Parathyroid hormone stimulates juxtaglomerular cell cAMP accumulation without stimulating renin release

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Parathyroid hormone (PTH) is an 84-amino acid peptide released from the chief cells of the parathyroid gland. Traditionally, PTH has been thought of as a hypercalcemic hormone by stimulating release of Ca from bone and renal Ca reabsorption (23). These effects of PTH are mediated by the PTH receptor PTH1R and are coupled to the production of cAMP (1,18). The PTH1R is also the receptor for parathyroid hormone-related protein (PTHrP; Refs. 1, 18 ); a protein produced in neoplastic syndromes (27), development (20), and ischemia (36) that exerts PTH-like hypercalcemic effects (27).

Recently, a second receptor for PTH has been cloned, called PTH2R (46). The tissue distribution of PTH2R differs significantly from PTH1R; PTH2R is expressed in the central nervous system, testes, pancreas, as well as the vascular pole of the glomerulus, and the role for the PTH2R is still being defined (45). Stimulation of the PTH2R by PTH also increases cellular cAMP (46). However, PTH has a much higher affinity for the PTH1R compared with PTH2R and stimulates the PTH1R at much lower concentrations (16, 44, 47). Furthermore, PTHrP does not stimulate the PTH2R receptor at all and is considered a PTH1R-selective agonist (43, 46). Because of this, PTH is not likely the natural ligand of PTH2R in vivo. The most likely natural ligand for PTH2R is tubuloinfundibular peptide of 39 residues (TIP-39; Refs. 17, 44, 47).

Renin is the rate-limiting enzyme in the renin-angiotensin system and is released from the juxtaglomerular (JG) cells of the afferent arteriole in the kidney (49). Renin release from JG cells is stimulated by substances that increase cAMP (22), such as catecholamines (12), ANG II inhibition (7), and prostaglandins (10, 24). It is thought that any substance that stimulates the production of JG cell cAMP increases renin release (8, 22). Substances that stimulate JG cell cAMP production do so by activating adenyl cyclase-V (AC-V), an isoform of adenyl cyclase inhibited by elevated intracellular calcium (13, 30, 31). AC-V has been localized on renin-containing granules in the JG cell (30).

Previously, it has been shown that PTH can stimulate renin secretion in vivo. Patients with primary hyperparathyroidism can have elevated plasma renin activity (PRA; Ref. 23), and PTH acutely increases PRA in anesthetized dogs (40). How PTH stimulates renin secretion in vivo is unknown. Thus the overall goal of this study was to determine whether PTH can directly stimulate renin release from JG cells via elevated JG cell cAMP and also to determine which PTH receptors mediate this effect.

It is possible that PTH could directly stimulate renin release from JG cells. PTH1R has been identified in isolated glomeruli with attached vessels, suggesting that it could be present in JG cells (50). However, this has not been definitively shown. Likewise, the PTH2R has also been identified in periglomerular vasculature but not specifically in JG cells (45). When stimulated, both PTH1R and PTH2R increase the production of cAMP, the stimulatory second messenger for renin secretion (1, 18, 45). However, since PTH is not the likely natural ligand for the PTH2R (16), we originally hypothesized that PTH directly stimulates renin release from JG cells by increasing cAMP via the PTH1R receptor.
Overall, we report that we found PTH1R, but not PTH2R, mRNA expressed in JG cells. We also unexpectedly found that stimulating PTH1R increased JG cell cAMP without increasing renin release from JG cells, suggesting compartmentalization of cAMP signaling within the JG cell.

METHODS AND EXPERIMENTAL PROTOCOLS

METHODS

Primary JG cell culture. Our primary culture of mouse JG cells has been described previously (25, 28–31). Four C57BL6 mice (8- to 9-wk-old) that had free access to food and water were killed by cervical dislocation to avoid the deleterious effects of anesthesia on JG cell survival in culture. With the use of a sterile technique, the kidneys were removed, decapsulated, and the renal cortex dissected. The cortical tissue was minced and then incubated with gentle stirring in an enzyme buffer containing the following (in mM): 130 NaCl, 5 KCl, 2 CaCl₂, 10 glucose, 20 sucrose, and 10 HEPES at pH 7.4 supplemented with 0.25% trypsin (Sigma-Aldrich, St. Louis, MO), 0.1% collagenase A (Roche Applied Science, Indianapolis, IN), and 0.0021% DNase (Sigma-Aldrich) at 37°C for 60 min. The enzyme buffer was replenished every 15 min. After enzymatic dissociation, the tissue was centrifuged at 200 g for 7 min and resuspended in 50 ml of the previously described buffer and passed first through a 74-μm nylon mesh sieve and subsequently through a 22-μm nylon mesh sieve. The cells retrieved from sieving were centrifuged at 200 g for 7 min and resuspended in 1 ml of buffer. The cell suspension was separated using 35 ml of a 40% isosmotic Percoll density gradient containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 5% FCS (GIBCO-Invitrogen, Grand Island, NY) and distributed in 250-μl aliquots into 4 wells of a 24-well culture plate precoated with (GIBCO-Invitrogen, Grand Island, NY) and distributed in 250-μl aliquots into 4 wells of a 24-well culture plate precoated with poly-λ-lysine (Millipore, Billerica, MA). The JG cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂ in air.

