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A N-terminal PTHrP peptide fragment void of a PTH/PTHrP-receptor binding domain activates cardiac ET_A receptors

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1 Adult ventricular cardiomyocytes show an unusual structure-function relationship for cyclic AMP-dependent effects of PTHrP. We investigated whether PTHrP(1–16), void of biological activity on classical PTHrP target cells, is able to mimic the positive contractile effect of PTHrP(1–34), a fully biological agonist on cardiomyocytes.

2 Adult ventricular cardiomyocytes were paced at a constant frequency of 0.5 Hz and cell contraction was monitored using a cell-edge-detection system. Twitch amplitudes, expressed as per cent cell shortening of the diastolic cell length, and rate constants for maximal contraction and relaxation velocity were analysed.

3 PTHrP(1–16) (1 μmol l^{-1}) mimicked the contractile effects of PTHrP(1–34) (1 μmol l^{-1}). It increased the twitch amplitude from 5.33 ± 0.72 to 8.95 ± 1.10 (% dl l^{-1}) without changing the kinetic of contraction.

4 PTH(1–34) (10 μmol l^{-1}) affected the positive contractile effect of PTHrP(1–34), but not that of PTHrP(1–16).

5 RpcAMPS (10 μmol l^{-1}) inhibited the positive contractile effect of PTHrP(1–34), but not that of PTHrP(1–16).

6 The positive contractile effect of PTHrP(1–16) was antagonized by the ET_A receptor antagonist BQ123.

7 Sarafotoxin 6b and PTHrP(1–16), but not PTHrP(1–34), replaced ^{3}H-BQ123 from cardiac binding sites.

8 We conclude that N-terminal PTHrP peptides void of a PTH/PTHrP-receptor binding domain are able to bind to, and activate cardiac ET_A receptors.
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Abbreviations: cyclic AMP, Adenosine 3′,5′-cyclic monophosphate; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; ET_A, endothelin A receptor

Introduction

Parathyroid hormone-related peptide (PTHrP) is a myocardially expressed peptide hormone that activates adenylate cyclase-dependent processes in ventricular cardiomyocytes (Bui *et al*., 1993; Schlüter *et al*., 1997). In many classical parathyroid hormone (PTH) target cells, both, PTHrP and PTH activate adenylate cyclase in a comparable way (reviewed in Schlüter, 1999). Ventricular cardiomyocytes from rat, although PTH and PTHrP targets, represent an exception to this rule (Schlüter *et al*., 1997). PTHrP, but not PTH, stimulates adenylate cyclase activity on these cells. Such a functional difference between PTHrP and PTH can also be found in whole heart preparations (Nickols *et al*., 1989). At present, no data are available on other species concerning the difference between PTHrP and PTH on the ventricular myocardium. Since the common adenylate cyclase activating domain of PTH and PTHrP is located on the first six amino acids, which are in fact similar in both peptide hormones, it is unlikely that this classical adenylate cyclase activating domain is involved in the described effects on cardiomyocytes. Indeed, it was found that deletion of the first six amino acids of PTHrP does not destroy its adenylate cyclase stimulating activity on ventricular cardiomyocytes (Schlüter *et al*., 1997). This indicates that its effect on ventricular cardiomyocytes involves another, not yet characterized adenylate cyclase activating domain on the PTHrP molecule.

On ventricular cardiomyocytes, PTHrP(1–34) is a fully active peptide fragment, simulating the effect of PTHrP on adenylate cyclase and cell contraction. This peptide is known to contain, at positions 18–32, a binding domain for the PTH/PTHrP-receptor found on classical target cells. The further truncated fragment PTHrP(1–16) has been used in negative control experiments aiming at this PTH/PTHrP-receptor, since PTHrP(1–16) does neither bind to, nor stimulate this receptor (Kemp *et al*., 1987). On non-classical target cells, however, PTHrP(1–16) has been found to antagonize the adenylate cyclase stimulating effect of PTHrP (Musso *et al*., 1989). This indicates that such a truncated PTHrP peptide can exert specific effects on non-classical target cells. *In vivo*, truncated peptides similar to

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PTHrP(1–16) can be generated since there is a proteolytic cleavage site between amino acids 21 and 22 in the PTHrP molecule (Diefenbach-Jagger *et al*., 1995). The protease kexin preferentially cleaves PTHrP(1–141) at this cleavage site, although the existence of seven other proteolytic cleavage sites.

