**Parathyroid Hormone/Adenylate Cyclase Coupling in Vascular Smooth Muscle Cells**

Alan S. Hanson, Stuart L. Linas

**Abstract** Parathyroid hormone (PTH) has been implicated in hypertension, but PTH infusion results in vasodilation. PTH activates adenylate cyclase in vascular smooth muscle, but little is known about the factors that regulate PTH receptor/adenylate cyclase coupling in vascular cells. To characterize hormone-receptor signaling, we measured cyclic AMP levels in rat arterial smooth muscle cells in culture exposed to PTH (bovine 1-34). PTH yielded time- and concentration-dependent increases in cyclic AMP levels. Compared with isoproterenol, PTH was more potent, with a threshold at $2\times10^{-9}$ versus $5\times10^{-8}$ mol/L and half maximal responses at $10^{-8}$ versus $2.4\times10^{-7}$ mol/L. PTH-induced increases in cyclic AMP were independent of extracellular calcium, cyclooxygenase metabolites, phospholipase C, and protein kinase C because PTH-induced increases in cyclic AMP were not prevented by variations in extracellular calcium, indomethacin, angiotensin II, vasopressin, and protein kinase C activators or inhibitors. PTH/adenylate cyclase coupling was $G$ protein-dependent because increases in cyclic AMP were prevented by preincubation with cholera toxin but not with pertussis toxin. Prolonged exposure to PTH resulted in time- and concentration-dependent homologous desensitization of cyclic AMP responses. Desensitization occurred proximal to $G$ protein/adenylate cyclase because after prolonged PTH, responses to forskolin and cholera toxin remained intact. Desensitization was independent of protein kinase A and receptor sequestration because cyclic AMP responses remained after prolonged exposure to forskolin and pretreatment with phenylarsine oxide, colchicine, and cytochalasin D. We conclude that in vascular smooth muscle cells, PTH is coupled to adenylate cyclase through a cholera toxin-sensitive $G$ protein. Prolonged exposure to PTH results in desensitization of PTH receptors. Because adenylate cyclase is a potent vasodilator signaling pathway, these cellular effects could account for the in vivo observation that PTH infusion results in vasodilation. In contrast, states of prolonged PTH excess may not be associated with vasodilation because PTH-induced cyclic AMP increases are desensitized. (Hypertension. 1994;23:468-475.)

**Key Words** • parathyroid hormones • adenyl cyclase • muscle, smooth, vascular • $G$ protein

Parathyroid hormone (PTH) is important in the regulation of calcium, phosphate, and bone metabolism. In addition, PTH has significant effects on vascular tone and has been implicated in the generation and maintenance of high blood pressure. Sustained increases in PTH have been implicated in hypertension, and parathyroidectomy has been found to reverse hypertension in some animal models. However, infusion of PTH results in a reduction in blood pressure and vasodilation in several animal models. These apparent discrepancies are difficult to resolve in vivo because the primary effects of PTH may be offset by counterregulatory responses that mask hormone action.

The cellular effects of PTH are mediated by receptors coupled to adenylate cyclase in nonvascular tissue such as bone. In vascular smooth muscle cells (VSMCs), adenylate cyclase is an important signaling system mediating vasodilation. $\beta$-Adrenergic agonists are major circulating vasodilators in vivo, and $\beta$-adrenergic receptor ($\beta$-AR)/adenylate cyclase coupling has been extensively studied. In addition to $\beta$-AR, PTH is coupled to adenylate cyclase in vascular smooth muscle tissue. However, in contrast to $\beta$-AR, data on PTH receptor/adenylate cyclase coupling in VSMCs are limited. The initial versus sustained effects of PTH and factors that regulate PTH receptor coupling to adenylate cyclase have not been reported.

Because the in vivo hemodynamic effects of PTH are not clear and PTH appears to be an important regulator of vasodilator adenylate cyclase signaling in vascular smooth muscle tissue, our purpose was to determine factors that regulate PTH receptor/adenylate cyclase coupling in vitro. To accomplish this goal, we compared PTH with isoproterenol, a well-recognized vasodilator and activator of $\beta$-AR and adenylate cyclase. In addition, we used VSMCs in culture to overcome problems inherent in vivo.

