The Hemodynamic Basis for the Cardiac Effects of Parathyroid Hormone (PTH) and PTH-Related Protein*

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ABSTRACT

PTH and PTH-related protein (PTHrP) have been regarded to have positive inotropic effects on the heart as well as positive chronotropic and vasodilator effects. However, inotropy due to a direct effect of these peptides has not heretofore been distinguished from an indirect inotropic effect as a result of altered heart rate or coronary flow. The aim of this study was to determine whether PTH and PTHrP have direct inotropic effects in isolated perfused rat hearts. Three groups of hearts were studied; in all groups, hearts contracted isovolumically and were perfused with a constant coronary pressure. In the control group, heart rate, coronary flow, peak pressure (LVP), peak rate of rise of LV pressure (dP/dt), and peak intracellular calcium (measured by aequorin) all increased with PTH and PTHrP in a dose-dependent manner. When heart rate was fixed by pacing in a second group of rats, PTH and PTHrP increased coronary flow, LVP, and dP/dt significantly, indicating that inotropic actions were not mediated solely by chronotropic effects. However, when heart rate was fixed by pacing and, additionally, coronary flow was held constant (by maximal vasodilation with nitroprusside) in a third group of rats, there was no significant effect of either PTH or PTHrP on LVP, dP/dt, or peak intracellular calcium. To demonstrate the responsiveness of this latter preparation to inotropic stimulation, the β-adrenergic agonist, isoproterenol, increased LVP, dP/dt, and peak calcium even when heart rate was fixed and vasodilation was maximal. Thus, PTH and PTHrP are inotropic agents by virtue of their influence on coronary flow and heart rate, but not by any direct effect on contractile elements in the heart.

PTH-RELATED protein (PTHrP) was discovered in a search for a circulating tumor-derived factor responsible for the hypercalcemia of malignancy (1-5). In terms of its hypercalcemic properties, PTHrP bears many similarities to PTH, the normal secretory product of the parathyroid cell. In contrast to PTH, PTHrP is not normally found in the circulation, but, rather, is expressed in a wide variety of different cells, an observation that has given rise to the concept of PTHrP as an autocrine or paracrine factor. Smooth muscle, a particularly rich source of PTHrP, responds to mechanical stress with a rapid increase in messenger RNA for the protein (6-12). By regulating smooth muscle tone, PTHrP may be an important local factor produced in response to biomechanical forces. The smooth muscle of the cardiovascular system is particularly responsive to PTHrP, as shown by enhanced expression of PTHrP messenger RNA when smooth muscle of rat aorta is distended (12).

Despite major differences between PTH and PTHrP in their genetics, regulation, and many physiological actions, PTH and PTHrP are both vasodilators and directly increase heart rate independent of autonomic reflexes (13-18). It has been reported that PTH stimulates G protein action and increases intracellular calcium in cardiac myocytes (19).

Materials and Methods

Surgical preparation

Experiments were performed on hearts of 43 male Sprague-Dawley rats, weighing 400-500 g. The procedures used to isolate and perfuse the rat heart were similar to those described previously (20). Briefly, the rat was given heparin (1000 U, ip) and then heavily anesthetized with ketamine HCl (40 mg, ip) and xylazine (2.0 mg, ip). After bilateral sternotomy, the heart was rapidly excised, and the aorta was cannulated for retrograde perfusion with a 16-gauge needle connected to a modified 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.
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The objective of these experiments was to assess the inotropic actions of PTH-(1-34) and PTHrP-(1-34) independent of their vasodilatory and chronotropic actions. To accomplish this, hearts were divided into three groups and further subdivided into two treatment subgroups according to whether they received PTH-(1-34) or PTHrP-(1-34). Five hearts were studied in each subgroup. Group 1 hearts received PTH-(1-34) or PTHrP-(1-34), and their impacts on heart rate, coronary flow (with constant coronary perfusion pressure of 80 mm Hg), LVP_{max} and dP/dt_{max} were recorded 1, 3, 5, 10, 15, 20, 25, and 30 min after each injection. This protocol was similar to those used in the past (15), and the results served as a point of comparison to previous studies. To eliminate the impact of varying heart rate on contractile state, hearts in group 2 were paced at a constant rate (230/min) before and after the administration of PTH-(1-34) or PTHrP-(1-34); this heart rate was greater than the maximal heart rate achieved in hearts of group 1 in response to the maximal doses of PTH-(1-34) and PTHrP-(1-34). Measurements of coronary flow (with constant coronary perfusion pressure of 80 mm Hg), LVP_{max} and dP/dt_{max} were made at the same time intervals. Finally, to eliminate the effects of both changing coronary vascular resistance and heart rate, group 3 hearts were paced (as in group 2), and the coronary bed was maximally vasodilated by the addition of sodium nitroprusside (0.1 mM) to the perfusate. Changes in all parameters are expressed as percent changes from baseline values before PTH-(1-34) and PTHrP-(1-34) infusion.

