Sex-specific differences in ventricular expression and function of parathyroid hormone-related peptide

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Abstract

Objective: Parathyroid hormone-related peptide (PTHrP) expression is modulated by estrogen. It is expressed in coronary endothelial cells and involved in the endothelium-dependent regulation of coronary resistance and cardiac function. In the present study, we hypothesized that endogenously synthesized and released PTHrP contributes to sex-specific differences in the regulation of cardiac function. Methods: The influence of sex on ventricular PTHrP expression in normotensive rats was determined via real-time PCR and immunoblot analysis. Sex-specific effects of exogenous PTHrP or endogenous released PTHrP were determined in vitro on isolated ventricular cardiomyocytes, Langendorff preparations on isolated hearts and in vivo using different agonistic or antagonistic PTHrP peptides. Results: Ventricular expression of PTHrP was elevated in hearts from female rats compared to male counterparts. Addition of PTHrP(1–36) did not increase left ventricular function in hearts from either sex, but increased coronary flow in hearts from female rats significantly greater than in those from males. 5Ile-PTHrP(1–36), which was used to antagonize endogenously released PTHrP, reduced left ventricular function in females but not males in vitro and in vivo. Under conditions of increased endogenous PTHrP release, i.e. ischemia–reperfusion, antagonization of PTHrP significantly reduced post-ischemic recovery in hearts from females but not in those from males. Conclusions: Sex determines the ventricular expression of PTHrP mRNA and protein. The results indicate that PTHrP may improve cardiac function to a greater extent in women than in men following a brief period of ischemia.

Keywords: Endothelium-derived factors; Sex; Vasculature; Ventricle; Regional blood flow

1. Introduction

Parathyroid hormone-related peptide (PTHrP), that was initially purified from tumors as a PTH-like factor of humoral hypercalcemia in malignancy, is expressed throughout the cardiovascular system [1–3]. In the ventricle, PTHrP seems to be part of the endothelium-dependent control of ventricular function. It exerts inotropic, chro-

notropic and hypertrophic effects on ventricular cardiomyocytes and dilates vessels. Within the ventricular myocardium, cardiomyocytes have been identified as potential PTHrP targets. PTHrP is expressed in the neighboring coronary endothelial cells from which it is released in a glycosylated form [4]. Authentic endothelium-derived PTHrP is able to exert a positive contractile effect on cardiomyocytes at nanomolar concentrations. Synthetic PTHrP peptides have also been shown to stimulate protein synthesis of cardiomyocytes and to decrease coronary resistance [5,6]. Thus, PTHrP improves cardiac performance and coronary perfusion. In addition, PTHrP is released under ischemic conditions and acts as a positive inotropic agonist specifically in the stunned myocardium [7]. Based on the above-mentioned characteristics of the
cardiovascular effects of PTHrP locally produced PTHrP might be considered as a protective agonist allowing the heart to maintain cardiac function.

At present, little is known about the ventricular expression of PTHrP under physiological and pathophysiological conditions, except of the findings that noradrenaline increases its expression and TGF-β1 downregulates it [8,9]. There is evidence that PTHrP expression is regulated also in an estrogen-dependent manner. Recent studies have shown that estrogen increases PTHrP mRNA expression in vivo in rat uterus and kidney [10,11]. The interest in studying the influence of sex on the cardiac expression and function of PTHrP is caused by the rapid increase of the risk of cardiovascular events after menopause [12–14] as well as the cardioprotective profile of PTHrP on the other hand.

It is unclear at present whether hearts from males or females express PTHrP and respond to PTHrP in a similar or different way. Our study was aimed to investigate in vivo and in vitro the impact of sex on cardiac PTHrP expression and we hypothesized that endogenously synthesized and released PTHrP contributes to sex-specific differences in the regulation of cardiac function. Therefore, we analyzed cardiac expression and function of PTHrP. As PTHrP is constantly released from coronary endothelial cells, we performed experiments with agonistic peptides to determine the impact of exogenous PTHrP on cardiovascular function and antagonists to antagonize endogenously released PTHrP. We used the following synthetic peptides: PTHrP(1–36), a classical functional active PTHrP peptide, and a cardiac-specific antagonist namely 5Ile-PTHrP(1–36), a hybride peptide that mimics the structure of PTH at the N-terminal part and that of PTHrP in the C-terminal part, and PTHrP(7–34), a classical PTH/PTHrP-receptor-1 (PTH-R1) antagonist. In the present study, we used two different PTHrP antagonists to limit possible side effects of the antagonists and to be as specific for the cardiac effects of PTHrP as possible. The impact of endogenously released PTHrP on left ventricular function in male and female rat hearts was determined under basal conditions, i.e. on isolated ventricular cardiomyocytes, in isolated perfused rat hearts and in vivo in anaesthesized rats. In addition, release of PTHrP was provoked by 30-min no-flow ischemia in isolated perfused rat hearts and the functional recovery during reperfusion was monitored during the next 30 min in absence or presence of a PTHrP receptor antagonist during ischemia.

