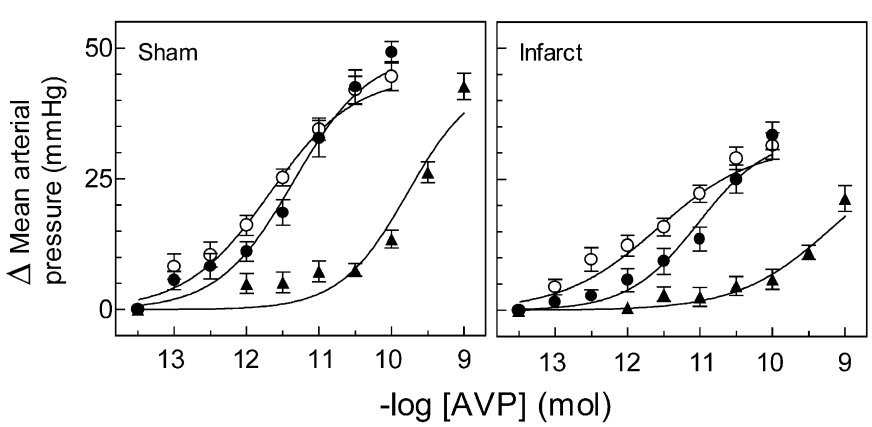

Fig. 1. Increase of mean arterial pressure by vasopressin in conscious, sham-operated ($n = 10$) and chronically infarcted ($n = 9$) rats treated with vehicle (open circles), V_{1a} receptor antagonist SR-49059 (1 mg/ml · kg; triangles) or the V_2 receptor antagonist OPC-31260 (2 mg/ml · kg; closed circles).

flow was extended over the next 30 min. Although maximal values were similar in infarct and sham rats, the response in infarct rats peaked early and the duration shortened, resulting in a 40% lower area under the curve.

No antidiuretic effect was observed during the vasopressin infusion. However, parallel experiments showed that the used dose of vasopressin was effective in increasing mean arterial pressure from 135 ± 7 mm Hg (baseline is high due to infusion of 150 μl/min) to 163 ± 4 mm Hg ($P < 0.001$). It remained at this level for the 2 h of infusion, and quickly returned to baseline after termination of the infusion.

Additional experiments in which the diuretic response to the V_2 receptor antagonist OPC-31260 was also studied without vasopressin infusion (Fig. 3) showed that, similar to the previous experiments, the diuretic response to OPC-31260 was accelerated and shortened in infarcted rats compared to sham. Moreover, when vasopressin was added to the NaCl infusion, the antidiuretic effect of vasopressin could be demonstrated as a significantly lower urine production than with infusion of NaCl alone (Fig. 3). Moreover, subtraction of the curves with and without vasopressin revealed an average net reduction of urine production during vasopressin infusion of 177 ± 50 μl in sham rats, which was significantly elevated in infarct rats; 350 ± 53 μl.

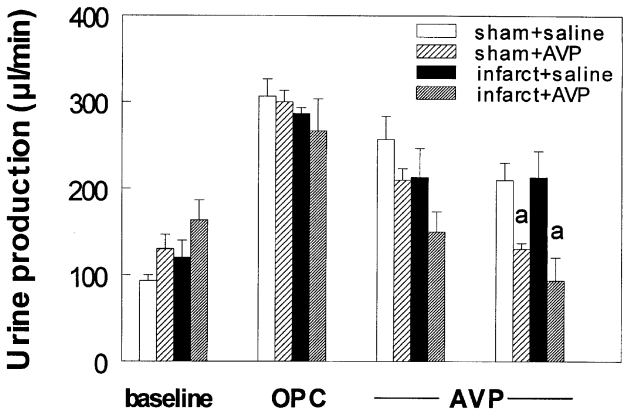

Fig. 3. Urine flow in sham-operated ($n = 6$) and chronically infarcted ($n = 4$) rats during continuous infusion of NaCl 0.077 M; 150 μl/min at baseline; during 30 min after administration of OPC-31260 (2 mg/ml·kg; OPC) and during the first and last 30 min of vasopressin infusion (vasopressin). (a) significant difference between vasopressin-and the respective saline infusion group.