All procedures were approved by the Henry Ford Health System Institutional Animal Care and Use Committee and adhere to the guiding principles in the care and use of experimental animals in accordance with the National Institute of Health’s guidelines. Henry Ford Hospital operates an Association for Assessment and Accreditation of Laboratory Animal Care-certified animal care facility.

Semiquantitative RT-PCR. Total JG cell RNA was extracted using Tri-reagent (Molecular Research Center, Cincinnati, OH). Custom rat-specific primers from TIB Molbiol (Adelphia, NJ) were used for all PCR reactions. The primer sequences for PTH1R were as follows: forward: 5'-gacctcaacaccagggac-3', and reverse: 5'-ctgctgaactcctcctg-3'. The primer sequences for PTH2R were as follows: forward: 5'-tggtaagctgtgsgagac-3', and reverse: 5'-actctttcctgtctgcatc-3'. Based on a BLAST search, we determined that the PTH2R primers share 100% identity for both mouse and rat samples (accession no.: mouse: NM_139270, rat: NM_031089). Semiquantitative RT-PCR was performed as follows: 1 μg of DNAse-treated total RNA sample was reverse transcribed using random primers and Omniscript reverse transcriptase (Qiagen, Valencia, CA) in a total volume of 20 μl for 1 h at 37°C followed by an inactivation step of 95°C for 5 min. Two microfilters of the reverse transcription reaction were then amplified in a thermal cycler with specific primers. Reactions were set up in a final volume of 20 μl, which contained 2 μl of sample and 1 μM each of both the primers. After an initial “hot start” at 94°C for 5 min, amplification occurred by denaturation at 94°C for 30 s, annealing at 58°C for 30 s for PTH1R or 60°C for PTH2R, and extension at 72°C for 1 min for a total of 40 cycles. A final extension was performed at 72°C for 5 min, after which samples were held at 4°C. At the end of PCR cycling, an aliquot of each sample was run on a 1% agarose gel in Tris-borate-EDTA and visualized by ethidium bromide staining.

Renin release. After isolated JG cells were incubated for 48 h, the culture medium was switched to serum-free minimum essential medium (SF-MEM; Gibco-Invitrogen). Unless otherwise specified, SF-MEM contained 100 μM of 3-isobutyl-1-methyl-xanthine (IBMX) to inhibit the degradation of cAMP as well as various experimental manipulations listed in the Experimental Protocols. After this, medium was aspirated and centrifuged, and the supernatant recovered for assay of renin concentration (ANG I generation) using 3-h incubation with rat angiotensinogen and assayed using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN) as previously described (2–4, 6, 28–31). The incubation of media and angiotensinogen occurred in the presence of 0.1 mg PMSE/ incubate to inhibit protease activity (2–4, 6, 28–31). Values for renin concentration were corrected for JG cell total protein and are referred to as renin release.

cAMP content. After the incubation medium was removed for renin determination, JG cells were harvested by gentle scraping the culture wells with 100 μl of PBS containing 1 mM IBMX plus 100 μl of 50% methanol. The cAMP content was determined from the harvested cells by an RIA kit (Biomedical Technology, Stoughton, MA). Values were corrected for JG cell total protein and expressed as fmol/mg protein.

Protein concentration. The protein concentration in JG cell lysates was determined using the Coomassie plus protein assay reagent kit (Pierce Biotechnology, Rockford, IL) according to the manufacturer’s instructions.

Experimental Protocols

Expression of PTH1R and PTH2R in JG cells. To determine if JG cells express either the PTH1R or PTH2R, semiquantitative RT-PCR for PTH1R and PTH2R was performed on freshly harvested, isolated mouse JG cells that were not placed into primary culture.