**Methods**

**Cell Culture**

Mesenteric arterial and aortic VSMCs from 8- to 11-week-old male Sprague-Dawley rats (140 to 245 g) were grown in culture using minimum essential medium (MEM) with Earle’s salts supplemented with 10% fetal bovine serum, as described by our laboratory. The procedures followed were in accordance with institutional guidelines. Studies were carried out on passages 2 through 10 in 24-well culture plates after cell confluence was attained (4 to 8 days). Uniformity of cell growth was confirmed by initial studies demonstrating low well-to-well cell protein content variation, with SD = 12% of the mean by the Lowry protein assay. We used two VSMC types to assess differential effects in conduit (aortic) and resistance (mesenteric) vessels, but as the results were similar in both cell types, the data are reported in combined form.

**Hormone Incubations and Cyclic AMP Assay**

All studies were carried out at 37°C. Bovine PTH, fragment 1-34, was used to perform two types of studies: brief hormone
exposure and prolonged exposure. Brief incubations were carried out in 250 μL phosphate-buffered saline (PBS) (mmol/L: NaCl 149, NaHPO4 6, KH2PO4 4) at pH 7.4, to which bacitracin (25 μM/mL) was added as a proteolysis inhibitor and isobutylmethylxanthine (IBMX) (0.5 mmol/L) as a phosphodiesterase inhibitor. Prolonged exposures to agents were performed directly in 500 μL MEM followed by aspiration and rinsing with PBS and subsequent brief hormone exposure. After hormone exposure, incubations were terminated, and cellular cyclic AMP (cAMP) was extracted by addition of HCl to a final concentration of 0.01N. cAMP was assayed immediately or after brief storage at −20°C using a single-range 125I radiomimunoaassay kit (Amersham); 125I was counted on a Packard 5330 gamma counter.

**Measurement of Inositol Trisphosphate**

The methods for measurement of inositol 1,4,5-trisphosphate (IP3) have been described previously in detail.9 Subconfluent VSMCs were incubated with 10% fetal calf serum in inositol-deficient MEM containing 2.4 μCi/mL myo-[2-3H]-inositol for 40 hours at 37°C. Wells were then washed with PBS at 21°C to remove unincorporated tracer. VSMC monolayers were incubated with hormones or assay buffer (30 seconds, 21°C), and reactions were terminated by addition of 20% trichloroacetic acid. The aqueous phase was extracted with diethyl ether, and pH was adjusted to 7.0 with 50 mmol/L Tris base. Samples were applied to Dowex AG1-X8 anion-exchange resin columns, and inositol phosphates were measured by anion-exchange column chromatography. IP3 values were expressed as the percentage of baseline (ratio of IP3 content in agonist-exposed cells to IP3 content in cells exposed only to assay buffer).

**Statistical Analysis**

For each experiment, concomitant unstimulated wells were included, and all results are expressed relative to basal cAMP levels. Experiments were repeated two to seven times, the results from each being the mean of two to four duplicate points. All results are expressed as mean±SEM. Statistical significance was determined by the two-tailed t test for unpaired samples.

**Reagents**

The following were obtained from Sigma Chemical Co: bPTH-(1-34), pertussis toxin, cholera toxin, 3,4,5-trimethoxybenzoc acid 8-(diethylamino)octyl ester (TMB-8), phorbol 12-myristate 13-acetate (PMA), angiotensin II (Ang II), forskolin, colchicine, phenylarsine oxide, cytochalasin D, prostaglandin E2 (PGE2), trypsin EDTA, penicillin/streptomycin, nonessential amino acids, and Eagle’s MEM. Indomethacin, 1-oleoyl-2-acetyl glycerol (OAG), staurosporine, and IBMX were from Calbiochem. Isoproterenol was from Abbott. Fetal bovine serum was from Hyclone.

**Results**

**Brief Hormone Exposure**

Basal cAMP values were 2.5±0.1 pmol per well (n=10) and were highly constant between individual experiments. Fig 1 shows the time course of cAMP generation in response to PTH and the β-AR agonist (isoproterenol) both without and with the phosphodiesterase inhibitor IBMX. Both agents elicited rapid and sustained increases in cAMP that were potentiated by IBMX. Increases in cAMP were noted by 30 seconds, peaked by 2 to 5 minutes, and remained elevated beyond 10 minutes. Fig 2 shows the dose-response curve to both agents after a 2-minute exposure. Threshold PTH response was seen at 2×10−8 mol/L and reached a plateau response of 6.75-fold of basal levels at 10−7 to 10−6 mol/L PTH. Comparison of the concentrations necessary to elicit half-maximal responses for PTH (10−8 mol/L) and isoproterenol (2.4×10−7 mol/L) showed that PTH was approximately 24 times more potent than isoproterenol.

