Cardiac-specific effects of parathyroid hormone-related peptide: Modification by aging and hypertension

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Abstract

Objective: Parathyroid hormone-related peptide (PTHrP) improves heart function of post-ischemic and stunned myocardium and is released from the heart under ischemic conditions. Hypertrophic hearts from spontaneously hypertensive rats (SHR) develop a reduced ischemic tolerance, show reduced expression of PTHrP and develop paradoxical effects in regard to PTHrP. We hypothesized that PTHrP is causally involved in reduced ischemic tolerance of hypertrophied hearts. This hypothesis was tested by addition of a cardiac-specific PTHrP agonist or antagonist during ischemia and investigation of the functional recovery during the early phase of reperfusion.

Methods: Hearts from male normotensive adult (6 months) or old (12 months) Wistar and SHR rats were perfused at a constant flow for 20 min and then exposed for 30 or 15 min to global zero-flow ischemia followed by 30 min reperfusion. PTHrP agonist (PTHrP(1–36)) or antagonist (5\textsuperscript{Ile},23\textsuperscript{Trp},36\textsuperscript{Tyr}-PTHrP(1–36)) (each 100 nmol/l) were added briefly before the onset of ischemia to ensure that they were present at the beginning of reperfusion. Heart function was determined by insertion of a balloon catheter into the left ventricle. Left ventricular developed pressure (LVDp), dP/dt\textsubscript{max}, dP/dt\textsubscript{min}, left ventricular end-diastolic pressure (LVEDP), heart rate (HR) and coronary resistance (CR) were recorded.

Results: Reduced post-ischemic recovery in old SHR was confirmed. Hearts from all four groups responded normally to exogenous PTHrP with a positive chronotropic effect under non-ischemic conditions. In hearts from adult normotensive rats, a beneficial effect of released endogenous PTHrP was confirmed. However, addition of the cardiac-specific PTHrP antagonist during ischemia significantly improved post-ischemic recovery in hearts from old normotensive rats and SHR. This beneficial effect of the antagonist was accompanied by a significant reduction in post-ischemic LVEDP and was more pronounced in adult SHR. This effect was also observed when the hearts were paced (4 Hz).

Conclusion: In summary, a protective effect of released endogenous PTHrP was confirmed for hearts from adult normotensive rats. This effect is converted into an opposite effect in hearts from SHR and old normotensive rats. Therefore, released endogenous PTHrP can contribute to reduced ischemic tolerance in hypertrophied hearts and during aging.

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Keywords: Coronary flow; Reperfusion; Hypertrophy; Heart rate

1. Introduction

Parathyroid hormone-related peptide (PTHrP) was initially identified as a factor responsible for the syndrome of humoral hypercalcemia of malignancy (reviewed in [1]). However, PTHrP is also expressed in the cardiovascular system. Smooth muscle cells, endothelial cells and atrial myocytes express PTHrP. Moreover, the peptide contributes to blood pressure control [2–4]. PTHrP can be processed by
members of the prohormone convertase family. At least three fragments have been identified: amino-terminal PTHrP(1–36), mid-regional PTHrP(38–94) and carboxy-terminal PTHrP(107–139). Carboxy-terminal PTHrP fragments seem to act via specific receptors distinct from those that are activated by the N-terminal PTHrP fragment [5].

The impact of PTHrP(1–36) on cardiac function is still under investigation. Specific attempts have been made to clarify the role of PTHrP during ischemia and reperfusion. We demonstrated that PTHrP is released from endothelial cells during ischemia [4]. Moreover, it increases cardiac function of stunned myocardium but has a limited impact on non-ischemic myocardium [6]. We further showed in vivo that a cardiac-specific antagonist of PTHrP, $^5$Ile,$^{23}$Trp,$^{36}$Tyr-PTHrP(1–36), reduces cardiac function in females. This led us to conclude that constantly released PTHrP contributes to maintenance of cardiac function in females [7]. In male hearts, PTHrP expression is less pronounced compared to females, but during ischemia PTHrP is also released. As a consequence, PTHrP levels are elevated at the onset of reperfusion in hearts from either sex. Indeed, inhibition of endogenously released PTHrP impaired post-ischemic recovery of rat hearts from normotensive females or males [6,7]. These data have established that PTHrP contributes to the ischemic tolerance of the heart.