Effect of PTH on cAMP and renin release. To determine if PTH can stimulate JG cell cAMP and renin release, cells were incubated with SF-MEM containing 10–6, 10–5, or 10–4 M human PTH 1–34 (Sigma-Aldrich) or PTH-free SF-MEM (control) for 2 h. JG cell cAMP content and renin release were measured (n = 11). Doses of PTH were similar to those used previously (1, 46).

Effect of PTHrP stimulation on cAMP and renin release. To determine if PTH1R-specific stimulation can increase JG cell cAMP and renin release, cells were incubated with SF-MEM containing 10–6 or 10–5 M PTHrP-1–37 (Bachem) or PTHrP-free SF-MEM (control) for 2 h. JG cell cAMP content and renin release were measured (n = 13). Doses of PTHrP are similar to those used previously (1, 46).

To demonstrate that the stimulation of cAMP by PTHrP was not an artifact due to the presence of IBMX, cells were incubated with SF-MEM containing 10–6 M PTHrP-1–37 or PTHrP-free media (control) for 2 h in the absence of IBMX. JG cell cAMP and renin release were measured. IBMX is commonly used to inhibit the degradation of cAMP in the presence of PTH (1). Renin release and cAMP were measured (n = 6).

Additionally, to determine if the duration of PTH1R stimulation could affect renin release, cells were incubated in the presence and absence of 10–4 M PTHrP-1–37 for 6 h. Renin release was measured (n = 8).

Effect of PTHrP stimulation on cAMP and renin release. To determine whether PTH2R-specific stimulations increased JG cell cAMP and renin release, cells were incubated with SF-MEM containing 10–6, 10–5, or 10–4 M of the PTHrP-2R-selective agonist (TIP-39) or TIP-39-free SF-MEM (control) for 2 h. JG cell cAMP and renin release were measured (n = 10). Doses of TIP-39 were similar to those used previously (47).

Effect of extracellular calcium and AC-V inhibition on PTH1R-stimulated cAMP. To determine if PTH1R-stimulated cAMP accumulation was calcium sensitive, JG cells were incubated in low (0.8 mM)

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or high (1.5 mM) calcium SF-MEM in the presence or absence of 10^{-6} M PTHrP-1–37 for 2 h. JG cell cAMP and renin release were measured (n = 12). Doses of media calcium were similar to those used previously (28).

To determine if PTH1R-mediated cAMP was mediated by AC-V, PTH1R was stimulated with 10^{-6} M PTHrP-1–37 for 2 h in the presence or absence of 20 μM 2-amino-7-(furanyl)-7,8-dihydro-5(6H)-quinazolinone (NKY 80), a selective inhibitor of AC-V. JG cell cAMP and renin release were measured (n = 12). The dose of NKY80 used was identical to one used previously (30).

Effect of forskolin on JG cell cAMP and renin release. To show our JG cell preparation could produce cAMP and concomitantly increase renin release, cells were incubated with SF-MEM containing 10^{-6} M forskolin (Sigma) or forskolin-free SF-MEM (control) for 2 h. Renin release was measured in all experiments (n = 7), and cAMP was determined in n = 4 experiments. The dose of forskolin used was identical to one used previously (31).

**Statistics**

The n-values from Experimental Protocols refer to the number of JG cell preparations that were treated with the corresponding protocol. In each JG cell preparation, the described treatments from each protocol were applied to separate wells. Each treatment was applied to one well per preparation.

Due to in vitro variations in renin release, all experimental data were considered dependent on their respective control values, necessitating paired protocols (5). For each protocol, wells of JG cells receiving treatments were paired to the untreated control well from the same specific preparation. Consistent with previous publications, renin release and JG cell cAMP were log_{10} transformed for statistical analyses to ensure the normality of distributions (14). Single intragroup comparisons were performed with paired Student’s t-tests. Multiple comparisons of single factors were performed with one-way repeated-measures ANOVA with a Student-Newman-Keuls post hoc test. Two-way repeated-measures ANOVA was performed on nested designs where we tested for a two-way interaction. A one-way design using Student-Newman-Keuls was used to examine individual pairwise comparisons. P < 0.05 was considered statistically significant. Data in RESULTS are presented as means ± SE. The absolute numbers for all data are provided in RESULTS.

The pretest power of our analyses is a function of the sample size and the number of tests we are examining. The sample size ranges from a low of 6 for the protocol done in the absence of IBMX to 13 for the effect of PTH1R stimulation or cAMP and renin release. The number of tests ranged from 1 (α-value 0.05) to 3 (α-value 0.0167). Based on previous experience, we anticipated that an effect size of 1.5 would be relevant. For all protocols using a paired t-test, the power exceeds 90% except for the IBMX exclusion protocol where the power was 84%. The two-way repeated-measures ANOVA had power exceeding 90% for tests of both main effects and the interaction. The high power lends credence to our observations of no change.