We measured aequorin luminescence to test the effects of PTH-(1-34) and PTHrP-(1-34) (3.0 μg: 740 pmol) on the intracellular calcium transients using the same protocols as those in groups 1 and 3. In addition, each of these hearts received acetic acid infusion (1 mM; placebo control) and isoproterenol infusion (15 and 150 pmol; positive control). Five hearts were studied in each of the two groups, with each heart receiving all drugs (i.e. PTH-(1-34), PTHrP-(1-34), acetic acid, and isoproterenol), which were infused in random order.

**Statistical analysis**

All data are expressed as the mean ± SEM. One-way analysis of variance with Fisher’s exact test was used to analyze differences between the values before and after each injection. The Wilcoxon single rank test was used for analysis of aequorin experiments. *P < 0.05* was considered statistically significant.

**Results**

Table 1 summarizes heart weight and the baseline values of heart rate, coronary flow, LVP_{max}, and dP/dt_{max} in the three groups of hearts. In this analysis, data from the PTH-(1-34) and PTHrP-(1-34) subgroups are pooled, as further analysis revealed no significant difference between subgroups. As shown, there were statistically significant differences among the groups in some of these parameters; the magnitude of these differences, however, was very small and would not be expected to contribute to the differences in response to PTH-(1-34) or PTHrP-(1-34) infusions, which will be discussed below.

**Group 1 hearts**

The impacts of varying doses of PTH-(1-34) and PTHrP-(1-34) on heart rate, coronary flow, LVP_{max} and dP/dt_{max} are shown in Fig. 1, with coronary perfusion pressure fixed at 80 mm Hg. Heart rate and coronary flow increased in a dose-dependent manner. At the highest dose, heart rate increased an average of 47.3% with PTH-(1-34) and 55.6% with PTHrP-(1-34).

**Table 1. Baseline values of heart weight, heart rate, coronary flow, LVP_{max}, and dP/dt_{max}**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 10)</th>
<th>Group 2 (n = 10)</th>
<th>Group 3 (n = 10)</th>
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<tbody>
<tr>
<td>Heart wt (g)</td>
<td>1.27 ± 0.06</td>
<td>1.32 ± 0.05</td>
<td>1.28 ± 0.08</td>
</tr>
<tr>
<td>Heart rate</td>
<td>118.3 ± 3.3</td>
<td>Controlled</td>
<td>Controlled</td>
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<td></td>
<td></td>
<td>12.66 ± 0.36&quot;</td>
<td>Controlled</td>
</tr>
<tr>
<td>Coronary flow</td>
<td>116.8 ± 2.7</td>
<td>106.0 ± 1.9&quot;</td>
<td>114.8 ± 2.4&quot;</td>
</tr>
<tr>
<td>(ml/min)</td>
<td>(mm Hg)</td>
<td>(mm Hg)</td>
<td>(mm Hg)</td>
</tr>
<tr>
<td>LVP_{max}</td>
<td>2044 ± 34</td>
<td>2183 ± 49</td>
<td>2389 ± 82&quot;</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>(mm Hg)</td>
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* P < 0.05 vs. group 1.  
* P < 0.05 vs. group 2.
PTHrP-(1-34), whereas corresponding coronary flows increased by 64.6% and 54.1%, respectively. LVP\textsubscript{max} and dP/dt\textsubscript{max} also increased significantly: at the highest doses, LVP\textsubscript{max} increased 14.8% with PTH-(1-34) and 9.8% with PTHrP-(1-34), with corresponding increases in dP/dt\textsubscript{max} of 29.2% and 25.0%, respectively. These values did not change significantly when the vehicle, acetic acid, was perfused. The magnitude of the changes in these hemodynamic parameters was similar for PTH-(1-34) and PTHrP-(1-34). The onset of action induced by either agent was rapid, reaching a maximum between 3-5 min after injection. Measured parameters returned to their baseline values by about 30 min after injection. These effects are similar to those reported previously in isolated rat hearts (15) and indicate an increase in contractile state after PTH-(1-34) and PTHrP-(1-34) infusion. This particular protocol, however, does not elucidate the mechanism of inotropism, as the independent effects of heart rate and coronary flow are not accounted for.