Post-ischemic recovery is impaired in hypertrophied hearts [8]. The reason for this is still unclear. Several possibilities have been ruled out, i.e. a different Ca$^{2+}$ sensitivity, an altered energy generation or an activation of ATP-sensitive K$^+$ channels [9–11]. Instead altered reactivity to ischemia-elicited factors might play a role [12]. We showed that, in hearts from spontaneously hypertensive rats (SHR), expression of PTHrP drops in a TGF-$\beta$-dependent way [13]. TGF-$\beta$ expression is transiently induced in ventricles from pressure-overloaded hearts [14]. In addition to these alterations in cardiac specific expression of PTHrP, it was shown that smooth muscle cells from SHR develop a paradoxical effect when exposed to exogenous PTHrP [15]. Taking into account that PTHrP contributes to post-ischemic recovery in adult normotensive rats, we hypothesized that either reduced PTHrP expression or different responsiveness of PTHrP receptors in old hypertrophied hearts from SHR are causally involved in a reduced post-ischemic recovery of these hearts. If reduced PTHrP expression in hearts from SHR is responsible for the reduced post-ischemic recovery, addition of biologically active PTHrP peptide and another group received $^5$Ile,$^{23}$Trp,$^{36}$Tyr-PTHrP(1–36), known to inhibit cardiac-specific effects of PTHrP [5]. Both peptides were given shortly before the onset of ischemia.

In conclusion, our study investigates the impact of cardiac-specific PTHrP agonists or antagonists on the early phase of post-ischemic recovery on hearts from adult or old normotensive or hypertensive rats. Our data show that hearts from old normotensive rats and from adult hypertensive rats do not benefit from endogenous PTHrP but from inhibition of cardiac-specific PTHrP effects.

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 and analysis of left ventricular developed pressure

Either adult (6 months) or old (12 months) male normotensive Wistar or spontaneously hypertensive rats were used to investigate the impact of PTHrP on post-ischemic recovery. Experiments were performed on isolated hearts from these 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 the 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 consisted of a warmed storage container for perfusate solutions, a rotary pump and a temperature-controlled chamber in which the hearts were mounted.
Table 1
Peak systolic blood pressure and heart rate in vivo

<table>
<thead>
<tr>
<th></th>
<th>P_systolic</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>138±2</td>
<td>354±2</td>
</tr>
<tr>
<td>Wistar</td>
<td>139±5</td>
<td>318±9*</td>
</tr>
<tr>
<td>SHR</td>
<td>201±7†</td>
<td>415±13‡</td>
</tr>
<tr>
<td>SHR</td>
<td>215±7‡</td>
<td>439±24§</td>
</tr>
</tbody>
</table>

Data are means±S.E.M. from n=4 animals for each group.
* Indicates significant differences between adult (6 months) old (12 months) SHR.
† Indicates significant differences between adult (6 months) and old (12 months) Wistar rats.
‡ Indicates significant differences between adult (6 months) Wistar and SHR.
§ Indicates significant differences between old (12 months) Wistar and SHR.

3. Results

3.1. Hypertension, hypertrophy and post-ischemic recovery in hearts from adult and old normotensive and spontaneously hypertensive rats

Before starting the experiments, four animals from each group were characterized in vivo in terms of peak systolic blood pressure and heart rate. This was done to prove that SHR had indeed developed hypertension and to investigate possible changes in all four groups during aging. On average, systolic blood pressure was higher in SHR compared to normotensive rats with no further increase between 6 and 12 months of age (Table 1). Animals older than 12 months were not included in this study because only very few male SHR survived longer. Normotensive rats had stable blood pressure values during the time span investigated in this study.

Myocardial hypertrophy, as indexed by an increased heart weight to body weight ratio, developed in adult SHR compared to adult normotensive rats. In contrast to the peak systolic blood pressure, which did not further increase during aging, heart weight/body weight increased in aging hearts from normotensive rats (Table 2).