**RESULTS**

Expression of PTH1R and PTH2R in JG Cells

To test if PTH1R and PTH2R are expressed in JG cells, we performed real-time RT-PCR on primary cultures of mouse JG cells. Using RT-PCR, we detected a transcript for PTH1R at the expected molecular size of 148 bp in mouse JG cells (Fig. 1). Transcripts for PTH1R were also detected in positive control samples from mouse heart and rat brain. A transcript for PTH2R was detected at the expected size of 150 bp in rat brain as a positive control, but no transcript was detected in JG cells. These data suggest that JG cells express the PTH1R but not PTH2R.

Effect of PTH on cAMP and Renin Release

Control means ± SE log_{10} transformed cAMP levels were 3.27 ± 0.06 fmol/mg protein. Means ± SE log_{10} transformed cAMP in the 10^{-10}, 10^{-8}, and 10^{-6} M PTH groups were 3.23 ± 0.16, 3.58 ± 0.17, and 3.92 ± 0.12 fmol/mg protein (P < 0.001, Fig. 2A), respectively. Control means ± SE log_{10} transformed renin release levels were 2.70 ± 0.12 ng ANG I·mg prot⁻₁·h⁻¹, 10^{-10} M, 10^{-8} M, and 10^{-6} M PTH did not increase renin release (log_{10} transformed: 2.65 ± 0.10, 2.67 ± 0.09, and 2.73 ± 0.11 ng ANG I·mg prot⁻₁·h⁻¹, respectively; Fig. 2B). These data demonstrate that PTH unexpectedly increased JG cell cAMP without affecting renin release.

Effect of PTH1R Stimulation on cAMP and Renin Release

To selectively stimulate the PTH1R receptor, PTHrP-1–37 was administered to primary cultures of JG cells and cAMP and renin release were measured. Means ± SE of the log_{10} transformed control cAMP were 3.13 ± 0.09 fmol/mg protein and was increased to 3.79 ± 0.05 (P < 0.01) and 3.93 ± 0.09 fmol/mg protein by 10^{-8} M and 10^{-6} M PTHrP, respectively (P < 0.001; Fig. 3A). Control means ± SE log_{10} transformed renin release were 2.36 ± 0.10 ng ANG I·mg prot⁻₁·h⁻¹, 10^{-8} M and 10^{-6} M PTHrP did not significantly change renin release (log_{10} transformed: 2.36 ± 0.11 and 2.41 ± 0.10 ng ANG I·mg prot⁻₁·h⁻¹, respectively; Fig. 2B). These data confirm that selective stimulation of the PTH1R increases JG cell cAMP without affecting renin release.

Next, we tested whether the PTH1R-mediated stimulation of cAMP, but not renin release, was solely due to the presence of IBMX in our solutions. Control means ± SE log_{10} transformed cAMP were 3.28 ± 0.22 fmol/mg protein; 1 × 10^{-6} M PTHrP significantly increased the log_{10} transformed cAMP in the absence of IBMX to 3.60 ± 0.16 fmol/mg protein (P < 0.05). Means ± SE log_{10} transformed control renin release were 2.61 ± 0.15 ng ANG I·mg prot⁻₁·h⁻¹ and did not significantly change with the administration of PTHrP in the absence of IBMX.
0.12 ng ANG I·mg prot⁻¹·h⁻¹; Fig. 4B). In the absence of any receptor expression, or any effect of PTH2R stimulation on either renin release or cAMP, these data suggest that the effect of PTH on cAMP is completely mediated by PTH1R.

**Effect of Extracellular Calcium and AC-V Inhibition on PTH1R-Stimulated cAMP**

Elevated extracellular calcium inhibits both stimulated renin release and JG cell cAMP accumulation via the calcium-sensing receptor (CaSR; Refs. 28, 29) and resultant inhibition of AC-V (30, 31). To test if elevated extracellular calcium inhibits PTH1R-mediated cAMP production, we applied low or high calcium SF-MEM to primary cultures of JG cells in the presence of absence of PTHrP. Means ± SE log₁₀ transformed cAMP in the control + low calcium group were 3.31 ± 0.17 fmol/mg protein. The addition of 10⁻⁶ M PTHrP increased cAMP in the presence of low calcium to 3.83 ± 0.20 fmol/mg protein (P < 0.01; Fig. 5A). Means ± SE log₁₀ transformed cAMP in the control + high calcium group were 3.29 ± 0.18 fmol/mg protein, and the addition of 10⁻⁶ M PTHrP increased cAMP in the presence of high calcium to 3.63 ± 0.22 fmol/mg protein (P < 0.05; Fig. 5A). No statistically significant interaction between calcium and PTHrP was detected. Means ± SE (2.59 ± 0.13 ng ANG I·mg prot⁻¹·h⁻¹). These data demonstrate that the PTHrP-mediated dissociation of elevated cAMP from renin release is not an artifact due to the presence of IBMX.