2. Materials and methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Animals

Adult male or female Wistar rats (250–300 g) were used to investigate the expression of PTHrP in normotensive animals and the influence of PTHrP on ventricular function. Coronary endothelial cells were isolated from ventricles of adult male rats as described before [4].

2.2. Polymerase chain reaction (PCR)

PCR for expression of PTHrP mRNA was performed as described before [4] as real time PCR. The expression was normalized to hypoxanthine-phosphoribosyl-transferase (HPRT) as a house-keeping gene.

2.3. Western blots

Cells or tissue samples were treated with lysis buffer as described before [4]. Samples (100 μg protein) were loaded on a 12.5% SDS-PAGE and blotted onto membranes as described before [4]. Blots were incubated first with a monoclonal antibody against the amino acid residues 38–64 of PTHrP (antibody GF08, Oncogene Research Products) and second with an anti-mouse IgG antibody coupled to alkaline phosphatase. Samples were re-blotted and incubated with amido black. The actin band was used as a loading control.

2.4. Functional analysis

2.4.1. Cell contraction of isolated ventricular cardiomyocytes

Ventricular cardiomyocytes were isolated from male or female rat hearts as described before [4,7]. Cell contraction was monitored via a cell edge detection system as described before [4]. Briefly cells were scanned via a line camera and the actual cell length was recorded at a reading frequency of 500 Hz. The total time of recording was 1000 ms starting with the trigger. Cells were paced at a constant rate of 2 Hz. Cell lengths are expressed as cell length normalized to the diastolic cell length.

2.4.2. Determination of left ventricular pressure

Experiments were performed on isolated hearts from male and female Wistar rats. Hearts were rapidly excised and the aorta was cannulated for retrograde perfusion with a 16-gauge needle connected to a Langendorff perfusion system. A polyvinyl chloride balloon was inserted into the left ventricle through mitral valve and held in place by a suture tied around the left atrium. The other end of the tubing was connected to a pressure transducer for continuous measurement of left ventricular pressure. A second transducer connected to the perfusion line just before the heart was used to measure coronary perfusion pressure. The perfusion system was consisted of a warmed storage vat for
were performed. Hearts were perfused with a modified Tyrode solution as described before [15,16]. After attachment to the Langendorff system, the hearts were allowed to stabilize for at least 20 min. The intravenous balloon was inflated to give a diastolic pressure of 10 mm Hg and balloon volume was held constant thereafter. The flow was adjusted to give a perfusion pressure of 50 mm Hg. Within the time of investigation no release of lactate dehydrogenase could be measured [17]. Two hearts were perfused at the same time, one of them received PTHrP peptides as indicated and the other one was used as control. In this set of experiments, we focused on the effects of PTHrP on left ventricular developed pressure (LVDP). To avoid hypoperfusion due to the vasoconstrictor effect produced by antagonists, hearts were perfused at a constant flow. To control that the antagonists and agonists are fully active in a comparable way hearts were not paced and the positive chronotropic effect was used to control efficacy.

2.4.3. Determination of coronary flow

In another set of experiments, coronary flow was determined. In this set of experiments, pacing electrodes were placed at the right ventricular outflow tract and the left ventricular apex. Hearts were perfused at a constant perfusion pressure of 55 mm Hg. Perfusate was not recirculated and the effluents were collected every 30 s to quantify coronary flow. In this set of experiments, we focused on the influence of PTHrP on coronary resistance. To avoid strong variations in shear stress caused by vasoconstriction or vasodilatation of the antagonists or agonists and to reduce the impact of frequency variations on the coronary resistance due to different times of diastole length, hearts were paced in this set of experiments and perfused at a constant pressure.

