Parathyroid hormone-related peptide improves contractile function of stunned myocardium in rats and pigs

JOHANNA JANSEN,1 PETRA GRES,1 CHRISTIAN UMSCHLAG,1 FRANK R. HEINZEL,1 HEIKE DEGENHARDT,2 KLAUS-DIETER SCHLÜTER,2 GERD HEUSCH,1 AND RAINER SCHULZ1

1Institute of Pathophysiology, University of Essen, 45122 Essen; and 2Institute of Physiology, Justus-Liebig University, 35392 Giessen, Germany

Submitted 28 November 2001; accepted in final form 22 August 2002

Jansen, Johanna, Petra Gres, Christian Umschlag, Frank R. Heinzel, Heike Degenhardt, Klaus-Dieter Schlüter, Gerd Heusch, and Rainer Schulz. Parathyroid hormone-related peptide improves contractile function of stunned myocardium in rats and pigs. Am J Physiol Heart Circ Physiol 284: H49–H55, 2003; 10.1152/ajpheart.01037.2001.—The effect of synthetic parathyroid hormone (PTH)-related peptide [PTHrP(1–34)] on regional myocardial function was studied in 11 anesthetized pigs. Intracoronary infusion of PTHrP (cumulative dose: 14 ± 1 μg) decreased coronary resistance to 33 ± 2% of baseline (P < 0.05) and regional myocardial function to 90 ± 3% of baseline (not significant). Ischemia-reperfusion alters the activity of several kinases and therefore possibly the myocardial effects of PTHrP. In stunned myocardium, induced by 20-min ischemia and 30-min reperfusion, the dose of PTHrP reducing coronary resistance to a minimum of 29 ± 2% was decreased to 8 ± 2 μg (P < 0.05). Regional myocardial function was no longer decreased but increased to 132 ± 9% (P < 0.05). The increase in regional myocardial function during PTHrP infusion was inversely related to baseline function at 30-min reperfusion in vivo (r = 0.9) as well as in myocytes isolated from stunned pig hearts (r = 0.7). In isolated rat hearts subjected to 30-min global ischemia followed by 30-min reperfusion, blockade of endogenous PTHrP by D-Trp12-Tyr34-PTH(7–34) attenuated the recovery of left ventricular developed pressure by 30 ± 14% (P < 0.05). Thus endogenous and exogenous PTHrP impact on the function of stunned myocardium.

ischemia; reperfusion; coronary blood flow; hormones; inotropy

PARATHYROID HORMONE (PTH)-related peptide (PTHrP) is structurally related to PTH. In contrast to PTH, which is exclusively expressed in and secreted by the parathyroid glands, PTHrP is expressed and released by vascular smooth muscle cells and endothelial cells, including coronary endothelial cells, but not by ventricular myocytes (20). PTHrP has been found in the cardiovascular system of several mammalian species, including rats, rabbits, pigs, and humans (3).

In vitro and in vivo, exogenous PTH and PTHrP are potent dilators of the arterial bed (15, 18), and both PTH and PTHrP dose dependently increase regional myocardial blood flow (18). PTH and PTHrP also dose dependently exert positive chronotropic and inotropic effects, PTHrP with greater potency than PTH (13). In vivo studies directly assessing the effects of PTHrP on regional myocardial function, however, are not available at present.

In isolated rat (21) and rabbit cardiomyocytes (11), PTHrP activates adenylyl cyclase and increases cAMP levels, subsequently activating PKA/PKC-dependent pathways (21). These intracellular signal transduction pathways are altered during ischemia-reperfusion in that adenylyl cyclase activity is increased initially during ischemia in isolated rat hearts (2, 28) and activation of PKC occurs during and after myocardial ischemia (16, 17).

Therefore, we investigated the functional effects of PTHrP in normal and stunned myocardium. In the first series, in pigs, exogenous PTHrP was infused, and changes in myocardial blood flow, function and oxygen consumption were assessed. In the second series, the effect of PTHrP on shortening fraction of myocytes isolated from stunned pig hearts was investigated. In the third series, the importance of endogenously released PTHrP on left ventricular (LV) function was studied in isolated rat hearts by selectively inhibiting the PTHrP-receptor using D-Trp12,Tyr34-PTH(7–34) (5).