To test if prolonged exposure to PTHrP could increase renin release, we incubated primary cultures of JG cells with 10⁻⁶ M PTHrP-1–37 for 6 h. Means ± SE log₁₀ transformed control renin release were 3.65 ± 0.11 ng ANG I·mg prot⁻¹·h⁻¹ and did not significantly change in the presence of PTHrP (log₁₀ transformed: 3.40 ± 0.18 ng ANG I·mg prot⁻¹·h⁻¹).

**Effect of PTH2R Stimulation on cAMP and Renin Release**

To test if the accumulation of JG cell cAMP was due to the PTH2R receptor, we used a PTH2R-specific agonist TIP-39. Means ± SE log₁₀ transformed control cAMP were 3.33 ± 0.04 fmol/mg protein; 10⁻¹⁰ M, 10⁻⁸ M, and 10⁻⁶ M TIP-39 had no significant effect on JG cell cAMP (log₁₀ transformed: 3.39 ± 0.05, 3.34 ± 0.07, 3.36 ± 0.08 fmol/mg protein, respectively, Fig. 4A). Control means ± SE log₁₀ transformed renin release were 2.43 ± 0.15 ng ANG I·mg prot⁻¹·h⁻¹; 10⁻¹⁰ M, 10⁻⁸ M, and 10⁻⁶ M TIP-39 did not increase renin release (log₁₀ transformed: 2.46 ± 0.12, 2.52 ± 0.17, 2.48 ± 0.01 ng ANG I·mg prot⁻¹·h⁻¹). These data demonstrate that the PTHrP-mediated dissociation of elevated cAMP from renin release is not an artifact due to the presence of IBMX.

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log₁₀ transformed renin release in the control low calcium group were 2.48 ± 0.11 ng ANG I·mg prot⁻¹·h⁻¹ and did not change in the presence of 10⁻⁶ M PTHrP (log₁₀ transformed: 2.49 ± 0.13 ng ANG I·mg prot⁻¹·h⁻¹; Fig. 5B). Means ± SE log₁₀ transformed control renin release was 2.33 ± 0.13 ng ANG I·mg prot⁻¹·h⁻¹. PTHrP did not significantly increase renin release in the absence or presence of NKY80 (log₁₀ transformed: 2.44 ± 0.11 and 2.48 ± 0.09 ng ANG I·mg prot⁻¹·h⁻¹, respectively; Fig. 6B). No statistically significant interaction between NKY80 and PTHrP using two-way ANOVA was detected for either cAMP or renin. Thus PTHrP is increasing JG cell cAMP, predominantly through non-AC-V sources.

To confirm these results, we also determined if PTHrP increases JG cell cAMP via AC-V. We exposed primary cultures of JG cells to PTHrP in the presence of a selective AC-V inhibitor, NKY-80. Means ± SE log₁₀ transformed control cAMP were 3.05 ± 0.11 fmol/mg protein and was significantly elevated in the presence of 10⁻⁶ M PTHrP (3.65 ± 0.15 fmol/mg protein; P < 0.001; Fig. 6A). The administration of PTHrP in the presence of AC-V inhibition with NKY80 also significantly increased means ± SE log₁₀ transformed cAMP from 2.85 ± 0.17 fmol/mg protein in the control + NKY80 group to 3.44 ± 0.14 fmol/mg protein (P < 0.001; Fig. 6A). Means ± SE log₁₀ transformed control renin release was 2.33 ± 0.13 ng ANG I·mg prot⁻¹·h⁻¹. PTHrP did not significantly increase renin release in the absence or presence of NKY80 (log₁₀ transformed: 2.44 ± 0.11 and 2.48 ± 0.09 ng ANG I·mg prot⁻¹·h⁻¹, respectively; Fig. 6B). No statistically significant interaction between NKY80 and PTHrP was detected for either cAMP or renin. Thus PTHrP is increasing JG cell cAMP, predominantly through non-AC-V sources.