We analysed if PTHrP(1–16) exerts specific effects on the contractile response of electrically paced cardiomyocytes isolated from ventricles of adult rats. We found a concentration-dependent positive contractile effect. This effect was compared with the well described effects of PTHrP on cardiomyocytes. We found that the positive contractile effect caused by PTHrP(1–34) is cyclic AMP-dependent, but PTHrP(1–16) is not, which argues against binding to, and stimulation of cardiac PTHrP receptors by PTHrP(1–16). We therefore investigated, whether PTHrP(1–16) acts through cardiac receptors which are linked to positive contractile effects independently of cyclic AMP. Based on some structural homologies between PTHrP(1–16) and endothelin we hypothesized that PTHrP(1–16) acts through cardiac endothelin receptors. Endothelin and sarafotoxin 6b are both ET_A receptor agonists and share a common pattern between amino acids 6 and 9. A hydrophobic amino acid is followed by a variable one, an acidic amino acid and a basic amino acid. Sarafotoxin 6c, an ET_B receptor agonist, has a slightly different composition: a hydrophobic amino acid is followed by a variable and two acidic amino acids. Between amino acids 8 and 11, PTHrP resembles endothelin and sarafotoxin 6b. In fact, it shares the common sequence (Leu-X-Asp-Lys) with endothelin (Figure 1). We provide evidence that the contractile effects described for PTHrP(1–16) are, indeed, mediated through the binding to, and activation of the endothelin A (ET_A) receptor subtype.

Methods

Chemicals and supplies

hPTHrP(1–34), hPTHrP(1–16), sarafotoxin 6b, and BQ123 were obtained from Bachem (Heidelberg, Germany). ^{3}H-BQ123 was obtained from Amersham (Braunschweig, Germany). Tissue culture materials were obtained from Becton-Dickinson (Heidelberg, Germany), and glutamine free medium 199 and foetal calf serum from Boehringer Mannheim (Gemany). All other chemicals were of analytical grade.

ET-1 - Leu - Met - Asp - Lys -

PTHrP - Leu - His - Asp - Lys -

Sftx6b - Met - Thr - Asp - Lys -

Sftx 6c - Met - Thr - Asp - Glu -

Figure 1 Sequence homology between endothelin, sarafotoxin 6b (sftx 6b), PTHrP, and sarafotoxin 6c (sftx 6c). The figure shows the amino acids 6–9 for endothelin and the sarafotoxins and 8–11 for PTHrP.

Cell isolation

Male Wistar rats (weighing approximately 250–300 g) were used to isolate cardiomyocytes. The cells were isolated as described before (Piper *et al*., 1982). The remaining calcium tolerant rod shaped cells were attached on culture dishes using an attachment protocol with 4% foetal calf serum as described earlier. The cells were used 4 h after isolation. The final cell population consisted of 85% rod shaped and quiescent cells.

Analytical procedures

Cell contraction On isolated cells paced at a constant frequency of 0.5 Hz, cell contraction of adult ventricular cardiomyocytes was investigated as described before (Schlüter *et al*., 1997). Cell shortening was monitored using a cell-edge-detection system from which the following parameters were calculated using the software MUCEL (Heidelberg, Germany): Cell shortening is expressed as percentage of systolic cell length relative to diastolic cell length. The kinetic of contraction is expressed as rate constants for maximal contraction and relaxation velocity. It was calculated by normalizing maximal contraction and relaxation velocities to peak contraction. In each case cells were constantly paced and five contractions were recorded every 15 s. The average of these recordings was used as one data point. The measurements on individual cells were repeated four times and the average of these four measurements was used as the average contractile performance of individual cells. From each preparation, three cells were analysed for each condition and the same experiments were repeated with three other preparations.