MATERIALS AND METHODS

In Vivo Pig Preparations

Eleven anesthetized Göttinger minipigs were instrumented for the measurement of LV pressure (LVP) and wall thickness (22). The left anterior descending coronary artery (LAD) and vein were cannulated, and the artery was perfused by an extracorporeal circuit, including an occlusive roller pump and two side ports. One side port was used for the injection of radiolabeled microspheres and the other was used for repetitive and controlled application of PTHrP(1–34) or PTH(1–34), respectively. Coronary arterial pressure was measured through a distal side arm of the cannula. Regional coronary resistance was calculated by dividing...
mean coronary arterial pressure by mean coronary blood flow. Heart rate was held constant by left atrial pacing.

**Regional myocardial function, blood flow, and oxygen consumption.** End diastole was defined as the point when the first derivative of LVP (LV dP/dt) started its rapid upstroke after crossing the zero line. Regional end systole was defined as the point of maximal wall thickness within 20 ms before peak negative LV dP/dt. Systolic wall thickening was calculated as end-systolic wall thickness minus end-diastolic wall thickness divided by the end-diastolic wall thickness.

Because ischemia-reperfusion alters the contraction pattern of the myocardium, the regional myocardial work performed by the LAD-perfused myocardium was calculated in addition to systolic wall thickening. Regional myocardial work was determined as the sum of the instantaneous LVP-wall thickness product over the time of the cardiac cycle with the equation

\[ W_t = \sum_{t=1}^{t_{end}} (LVP_{a,m} - LVP_{a,\min}) \cdot (WTh_{a,m} - WTh_{a,m-1}) \]

where ed is end diastole, n is the actual cardiac cycle, m is a sampling point within cardiac cycle n at a sampling frequency of 5 ms, LVP_{a,m} is the instantaneous LVP within cardiac cycle n and at sampling point m, LVP_{a,\min} is the minimum LVP within cardiac cycle n, and WTh is wall thickness. The maximal work value during systole is reported as WI (8).

Regional myocardial blood flow was measured using radiolabeled microspheres, and myocardial oxygen consumption was calculated by multiplying the arterial-coronary venous oxygen difference by the transmural blood flow at the crystal site.

**Morphology.** Five transverse slices from each heart were incubated in a triphenyltetrazolium chloride solution to exclude myocardial infarction.

**Experimental protocol. NORMAL CONDITIONS.** To maximize regional effects and minimize systemic effects, PTHrP and PTH were administered directly into the LAD. After measurements of systemic hemodynamics, regional myocardial function, and blood flow at baseline were completed, a nonactive fragment of PTHrP, i.e., PTHrP(140–173), was administered to exclude unspecified protein-related side effects. Thirty minutes later, a continuous intracoronary infusion of synthetic PTHrP(1–34) was started at a dose of 2 μg/min. The perfusion pump was adjusted so that minimum coronary arterial pressure did not fall below 70 mmHg. The dose of PTHrP was doubled every minute until maximum coronary dilation was achieved, i.e., increasing the dose of PTHrP further did not result in any further increase in coronary blood flow. When parameters were stable for at least 20 s, measurements were repeated, and the infusion was stopped. Measurements of regional myocardial blood flow and thus the duration of maximal hyperemia averaged 3.0 ± 0.2 min.

Parameters were then allowed to return to baseline. After a further set of measurements, the same protocol was performed with PTH(1–34). Regional myocardial blood flow was measured in six pigs receiving PTHrP and in five pigs receiving PTH.

**MYOCARDIAL STUNNING.** To induce myocardial stunning, coronary inflow was reduced to achieve a 90% reduction in the regional myocardial work index (23). Ischemia was continued for 20 min before the myocardium was reperfused for 30 min. Thereafter, the same set of measurements as under control conditions was obtained, and reperfusion was extended to 2 h for TTC staining.