2.4.4. Determination of left ventricular developed pressure in vivo

For determination of the effect of $^5$Ile-PTHrP in intact animals, rats were anaesthetized with 1.5% isoflurane. A Millar tip catheter (Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle. Drug injections were given via the left jugular vein. Continuous pressure monitoring and data analysis were performed using a powerlab AD-interface and the Chart 4.2 software (ADInstruments, Spechbach, Germany).

2.5. Statistics

Quantitative results were expressed as means ± S.E.M. In experiments in which more than two groups were compared to each other, ANOVA was used, with Student–Newman–Keuls test for post-hoc analysis. In cases in which two groups were compared, conventional $t$-tests were performed. $p<0.05$ was used as a level of significance. The EC$_{50}$ values were calculated from the derived means values of the experiments and analyzed by the GraphPad Prism 3.0 program.

3. Results

3.1. Sex-specific differences of ventricular expression of PTHrP

In the first set of experiments, sex-specific differences in ventricular expression of PTHrP mRNA were investigated. Expression of PTHrP mRNA normalized to HPRT as loading control in females exceeded that of males by 85.0 ± 5.4%. Similar expression of PTHrP protein normalized to actin as a loading control was higher in ventricles from female than from male rats (± 68 ± 7%, means ± S.E.M., $n=4$, $p<0.05$). A representative immunoblot is given in Fig. 1. Isolated coronary endothelial cells from female rat hearts showed also elevated PTHrP protein expression normalized to actin as a loading control compared to coronary endothelial cells from male rats (Fig. 1). On average, PTHrP protein levels in coronary endothelial cells isolated from female hearts was elevated by 81 ± 12% ($n=4$, means ± S.E., $p<0.05$) compared to cells from male hearts. According to the increased expression of PTHrP in isolated coronary endothelial cells from female hearts versus cells from male hearts, a significant higher release of PTHrP into the perfusate was determined. In the perfusate obtained from female rat hearts we found 0.65 ± 0.07 µg PTHrP ml$^{-1}$ g heart weight$^{-1}$ compared to 0.47 ± 0.04 µg PTHrP ml$^{-1}$ g heart weight$^{-1}$ in the perfusate obtained from male hearts ($p<0.05$, $n=4$ hearts).

3.2. Sex-specific differences of the cardiac effects of PTHrP on rats in vitro

3.2.1. Impact of PTHrP on chronotropy

Since the data on the differential expression of PTHrP in females and males suggest a different role for PTHrP in
cardiac function, we investigated in a second step, whether the hearts of male or female rats respond differentially to PTHrP. Cumulative concentration–response curves were obtained for the chronotropic effects of synthetic PTHrP (1–36) or 5Ile-PTHrP(1–36) in hearts from either sex. No significant differences were obtained for both peptides in regard to the chronotropic effect (Fig. 2). Based on the concentration–response relationships shown on Fig. 2, a concentration of 100 nmol/l was used in all subsequent in vitro experiments for all peptides. Both peptides increased beating frequency in a comparable way within 5 min (Fig. 3). PTHrP(7–34), a classical receptor antagonist void of intrinsic activity, did not influence beating frequencies in female or male rat hearts (data not shown).

3.2.2. Impact of PTHrP on coronary resistance

PTHrP(1–36) did not significantly influence basal coronary resistance in saline perfused rat hearts (data not shown). However, when coronary vessels were preconstricted by phenylephrine, PTHrP decreased coronary resistance in a concentration-dependent way (Fig. 4). The EC50 for the dilating effect of PTHrP was calculated to 0.74 nmol/l in female rat hearts and 1.03 nmol/l in male rat hearts based on cumulative concentration response curves. On average, the maximal effect by which 100 nmol/l PTHrP(1–36) decreased coronary resistance in hearts isolated from either male or female rats amounted to 13.1% and 20.0%, respectively (Table 1). In hearts from females and males, PTHrP reduced coronary resistance within 5 min (Fig. 5). 5Ile-PTHrP(1–36) was used to antagonize the effect of endogenously released PTHrP. It increased basal coronary resistance within 5 min (Fig. 6). On average, at 100 nmol/l 5Ile-PTHrP(1–36) increased coronary resistance in male and female hearts by 16.2% and 39.9%, respectively (Table 1). Similar to 5Ile-PTHrP(1–36), addition of the classical PTH/PTHrP receptor antagonist PTHrP(7–34) (100 nmol/l) led to an increase in coronary resistance (+11.3 ± 4.2%, n = 4, p < 0.05).