All hearts were analyzed for heart function in a Langendorff perfused heart model. Perfusion pressure was set to approximately 50 mm Hg and left ventricular end-diastolic pressure (LVdP) was set to 10 mm Hg. Under these conditions, no significant differences in terms of left ventricular developed pressure (LVDP), dP/dt_max and dP/dt_min and heart rate were seen between hearts from either adult normotensive or hypertensive rats. However, hearts from old SHR had higher LVDP, dP/dt_max and dP/dt_min under these conditions. Hearts from old normotensive rats and those from adult and old SHR developed a regression in coronary resistance (Table 3).

After characterization of baseline parameters, hearts were exposed to 30 min of zero-flow ischemia, followed by reperfusion for 30 min. Time courses of the experiments indicating the development of LVDP and LVdP during ischemia and reperfusion are shown in Fig. 1. As indicated, hearts from normotensive rats rapidly recovered from zero-flow ischemia during the first 5 min. Thereafter, recovery of LVDP dropped down again but recovered...
during the 30-min period (Fig. 1A). Measurements of LVeDP revealed an earlier onset of rigor contraction during ischemia in hearts from adult versus old normotensive animals (Fig. 1A). At the end of reperfusion (30 min), LVeDP was elevated in both groups to a similar degree. In hearts from old SHR, the initial recovery during the first 5 min was impaired compared to hearts from adult SHR (Fig. 1B). The data on LVeDP indicate an earlier onset of rigor contraction during ischemia in hearts from old versus adult SHR. Again, no significant difference occurred between the LVeDP at the end of reperfusion, but the values remained significantly elevated compared to pre-ischemic values in both groups. The 30-min recovery of the other parameters are summarized in Fig. 2. $dP/dt_{\text{max}}$ and $dP/dt_{\text{min}}$ remained lower than the pre-ischemic values in all groups. They were significantly lower in the old SHR compared to the three other groups (Fig. 2A and B). Coronary resistances were elevated in all four groups compared to pre-ischemic values. In hearts from adult SHR, coronary resistance after 30 min of reperfusion was higher than in the three other groups (Fig. 2C). Heart rates were not different between the groups, except for hearts from old SHR in which heart rates were slightly higher after 30 min reperfusion (Fig. 2D).

### Table 3
Heart function in vitro (pre-ischemic baseline values)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>LVDP (mm Hg)</th>
<th>$dP/dt_{\text{max}}$ (mm Hg/s)</th>
<th>$dP/dt_{\text{min}}$ (mm Hg/s)</th>
<th>HR (min⁻¹)</th>
<th>CR (Hg min/ml g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar 6</td>
<td>81±14</td>
<td>6511±751</td>
<td>4308±608</td>
<td>276±33</td>
<td>4.3±0.6</td>
</tr>
<tr>
<td>Wistar 12</td>
<td>86±16</td>
<td>5830±1823*</td>
<td>2975±756*</td>
<td>238±21*</td>
<td>2.1±0.3*</td>
</tr>
<tr>
<td>SHR 6</td>
<td>86±12</td>
<td>5200±707§</td>
<td>3460±320§</td>
<td>215±34§</td>
<td>2.5±0.7§</td>
</tr>
<tr>
<td>SHR 12</td>
<td>114±15§</td>
<td>5250±541§</td>
<td>3700±394§</td>
<td>198±30*</td>
<td>2.0±0.4</td>
</tr>
</tbody>
</table>

Data are means±S.D. of $n=24$ animals for each group. Each $p<0.05$. The explanation of symbols is given in Table 1.

![Fig. 1. Time course of experiments in which hearts were challenged by 30-min zero-flow ischemia followed by 30 min of recovery. Left ventricular developed pressure (LVDP, left) and left ventricular end-diastolic pressure (LVeDP, right) are shown for adult (6 months) and old (12 months) hearts from normotensive rats (A) or SHR (B). Data are expressed as means±S.E.M. in % recovery from pre-ischemic values. * $p<0.05$ adult (6 months) vs. old (12 months); $n=12$ hearts in each group.](https://academic.oup.com/cardiovascres/article-abstract/66/2/334/270668)
3.2. Impact of a PTHrP agonist or antagonist on post-ischemic recovery in hearts from adult and old normotensive and spontaneously hypertensive rats