Data analysis and statistics. All data are reported as means ± SE. Hemodynamic and functional parameters were digitized and recorded over a 20-s period with the use of CORDIT II software (25). Calculations of all hemodynamic parameters were done on a beat-to-beat basis, and data were then averaged. Statistical analysis was performed with SYSTAT software. A linear regression analysis was obtained between regional myocardial function at 30-min reperfusion (expressed as percent of function at baseline) and the increase in regional myocardial function during the subsequent PTHrP infusion.

Data were compared by two-way ANOVA. When significant differences were detected, individual mean values were compared by least-significant difference post hoc tests. A value of P < 0.05 was accepted as indicating a significant difference in mean values.

**Single Pig Cardiomyocytes**

Single myocytes were isolated from the stunned myocardium as previously described (27). Briefly, a wedge of ventricular tissue was perfused through its supplying artery in a Langendorff setup using Tyrode solution (5 min) followed by Ca²⁺-free modified Tyrode solution (30 min). Collagenase A (1.4 g/l, Roche; Mannheim, Germany) and protease type XIV (0.1 g/l, Sigma-Aldrich; Taufkirchen, Germany) were added, and perfusion was continued for 25 min. Enzyme solution was washed out with a 0.18 mmol/l Ca²⁺ solution. Thin slices were then cut from this wedge, and cells 30-min the perimyocordial layer were dispersed and resuspended. Measurements were performed within 24 h after isolation. Cells were superfused with Tyrode solution and studied under an inverted microscope (Axioskop S100TV, Zeiss; Jena, Germany) at 37°C during electric field stimulation (1 Hz). Tyrode solution contained (in mmol/l) 130 NaCl, 5.4 KCl, 1.18 NaHCO₃, 0.5 MgCl₂, 1.8 CaCl₂, and 10 glucose; pH 7.35. Cell shortening was measured in steady-state conditions using a video edge detector (Crescent Electronics; Sandy, UT) before and after washin of PTHrP(1–34) (100 nM). In four additional animals, myocytes were isolated from normoperfused myocardium.

**Data analysis and statistics.** All data are reported as means ± SE. Statistical analysis was performed with SYSTAT software. Data were compared by unpaired t-test. A linear regression analysis was obtained between baseline myocyte shortening and the functional response to PTHrP infusion. A value of P < 0.05 was accepted as indicating a significant difference in mean values.

**Isolated Rat Hearts**

Experiments were performed on hearts from twelve 3-mo-old Wistar rats. The hearts were removed and connected to a Langendorff perfusion system. LVP was measured via a balloon inserted into the LV, and perfusion pressure was monitored via a pressure transducer placed near the aorta. Hearts were perfused at a mean flow of 10 ml/min to maintain an initial perfusion pressure of 50 mmHg. LV diastolic pressure was set to 11 mmHg. After a 20-min stabilization period, 30 min of no-flow ischemia was followed by 30 min of reperfusion in the control (n = 6) and treated groups (n = 6). In the treated group, n-Trp¹²-Tyr³⁴-PTH(7–34) was continuously added to the perfusion buffer (100 nmol/l) starting just before ischemia.

Data analysis and statistics. All data are reported as means ± SE. Data were compared by unpaired t-test. A value of P < 0.05 was accepted as indicating a significant difference in mean values.
Table 1. Systemic hemodynamics, regional myocardial function, blood flow, oxygen consumption, and coronary resistance in normal and stunned myocardium with and without PTHrP(1–34)