**Constant heart rate protocol (group 2)**

The impacts of PTH-(1-34) and PTHrP-(1-34) on coronary flow, LVP\textsubscript{max}, and dP/dt\textsubscript{max} when heart rate was held constant (230/min) are shown in Fig. 2. Coronary flow increased significantly and in a dose-dependent manner by amounts similar to those in group 1 hearts. LVP\textsubscript{max} and dP/dt\textsubscript{max} also increased significantly. Changes in LVP\textsubscript{max} were somewhat greater than those in group 1, whereas changes in dP/dt\textsubscript{max} were slightly less than those in group 1 (discussed below). Responses to PTH-(1-34) were greater than those to PTHrP-(1-34). Thus, even with heart rate fixed, the contractile state increased in response to PTH-(1-34) and PTHrP-(1-34). Baseline values of coronary flow, LVP\textsubscript{max}, and dP/dt\textsubscript{max} are shown in Table 1.
Constant heart rate and constant coronary vascular resistance protocol (group 3)

In group 3 hearts, heart rate was fixed at 230/min (similar to group 2), but the coronary bed was now maximally dilated with nitroprusside. Accordingly, there was no impact of either PTH-(1-34) or PTHrP-(1-34) on coronary perfusion pressure (Fig. 3) at a constant coronary flow [PTH-(1-34) group, 19.3 ± 0.7 ml/min; PTHrP-(1-34) group, 18.5 ± 1.5 ml/min], which confirms a state of maximal coronary vasodilation. In this case, there was no significant effect of either PTH-(1-34) or PTHrP-(1-34) on coronary perfusion pressure (Fig. 3) at a constant coronary flow [PTH-(1-34) group, 19.3 ± 0.7 ml/min; PTHrP-(1-34) group, 18.5 ± 1.5 ml/min], which confirms a state of maximal coronary vasodilation. In this case, there was no apparent inotropic action of either PTH-(1-34) or PTHrP-(1-34).

Isoproterenol infusion

To demonstrate that the isolated rat heart paced at 230/min and vasodilated with nitroprusside is still capable of mounting an inotropic response, we evaluated the effects of bolus injections of isoproterenol (15 and 150 pmol; n = 3). The results indicate that even when heart rate is fixed and coronary vasodilation is maximal, there is a significant inotropic response to isoproterenol, as indicated by increases in both $LVP_{max}$ and $dP/dt_{max}$ (Fig. 4).

Calcium measurements

The average effects of vehicle (acetic acid), isoproterenol, PTH-(1-34) and PTHrP-(1-34) on heart rate, $LVP_{max}$ and aequorin light for all hearts studied are summarized in Table 2 (recall that coronary perfusion pressure is fixed at 80 mm Hg in all hearts). Vehicle infusion did not affect any parameter in either of the two groups. $LVP_{max}$, heart rate, and the amplitude of the aequorin signal increased in group 1 hearts, and all changes were physiologically and statistically significant ($P < 0.05$, by Wilcoxon single rank test). Furthermore, the changes in each of the parameters due to PTH-(1-34) and PTHrP-(1-34) infusions were comparable to those observed with low dose isoproterenol. Figure 5, A and B, show representative tracings of aequorin luminescence and LVP from a group 3 heart (constant heart rate and constant coronary resistance) before and during PTH-(1-34) infusion. These tracings reveal the constancy of peak LVP and peak aequorin luminescence (after signal averaging of eight beats). On the average, neither $LVP_{max}$ nor aequorin signal amplitude changed in group 3 hearts with either PTH-(1-34) or PTHrP-(1-34) (Table 2). In contrast, isoproterenol infusion increased both $LVP_{max}$ and peak aequorin luminescence in group 3 hearts (Table 2 and Fig. 5, C and D), indicating that this experimental model retains its ability to respond to inotropic stimulation even when heart rate is fixed and coronary resistance is controlled.