In the next set of experiments, the impact of PTHrP on ischemia and functional recovery in the early phase of reperfusion was investigated. Experiments were performed on hearts from all four groups. According to our previous study, we used PTHrP(1–36) (100 nmol/l) as a biologically active PTHrP peptide and $^{5}$Ile,$^{23}$Trp,$^{36}$Tyr-PTHrP(1–36) (100 nmol/l) as a cardiac-specific PTHrP antagonist [7]. Although $^{5}$Ile,$^{23}$Trp,$^{36}$Tyr-PTHrP(1–36) antagonizes the cardiac specific effects of PTHrP(1–36), both peptides activate the classical PTH-1 receptor. This receptor is responsible for the positive chronotropic effect evoked by PTHrP. According to these assumptions, both peptides increased heart rates from all four groups by approximately 30% under non-ischemic conditions (Fig. 3). This indicates that equally efficacious concentrations were used for both peptides.

We confirmed our previous findings on hearts from adult normotensive rats. Addition of the antagonist $^{5}$Ile,$^{23}$Trp,$^{36}$Tyr-PTHrP(1–36) during ischemia impaired the functional recovery during the early phase of reperfusion. Recovery of LVDP was still lower at the end of 30-min reperfusion in these hearts (Fig. 4A). Rigor contraction during ischemia developed earlier and LVeDP remained elevated until the end of reperfusion. In the presence of PTHrP, hearts recovered rapidly within 7 min to pre-ischemic values, although even in these hearts LVeDP remained elevated (Fig. 4A). In contrast, hearts from old normotensive rats had an improved recovery when treated with the antagonist, although rigor contraction developed earlier (Fig. 4B). The improved LVDP during reperfusion was accompanied by a reduction in post-ischemic elevation of LVeDP. In a similar way, hearts from adult SHR recovered significantly better in the presence of the PTHrP antagonist (Fig. 4C). The effect was more pronounced than that in old normotensive rats. In contrast to old normotensive rats, the antagonist did not accelerate rigor contraction during ischemia in old SHR. In these animals, the antagonist also had a small positive effect on post-ischemic recovery (Fig. 4D). However, in old SHR, the antagonist did not reduce LVeDP, which is in contrast to its effects on adult SHR.

The main findings described above for LVDP and LVeDP were confirmed for other parameters as well. After 30 min of reperfusion, hearts from adult normotensive rats treated
Fig. 4. Time course of experiments in which hearts were challenged by 30-min zero-flow ischemia followed by 30 min of recovery. Effects of PTHrP(1–36) (PTHrP, 100 nmol/l) and ³⁴Ile-PTHrP(1–36) (Ile-rP, 100 nmol/l) on 30-min recovery are shown. Left ventricular developed pressure (LVDP, left) and left ventricular end-diastolic pressure (LVeDP, right) are shown for (A) hearts from adult (6 months) and (B) old (12 months) normotensive rats or (C) adult (6 months) and (D) old (12 months) SHR. Data are means±S.E.M. from n=6. *, p<0.05 PTHrP vs. Ile-rP in each group.
with the antagonist had reduced \( \frac{dP}{dt_{\text{max}}} \) and \( \frac{dP}{dt_{\text{min}}} \) compared to the agonist-treated group (Fig. 5A and B). However, these values increased in hearts from old normotensives and adult SHR. \( \frac{dP}{dt_{\text{min}}} \) was also enhanced by the antagonist in old SHR (Fig. 5B). Coronary resistance was not different in hearts from adult normotensive or hypertensive rats. However, in hearts from old normotensive rats, the antagonist led to a slightly reduced coronary resistance (Fig. 5C). Heart rates were also not different between agonist and antagonist groups in hearts from adult normotensive or hypertensive animals. However, in hearts from old normotensive and hypertensive rats, heart rates were slightly lower in the antagonist group (Fig. 5D).