<table>
<thead>
<tr>
<th>Table 1. Systemic hemodynamics, regional myocardial function, blood flow, oxygen consumption, and coronary resistance in normal and stunned myocardium with and without PTHrP(1–34)</th>
<th>Normal Myocardium</th>
<th>PTHrP(1–34)</th>
<th>Stunned Myocardium</th>
<th>PTHrP(1–34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>103 ± 2</td>
<td>102 ± 2</td>
<td>103 ± 2</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>LVPP, mmHg</td>
<td>97 ± 2</td>
<td>89 ± 2*</td>
<td>91 ± 3†</td>
<td>84 ± 2*</td>
</tr>
<tr>
<td>AoPmean, mmHg</td>
<td>76 ± 2</td>
<td>66 ± 2</td>
<td>71 ± 2</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>1,352 ± 52</td>
<td>1,409 ± 66</td>
<td>1,156 ± 61†</td>
<td>1,169 ± 58†</td>
</tr>
<tr>
<td>CAPmean, mmHg</td>
<td>121 ± 2</td>
<td>110 ± 4*</td>
<td>113 ± 2</td>
<td>102 ± 4*</td>
</tr>
<tr>
<td>CR, mmHg-min·ml⁻¹</td>
<td>3.08 ± 0.18</td>
<td>1.02 ± 0.13a</td>
<td>3.07 ± 0.31</td>
<td>0.83 ± 0.04a</td>
</tr>
<tr>
<td>AVI, mmHg-mm</td>
<td>294 ± 15</td>
<td>266 ± 18</td>
<td>105 ± 13†</td>
<td>127 ± 12†</td>
</tr>
<tr>
<td>AWT, %</td>
<td>37.1 ± 2.6</td>
<td>33.1 ± 2.6</td>
<td>12.5 ± 1.8†</td>
<td>15.5 ± 1.9†</td>
</tr>
<tr>
<td>AWTend, mm</td>
<td>12.9 ± 0.8</td>
<td>13.8 ± 0.7</td>
<td>13.1 ± 0.8</td>
<td>14.1 ± 0.7</td>
</tr>
<tr>
<td>PWT, %</td>
<td>20.0 ± 2.5</td>
<td>25.6 ± 2.3</td>
<td>19.1 ± 1.5</td>
<td>20.7 ± 1.9</td>
</tr>
<tr>
<td>MV̇O₂, µl·min⁻¹·g⁻¹</td>
<td>57 ± 3</td>
<td>67 ± 7</td>
<td>43 ± 3</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>AV̇O₂, ml/100 ml</td>
<td>7.8 ± 0.7</td>
<td>2.6 ± 0.2*</td>
<td>6.3 ± 0.9</td>
<td>2.1 ± 0.2*</td>
</tr>
<tr>
<td>TMF, ml·min⁻¹·g⁻¹</td>
<td>0.74 ± 0.07</td>
<td>2.56 ± 0.17*</td>
<td>0.73 ± 0.09</td>
<td>2.54 ± 0.15*</td>
</tr>
<tr>
<td>CD, µg</td>
<td>14.3 ± 0.9</td>
<td>4.3 ± 0.9</td>
<td>8.3 ± 1.9†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. PTHrP, parathyroid hormone (PTH)-related peptide; HR, heart rate; LVPP, left ventricular (LV) peak pressure; AoPmean, mean aortic pressure; dP/dtmax, maximum of the first derivative of LV pressure; CAPmean, mean coronary arterial pressure; CR, coronary resistance; AVI, anterior work index; AWT, anterior systolic wall thickening; AWTend, end-diastolic anterior wall thickness; PWT, posterior systolic wall thickening; MV̇O₂, myocardial oxygen consumption; AV̇O₂, arterial-coronary venous oxygen difference; TMF, transmural blood flow; CD, cumulative dose. Statistical analysis was by two-way ANOVA and least-significant difference post hoc test. *P < 0.05 vs. baseline; †P < 0.05 vs. normal myocardium.

Chemicals

PTHrP(1–34), PTH(1–34), D-Trp₁²-Tyr³⁴-PTH(7–34) and PTHrP(140–173) were obtained from Bachem (Heidelberg, Germany).

RESULTS

In Vivo Pig Studies

Systemic hemodynamics. Bolus injection of PTHrP(140–173) did not exert any effects. Intracoronary infusion of PTHrP(1–34) or PTH(1–34) at the highest concentration decreased LV peak pressure (Tables 1 and 2).