In a different set of experiments, hearts were preconstricted by phenylephrine and subsequently dilated by addition of isoprenaline (100 nmol/l). Coronary resistance was reduced by isoprenaline from 14.8 ± 0.9 to 11.2 ± 0.5 mm Hg min ml⁻¹ g⁻¹ (p < 0.05, n = 6). PTHrP(1–36) did not further reduce coronary resistance (11.7 ± 0.7 mm Hg min ml⁻¹ g⁻¹). However, 5Ile-PTHrP(1–36) raised coronary resistance from 11.2 ± 0.4 to 13.6 ± 0.9 mm Hg min ml⁻¹ g⁻¹ in presence of isoprenaline (p < 0.05, n = 6). The vasodilating effect of PTHrP was not attenuated by infusion of L-nitro arginine (L-NA, 100 μmol/l), which was used to inhibit nitric oxide production. PTHrP(1–36) was able to increase coronary resistance in presence of L-NA in hearts from either males or females (Fig. 7).
3.2.3. Impact of PTHrP on inotropy

In a separate set of experiments, we investigated the effect of PTHrP on the LVDP. Similar to the inhibitory effect of 5Ile-PTHrP(1–36) on coronary resistance, the antagonist reduced basal LVDP in females by 12.5 ± 4.1% (p < 0.05). No such effect was observed in males (Table 2). Addition of PTHrP(1–36) did not increase LVDP in hearts from either sex (Table 2). Fig. 8 shows representative single heart records from such experiments. As expected from the concentration–response curves described above, both peptides equally increased heart rate (Table 2). To decide whether the antagonist 5Ile-PTHrP(1–36) exerts a direct negative inotropic effect or blocks endogenously released PTHrP, experiments were performed on isolated ventricular cardiomyocytes from male or female rat hearts. Isolated cardiomyocytes from male and female rat hearts responded with a similar contractile response to synthetic PTHrP(1–

![Graph](image_url)

Fig. 3. Time curve indicating the positive chronotropic effects of PTHrP(1–36) or 5Ile-PTHrP(1–36) in isolated perfused hearts from male or female rats. Hearts were allowed to beat ad libitum and perfused under flow-constant conditions. Data are means ± S.E.M. from n = 6 experiments.

![Graph](image_url)

Fig. 4. Cumulative concentration response–curve for the vasodilating effect of exogenous PTHrP(1–36). Hearts were preconstricted by addition of phenylephrine (10 μmol/l) and subsequently perfused with increased concentrations of the peptide. Hearts were paced and perfused under pressure constant conditions. Data are means ± S.E.M. from n = 6 experiments. *p < 0.05 vs. basal value.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Coronary resistance (mm Hg min ml⁻¹ g⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>Basal</td>
<td>n = 6</td>
</tr>
<tr>
<td></td>
<td>10.88 ± 1.21</td>
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<tr>
<td>PTHrP(1–36)</td>
<td>n = 6</td>
</tr>
<tr>
<td></td>
<td>8.70 ± 0.78*</td>
</tr>
<tr>
<td></td>
<td>(−20.0%)</td>
</tr>
<tr>
<td>5Ile-PTHrP(1–36)</td>
<td>n = 6</td>
</tr>
<tr>
<td></td>
<td>8.66 ± 1.31</td>
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<tr>
<td>Basal</td>
<td>n = 6</td>
</tr>
<tr>
<td></td>
<td>10.06 ± 1.40*</td>
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<td></td>
<td>(−39.9%)</td>
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Isolated hearts were perfused with a pressure of 55 mm Hg and constantly paced at 3 Hz. To determine vasodilative effects by PTHrP, hearts were preconstricted by addition of phenylephrine (10 μmol/l). Coronary flow was monitored constantly. Coronary resistance was calculated as the ratio of perfusion pressure and coronary flow and normalized to heart weight. *p < 0.05 vs. corresponding basal values.
36) while 5Ile-PTHrP(1–36) did not interfere with cell contraction (Fig. 9). PTHrP increased cell contractile responsiveness on cardiomyocytes isolated from rat hearts of males from 4.87 ± 0.29% of diastolic cell length to 7.31 ± 0.70% ( + 50.1% vs. control, \( n = 12 \) cells from three preparations, \( p < 0.05 \)). Maximal contraction velocity was accelerated from 143 ± 15 to 299 ± 86 μm/s and the corresponding relaxation velocity from 118 ± 13 to 226 ± 36 μm/s. The same peptide increased contractile responsiveness on cardiomyocytes from hearts of females from 5.31 ± 1.56% to 7.62 ± 1.06% ( + 43.4% vs. control, \( n = 16 \) from two preparations, \( p < 0.05 \) vs. control). Neither was the contraction or relaxation velocity different in cells from either male or female rats.