3.3. Effect of a PTHrP agonist or antagonist on post-ischemic recovery in hearts from adult spontaneously hypertensive rats paced at 4 Hz

After observing the strong effect of the PTHrP antagonist on hearts from adult SHR, we repeated these experiments and paced the hearts before the onset of ischemia and after 30 min of reperfusion. As long as the hearts beat

![Fig. 5. Effects of PTHrP(1–36) (PTHrP, 100 nmol/l) and 5Ile-PTHrP(1–36) (Ile-rP, 100 nmol/l) on 30-min recovery after 30 min of ischemia. Thirty-minute recovery of hearts from adult normotensive rats (W-6), old normotensive rats (W-12), adult SHR (S-6) and old SHR (S-12) are shown. (A) Maximal contraction velocity \( \frac{dP}{dt_{\text{max}}} \). (B) Maximal relaxation velocity \( \frac{dP}{dt_{\text{min}}} \). (C) Coronary resistance (CR). (D) Heart rate (HR). All data are expressed as % of pre-ischemic values. Data are means±S.E.M. from \( n=6 \). *, \( p<0.05 \) PTHrP vs. Ile-rP in each group.]

![Fig. 6. Influence of pacing on heart rate (A) and LVDP (B) of hearts from adult SHR that were challenged by 30 min of ischemia followed by 30 min of reperfusion. Data were evaluated before ischemia (pre-ischemic) and at the end of 30-min reperfusion for hearts treated either with PTHrP(1–36) (PTHrP, 100 nmol/l) or 5Ile-PTHrP(1–36) (Ile-rP, 100 nmol/l). HR-s and LVDP-s indicate the values of hearts allowed to beat freely; HR-p and LVDP-p indicate values from hearts paced at 4 Hz. Data are means±S.E.M. from \( n=5 \). *, \( p<0.05 \) PTHrP vs. Ile-rP. #, \( p<0.05 \) vs. pre-ischemic values.]
Fig. 7. Time course of experiments in which hearts were challenged by 15-min zero-flow ischemia followed by 30 min of recovery. Effect of PTHrP(1–36) (PTHrP, 100 nmol/l) and 5Ile-PTHrP(1–36) (Ile-rP, 100 nmol/l) on 30 min recovery. Left ventricular developed pressure (LVDP) is shown for (A) hearts from adult (6 months) and (B) old (12 months) normotensive rats or (C) adult (6 months) and (D) old (12 months) SHR. Data are means ± S.E.M. from n=5. *, p<0.05 PTHrP vs. Ile-rP in each group.

Fig. 8. Effect of PTHrP(1–36) (PTHrP, 100 nmol/l) and 5Ile-PTHrP(1–36) (Ile-rP, 100 nmol/l) on 30-min recovery after 15 min of ischemia. Thirty-minute recovery of hearts from adult normotensive rats (W-6), old normotensive rats (W-12), adult SHR (S-6) and old SHR (S-12) are shown. (A) Maximal contraction velocity (dP/dt max). (B) Maximal relaxation velocity (dP/dt min). (C) Coronary resistance (CR). (D) Heart rate (HR). All data are expressed as % of pre-ischemic values. Data are means ± S.E.M. from n=6. *, p<0.05 PTHrP vs. Ile-rP in each group. Comparisons were made only within groups but not between groups.
spontaneously, no significant heart rate differences were seen between the groups and heart rates returned to pre-ischemic values (Fig. 6A). Under these conditions, LVDP was again elevated in the antagonist-treated group (Fig. 6B). When the hearts were paced at 4 Hz, the agonist-treated hearts developed lower LVDP, but the antagonist-treated group had significantly elevated LVDP values compared to the agonist-treated group, although still slightly lower than pre-ischemic values (Fig. 6B).

3.4. Effect of a PTHrP agonist or antagonist on post-ischemic recovery in hearts from adult and old normotensive and spontaneously hypertensive rats during short-term ischemia

Finally, the impact of PTHrP agonists or antagonists was investigated on hearts during a short-term ischemia of 15 min. Under these conditions, the hearts did not develop rigor contraction. In none of the groups did LVeDP increase at any time point (data not shown). Hearts from all four groups fully recovered from ischemic insults. No differences were observed between the agonist and antagonist groups (Fig. 7A,C,D), except that in hearts from old normotensive rats. In these hearts, LVDP recovered significantly better during reperfusion compared to the agonist-treated hearts (Fig. 7B). This correlated with increases in $dP/dt_{\text{max}}$ and $dP/dt_{\text{min}}$ and coronary resistance (Fig. 8A,B,C). Hearts rates were not different in any of the groups (Fig. 8D). The findings for hearts from old normotensive rats were confirmed under pacing conditions, indicating that heart rate variations are not responsible for the observed effect on post-ischemic recovery (Fig. 9).