Fig. 5. Effect of extracellular calcium on PTH1R-stimulated cAMP and renin release. A: 10⁻⁶ M PTHrP increased cAMP in the presence of both low and high media calcium. B: 10⁻⁶ M PTHrP did not increase renin release in the presence of high or low media calcium. Renin release values for control + high calcium and PTHrP + high calcium are significantly less than their low calcium counterparts (P < 0.001), consistent with the inhibitory effects of calcium on renin release. With the use of two-way ANOVA, no statistically significant interaction between calcium and PTHrP was detected. These data demonstrate that the PTH1R-mediated accumulation of cAMP is not calcium sensitive and is likely from a non-AC-V source.

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Fig. 4. Effect of PTH2R stimulation on cAMP and renin release. To selectively stimulate the PTH2R receptor, TIP-39 was administered to primary cultures of JG cells and cAMP and renin release were measured. A: none of the various concentrations of TIP-39 administered affected cAMP. B: none of the various concentrations of TIP-39 affected renin release.
Effect of Forskolin on JG Cell cAMP and Renin Release

As a positive control, we exposed primary cultures of JG cells to $10^{-6}$ M forskolin for 2 h and measured cAMP and renin release. Forskolin significantly increased JG cell means ± SE log$_{10}$ transformed cAMP from 3.39 ± 0.13 to 4.48 ± 0.07 fmol/mg protein ($P < 0.01$). Forskolin also significantly increased log$_{10}$ transformed renin release from 2.96 ± 0.10 to 3.29 ± 0.08 ng ANG I/mg prot$^{-1}$·h$^{-1}$ ($P < 0.01$). Based on these data, we demonstrated our JG cell preparations are able to increase renin release in response to "classical" stimuli that increase JG cell cAMP (26, 31, 39).

DISCUSSION

We hypothesized that PTH1R stimulation would increase renin release due to increased cAMP accumulation. We demonstrated with RT-PCR that PTH1R mRNA is expressed in JG cells, while PTH2R mRNA is not. Surprisingly, PTH stimulated JG cell cAMP accumulation but did not affect renin release. This was due to PTH1R stimulation, as the PTH1R agonist PTHrP mimicked these effects, while the PTH2R agonist TIP-39 had no effect. PTHrP-mediated cAMP accumulation occurred in the presence of high media calcium and also AC-V inhibition, indicating that PTH1R stimulation increases cAMP through non-calcium-sensitive, non-AC-V signaling pathways. PTHrP did not increase JG cell renin expression. These data support the notion that PTH stimulates JG cell cAMP production, without affecting renin release, presumably through different AC isoforms than AC-V. Importantly, our data demonstrate for the first time that elevated JG cell cAMP can be dissociated from increased renin release and suggest that compartmentalized cAMP signaling exists within JG cells.

The most significant finding from our study was that stimulating the PTH1R increased JG cell cAMP without increasing renin release. cAMP is formed by the enzymatic conversion of ATP to cAMP, mediated by adenylyl cyclase. cAMP is the key stimulatory second messenger for renin secretion (8, 22). Pathways that stimulate renin secretion, such as the β$_{1}$-adrenergic agonists (12), ANG II inhibition (7), and prostaglandins (10, 24), do so by increasing JG cell cAMP. Furthermore, stimuli that play a secondary role in regulating renin release, such as calcium (2, 3, 13, 28–31), endothelin (33), or nitric oxide (6, 37), also regulate renin release via changes in cellular cAMP. All of these stimuli affect JG cell cAMP levels and, as such, modulate renin secretion. Thus it was a surprise that both PTH and PTHrP were able to greatly increase JG cell cAMP without increasing renin release. These data suggest that the PTH1R receptor may be contained in a different intracellular compartment from other G protein-coupled receptors that regulate JG cell cAMP. Previously, it has been shown (30) that a threefold increase in JG cell cAMP induces a 60% increase in renin release. Many of the doses of PTH or PTHrP that we used increased cAMP more than threefold. Thus it is unlikely that PTH1R stimulation failed to increase renin release because intracellular cAMP was insufficiently elevated. While cAMP is produced via adenylyl cyclase, it is enzymatically degraded by phosphodiesterases (PDEs). Thus the content of cAMP within a cell is a function of the stimulatory effects of adenylylate cyclase on cAMP, as well as the inhibitory effects of PDEs due to increased cAMP degradation (11, 28). JG cells express numerous PDE isoforms that rapidly degrade cAMP (11, 28). Because of this, and consistent with the literature, we used the nonspecific, noncompetitive PDE inhibitor IBMX to prevent the rapid degradation of cAMP in our JG cell samples (28–31). IBMX is known to increase cAMP in JG cell cultures (28). However, to demonstrate that the production of cAMP by PTHrP in our JG cells was not an artifact of impaired PDE activity by IBMX, we applied PTHrP to primary cultures of JG cells in IBMX-free media and measured JG cell cAMP and renin release. PTHrP increased JG cell cAMP in IBMX-free media, while it had no effect on renin release. These data demonstrated that the ability of PTHrP to increase JG cell cAMP is not an artifact of IBMX in our cell media. We tested whether prolonged exposure of JG cells to PTHrP could increase renin release. Exposure of JG cells to PTHrP for 6 h had no effect on renin release, indicating that prolonged exposure to PTHrP also has no effect on renin release.