Binding assay The binding of PTHrP(1–16), PTHrP(1–34), and sarafotoxin 6b was determined on cultured cells. Cells were incubated with the indicated concentration of agonists, and 1500 c.p.m. ml^{-1} ^{3}H-BQ123 was added to the cultures. The cultures were treated with the indicated agonists at room temperature for 2 h, washed thereafter with ice-cold PBS and the ratio between free ^{3}H-BQ123 and bound ^{3}H-BQ123 was determined in a radioactive-counter.

Statistics

All data are expressed as mean ± s.e.mean. Experiments in which more than two groups were compared, ANOVA and the Student-Newman-Keuls test for *post hoc* analysis was used. Cases in which two groups were compared, conventional *t*-tests were performed. $P<0.05$ was used as the level of significance.

Results

The positive contractile effect of PTHrP(1–16)

It was investigated whether PTHrP(1–34), PTHrP(1–16), and sarafotoxin 6b influences the contractile response of electrically driven ventricular cardiomyocytes. The cells were paced at a constant frequency of 0.5 Hz in absence or presence of agonists. It was found that all three agonists exert

a positive contractile effect. Figure 2 shows a representative single cell recording of contracting cardiomyocytes paced under control conditions without further addition or after addition of agonists. PTHrP(1–34) (1 μmol l^{-1}) increased the contraction amplitude, time to peak contraction, time to 90% relaxation, and increased rate constants for maximal contraction and relaxation velocity (Table 1). This positive contractile effect of PTHrP(1–34) was compared to PTHrP(1–16). When applied at 1 μmol l^{-1}, PTHrP(1–16) caused a comparable increase in cell shortening, and a significant increase in time to peak, but not in rate constants (Table 1). Under the apparent conditions, PTHrP(1–34) caused 50% of its maximal effect within 11.8 s and PTHrP(1–16) within 19.8 s. The positive contractile effect of PTHrP(1–16) was further compared to selective ET_A receptor stimulation caused by sarafotoxin 6b (10 μmol l^{-1}). Again, an increase in contraction amplitude was found without changes in rate constants (Table 1). In summary, the efficacy of PTHrP(1–34), PTHrP(1–16), or sarafotoxin 6b to increase contraction amplitudes was comparable. PTHrP(1–34), but not PTHrP(1–16) or sarafotoxin 6b, changed the kinetics of contraction. The potency of PTHrP(1–34) to increase contraction amplitudes was slightly stronger than that of PTHrP(1–16) (Figure 3). The EC_{50} values for PTHrP(1–34) was calculated at 31 nmol l^{-1} and at 64 nmol l^{-1} for PTHrP(1–16).

Effect of receptor antagonists on the positive contractile effects of PTHrP(1–16)

On cardiomyocytes, PTH(1–34) is known to antagonize the positive contractile effect of PTHrP (Schlüter *et al*., 1997). It was investigated whether a tenfold addition of excess PTH(1–34) antagonizes the positive contractile effects of PTHrP(1–16). PTH(1–34) did not change the contraction amplitude of cardiomyocytes but, as expected, antagonized the positive contractile effect of PTHrP(1–34). The positive contractile effect of PTHrP(1–16) was not influenced by the co-presence of PTH(1–34) (Table 2). This result argues against the possibility that PTHrP(1–16) exerts its effect *via* binding to, and activation of cardiac PTHrP receptors. Accordingly, Rp-cyclic AMPS (10 μmol l^{-1}) attenuated the positive contractile effect of PTHrP(1–34), indicating that the positive contractile effect of PTHrP(1–34) is mediated in a cyclic AMP-dependent way, but not that of PTHrP(1–16) (Table 3).