Discussion

It is well documented that PTH and PTHrP have several effects on the cardiovascular system (13-18, 22, 23). These peptides dilate the vasculature and increase the heart rate independent of autonomic reflexes. Both of these effects were observed in the present study (group 1). Although original studies from one group of investigators showed no inotropic effect in studies of ventricular (24) and atrial tissue (14), the results of several more recent studies have been interpreted as indicating that both PTH and PTHrP have direct positive inotropic effects (15,16,22). The conditions under which such positive inotropy was observed previously were seen in our group 1 hearts; these peptides were administered to isolated rat hearts with a constant perfusion pressure while coronary flow and heart rate were allowed to vary. Under these conditions, two indices of contractile state ($LVP_{max}$ and $dP/dt_{max}$) increased in a dose-dependent manner after PTH-(1-34) or PTHrP-(1-34) administration. However, several factors complicate the interpretation of these data. First, it is well known that both heart rate and coronary flow exert independent influences on the contractile state of the ventricle. Increases in heart rate can either increase or decrease contractile strength depending on the range over which heart rate is varied, the species studied, and the nature of the experimental model (25, 26). Mechanisms contributing to the dependence of contractile
FIG. 4. Effects of isoproterenol on LVP\textsubscript{max} and dP/dt\textsubscript{max}, with constant heart rate (230/min) and constant coronary flow, with coronary bed dilated with nitroprusside (and, thus, constant coronary perfusion pressure; n = 3). Isoproterenol increased LVP\textsubscript{max} and dP/dt\textsubscript{max} in a dose-dependent manner, indicating that even with constant heart rate and coronary vasodilation, this experimental model is capable of mounting an appropriate response to a positive inotropic agent. All parameters are expressed as percent changes from baseline after peptide infusion.

TABLE 2. Effects of PTH, PTHrP, and isoproterenol on heart rate, LVP\textsubscript{max}, and aequorin luminescence

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (acetic acid)</th>
<th>PTH (3.0 µg)</th>
<th>PTHrP (3.0 µg)</th>
<th>Isoproterenol (15 pmol)</th>
<th>Isoproterenol (150 pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (n = 5)</strong></td>
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<tr>
<td>Heart rate (%)</td>
<td>0.4 ± 0.8</td>
<td>41.8 ± 11.4*</td>
<td>42.0 ± 9.8*</td>
<td>32.7 ± 11.0*</td>
<td>63.3 ± 12.3*</td>
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<tr>
<td>LVP\textsubscript{max} (%)</td>
<td>-1.6 ± 0.9</td>
<td>6.3 ± 1.4*</td>
<td>8.5 ± 1.7*</td>
<td>5.0 ± 1.0*</td>
<td>13.3 ± 1.3*</td>
</tr>
<tr>
<td>Light (%)</td>
<td>-4.0 ± 2.1</td>
<td>21.8 ± 7.5*</td>
<td>18.1 ± 4.7*</td>
<td>31.4 ± 13.1*</td>
<td>126.6 ± 18.7*</td>
</tr>
<tr>
<td><strong>Group 3 (n = 5)</strong></td>
<td></td>
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<tr>
<td>LVP\textsubscript{max} (%)</td>
<td>0.2 ± 0.2</td>
<td>2.8 ± 1.2</td>
<td>1.1 ± 0.9</td>
<td>8.8 ± 2.5*</td>
<td>39.3 ± 9.0*</td>
</tr>
<tr>
<td>Light (%)</td>
<td>-4.4 ± 4.5</td>
<td>-3.1 ± 2.5*</td>
<td>1.7 ± 2.7</td>
<td>19.8 ± 10.3*</td>
<td>205.4 ± 25.7*</td>
</tr>
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</table>

Values are the percent change in response to infusions from the immediately preceding preinjection values.

* P < 0.05 vs. preinjection values, by Wilcoxon single rank test.

A further complication in the interpretation of results from previous studies is that studies of whole hearts employed dP/dt\textsubscript{max} as the sole index of ventricular contractility. However, different indexes of contractile state will vary in their responses to inotropic agents depending on whether heart rate is fixed or allowed to vary. As shown in Fig. 1, the percent changes in LVP\textsubscript{max} and dP/dt\textsubscript{max} in response to PTH-(1-34) administration are different, the same is true for PTHrP-(1-34) administration. This is because when heart rate increases, there is a decrease in the duration of contraction due to alterations in calcium cycling during the beat. As a result, there will necessarily be an increase in dP/dt\textsubscript{max} even if the peak pressure does not change.