**Interactions With Other Messengers**

In some target tissues, PTH decreases entry of extracellular calcium into cells and increases calcium efflux from cells.11,12 To determine the role of calcium on PTH/adenylate cyclase coupling in VSMCs, we incubated cells with PTH in buffer containing various concentrations of calcium (nominally 0, 0.25, 0.75, and 1.25 mmol/L calcium), verapamil (10−5 mol/L), or TBM-8 (50 μmol/L). The results are listed in Table 1 and reveal that cAMP responses to PTH were unaffected by extracellular calcium concentration, calcium channel antagonist, or internal calcium distribution.

Because prostaglandins activate adenylate cyclase in VSMCs,8,9 we investigated the possibility that cyclooxygenase metabolites mediated PTH-induced increases in cAMP. Cells were pretreated with indomethacin (10−7 mol/L, 12 minutes) before PTH exposure. As can be...
TABLE 1. Effect of Pretreatment with Various Agents on Acute Response to Parathyroid Hormone (10^{-7} mol/L)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Response Without Pretreatment</th>
<th>Response With Pretreatment</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (0.00 mmol/L)</td>
<td>...</td>
<td>3.63±1.04</td>
<td>5</td>
</tr>
<tr>
<td>Calcium (0.25 mmol/L)</td>
<td>...</td>
<td>3.7±5.6</td>
<td>2</td>
</tr>
<tr>
<td>Calcium (0.75 mmol/L)</td>
<td>...</td>
<td>3.4±5.2</td>
<td>2</td>
</tr>
<tr>
<td>Calcium (1.25 mmol/L)</td>
<td>...</td>
<td>3.4±4.5</td>
<td>2</td>
</tr>
<tr>
<td>Verapamil (10^{-9} mol/L)</td>
<td>2.84±0.71</td>
<td>3.15±0.98</td>
<td>3</td>
</tr>
<tr>
<td>TMB-8 (5×10^{-9} mol/L)</td>
<td>3.31±0.55</td>
<td>3.08±0.70</td>
<td>3</td>
</tr>
<tr>
<td>Indomethacin (10^{-7} mol/L)</td>
<td>2.1,3.9</td>
<td>3.9,4.8</td>
<td>2</td>
</tr>
<tr>
<td>Ang II (10^{-10} mol/L)</td>
<td>3.64±0.64</td>
<td>3.18±0.77</td>
<td>4</td>
</tr>
<tr>
<td>Ang II (10^{-7} mol/L)</td>
<td>1.8,4.2</td>
<td>2.9,3.5</td>
<td>2</td>
</tr>
<tr>
<td>AVP (10^{-10} mol/L)</td>
<td>3.93±0.88</td>
<td>3.17±1.11</td>
<td>4</td>
</tr>
<tr>
<td>AVP (10^{-7} mol/L)</td>
<td>1.8,4.2</td>
<td>2.3,5.1</td>
<td>2</td>
</tr>
<tr>
<td>OAG (10^{-4} mol/L)</td>
<td>1.4,2.6</td>
<td>2.6,2.4</td>
<td>2</td>
</tr>
<tr>
<td>PMA (10^{-8} mol/L)</td>
<td>1.98±0.59</td>
<td>2.03±0.56</td>
<td>5</td>
</tr>
<tr>
<td>PMA (10^{-7} mol/L)</td>
<td>1.98±0.59</td>
<td>2.21±0.40</td>
<td>5</td>
</tr>
<tr>
<td>Staurosporine (10^{-7} mol/L)</td>
<td>1.1,1.7</td>
<td>1.2,1.6</td>
<td>2</td>
</tr>
</tbody>
</table>

TMB-8 indicates 3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester; Ang II, angiotensin II; AVP, arginine vasopressin; OAG, 1-oleoyl-2-acetyl glycerol; and PMA, phorbol 12-myristate 13-acetate. Values listed are fold increases above basal levels. Cells were preexposed to buffer or indomethacin (2 minutes), verapamil (5 minutes), Ang II (4 minutes), AVP (4 minutes), OAG (12 minutes), PMA (12 minutes), or staurosporine (5 minutes). Then parathyroid hormone (10^{-7} mol/L, 2 minutes) responses were determined. Data are reported as responses without or with pretreatment. When more than two studies were performed, data are mean±SEM; otherwise, individual studies are reported. Values listed are stimulated/basal cyclic AMP levels. Basal levels averaged 2.5±0.1 pmol per well (n=10).

seen in Table 1, cAMP responses to PTH were unaffected by pretreatment with indomethacin.