4. Discussion

4.1. Main findings

The main finding of the present study is that PTHrP develops a paradoxical effect on post-ischemic recovery in hearts from SHR and also in hearts from old normotensive rats. Based on our previous findings for adult normotensive rat heart preparations, we hypothesized that PTHrP improves post-ischemic recovery. We were able to confirm that hearts isolated from hypertensive animals have an impaired post-ischemic recovery. The present study was undertaken to evaluate whether released endogenous PTHrP contributes to this phenomenon. Our study indicates that, in the setting of either aging or hypertension, the peptide acts in a paradoxical way. This conclusion is based on our experiments in which $^5$Ile-$^23$Trp-$^36$Tyr-PTHRP(1–36), a cardiac-specific PTHrP antagonist, improved post-ischemic recovery in hearts from SHR. Similarly, the antagonist improved the early functional recovery during the initial reperfusion. In light of these new data, we conclude that the development of such a paradoxical effect of PTHrP may be part of the scenario leading to reduced ischemic tolerance in hearts from SHR. Paradoxical effects of PTHrP on renal vascular smooth muscle cells derived from SHR have been described previously [15]. However, as both peptides exert a positive chronotropic effect in all four experimental groups, it is clear that the currently observed paradoxical effect of PTHrP on the reperfused myocardium of SHR does not indicate a general paradoxical coupling of PTHrP receptors. The development of such paradoxical effects seems to be specific for cardiac effects of PTHrP.

4.2. Cardiovascular effects of PTHrP

Characterization of the cardiovascular effects of PTHrP has been addressed in several studies during recent years. In general, these studies were performed on cell cultures and organs from adult normotensive animals. PTHrP has been described to have receptor-dependent and intracrine effects on endothelial cells and smooth muscle cells [17,18]. In a recent study on smooth muscle cells from the kidneys of...
SHR, a paradoxical effect was shown [16]. On these cells, PTHrP normally acts as a growth stimulus and acts in a cAMP-dependent manner [18]. However, on smooth muscle cells from SHR, PTHrP inhibited the growth of the cells. The authors suggested that this paradoxical effect of PTHrP is caused by a coupling of PTHrP receptors to G_i proteins instead of G_s [15]. If the same mechanisms are responsible for our currently described effect on cardiac tissue, this might explain the reduced post-ischemic heart function. The precise mechanism for this switch, however, remains to be elucidated. On hearts from adult normotensive rats, PTHrP improves post-ischemic recovery. This was shown previously by using two different PTHrP antagonists and confirmed in this study. PTHrP can activate adenyl cyclase. The classical adenyl cyclase-activating domain of the peptide comprises amino acids 1–6 [1]. The fact that a point mutation in this domain (amino acids 1–6) is sufficient of the peptide comprises amino acids 1–6 [1]. The fact that a point mutation in this domain (amino acids 1–6) is sufficient to convert the effects of PTHrP to those of an antagonist, as shown by the use of ^3Ile,^23Trp,^36Tyr-PTHRP(1–36) ([7] and this study), further suggests that the so-called adenyl cyclase-activating domain of the peptide is involved in the effects described here.