3.3. Sex-specific differences in the antagonistic effect of 5Ile-PTHrP in situ

As the in vitro experiments on Langendorff preparations had suggested a role for endogenously released PTHrP in female but not male rats on cardiac function, we performed experiments on anaesthetized normotensive rats to evaluate its influence in vivo. Increasing the dose of 5Ile-PTHrP as

![Fig 5. Time curve indicating the vasoconstrictor effect of 5Ile-PTHrP(1–36) in isolated perfused rat hearts from females or males. Hearts were preconstricted by addition of phenylephrine (10 μmol/l) and subsequently perfused at 100 nmol/l of the peptide. Coronary resistance was determined during 5 min after addition of PTHrP. Hearts were paced and perfused under pressure constant conditions. Data are means ± S.E.M. from \( n = 6 \) experiments. * \( p < 0.05 \) vs. each other.](image1)

![Fig 6. Time curve indicating the vasoconstrictor effect of 5Ile-PTHrP(1–36) (100 nmol/l) in isolated perfused rat hearts from females or males. Hearts were paced and perfused under pressure constant conditions. Data are means ± S.E.M. from \( n = 6 \) experiments.](image2)

![Fig 7. Time curve indicating the vasodilatative effect of PTHrP(1–36) in isolated perfused rat hearts from females or males. Hearts were preconstricted by addition of L-NA (100 μmol/l) and subsequently perfused with 100 nmol/l of the peptide. Coronary resistance (CR) was calculated during the first 5 min after addition of PTHrP. Data are means ± S.E.M. from \( n = 6 \) experiments. * \( p < 0.05 \) vs. basal value.](image3)

![Fig 6. Time curve indicating the vasoconstrictor effect of 5Ile-PTHrP(1–36) (100 nmol/l) in isolated perfused rat hearts from females or males. Hearts were preconstricted by addition of phenylephrine (10 μmol/l) and subsequently perfused at 100 nmol/l of the peptide. Coronary resistance was determined during 5 min after addition of PTHrP. Hearts were paced and perfused under pressure constant conditions. Data are means ± S.E.M. from \( n = 6 \) experiments. * \( p < 0.05 \) vs. each other.](image4)

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LVDP</td>
<td>HR</td>
</tr>
<tr>
<td>Basal</td>
<td>94.6 ± 3.3</td>
<td>271 ± 4</td>
</tr>
<tr>
<td>PTHrP (1–36)</td>
<td>94.6 ± 2.8</td>
<td>326 ± 10*</td>
</tr>
<tr>
<td>5Ile-PTHrP (1–36)</td>
<td>82.8 ± 3.9*</td>
<td>341 ± 33*</td>
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Isolated hearts were perfused in the Langendorff mode with a constant flow of 10 ml and allowed to beat frequently. Left ventricular developed pressure was determined via a balloon catheter placed in the left ventricle. Beating frequency was calculated from the number of left ventricular peak pressures per minute. Measurements were performed 5 min after addition of the drugs. * \( p < 0.05 \) vs. corresponding basal values.
an PTHrP antagonist from 1 to 10 μg/kg body weight, a
dose-dependent decrease in left ventricular systolic pres-
ture, $dP/dt_{max}$ and $dP/dt_{min}$ was found in females but not
males (Fig. 10). There was no significant effect of $^5$Ile-
PTHrP on beating frequency within the dose range under
investigation.

Fig. 8. Representative single heart records of either perfused male or female hearts before (control) or five minutes after addition of PTHrP(1–36) (PTHrP, 100
nmol/l) or Ile-PTHrP(1–36) (Ile-PTHrP, 100 nmol/l). Data are left ventricular pressure (LVP) following one second of perfusion.