PTH activates the phospholipase C (PLC) system in some systems. In many cell types, the adenylate cyclase and PLC signaling systems interact. We investigated the possibility of this interaction by measuring PTH-induced IP$_3$ formation as well as by pretreating cells with Ang II (10^{-7} mol/L, 4 minutes) or arginine vasopressin (AVP) (10^{-7} mol/L, 4 minutes), two known PLC agonists in VSMCs. PTH (10^{-7} to 10^{-5} mol/L, n=4) did not increase IP$_3$, whereas both AVP (152±3%, n=4) and Ang II (165±4%, n=4) increased IP$_3$ formation. Table 1 shows the effects of pretreatment with Ang II or AVP on basal and PTH-stimulated cAMP levels. The results demonstrated that PLC activation had no effect on PTH-induced increases in cAMP.

To determine whether protein kinase C (PKC) regulated PTH-induced increases in cAMP, cells were pretreated with the PKC agonist OAG (10^{-7} mol/L, 12 minutes) or PMA (10^{-6} to 10^{-7} mol/L, 12 minutes) or with the PKC antagonist staurosporine (10^{-7} mol/L, 5 minutes). Table 1 indicates that PTH-induced cAMP increases were unaffected by PKC agonists or antagonists.

G Protein Coupling

PTH receptors are coupled to effectors by both pertussis toxin-sensitive and cholera toxin-sensitive mechanisms in nonvascular tissue. To determine G protein coupling between PTH receptors and adenylate cyclase in VSMCs, we measured the effect on basal and stimulated cAMP of 16 hours of preincubation with pertussis toxin (10^{-8} to 10^{-6} g/mL) or cholera toxin (10^{-8} to 10^{-6} g/mL) (Fig 3). Pertussis toxin had no effect. In contrast, cholera toxin caused increases in basal cAMP but abrogated agonist-stimulated responses. At 10^{-6} cAMP

Fig 3. Line graph shows effect of 16-hour preincubation with pertussis toxin or cholera toxin on parathyroid hormone (PTH)-stimulated cyclic AMP (cAMP). Data are expressed as PTH-induced minus toxin-induced increases in cAMP and are mean±SEM. Isobutylmethylxanthine (0.5 mmol/L) was present with PTH. Pertussis toxin (n=2) had no effect on basal cAMP. * indicates effect of pertussis toxin on PTH (10^{-7} mol/L, 2 minutes)-stimulated cAMP (n=2). Cholera toxin (n=3) increased basal cAMP by 7.2-, 8.1-, and 9.4-fold at 10^{-8}, 10^{-7}, and 10^{-6} g/mL, respectively. • indicates effect of cholera toxin on PTH-stimulated cAMP (n=3).
Prolonged Hormone Exposure: Desensitization

Many hormone-receptor systems can be desensitized after prolonged hormonal exposure. To assess the susceptibility of PTH-stimulated cAMP production to desensitization in VSMCs, we exposed cells to varying concentrations of PTH (10⁻¹⁰ to 10⁻⁷ mol/L) for 24 hours before acute stimulation. Fig 5 shows dose-dependent losses of acute responsiveness to PTH stimulation. The time dependency of this desensitization using a threshold concentration of PTH (3×10⁻⁹ mol/L) is shown in Fig 6. After 5 hours of PTH preincubation, there was 88±4% loss of subsequent PTH responsiveness (P=.003, n=3).

To study the specificity of PTH-induced loss of adenylate cyclase responsiveness, 5-hour exposure to low-concentration PTH (3×10⁻⁹ mol/L) was followed by acute stimulation with other known adenylate cyclase agonists. These results are shown in Table 2. In contrast to the blunted effect on PTH stimulation, subsequent responses to isoproterenol (10⁻⁶ mol/L, 2 minutes) or PGE₂ (10⁻⁷ mol/L, 2 minutes) were unaffected, indicating that PTH desensitization was specific for PTH.