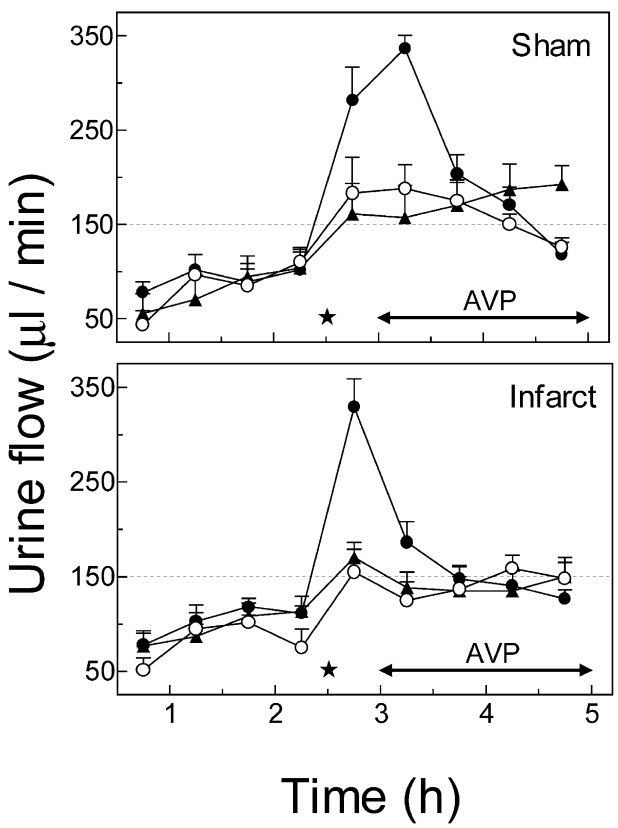

Fig. 2. Urine flow in sham-operated ($n = 9$–10) and chronically infarcted ($n = 10$–12) rats during constant infusion (0.077 M NaCl; 150 μl/min). At $t = 2\ 1/2$ h (★), a bolus injection of vehicle (open circles), the V_{1a} receptor antagonist SR-49059 (1 mg/ml·kg; triangles) or the V_2 receptor antagonist OPC-31260 (2 mg/ml·kg; closed circles) were administered. At $t = 3$ h, the infusion was switched to NaCl containing vasopressin (24 pmol/min; 150 μl/min) for 2 h.

4. Discussion

4.1. General

The vasopressin system is activated after myocardial infarction to help restore the fall in mean arterial pressure and cardiac output by eliciting vasoconstriction and fluid retention. In the present study, we evaluated the vasoconstrictor and anti-diuretic effects of vasopressin in infarcted versus sham-operated rats, and examined the role of V_{1a} and V_2 receptors, by using SR-49059 and OPC-31260, respectively.

The major vascular findings were: (i) vasopressin induced a dose-dependent increase in mean arterial pressure; (ii) baseline mean arterial pressure and maximal increase in mean arterial pressure were significant lower in infarcted rats than in sham rats; (iii) SR-49059 caused a significant rightward shift in both sham and infarcted rats.

The major renal findings were: (i) OPC-31260 induced an increased diuresis; (ii) the antidiuretic effect of exogenous vasopressin could only be demonstrated after partial blockade of V_2 receptors; (iii) the diuretic effects of OPC-31260 were reduced in infarcted rats, while the antidiuretic response to vasopressin was enhanced.

4.2. Vascular experiments

Vasopressin induced dose dependent increases in mean arterial pressure and decreases in heart rate. The increase in mean arterial pressure is due to constriction of resistance vessels. These findings are in agreement with our

previous study (Lankhuizen et al., 2000) and the observations of several other research groups (Webb et al., 1986; Laycock and Lightman, 1989; Walker et al., 1989), where also a decrease in cardiac output and an increase in total peripheral resistance were observed.