Fig. 9. Effect of PTHrP(1–36) or $^5$Ile-PTHrP(1–36) (100 nmol/l each) on the contractile responsiveness of isolated ventricular cardiomyocytes which were
isolated from either male or female rat hearts. Cells were paced at 2 Hz and cell lengths were monitored via a cell edge detection system. Data are % cell length
during contraction normalized to diastolic cell length. Original single cell recordings are given.
3.4. Sex-specific differences in the functional recovery during reperfusion in presence of PTHrP receptor antagonists

As shown previously, PTHrP is released from the coronary system during ischemia [7]. The functional impact of PTHrP was investigated by treatment of the hearts with the PTHrP receptor antagonist before a 30-min no-flow ischemia was performed. The functional recovery was monitored during the reperfusion time. As shown on Fig. 11A, hearts from female rats showed a significant impaired functional recovery in the presence of $^5$Ile-PTHrP. In contrast, on hearts from male rats, the inhibitor did not significantly impair post-ischemic recovery (Fig. 11B). After 30 min of reperfusion, LVDP in male control hearts was $85 \pm 18\%$ of pre-ischemic values, $62 \pm 5\%$ in hearts treated with $^5$Ile-PTHrP and $95 \pm 15\%$ in hearts treated with a classical receptor antagonist (PTH(7–34)). However, control hearts from female rats had a $72 \pm 28\%$ recovery but those pre-treated with PTHrP receptor antagonists showed a significant impairment ($^5$Ile-PTHrP: $49 \pm 16\%$, PTH(7–34): $47 \pm 16\%$; $p < 0.05$ vs. control, $n = 6$). Basal release of PTHrP was higher in female compared to male rat hearts (see above) and remained higher at the beginning of reperfusion.

4. Discussion

4.1. Main findings

There are significant sex-specific differences in the development and progression of cardiovascular diseases. Previous studies have demonstrated that expression of PTHrP is regulated in an estrogen-dependent way in the rat uterus and the kidney. According to these studies, we hypothesized that sex influences ventricular expression of PTHrP and evaluated the functional consequence of this. The main findings of our study are: first, that ventricular...
expression of PTHrP is indeed higher in females than in males; second, that PTHrP reduces coronary resistance significantly more effective in females than in males; and third, that inhibition of endogenously released PTHrP reduces left ventricular developed pressure stronger in females than in males.

4.2. Sex and PTHrP expression

Mechanisms, which are involved in the regulation of ventricular expression of PTHrP, have not been studied in great detail. We hypothesized that sex may influence its level of expression, because earlier studies have suggested that PTHrP mRNA expression may be dependent on estrogen status both in vitro and in vivo [10,11]. Our findings on ventricular expression of PTHrP are in agreement with these studies focused on the regulation of PTHrP mRNA expression in vivo in various non-myocardial tissues. The different male vs. female expression of cardiac PTHrP cannot be attributed, however, to a different vascularization pattern, because it holds for isolated preparations of coronary endothelial cells. It was already shown before that coronary endothelial cells represent the main source for PTHrP in the ventricle [2,4]. Thus, the expression of PTHrP seems to be higher in ventricles from females compared to males. PTHrP is released from coronary endothelial cells in a mechanosensitive way [17]. In the present study, we found that the increased expression of PTHrP in hearts from females goes along with an elevated release of PTHrP. This led us to hypothesize that endogenously released PTHrP contributes to a higher extent to heart function in females than in males.

4.3. Sex-specific differences on the impact of endogenously released PTHrP on cardiac function

Special attempts were made in our study to investigate the influence of PTHrP on cardiac function in female or male rats. In general, PTHrP exerts positive chronotropic, inotropic and dilating effects. The chronotropic effect is mediated via stimulation of the classical PTH-R1 that can be activated by PTHrP and PTH [18]. In contrast, the ventricular effects of PTHrP on inotropy and coronary resistance cannot be mimicked by PTH [6]. Therefore, the term PTHrP is misleading, as the hormone differs markedly from parathyroid hormone (PTH): First, except a small N-terminal region, its primary structure is different from PTH. Second, PTHrP is a paracrine factor expressed throughout the body, whereas PTH is expressed and released mainly from the parathyroid gland. Third, most of the effects of PTHrP are not related to those of PTH, e.g. those effects on ventricular cardiomyocytes. Thus, PTHrP is not only a PTH-like calcitropic hormone but exerts its own physiological functions. Since the active domain responsible for these effects is located within the first six amino acids, it is reasonable to suggest that the structural basis for this difference between both peptides must be located within this domain. Since both agonists vary only at position 5 (histidine vs. isoleucine), we used the hybride peptide (5Ile-PTHrP) to antagonize specifically the ventricular effects of endogenously released PTHrP.