Due to the structural homologies between PTHrP(1–16) and endothelin, we further investigated the influence of ET_A inhibition on the positive contractile effect of PTHrP(1–16). BQ123 (1 μmol l^{-1}) was used as an ET_A receptor antagonist. It did not influence the contraction, but antagonized sarafotoxin 6b, an ET_A receptor agonist. BQ123 also antagonized the positive contractile effect of PTHrP(1–16), but not of PTHrP(1–34) (Table 4). It is known that endothelin *via* binding to ET receptors, can exert a positive contractile response that persists after washout of the agonist. In contrast, stimulation of β-adrenoceptors leads to a cyclic AMP-dependent positive contractile effect that is reversed upon washout of the drug. Indeed, we found, that the

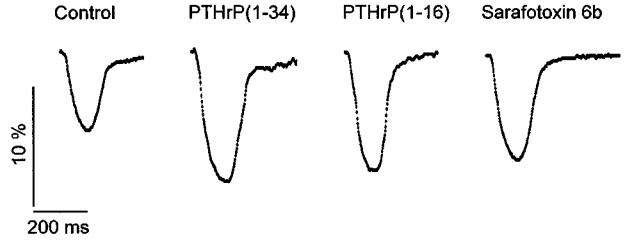

Figure 2 Representative tracings of single cell contraction curves. Cardiomyocytes were paced at a constant frequency of 0.5 Hz under basal conditions (control), under addition of PTHrP(1–34) (1 μmol l^{-1}), PTHrP(1–16) (1 μmol l^{-1}) or sarafotoxin 6b (10 μmol l^{-1}). The horizontal bar indicates the time and the vertical bar indicates per cent cell contraction normalized to the resting cell length.

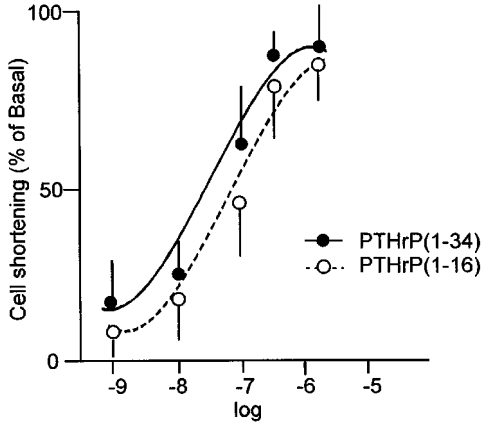

Figure 3 Concentration response curve for the positive contractile effects of PTHrP(1–34) and PTHrP(1–16). The change in contraction amplitude (normalized to diastolic cell length) is plotted as per cent of basal against the concentrations. Data represent mean ± s.e.mean from $n=12$ cells.

Table 1 Comparison of the positive contractile effects of PTHrP(1–34), PTHrP(1–16), and sarafotoxin 6b

	n	*Cell length* (μm)	*Cell shortening* (μm)	*dl l^{-1}* (%)	*TTP* (ms)	*Con_{max}* (s^{-1})	*R90* (ms)	*Rel_{max}* (s^{-1})
Control	36	106.74 ± 26.69	5.69 ± 0.77	5.33 ± 0.72	162 ± 6	25.13 ± 2.64	203 ± 7	20.74 ± 2.28
PTHrP(1–34)	12	102.45 ± 35.41	8.25 ± 1.19*	8.05 ± 1.16*	116 ± 9*	32.97 ± 5.70*	154 ± 23*	26.91 ± 2.91*
PTHrP(1–16)	12	114.41 ± 26.31	10.24 ± 1.26*	8.95 ± 1.10*	137 ± 5	22.46 ± 2.83	192 ± 2	19.04 ± 0.39
Sarafotoxin 6b	12	110.74 ± 22.15	9.06 ± 2.43*	8.18 ± 2.19*	152 ± 7	24.83 ± 1.55	186 ± 11	21.41 ± 1.55