Coronary resistance and myocardial blood flow. In normal myocardium, intracoronary infusion of PTHrP(1–34) decreased coronary resistance to 33 ± 2% of baseline (P < 0.05; Fig. 1A). The cumulative dose to achieve maximum coronary dilation was 14.3 ± 0.9 µg. At this dose, PTHrP(1–34) increased myocardial blood flow to 360 ± 27% of baseline (P < 0.05). After termination of the PTHrP infusion, coronary resistance returned to baseline values within 16.7 ± 2.4 min.

In stunned myocardium, the cumulative dose decreasing coronary resistance to 29 ± 2% of baseline (P < 0.05; Fig. 1A) was significantly decreased (8.3 ± 1.9 µg, P < 0.05 vs. normal myocardium). This dose increased myocardial blood flow to 395 ± 48% of baseline (P < 0.05). After termination of the PTHrP infusion, coronary resistance returned to baseline values within 8.8 ± 1.6 min.

PTH(1–34) decreased coronary resistance to 44 ± 5% of baseline in normal and to 39 ± 3% of baseline in

Table 2. Systemic hemodynamics, regional myocardial function, blood flow, oxygen consumption, and coronary resistance in normal and stunned myocardium with and without exogenous PTH(1–34)

<table>
<thead>
<tr>
<th>Table 2. Systemic hemodynamics, regional myocardial function, blood flow, oxygen consumption, and coronary resistance in normal and stunned myocardium with and without exogenous PTH(1–34)</th>
<th>Normal Myocardium</th>
<th>PTH(1–34)</th>
<th>Stunned Myocardium</th>
<th>PTH(1–34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>103 ± 2</td>
<td>103 ± 2</td>
<td>103 ± 2</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>LVPP, mmHg</td>
<td>97 ± 2</td>
<td>89 ± 2*</td>
<td>91 ± 3†</td>
<td>84 ± 2*</td>
</tr>
<tr>
<td>AoPmean, mmHg</td>
<td>76 ± 2</td>
<td>67 ± 2*</td>
<td>71 ± 2</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>1,395 ± 57</td>
<td>1,434 ± 75</td>
<td>1,156 ± 61†</td>
<td>1,132 ± 50†</td>
</tr>
<tr>
<td>CAPmean, mmHg</td>
<td>118 ± 2</td>
<td>106 ± 5</td>
<td>111 ± 2</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>CR, mmHg-min·ml⁻¹</td>
<td>2.59 ± 0.15</td>
<td>1.15 ± 0.15*</td>
<td>2.95 ± 0.28</td>
<td>1.09 ± 0.13*</td>
</tr>
<tr>
<td>AVI, mmHg-mm</td>
<td>300 ± 18</td>
<td>264 ± 16</td>
<td>101 ± 12†</td>
<td>120 ± 14†</td>
</tr>
<tr>
<td>AWT, %</td>
<td>36.6 ± 2.8</td>
<td>33.2 ± 2.9</td>
<td>11.5 ± 1.7†</td>
<td>15.3 ± 2.4†</td>
</tr>
<tr>
<td>AWTend, mm</td>
<td>13.0 ± 0.7</td>
<td>13.7 ± 0.6</td>
<td>13.3 ± 0.8</td>
<td>14.2 ± 0.8</td>
</tr>
<tr>
<td>PWT, %</td>
<td>20.3 ± 2.3</td>
<td>24.3 ± 2.4</td>
<td>18.5 ± 1.4</td>
<td>20.7 ± 2.0</td>
</tr>
<tr>
<td>MV̇O₂, µl·min⁻¹·g⁻¹</td>
<td>51 ± 7</td>
<td>65 ± 8</td>
<td>41 ± 8</td>
<td>46 ± 8</td>
</tr>
<tr>
<td>AV̇O₂, ml/100 ml</td>
<td>7.0 ± 1.4</td>
<td>3.5 ± 0.7*</td>
<td>5.8 ± 1.2</td>
<td>2.4 ± 0.4*</td>
</tr>
<tr>
<td>TMF, ml·min⁻¹·g⁻¹</td>
<td>0.77 ± 0.08</td>
<td>2.04 ± 0.35*</td>
<td>0.73 ± 0.00</td>
<td>2.0 ± 0.3*</td>
</tr>
<tr>
<td>CD, µg</td>
<td>18.8 ± 10.3</td>
<td>12.5 ± 6.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical analysis was by ANOVA and least-significant difference post hoc test. *P < 0.05 vs. baseline; †P < 0.05 vs. normal myocardium.
stunned myocardium \((P < 0.05; \text{Fig. 1B})\), and it increased myocardial blood flow to 271 ± 30% and 274 ± 24% of baseline, respectively.