Because adenylate cyclase is the major signaling pathway of PTH action in VSMCs, we questioned whether protein kinase A (PKA) stimulation was responsible for the subsequent loss of adenylate cyclase responses. Cells were pretreated for 5 hours with either isoproterenol (10⁻⁶ mol/L) or the direct adenylate cyclase stimulant forskolin (10⁻⁶ mol/L) before acute PTH exposure. Fig 7 shows that neither maneuver blunted PTH responsiveness, thereby excluding desensitization by PKA.

Receptor desensitization can occur at any level in the signaling cascade, including receptors, G proteins, or adenylate cyclase. To test the latter possibility, we desensitized cells by 5 hours of PTH preincubation followed by acute exposure to forskolin (10⁻⁵ mol/L, 4 minutes). Table 2 shows that the brisk cAMP response to forskolin remained intact, demonstrating that desensitization was mediated by events proximal to adenylate cyclase. To test whether desensitization was due to alteration in G protein/adenylate cyclase coupling, we desensitized cells with PTH and then exposed them to cholera toxin (10⁻⁶ g/mL, 40 minutes). As shown in Table 2, responses to cholera toxin were not desensitized by PTH, indicating that desensitization occurred proximal to G protein/adenylate cyclase coupling.

To determine the role of receptor sequestration and loss of cell surface receptors in PTH-induced desensitization, we pretreated cells with cytochalasin D (10⁻⁷ g/mL cholera toxin, the PTH-stimulated rise of cAMP above baseline was blunted by more than 70% (P<.01 versus 0 cholera toxin). Similar responses to pertussis toxin and cholera toxin were observed with isoproterenol as the agonist (Fig 4). Pertussis toxin was without effect, and cholera toxin (10⁻⁶ g/mL) reduced isoproterenol-stimulated cAMP by 67% (P<.05 versus 0 cholera toxin). These results are consistent with Gi coupling of both the β-AR and PTH receptor to adenylate cyclase in VSMCs.
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Table 2. Effect of 5-Hour Parathyroid Hormone (3×10⁻⁸ mol/L) Exposure on Acute Response to Other Adenylate Cyclase Agonists

<table>
<thead>
<tr>
<th>Acute Stimulation</th>
<th>Response Without 5-Hour PTH</th>
<th>Response With 5-Hour PTH</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO (10⁻⁷ mol/L)</td>
<td>18, 26</td>
<td>14, 24</td>
<td>2</td>
</tr>
<tr>
<td>PGE₂ (10⁻⁵ mol/L)</td>
<td>2.8, 5.2</td>
<td>3.2, 4.4</td>
<td>2</td>
</tr>
<tr>
<td>FSK (10⁻⁵ mol/L)</td>
<td>9.13±0.75</td>
<td>8.55±0.97</td>
<td>4</td>
</tr>
<tr>
<td>CT (10⁻⁶ g/mL)</td>
<td>14.17±4.38</td>
<td>14.49±5.07</td>
<td>4</td>
</tr>
</tbody>
</table>

PTH indicates parathyroid hormone; ISO, isoproterenol; PGE₂, prostaglandin E₂; FSK, forskolin; and CT, cholera toxin. Cells were pretreated with PTH (3×10⁻⁹ mol/L, 5 hours) or buffer. Pretreatment solutions were removed, and cells were washed with incubation buffer and then exposed to acute stimulation with ISO (2 minutes), PGE₂ (2 minutes), FSK (4 minutes), or CT (40 minutes). Data are expressed as Table 1. Values listed are stimulated/basal cyclic AMP values.

Table 3. Effect of Pretreatment with Colchicine or Phenylarsine Oxide on Parathyroid Hormone-Induced Desensitization to Acute Parathyroid Hormone (10⁻⁷ mol/L, 2 Minutes) Stimulation

<table>
<thead>
<tr>
<th>5-Hour Pretreatment</th>
<th>Response</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>1.12±0.04</td>
<td>3</td>
</tr>
<tr>
<td>PTH+colchicine</td>
<td>1.17±0.20</td>
<td>3</td>
</tr>
<tr>
<td>PTH</td>
<td>0.88±0.02</td>
<td>4</td>
</tr>
<tr>
<td>PTH+PAO</td>
<td>0.89±0.12</td>
<td>4</td>
</tr>
<tr>
<td>PTH</td>
<td>1.35±0.03</td>
<td>4</td>
</tr>
<tr>
<td>PTH+cytochalasin D</td>
<td>1.42±0.06</td>
<td>4</td>
</tr>
</tbody>
</table>

PTH indicates parathyroid hormone; and PAO, phenylarsine oxide. Data are mean±SEM. Cells were pretreated with PTH (3×10⁻⁹ mol/L, 5 hours) and buffer or colchicine (10⁻⁷ mol/L), cytochalasin D (10⁻⁷ mol/L) or PAO (10⁻⁷ mol/L). Pretreatment solutions were removed, and cells were washed with incubation buffer and then exposed to acute stimulation with PTH (10⁻⁷ mol/L, 2 minutes). Data are expressed as in Table 1. Values listed are fold increases and show no significant effect of any agent.