Isolated cardiomyocytes were paced at a constant frequency of 0.5 Hz without further additions (control), or in the presence of PTHrP(1–34) (1 μmol l^{-1}), PTHrP(1–16) (1 μmol l^{-1}), or sarafotoxin 6b (10 μmol l^{-1}). Cell length is given in μm, cell shortening is expressed as maximal cell shortening in absolute values (μm) and per cent of the diastolic cell length (dl l^{-1}). Time to peak (TTP) and time to reach 90% relaxation (R90) are given in ms. The kinetics of contraction (Con_{max} and Rel_{max} are given in rate constants (s^{-1}). Data represent mean ± s.e.mean. *$P<0.05$ vs control.

Table 2 Influence of PTH(1–34) on the positive contractile effects of PTHrP(1–34) and PTHrP(1–16)

	Cell shortening dl l^{-1} (%)
Control	4.12±0.25
PTH(1–34)	4.35±0.44
PTHrP(1–34)	7.92±1.50*
PTHrP(1–34)+PTH(1–34)	3.67±0.87
PTHrP(1–16)	8.67±1.10*
PTHrP(1–16)+PTH(1–34)	7.10±0.62*

Isolated cardiomyocytes were paced at a constant frequency of 0.5 Hz without further additions (control), or in the presence of PTHrP(1–34) (1 μmol l^{-1}), PTHrP(1–16) (1 μmol l^{-1}), or PTHrP(1–34) and PTHrP(1–16) in the co-presence of PTH(1–34) (10 μmol l^{-1}). Data represent contractile parameters from $n=12$ cells. Cell shortening is expressed as maximal cell shortening in per cent of the diastolic cell length. Data represent mean±s.e.mean. *$P<0.05$ vs control.

Table 3 Influence of Rp-cyclicAMPS on the positive contractile effects of PTHrP(1–34) and PTHrP(1–16)

	Cell shortening dl l^{-1} (%)
Control	4.12±0.25
Rp-cyclicAMPS	5.55±0.63
PTHrP(1–34)	7.92±1.50*
PTHrP(1–34)+Rp-cyclicAMPS	3.67±0.87
PTHrP(1–16)	8.67±1.10*
PTHrP(1–16)+Rp-cyclicAMPS	7.10±0.62*

Isolated cardiomyocytes were paced at a constant frequency of 0.5 Hz without further additions (control), or in the presence of PTHrP(1–34) (1 μmol l^{-1}), PTHrP(1–16) (1 μmol l^{-1}), or PTHrP(1–34) and PTHrP(1–16) in the co-presence of Rp-cyclicAMPS (10 μmol l^{-1}). Data represent contractile parameters from $n=12$ cells. Cell shortening is expressed as maximal cell shortening in per cent of the diastolic cell length. Data represent mean±s.e.mean. *$P<0.05$ vs control.

Table 4 Influence of BQ123 on the positive contractile effects of sarafotoxin 6b and PTHrP(1–16)

	Cell shortening dl l^{-1} (%)
Control	4.88±1.13
BQ123	4.51±0.35
Sarafotoxin 6b	7.75±0.31*
Sarafotoxin 6b+BQ123	5.63±0.44
PTHrP(1–16)	8.78±0.50*
PTHrP(1–16)+BQ123	3.63±0.19
PTHrP(1–34)	7.88±1.31*
PTHrP(1–34)+BQ123	8.14±1.42*

Isolated cardiomyocytes were paced at a constant frequency of 0.5 Hz without further additions (control), or in the presence of sarafotoxin 6b (10 μmol l^{-1}), PTHrP(1–16) (1 μmol l^{-1}), PTHrP(1–34) (1 μmol l^{-1}), or sarafotoxin 6b, and PTHrP(1–16), and PTHrP(1–34) in the co-presence of BQ123 (1 μmol l^{-1}). Data represent contractile parameters from $n=12$ cells. Cell shortening is expressed as maximal cell shortening in per cent of the diastolic cell length. Data represent mean±s.e.mean. *$P<0.05$ vs control.

positive contractile effect evoked by isoprenaline (1 μmol l^{-1}), a β-adrenoceptor agonist, was blunted, while PTHrP(1–34) and PTHrP(1–16) persisted after washout of the drug (Table 5).