**Regional myocardial function and myocardial oxygen consumption.** PTHrP(1–34) tended to decrease the regional myocardial function of normal myocardium \([90 ± 3\%, \text{not significant (NS); Fig. 2A}]\). Myocardial oxygen consumption did not change significantly. In contrast, in stunned myocardium, PTHrP(1–34) significantly increased the regional myocardial function to 132 ± 9% of baseline \((P < 0.05; \text{Fig. 2A})\). Regional myocardial oxygen consumption also increased, but such an increase did not reach statistical significance. The increase in regional myocardial function during PTHrP(1–34) was inversely related \((y = -1.9701x + 200.66, r = 0.9)\) to postischemic function before PTHrP.

PTH(1–34) also tended to decrease the regional myocardial function under normal conditions \((89 ± 4\% \text{ of baseline value; Fig. 2B})\) but increased the regional myocardial function of stunned myocardium to 130 ± 14% of baseline \((P < 0.05; \text{Fig. 2B})\).

**Effect of PTHrP on Isolated Pig Ventricular Myocytes**

Baseline shortening of myocytes isolated from stunned myocardium was significantly reduced compared with that of myocytes isolated from normal myocardium \((8.6 ± 0.4\%, n = 8, \text{vs. } 6.0 ± 0.3\%, n = 21, P < 0.05)\). As in vivo, the change in the myocyte shortening fraction during exposure to PTHrP(1–34) was inversely related \((y = -5.5012x + 0.291, r = 0.7, n = 21)\) to the baseline shortening fraction.

**Blockade of Endogenous PTHrP in Isolated Rat Hearts**

With blockade of endogenous PTHrP, myocardial function of postischemic-reperfused rat hearts was significantly lower than in control hearts. After 30 min of reperfusion, LV developed pressure averaged 70 ± 5 mmHg in the control group versus 49 ± 7 mmHg in the treated group \((P < 0.05; \text{Fig. 3})\). Although a significant decrease in heart rate occurred in the early phase of reperfusion in the treated group \((213 ± 75 \text{ vs. } 143 ± 41 \text{ beats/min}, P < 0.05 \text{ at 4 min of reperfusion})\), there was no difference in heart rate between both groups from 5
DISCUSSION

Until 30 min of reperfusion (300 ± 65 beats/min in the control group vs. 270 ± 77 beats/min in the treated group at 30 min of reperfusion).

**Effects of PTHrP and PTH on Coronary Resistance**

PTHrP is constantly released from the coronary vascular bed of rats and pigs (5) and participates in the regulation of coronary blood flow because blockade of endogenous PTHrP increases coronary resistance in rats (5). The release of biologically active PTHrP is increased during hypoxic perfusion of isolated rat hearts (19). It is assumed that the vasodilator effects of PTHrP and PTH are mediated by one receptor, i.e., the PTH/PTHrP receptor, which is coupled to adenyl cyclase. Activation of this receptor increases cAMP levels in vascular smooth muscle cells; this effect is endothelium independent (15).

Exogenous PTHrP dose dependently increased the regional myocardial blood flow in rats (18). The novel finding of the present study, however, is that the dose of PTHrP(1–34) causing maximum vasodilation is significantly reduced. More importantly, exogenous and endogenous PTHrP(1–34) improve myocardial function in stunned myocardium without altering its oxygen consumption. PTH(1–34) exerts similar effects as PTHrP(1–34).

**Critique of Methods**

The strengths and limitations of the present model have been discussed in detail before (8).