The observation that the vasopressin-induced vasoconstriction was potently antagonised by SR-49059 corresponds with previous studies (Serradeil-Le Gal et al., 1993; Bax et al., 1995; Liu et al., 1995) and supports the contention that the V_{1a} receptor is involved in the vasoconstrictor response to vasopressin. The antagonism by OPC-31260 appears contradictory, since V_2 receptors only play a minor role in the vascular response to vasopressin and, that too, is vasodilatation rather than vasoconstriction (Walker, 1986; Walker et al., 1988; Tagawa et al., 1995; Wang et al., 1996). In our previous study, we also reported that OPC-31260 could antagonise V_{1a} receptor mediated responses (Lankhuizen et al., 2000). OPC-31260 has a V_2/V_{1a} receptor selectivity ratio of 25:1, indicating only a relative V_2 receptor selectivity (Burrell et al., 1994a,b). Therefore, we conclude that the antagonism of the vasoconstrictor response to vasopressin by OPC-31260, as observed in infarcted rats in the present study, can most probably be attributed to V_{1a} receptor blockade.

Myocardial infarction evokes the activation of the arginine–vasopressin system, which would result in (temporally) increased circulating levels of vasopressin, which in turn could change responsiveness to vasopressin. Indeed, in a previous in vitro study in different vascular beds, we showed regionally different changes in responsiveness to vasopressin (Lankhuizen et al., 2000), which was also observed by Stassen et al. (1997). As the response was sensitive to SR-49059, it was mediated by the V_{1a} receptor (Lankhuizen et al., 2000).

In the present study, a significant difference in the in vivo pressure response to vasopressin between sham and infarcted rats was observed. Blood pressures of infarcted rats increased less than that of sham rats, and started at a lower baseline value, the latter being indicative for impaired cardiac function. pD_2 values were similar in sham and infarcted rats. Responses could effectively be antagonised by the V_{1a} receptor antagonist SR-49059, suggesting V_{1a} receptor involvement. Although we cannot exclude an increase in absolute or relative effect of vasodilatory V_2 receptors, a decrease in vasoconstrictory V_{1a} receptors would be more feasible to explain the reduced response in infarcted rats; infarct rats showed a reduced maximal response with similar pD_2 values, responses appeared more sensitive to V_{1a} receptor antagonism; even OPC-31260, with a relative selectivity for the V_2 receptor (25:1) could antagonise the response. In favour of this explanation is the vasopressin hyporesponsiveness in mesenteric resistance vessels, shown in vitro (Stassen et al., 1997; Lankhuizen et al., 2000) and the observation that in infarcted but not in sham rats V_1 inhibition decreased arterial pressure and caused venodilation (Raya et al., 1990).

Another explanation for the reduced responsiveness in infarcted rats could be that the heart, because of the limited left ventricular function, is not capable of developing more pressure at the higher vasopressin doses. This would be supported by the results of the pilot experiments, in which cardiac output and central venous pressure were measured as well, showing that total peripheral resistance (difference between arterial and venous pressure, divided by the cardiac output) was normally increased in response to vasopressin. However, this would be expected to change the EC_{50} value for the vasopressin dose–response curves.

Finally, changes in vessel structure and/or vascular smooth muscle cell phenotype, as discussed in our previous study (Lankhuizen et al., 2000), could have contributed as well.

Heart rate decreased significantly more in sham than in infarcted rats. Since direct effects of vasopressin on heart rate have not been recognised, the heart rate changes could rather be attributed to a baroreflex response on the blood pressure changes. A similar baroreflex activation in sham and infarct rats, as indicated by a similar slope of the delta pressure delta heart rate curve, would explain the lower heart rate response as a consequence of the lower blood pressure response in infarcted rats.

4.3. Renal experiments

Under normal conditions, urine excretion is about 12–18 ml per 24 h. Therefore, to investigate the effect of vasopressin and vasopressin antagonists on urine excretion, a higher constant output is needed. In the present study, at an input of 150 μl/min, urine flow had stabilised at 103 ± 7 μl/min, with no differences between the groups.