Furthermore, we investigated the influence of either β- or α-adrenoceptor inhibition on the positive contractile effect of PTHrP(1–16), using atenolol to antagonize β-adrenoceptors and prazosin to antagonize α-adrenoceptors. Atenolol (10 μmol l^{-1}) did neither change the contractile profile of cardiomyocytes nor influenced the contractile response to PTHrP(1–16) but antagonized isoprenaline (Table 6). Prazosin (10 μmol l^{-1}) did not exert an effect but antagonized the positive contractile effect of phenylephrine, an α-adrenoceptor agonist. Prazosin, however, did not inhibit the positive contractile effect of PTHrP(1–16) (Table 7).

In summary, the positive contractile effect of PTHrP(1–16) was antagonized by the co-presence of an ET_A receptor antagonist but not in the co-presence of either a PTHrP-receptor antagonist, a β-, or an α-adrenoceptor antagonist. This suggests that PTHrP(1–16) interacts with the ET_A receptor. Indeed, increasing concentrations of either

Table 5 Positive contractile effects of PTHrP(1–34), PTHrP(1–16), and isoprenaline on cardiomyocytes during exposure and after washout

	n	*Cell shortening dl l^{-1}* (%)
Control	12	6.12±1.25
PTHrP(1–34)	12	1.18±1.44*
PTHrP(1–34) 15 min after washout	12	12.74±1.16*
PTHrP(1–16)	12	11.26±2.44*
PTHrP(1–16) 15 min after washout	12	11.97±1.50*
Isoprenaline	12	14.83±2.87*
Isoprenaline 15 min after washout	12	7.14±1.13

Isolated cardiomyocytes were paced at a constant frequency of 0.5 Hz without further additions (control), in the presence of agonists as indicated (each 1 μmol l^{-1}), or 15 min after the agonists were washed out. Data represent contractile parameters from $n=12$ cells. Cell shortening is expressed as maximal cell shortening in per cent of the diastolic cell length. Data represent mean±s.e.mean. *$P<0.05$ vs control.

Table 6 Influence of atenolol on the positive contractile effects of isoprenaline and PTHrP(1–16)

	n	*Cell shortening dl l^{-1}* (%)
Control	12	4.88±1.17
Atenolol	12	5.33±0.64
Isoprenaline	12	12.24±2.98*
Isoprenaline+Atenolol	12	5.46±0.85
PTHrP(1–16)	12	9.10±1.16*
PTHrP(1–16)+Atenolol	12	9.21±0.19*

Isolated cardiomyocytes were paced at a constant frequency of 0.5 Hz without further additions (control), or in the presence of atenolol (10 μmol l^{-1}), isoprenaline (1 μmol l^{-1}), PTHrP(1–16) (1 μmol l^{-1}), or isoprenaline and PTHrP(1–16) in the co-presence of atenolol (1 μmol l^{-1}). Data represent contractile parameters from $n=12$ cells. Cell shortening is expressed as maximal cell shortening in per cent of the diastolic cell length. Data represent mean±s.e.mean. *$P<0.05$ vs control.

PTHrP(1–16) or sarafotoxin 6b, but not PTHrP(1–34), reduced ^{3}H-BQ123 binding to cardiomyocytes (Figure 4).

Discussion

The main finding of this study is that PTHrP(1–16), which is inert on classical PTH/PTHrP target cells, binds to, and activates cardiac ET_A receptors and thereby evokes a positive contractile effect on ventricular cardiomyocytes.