In rat vascular smooth muscle cells, PTHrP and PTH bind to the same receptor. Specific receptor antagonists blocking the cardiovascular actions of PTHrP in the rat, such as D-Trp 12 Tyr 34-PTH(7–34) (5, 6) or Ile 5-, Trp 23-, Tyr 36- PTHrP(1–36), however, did not exert any inhibitory effects on flow and function in pigs, as measured in preliminary experiments (data not shown). Such species-specific differences between binding of NH2-terminal-truncated peptides have been described, although they did not occur for nontruncated peptides (9). Because data on the pig receptor system are lacking, the data suggest that the coronary and myocardial PTHrP/PTH receptor and/or the specific antagonists are species specific. The observed positive inotropic effects of PTHrP(1–34) or PTH(1–34) in the present study were not related to their positive chronotropic properties, as postulated by Ogino et al. (14), because heart rate was held constant by left atrial pacing. Although increases in myocardial blood flow have been proposed to increase regional myocardial function after repetitive periods of ischemia/reperfusion (26), it appears unlikely that the PTHrP-induced increase in the regional function of stunned myocardium in the present study was secondary to an increase in regional myocardial blood flow for two reasons. First, we have previously shown in the same experimental model of myocardial stunning, induced by a single period of ischemia, that coronary hyperperfusion per se does not alter regional myocardial function (22) and, second, PTHrP exerts a similar functional effect on cardiomyocytes isolated from stunned hearts.

**Effects of PTHrP and PTH on Regional Myocardial Function and Oxygen Consumption**

In isolated rat hearts both, PTHrP and PTH exert positive chronotropic and inotropic effects (13, 14), PTHrP being more potent. Whereas the positive chronotropic effect can be explained by increases in the pacemaker current and the slope of the pacemaker potential (7), explanations concerning the inotropic response of PTHrP and PTH are controversial but have been related to an increase in cellular cAMP levels (21).

In the present study, PTHrP and PTH increased regional myocardial function of stunned myocardium. The increase in regional myocardial function during PTHrP was inversely related to baseline function; i.e.,
a reduction in regional myocardial function to <50% of normal myocardium was required to achieve an increase in regional myocardial function during PTHrP. A similar phenomenon was also observed in isolated myocytes. Whereas PTHrP did not increase the contractile function in myocytes isolated from normal rabbit hearts (11) or pig hearts (present study), the shortening fraction in myocytes from stunned myocardium increased in those with more pronounced baseline dysfunction, pointing toward a direct action of PTHrP on cardiomycocytes.

There are reports of unique effects of other agents in stunned myocardium when given exogenously. In sheep, intravenous infusion of the phosphodiesterase inhibitor milrinone increased the preload recruitable stroke work in stunned myocardium without having effects on normal myocardium (4). Similarly, in dogs, the A2a receptor agonist CGS-21680, when given at 2 h of reperfusion after a 15-min coronary artery occlusion, increased preload recruitable stroke work without having a significant effect on contractile function before ischemia (10). Whereas milrinone increased regional myocardial function within minutes (a time course similar to that observed with PTHrP), the maximal increase in regional myocardial function during CGS-21680 was obtained after a 30-min infusion.

The mechanism involved in the increase in myocardial function during PTHrP infusion is unclear at present and deserves further study.

The release of biologically active PTHrP is increased during hypoxic perfusion of isolated rat hearts (19). Such increased release of PTHrP mediates functional effects in the reperfused “stunned” myocardium, because blockade of endogenous PTHrP significantly decreased LV developed pressure in postischemic-reperfused isolated rat hearts. Thus not only exogenous but also endogenous PTHrP appears to be of importance for the function of stunned myocardium.

In conclusion, the present study shows, for the first time, in vitro and in vivo, that PTHrP impacts on the function of stunned myocardium. Further studies will be necessary to elucidate which receptors and which intracellular pathways are involved in this effect.

Data have been presented in part at the American Heart Association Scientific Sessions in Anaheim, Germany, November 2001. This study was supported by the IFORES program of the Medical Faculty of the University of Essen (170 100-0). This paper represents the M.D. thesis of J. Jansen.

REFERENCES