During the vasopressin infusion, no antidiuretic effect was observed. This was surprising, since practically the same protocol was used as Windle et al. (1995). Windle et al. observed a statistically significant decrease in urine excretion after switching the 150 μl/min NaCl infusion to a 2 h NaCl infusion containing 24 pmol/150 μl vasopressin. Two differences might explain the different observations. Windle et al. used Long Evans instead of the Wistar rats used in the present study. Secondly, in Windle et al.'s experiments, infusion was switched immediately after spontaneous urination, whereas in the present study, the infusion was switched at prior determined timepoints. However, the antidiuretic effect of vasopressin would then be delayed, rather than absent.

V_2 receptor mediated effects of vasopressin occur at concentration far lower than are required to engage the V_1 receptor mediated actions, although affinity for cloned rat V_{1a} and V_2 receptors are similar. Moreover, Smyth et al. (1985) observed in isolated perfused kidneys at a vasopressin perfusate concentration of 10 μU/ml a statistically significant decrease in urine excretion, but no renal vascular changes yet. To exclude that the vasopressin and/or the used dose in the present study was not effective, in a

parallel experiment, mean arterial pressure was measured during vasopressin infusion. Since mean arterial pressure was significantly increased during the infusion period, ineffectiveness of (the dose of) vasopressin could not explain the absence of an antidiuretic effect. The antidiuretic effect of vasopressin could, however, be demonstrated in the additional experiments. Urine flow during NaCl infusion containing vasopressin did not reach below baseline, but was significantly lower than urine flow from animals that received NaCl infusion without vasopressin. It could be that the endogenous levels of vasopressin in our (sham as well as infarcted) rats are to high to demonstrate the antidiuretic effect of exogenous vasopressin. Only when the effects of endogenous vasopressin are merely blocked by an antagonist, effects of exogenous administered vasopressin become unmasked. An attempt to measure vasopressin levels revealed similar values in anaesthetised sham and infarcted rats (22.9 ± 2.4 and 22.4 ± 2.0 pg/ml, respectively), which is in agreement with studies of Raya et al. (1990) in anaesthetised but not in conscious rats; the latter showing increased levels after infarction.

The observed increase in diuresis after administration of OPC-31260 is in agreement with Tsuboi et al. (1994), who observed a huge increase in urinary volume after oral administration of OPC-31260 in conscious rats with water available ad libitum. Even in rats that were deprived of water for 24 h, OPC-31260 induced an increase in diuresis. Although in the present study, maximal response to OPC-31260 was similar in sham and infarcted rats, in infarcted rats maximal urine flow immediately returned to baseline after the vasopressin infusion was started, whereas in sham rats the increase of urine flow was prolonged over the next 30 min. Similarly, the response was accelerated and shortened in infarcted rats when no vasopressin was added to the NaCl infusion. A 40% smaller Area under the Curve of infarcted rats suggests that infarcted rats are less sensitive to the V_2 antagonist. Corresponding to the V_{1a} receptor discussion, this would then imply the presence of more V_2 receptors in infarcted rats. The slope of the decrease in urine production during vasopressin infusion as well as the calculated net reduction of urine production indicate a higher response to vasopressin, supporting the previous suggestion.

The major renal effect of diuretics is excretion of sodium and water. However, at variance with classical natriuretic agents, the nonpeptide vasopressin V_2 receptor antagonists, such as OPC-31260, only induce free water without modifying electrolytes (Thibonnier, 1998). The term ‘aquaretics’ has been coined to describe this class of new agents.

4.4. Conclusions

Data from the present study indicate that the blood pressure response to vasopressin in infarcted rats is depressed compared to sham animals. Since this response is discussed to be mediated by V_{1a} receptors, a reduced V_{1a} receptor density seems feasible.

A prolonged renal response to the V_2 antagonist as well as a calculated enhanced reduction of urine production during vasopressin infusion provides evidence for an increase in V_2 receptor density in infarcted rats. Effects may indicate a shift in V_{1a}/V_2 receptor ratio after myocardial infarction.

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