In this study we found a biological activity for PTHrP(1–16) on ventricular cardiomyocytes, i.e. a positive contractile effect. Even though this seems similar to the effect of PTHrP(1–34), but the mechanism is different. A different mode of action can be concluded from the following facts: First, PTH(1–34) does not antagonize the effect of PTHrP(1–16) but antagonizes PTHrP(1–34). Second, Rp-cyclic AMPS does not antagonize the effect of PTHrP(1–16) but PTHrP(1–34), which indicates that different second messengers are involved in the positive contractile effects of PTHrP(1–16) and PTHrP(1–34). Third, PTHrP(1–16) has no biological effects on classical PTH and PTHrP target cells, which express the known PTH/PTHrP-receptor, but has effects on cardiomyocytes, as shown in our study. These results rule out the possibility that PTHrP(1–16) acts on contractility through the same type of receptors as PTHrP(1–34).

Table 7 Influence of prazosin on the positive contractile effects of phenylephrine and PTHrP(1–16)

	n	*Cell shortening* $dl\ l^{-1}$ (%)
Control	12	5.96±0.81
Prazosin	12	4.88±1.13
Phenylephrine	12	9.65±0.31*
Phenylephrine + Prazosin	12	6.39±0.44
PTHrP(1–16)	12	8.81±0.50*
PTHrP(1–16) + Prazosin	12	8.23±0.21*

Isolated cardiomyocytes were paced at a constant frequency of 0.5 Hz without further additions (control), or in the presence of prazosin (10 μmol l^{-1}), phenylephrine (10 μmol l^{-1}), PTHrP(1–16) (1 μmol l^{-1}), or phenylephrine and PTHrP(1–16) in the co-presence of prazosin. Data represent contractile parameters from $n=12$ cells. Cell shortening is expressed as maximal cell shortening in per cent of the diastolic cell length. Data represent mean±s.e.mean. *$P<0.05$ vs control.

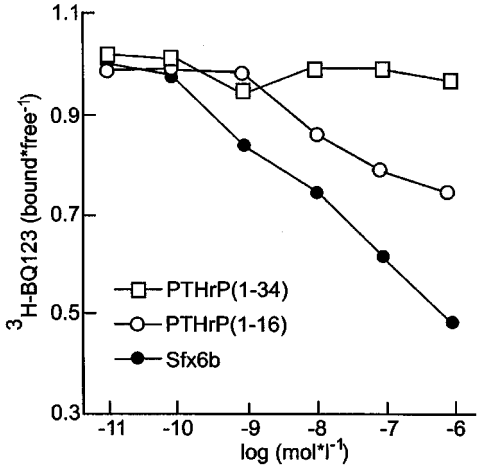

Figure 4 Binding of sarafotoxin 6b and PTHrP(1–16) to ventricular cardiomyocytes. Cells were incubated for 2 h in the presence of ^{3}H-BQ123 at room temperature and the amount of bound and free ^{3}H-BQ123 was determined in the presence of the indicated concentrations of sarafotoxin 6b (○), PTHrP(1–16) (●), or PTHrP(1–34) (□). Data represent the means from three culture dishes. The experiments were performed twice with identical results.

For the reasons mentioned above, we hypothesized that PTHrP(1–16) binds to, and activates another type of receptor. We considered several types of receptors which are expressed on adult cardiomyocytes and can mediate a positive contractile effect. Among others, the endothelin receptors represented possible candidates. The structural similarities, together with the known functional effects of ET_A receptor activation in cardiomyocytes, led us to hypothesize that PTHrP(1–16) binds to, and acts through the ET_A receptor. The results demonstrate, that this is indeed the case: First, pharmacological activation of ETA receptors by sarafotoxin 6b increases cell shortening as found for PTHrP(1–16). Second, inhibition of ET_A receptors with BQ123 antagonized the effects of both, sarafotoxin 6b and PTHrP(1–16). Third, the effect of PTHrP(1–16) on contraction amplitude is, in contrast to PTHrP(1–34), not accompanied by a strong effect on the kinetic of contraction. Earlier studies describing the positive contractile effect of endothelin also found no effect on time to peak force or time to reach 50% of relaxation (Ishikawa *et al*., 1988; Mebazaa *et al*., 1990). Fourth, the effect of PTHrP(1–16) persisted during washout of the drug, another characteristic feature of the effect of endothelin (Mebazaa *et al*., 1990), but not for agonists acting *via* activation of adenylate cyclase, such as isoprenaline. Finally, sarafotoxin 6b and PTHrP(1–16), but not PTHrP(1–34), replaced ^{3}H-BQ123 from cellular binding sites.