The main purpose of this study was to determine whether PTH-(1-34) and/or PTHrP-(1-34) have direct inotropic actions on the heart. When indirect effects of PTH-(1-34) and PTHrP-(1-34) on inotropy (heart rate and coronary flow) were controlled for, neither peptide could be shown to have direct positive inotropic effects in isolated rat hearts (group 3 hearts). Thus, the increases in LVP\textsubscript{max} and dP/dt\textsubscript{max} seen in our investigation and in previous studies are most likely due to secondary increases in coronary flow and heart rate.

To eliminate the possibility that pacing and vasodilation with nitroprusside prevent the heart from mounting an inotropic response, we confirmed that under such conditions, isoproterenol, a known positive inotrope, was active.

It was previously reported that PTH increases resting intracellular Ca\textsuperscript{2+} in isolated quiescent myocytes (19), thus suggesting a mechanism by which PTH could directly increase the contractile state. In the present study, LVP\textsubscript{max} and aequorin luminescence increased with PTH-(1-34) and PTHrP-(1-34) in group 1 hearts. As we have shown, these
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Effects are related to the inotropic actions of both heart rate and increased coronary flow. Although the fundamental mechanisms of action of these two factors are not fully understood, they are both known to increase contractile performance by raising the peak intracellular calcium concentration (27, 28) and, thus, underlying the observations in group 1 hearts. On the other hand, there were no effects of either PTH-(1-34) or PTHrP-(1-34) on aequorin luminescence in group 3 hearts, which correlated with a lack of effect on contractility. Furthermore, the concentration range of PTH-(1-34) and PTHrP-(1-34) used in the present study (5-50 nM) is well below the range over which these agents have been shown to affect intracellular calcium directly in isolated myocytes (100-1000 nM) (19).

The molecular mechanisms underlying the cardiovascular actions of PTH-(1-34) and PTHrP-(1-34) were not investigated in the present study. Several classes of compounds are known to act as vasodilators and chronotropes, but have no effect on the ventricular contractile state. For example, β2-adrenergic receptors can mediate vasodilatory and chronotropic effects without significant inotropy (29, 30). PTH is known to increase intracellular cAMP and calcium levels (13, 31, 32); it also activates L-type calcium channels via stimulation of G proteins (19), although this latter effect is observed at high concentrations of PTH. It is not clear whether these cellular effects of PTH are related to its cardiovascular properties. On the other hand, pacemaker activity is generated via high concentrations of PTH. It is not clear whether these cellular effects of PTH are related to its cardiovascular properties. For example, β2-adrenergic agonists can also account for the actions of PTH and PTHrP. Further studies will be needed to clarify the precise mechanisms by which PTH and PTHrP influence vascular and chronotropic properties in the heart.

The 1-34 peptides of PTH and PTHrP selected for this study are those classically associated with their biological actions on bone. Similar to bone, the cardiovascular actions of the 1-34 peptides of PTH and PTHrP also show great potency, with major effects on the heart at the lower end of the dose-response range for both peptides. These low concentrations are certainly within the range expected for a physiological action of a circulating (PTH) or locally active (PTHrP) peptide. Thus, the effects of PTH-(1-34) and PTHrP-(1-34) on heart rate and coronary flow are of potential physiological importance.

The lack of a direct inotropic effect of PTH-(1-34) or PTHrP-(1-34) on the heart at the low concentrations employed in this study does not exclude the possibility that other fragments of these compounds are directly inotropic. PTH and PTHrP contain within their linear sequences sites for peptide cleavage that lead to fragments with unusual biological properties (34). In one example, a fragment in the carboxy-terminal region of PTHrP has been reported to have an effect (inhibition of bone resorption) opposite to that of an amino-terminal fragment (35, 36). Local peptidic cleavage of PTHrP to release active and counteractive fragments on the cardiac cell or its vasculature are areas for future investigation.

Recent studies have demonstrated that although PTHrP is present throughout the cardiovascular system, the amount of PTHrP in the ventricle is much less than that in the atria and aorta (11, 37). This may suggest that the physiologically important functions of PTHrP are chronotropy and vascular dilation rather than inotropy. The results of the present study support this concept.

Acknowledgment

We thank Dr. Michael Rosen for helpful discussions and advice.

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