Discussion

The major findings of our studies are that in VSMCs, PTH caused rapid time- and concentration-dependent increases in cAMP that were abrogated by cholera toxin but not pertussis toxin and were independent of the PLC, PKC, cyclooxygenase, and calcium signaling systems. PTH exposure resulted in time- and concentration-dependent homologous desensitization of cAMP responses. Desensitization appeared to be independent of PKA-, PKC-, and receptor-mediated endocytosis.

Acute Effects

Intravenous administration of PTH results in decreases in blood pressure that are mediated by reductions in vascular tone.9-23 The vasodilator effect of PTH is endothelium independent23 and mediated at least in part by increases in the cAMP content of VSMCs. Our data showing brisk PTH-mediated cAMP responses in VSMCs in culture are in agreement with other studies in VSMC cultures as well as vascular tissues such as rat aorta, rabbit aorta, bovine pulmonary artery, and rabbit renal microvessels.8,9,26,28 Of importance is the observation that the kinetics of cAMP responses to PTH were similar to those obtained with the classic vasodilator isoproterenol. Moreover, PTH appeared to be a more potent activator of cAMP in VSMCs because threshold responses to PTH were elicited at 2×10⁻⁹ mol/L compared with 5×10⁻⁸ mol/L with isoproterenol, and 50% maximal responses were obtained with 10⁻⁸ mol/L PTH and 2.4×10⁻⁷ mol/L isoproterenol. This information confirms in vivo studies demonstrating that PTH was more potent than isoproterenol in relaxing constricted vessels.30

In vivo studies suggest that the vasodilator effect of PTH is not direct but is mediated by prostaglandins. For example, administration of cyclooxygenase inhibitors blocked PTH-induced vasorelaxation, whereas in hind limb preparations, cyclooxygenase inhibitors reversed PTH antagonism of the pressor effects of norepinephrine and Ang II.4 Of interest is the possibility that the prostaglandin-dependent vasodilator effects of PTH may be endothelium dependent, because the restoration of norepinephrine responsiveness after parathyroidectomy was observed only in the presence of an intact...
endothelium.31 Because prostaglandins activate adenylate cyclase in cell culture systems, including VSMCs,12 we questioned whether PTH activation of adenylate cyclase could be prostaglandin dependent. In preliminary studies, we found that PGE2 caused time- and concentration-dependent increases in cAMP (data not shown). However, our results indicate that PTH-induced cAMP in VSMCs was independent of prostaglandin production because the cyclooxygenase inhibitor indomethacin failed to block this response (Table 1).

The vasodilator effects of PTH in vivo have been attributed to PTH-mediated reductions in cytosolic calcium. In vascular tissue, PTH has been reported to decrease calcium influx and increase calcium efflux11,12 as well as inhibit L-type calcium channels.32 In apparent contrast, increases in intracellular calcium have also been reported.33 In aorta,12 mesenteric artery, and tail artery strips,11 PTH has been reported to decrease calcium influx and increase calcium efflux. The effect of intracellular and extracellular calcium concentrations on adenylate cyclase varies among tissues and agonists. In VSMCs, we found that a calcium channel blocker, an inhibitor of intracellular calcium mobilization, as well as varying extracellular calcium from normal levels (1.25 mM mol/L) to nominally zero did not influence PTH-induced increases in cAMP formation (Table 1). These results suggest that PTH-induced cAMP is independent of extracellular calcium.

PTH activates PLC and PKC in a number of nonvascular cells.12-14 PKC activators have been found to modulate PTH/cAMP interactions in cultured renal proximal tubular epithelial cells.34 To determine the role of the PLC and PKC signaling pathways in PTH-activated adenylate cyclase activation in VSMCs, we performed a number of studies. PTH did not increase IP3 formation in VSMCs. Activation of PLC by Ang II or AVP in concentrations shown to activate PLC did not effect PTH-induced cAMP formation (Table 1). Moreover, neither activation of PKC with phorbol ester or OAG nor inhibition of PKC with staurosporine altered this response. These data indicate that in contrast to nonvascular tissue, PTH-induced cAMP stimulation in VSMCs is independent of the PLC/PKC signaling system.