Hearts from SHR, specifically hearts from adult SHR, benefit from treatment with the PTHrP antagonist used in our study. This might be due to the effect of locally released PTHrP during ischemia or due to an effect during the subsequent phase of reperfusion. Our study does not allow us to distinguish between these two possibilities. However, in all hearts from normotensive rats and also in hearts from old SHR, the PTHrP antagonist accelerated rigor contraction during ischemia. Despite this, the antagonist reduced post-ischemic recovery in hearts from adult normotensive rats but improved it in hearts from old normotensive rats and old SHR. Therefore, it is likely that the difference in the early phase of reperfusion between hearts from these animals is due to an effect of PTHrP during reperfusion. In line with these suggestions, the antagonist acted in a similarly in the absence of rigor contraction and during severe ischemic damage in hearts from old normotensive rats. In contrast, no effect of PTHrP antagonism was observed in adult SHR in experiments in which the time of ischemia was restricted to 15 min, so that no rigor contraction developed prior to reperfusion. The reason for this difference between Wistar normotensive rats and SHR remains to be elucidated. It should be noted, however, that PTHrP antagonism did not modify the onset of rigor contraction during prolonged ischemia in SHR either. As noted above, the impact of PTHrP on post-ischemic recovery can be considered as a combined effect of PTHrP during ischemia and during reperfusion. However, the precise impact of endogenous PTHrP in ischemia cannot be analyzed from this type of experiment. Future studies, including quantification of the amount of necrosis and probably apoptosis, are needed to clarify this point.

The situation in hearts from adult SHR might be different. The antagonist did not modify rigor contraction in these hearts. In addition, rigor contraction in these hearts developed later. Thus, PTHrP may not be relevant for the onset of rigor contraction in these hearts. A possible explanation might be that these hearts have reduced PTHrP expression, which is in accordance with previous findings for SHR [13]. However, this was not investigated in our study and must be considered as a speculation. Again, the effect of either PTHrP or PTHrP antagonist on early post-ischemic recovery seems to be mediated during reperfusion rather than during ischemia.

4.3. PTHrP and aging

A detailed analysis of cardiac-specific expression and function of PTHrP during aging has not been performed previously. Our study indicates modification of post-receptor coupling during aging that seem to be accelerated in pressure overload hearts. We did not investigate alterations in cardiac-specific expression in aging normotensive rats. However, we did observe in this study that the coupling of cardiac-specific receptors alters in terms of function. Our finding that endogenously released PTHrP improves post-ischemic recovery in adult normotensives but worsens recovery in old normotensives indicates that these changes are independent of pressure-induced hypertrophy. Indeed, the hearts from old normotensive rats mimicked a few characteristics seen in hearts from adult SHR: an elevated heart weight to body weight ratio, reduced coronary resistance and development of paradoxical effects of PTHrP. Based on our in vivo measurements, we must conclude that no general increase in blood pressure occurs in either normotensives or SHR during aging. Thus, the aforementioned changes seem to be more related to aging than to pressure-induced hypertrophy and are accelerated in SHR (Fig. 9).

4.4. Limitations of the study

This study was designed to investigate the impact of a PTHrP agonist or antagonist on the early phase of reperfusion. Based on these experiments, no general comment can be given about the long-term outcome of these interventions. However, our study clearly demonstrates that the previously observed local increase in PTHrP concentration contributes to the early phase of functional recovery of the hearts. Another limitation of our study is that we did not measure infarct sizes in these hearts. Infarct area may be variable and modified by PTHrP during ischemia. However, as explained above, the difference between the effect of the agonist and antagonist on rigor contraction during ischemia and on the early phase of reperfusion at least suggests that the effect of PTHrP may be independent of infarct sizes. In favor of this assumption, LVeDP values are not different between the groups irrespectively of the LVDP. Finally, under the conditions used in these experiments, some of the hearts did not develop severe post-ischemic injury. That is in part due to the fact that the perfusion pressure (about 50 mm
Hg) was relatively low. This was done, however, to void the development of edema during reperfusion, which is another risk in experiments using blood-free perfusion systems. As can be seen for hearts from adult SHR in this study, the post-ischemic recovery is also lower when adjusted to a fixed heart rate (4 Hz). However, in none of the experiments did these limitations affect the final conclusion from our experiments using the standard protocol. Thus, we believe that the conditions used in these experiments, which allowed the hearts to recover properly, were sufficient to detect the aforementioned effects of PTHrP on the early phase of reperfusion.

5. Conclusions

In summary, our study provides evidence for substantial age-dependent changes in the coupling of cardiac-specific receptors to heart function. This process seems to be accelerated in hearts from SHR. The development of such changes appears to reduce the endogenous anti-ischemic potential of the heart and may contribute to reduced post-ischemic recovery in these hearts.

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References