AC-V is one of two possible isoforms of adenylylate cyclase regulating renin release from JG cells (13, 30, 31). AC-V regulates the cAMP-mediated stimulation of renin by catecholamines, as the blockade of AC-V eliminates the increase in JG cell cAMP and renin release in response to adrenergic...
stimulation. Additionally, AC-V is found directly on the renin-containing granules in the JG cells, which is consistent with local, compartmentalized cAMP production. AC-V is a calcium-inhibitable isoform of adenylate cyclase. Elevations in extracellular calcium inhibit renin secretion and cAMP accumulation via an extracellular CaSR-mediated increase in intracellular calcium, leading to inhibition of AC-V (28–31). Thus, if PTHrP were increasing JG cell cAMP via AC-V, we expected that the cAMP-generating ability of PTHrP would be inhibited by elevated media calcium and AC-V inhibition. High media calcium and AC-V inhibition with NKY80 had no effect on the PTHrP-mediated increase in JG cell cAMP, suggesting that PTHrP was signaling through a non-AC-V isoform of adenylate cyclase. JG cells also express AC isoforms I, IV, and IX, suggesting that PTH1R is stimulating cAMP accumulation through one of these isoforms (13). The administration of the high calcium media was successful, as the high calcium media were able to decrease renin release in both the presence and absence of PTHrP.

The expression of AC-VI in the JG cell is controversial. While some groups have found its expression in JG cells (13), others have not (30, 31). However, all groups (13, 30, 31) that have studied AC-V have found it expressed in the JG cell and have shown that it regulates renin release. Thus we focused our studies on AC-V in relation to PTHrP. However, even if AC-VI were expressed in our population of JG cells, the effects of PTHrP on cAMP were unaffected by media calcium. Since AC-VI is also a calcium-inhibited isoform of adenylate cyclase, if PTHrP were acting through JG cell AC-VI, we would expect that the PTHrP-induced increase in JG cell cAMP would be blunted by elevated media calcium (28, 29). However, elevated calcium had no effect on PTHrP-induced cAMP in the JG cell. This suggests that calcium-inhibitable isoforms of adenylate cyclase are not involved in the PTH1R-mediated increase in JG cell cAMP.

We also measured the effects of forskolin on JG cell cAMP production and renin release. Forskolin is a potent adenylate cyclase activator and is commonly used to increase cAMP levels (26, 31, 39). Forskolin significantly increased JG cell cAMP and also stimulated renin release. This is important, as it demonstrates that our JG cell preparations were still sensitive to classical stimuli that increase renin release via elevated cAMP. Similar to previously published data, forskolin only moderately increased renin release (twofold) relative to the increase in JG cell cAMP (10-fold increase; Ref. 31). This is because forskolin is a nonspecific activator of adenyl cyclases (26) and activates all adenyl cyclase subtypes expressed in JG cells. Previously, we (30) have shown that stimulation of AC-V can increase renin release 60% with only a threefold increase in cAMP. This is most likely because AC-V localizes directly to the renin-containing granules, implying that cAMP generated specifically from AC-V would be more "efficient" at stimulating renin release.

In contrast to our findings that PTH and PTHrP increase JG cell cAMP without affecting renin release, an earlier report (34) found that PTH did increase renin release from primary cultures of rat JG cells. Initially, these data are difficult to reconcile with ours. However, the authors of this other study (34) detail how they were unable to demonstrate an effect of PTH on renin release unless they added the serine-protease inhibitor PMSF to their JG cell perfusion solutions. Furthermore, PMSF stimulated renin release in the absence of PTH, suggesting that the stimulatory effect of their preparation on renin release was solely due to the presence of PMSF (34). Additionally, the authors of this study did not measure cAMP (34). As such, it is unknown whether their administration of PTH to JG cells actually stimulated JG cell cAMP production, while we used PMSF when we incubated our renin. Thus, while this report stimulated our own interest in a PTH-renin interaction, we feel comfortable that our data are accurate.