In regard to the chronotropic effect of PTHrP, we showed that this effect is mediated via a stimulation of the classical PTH-R1. PTHrP(1–36) and 5Ile-PTHrP(1–36) revealed similar effects, but PTHrP(7–34) did not. There are no indications from our study for any sex-specific differences between male and female rats in regard to the chronotropic effect of PTHrP.

PTHrP also affects coronary resistance and cardiac inotropy. These effects were found to be more sensitive to PTHrP than to PTH [5,6]. Our results with 5Ile-PTHrP(1–36) confirmed such a non-classical structure–function relationship. In hearts from male and female rats in vitro, 5Ile-PTHrP(1–36) caused vasodilatation but PTHrP(1–36) did not further reduce coronary resistance, indicating that the amount of endogenously released PTHrP is already sufficient. However, when the vessels were preconstricted by phenylephrine, PTHrP exerted a strong vasodilative effect. The effects of PTHrP on coronary resistance were more pronounced in females than in males. For the aforementioned reasons, we used 5Ile-PTHrP(1–36) to inhibit endogenously released PTHrP. This peptide increased coronary resistance even under basal conditions. It is in line with these suggestions that PTHrP(7–34), another antagonist but void of any biological activity, also increased coronary resistance in these hearts. The vasodilating effect of PTHrP was found to be independent from nitric oxide production, as it persisted in the presence of l-NA, which was used to inhibit nitric oxide production. Thus, the different effect of PTHrP on coronary resistance in either male or female rat hearts seems not be indirectly mediated by different eNOS expression, which was shown to be differentially expressed [19]. The outcome of these experiments is also in agreement with former findings that endothelial cells do not express PTH/PTHrP receptors [20]. Therefore, it is highly unlikely that PTHrP exerts its dilating effect in an endothelium-dependent way.

In addition, inhibition of endogenously released PTHrP by 5Ile-PTHrP in female rat hearts reduced LVDP significantly but not in hearts from male rats. This suggests that endogenously released PTHrP contributes to the maintenance of cardiac function in females to a greater extent than in males. Specific attempts were performed to exclude a direct negative inotropic effect of 5Ile-PTHrP. On isolated cardiomyocytes from either male or female rat hearts PTHrP exerted a positive contractile effect that was not seen with 5Ile-PTHrP, which on the other hand did not reduce contractile responsiveness of the cells. Finally, the different responsiveness to 5Ile-PTHrP from male and female rats could be confirmed in vivo, in which 5Ile-PTHrP caused a dose-dependent decrease in...
cardiac function (left ventricular systolic pressure, dP/d\(t_{\text{max}}\), dP/d\(t_{\text{min}}\)) in females but not in males.

The release of PTHrP has been demonstrated to increase under conditions of ischemia both in vitro and in vivo [7]. As PTHrP contributes to ventricular function of female but not male hearts even under basal conditions, we expected that the impact of endogenously released PTHrP on post-ischemic recovery will be even more remarkable. Indeed, both PTHrP-receptor antagonists significantly impaired functional recovery of female hearts but not of male hearts. Therefore, due to a significant higher PTHrP release from female rat hearts, the participation of the PTHrP system in post-ischemic recovery seems to be more important in female than male rats.

The mechanism by which \(^{5}\)Ile-PTHrP(1–36) allows to antagonize the activity of endogenously released PTHrP remains to be elucidated. At present, one might speculate that the ventricle expresses a novel tissue-specific PTHrP receptor. However, such a receptor has never been found. Alternatively, \(^{5}\)Ile-PTHrP(1–36) might lead to an accelerated receptor desensitization compared to PTHrP and therefore inhibits the endogenously released PTHrP to act. Nevertheless, the present data clearly indicate that, in normotensive rats, the increased ventricular expression of PTHrP goes along with an increased responsiveness to PTHrP.

In summary, our study shows sex-specific differences in ventricular PTHrP expression and function in rats. Whether the differences between females and males hearts in regard to PTHrP are directly linked to estrogen was not the aim of this study and requires an additional de novo experimental setup confirmation. However, it is tempting to speculate that ventricular PTHrP expression in humans may be part of the scenario leading to an impaired cardiac function at least in some groups of post-menopausal women with cardiovascular disease.

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