This study was aimed to characterize the structure function relationship for PTHrP in regard to its positive contractile effect on cardiomyocytes. The results of this study, that PTHrP fragments void of their receptor binding domain cross-react with other cardiac receptor systems, is important for future peptide design. The discovery, that similar breakdown products of PTHrP can be generated by proteolytic cleavage gives rise to some considerations regarding the physiological or pathophysiological role of PTHrP. One of our previous studies (Schlüter *et al*., 2000) has shown that coronary endothelium releases PTHrP under hypoxia and energy-depleting conditions, suggesting that PTHrP influences vascular and contractile function of the heart during an early phase of reperfusion. Its positive inotropic effect may attenuate the extent of postischemic stunning. This study was the first to analyse not only expression of PTHrP in the ventricle but also identify conditions under which it is released. *In vivo*, PTHrP is also broken down into peptide fragments. As stated in the introduction, proteolytic cleavage of PTHrP leads to the generation of small N-terminal peptide fragments void of a PTH/PTHrP-receptor binding domain. However, there are at present no data directly showing that PTHrP breakdown products can be detected in the coronary perfusate. Never-

theless, the present study shows that such N-terminal fragments can exert contractile effects by interacting with a different cardiac receptor, the ET_A receptor.

The concentration of PTHrP(1–34) used in our study is in the same order of magnitude than concentrations used to study the effects of PTHrP on classical target cells like osteoblasts. Therefore, the concentration of the synthetic peptide PTHrP used in this study is in a normal range for *in vitro* systems. In a previous study we have shown, however, that coronary endothelial cells, the most likely source of PTHrP acting on ventricular cardiomyocytes, produce and release a posttranslationally modified PTHrP (Schlüter *et al*., 2000). The potency of this authentic and full length PTHrP is approximately 10 times higher than that of synthetic peptides. In addition, *in vitro*, coronary endothelial cells release up to 2.6 pg PTHrP/mg protein h^{-1}. The mechanism by which PTHrP(1–34) exerts its positive contractile effect on ventricular cardiomyocytes was not investigated in this study. PTH(1–34), void of adenylate cyclase activation on ventricular cardiomyocytes, antagonizes the positive contractile effects of full-length PTHrP (Schlüter *et al*., 2000) and PTHrP(1–34) (this study). Therefore, the most likely mechanism by which authentic PTHrP can exert a positive contractile effect is the binding to, and activation of a tissue specific PTH/PTHrP-receptor subtype.

The result of our study indicates that N-terminal PTHrP fragments like PTHrP(1–34) can not be further truncated at the C-terminus to study the structure function relationship, because they would lose their binding domain to the cardiac PTHrP receptors and exert effects related to binding to and activation of distinct receptor subtypes, i.e. the ET_A receptor. For any future design of partial peptides it is, therefore, important to know that they can not be too small, but should include the classical receptor binding domain.

In summary, our study shows a biological effect for PTHrP(1–16) on adult cardiomyocytes. This is an important finding, because proteolytic processing of PTHrP *in vivo* can result in the production of N-terminal peptide fragments which lack the classical PTH/PTHrP-receptor domain. These fragments were thought to be biologically inert, which they are, indeed, in respect to the PTH/PTHrP-receptors. But on cardiomyocytes they can exert biological effects through another peptide hormone receptor.

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