We also tested whether PTH regulated renin release from JG cells via the PTH2R. PTH2R expression has been demonstrated in the vascular pole of the glomerulus (45). However, it was unknown whether the PTH2R was expressed in JG cells. Our data demonstrate that the PTH2R is not expressed in JG cells and that the PTH2R-specific agonist TIP-39 has no effect on renin release. Thus it appears that the PTH2R is not expressed in the JG cell component of the vascular pole of the glomerulus. Its function in the juxtaglomerular apparatus is not understood.

PTH has been reported to stimulate renin secretion from the isolated-perfused kidney (15, 35) and also acutely elevate PRA in vivo (40). Also, patients with primary hyperparathyroidism can develop elevated PRA (23). Thus we originally speculated that PTH may be able to increase PRA via a direct stimulatory effect on JG cell renin release. However, even though we found the PTH1R expressed in JG cells, and PTH1R stimulation can increase JG cell cAMP, stimulation of the PTH1R did not increase renin release. This suggests that PTH regulates PRA via indirect mechanisms in vivo. In support of this notion, Smith et al. (41) have shown that PTH acutely increases PRA without affecting renin secretion from the kidney, consistent with our data. Recently, it has also been shown that many renal tubular segments also contain renin (19, 32). However, since the data of Smith et al. (41) suggest that PTH increases PRA without affecting renin secretion, this would argue against tubular renin release being a significant contributor to the PTH-mediated increase in PRA. Emerging data have also shown that chronically elevated plasma PTHrP levels can increase PRA as well (4). This appears to be due in large part to the anorexic effects of PTHrP on NaCl consumption: PTHrP significantly decreased food intake, and the replacement of NaCl in the drinking water normalized PTHrP-elevated PRA levels. Thus chronically elevated PTH/PTHrP probably indirectly regulates PRA through changes in NaCl balance. Whether PTH-mediated changes in PRA contribute to the elevated levels of cardiorenal morbidity seen in patients with primary hyperparathyroidism remains to be seen (51).

In conclusion, we tested the hypothesis that PTH could increase renin release directly from JG cells due to increased cAMP production via the PTH1R receptor. Contrary to our hypothesis, even though we found the PTH1R receptor expressed in JG cells, we found that stimulation of PTH1R receptors via PTH and PTHrP increased JG cell cAMP but not renin release. PTH2R is not expressed in JG cells and does not mediate renin release. The PTH1R-mediated stimulation of cAMP is not affected by changes in extracellular Ca and occurs even in the presence of AC-V inhibition. Last, we demonstrated that forskolin significantly elevated both cAMP and renin release. These data support the notion that PTH increases JG cell cAMP, but not renin release, by stimulating adenyl cyclases besides AC-V and/or AC-VI via the PTH1R receptor.
The implication of our data is that specific cAMP-signaling domains may exist in the JG cell.

Perspectives

To our knowledge, this is the first report demonstrating an instance in which elevated JG cell cAMP does not increase renin release. This observation seems to provide more questions than answers, which will be resolved by future investigations. First of all, why do PTH1R-mediated elevations in JG cell cAMP fail to stimulate renin release when all other substances that increase cAMP do? The most likely answer is that there is compartmentalization of cAMP signaling and AC isoforms within the JG cell. It is known that many of the important receptors that regulate JG cell CAMP accumulation (such as, AC-V and AC-VI, β-adrenergic receptors, endothelin receptors, CaSR, and ANG II-type 1 receptor) all interact with lipid microsIGNALing domains in the cell membrane (21, 38, 42, 48). Additionally, it is known that AC-V localized directly on renin-containing granules in the JG cell, supporting the notion of compartmentalization signaling within the JG cell (30). It could be that the PTH1R is not localized to these signaling domains in the JG cell, providing an explanation for why PTH1R-mediated increases in cAMP do not increase renin release. An additional question raised by our findings is: what is the purpose of elevated JG cell cAMP that does not stimulate renin release? Perhaps PTH-derived cAMP increases renin synthesis. PTHrP release is stimulated from the endothelium in response to hypoxia and may regulate cell survival in response to stress. cAMP is known to increase GLUT-1-mediated glucose transport and regulate the cell cycle and cell proliferation (9). Thus non-AC-V-derived cAMP may play an important role in renin synthesis, cell cycle regulation, cellular glucose homeostasis, and cell survival. While all of these ideas are provocative, they have yet to be tested, and no doubt will be the focus of future investigations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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