PTH receptors are coupled to adenylate cyclase by G proteins in a number of tissues. In renal cortex35,36 and renal microvessels,37 PTH-stimulated cAMP production is enhanced by G nucleotide analogues. Most studies show PTH to be coupled to adenylate cyclase by Go17,18,37 In OK cells, G, dependency of PTH action has been inferred from the observation that PTH-induced adenylate cyclase activation is chola toxin sensitive.26 However, there is also evidence that PTH receptors are coupled to Gq,39 G protein requirements for PTH/adenylate cyclase coupling have not been reported in VSMCs. Fig 5 indicates that PTH-stimulated cAMP production was prevented by cholera toxin and unaffected by pertussis toxin. Therefore, the results indicate that PTH is coupled to adenylate cyclase by Gq in VSMCs.

Sustained Effects

The sustained effects of PTH on adenylate cyclase in vascular tissue have not been reported. We found time- and concentration-dependent desensitization of cAMP responsiveness to PTH, notable for its magnitude (88%) with concentrations of PTH as low as 3 × 10−9 mol/L. The desensitization was homologous, because after exposure to PTH, cAMP responses to the other agonists (isoproterenol and PGE2) were preserved (Table 2). The site of desensitization appeared to be the PTH receptor. In this regard, after desensitization, cAMP responses to forskolin and chola toxin were retained (Table 2). Because forskolin acts directly on the catalytic unit of adenylate cyclase and chola toxin acts on the G protein/catalytic site, the results are consistent with the conclusion that PTH-induced desensitization occurs proximal to G protein at the PTH receptor.

Homologous PTH desensitization of adenylate cyclase has been demonstrated in nonvascular cells.39,42 In these cells, the PTH receptor has been identified as the site of desensitization because decreases in cAMP have been associated with decreases in ligand binding. We tried to determine whether decreases in PTH binding occurred in desensitized VSMCs. However, with the ligand 125I-[Nle8,Nle14,Tyr34]-PTH used for binding to nonvascular tissues,43 we were unable to demonstrate specific PTH binding to VSMCs in culture (data not shown).

In VSMCs, homologous desensitization of some hormone systems is caused by receptor sequestration. Sequestration is especially important in the rapid desensitization of Ang II-mediated PLC responses in VSMCs.10,22 However, in contrast to Ang II-mediated desensitization of PLC responses, receptor sequestration did not appear to mediate PTH-induced desensitization of adenylate cyclase. Table 3 demonstrates that phenylarsine oxide, a trivalent sulfhydryl-modifying agent that blocks Ang II receptor internalization in VSMCs;44 colchicine, an agent that interferes with microtubule assembly;45 and cytochalasin D, a microfilament disrupting agent,19 did not abrogate PTH-induced desensitization.

We questioned whether PKA or PKC could have mediated desensitization by phosphorylating the PTH receptor. Our results do not support a role for PKA because acute PTH responsiveness was preserved after exposure to forskolin or isoproterenol in concentrations shown to markedly increase cAMP (Table 3). Moreover, it seems unlikely that PKC is involved in desensitization because PTH-dependent cAMP responses were not affected by pretreatment with the direct PKC activators PMA or OAG or with hormone agonists that activate PKC in VSMCs such as Ang II and AVP (Table 1).

In summary, the mechanism of desensitization of PTH-induced adenylate cyclase responsiveness in VSMCs appears to be independent of receptor sequestration and receptor phosphorylation by PKA- or PKC-dependent mechanisms. Although alternative explanations are possible, the mechanism of PTH-mediated desensitization may be analogous to the homologous desensitization of the β-AR system. Rapid desensitization of the adenylate cyclase response has been found to be mediated by a kinase intrinsic to the β-AR that is activated by ligand occupancy and subsequent phosphorylation of the β-AR.44

Relation to Blood Pressure

The administration of PTH results in reduction in blood pressure.4-6 The hypotensive effect of PTH is
direct on VSMC tissue because it has been demonstrated in isolated vessel preparations and is endothelium independent. Our results in VSMCs support the contention that the cellular mechanism of PTH-induced vasodilation is mediated by the adenylate cyclase signaling system. Compared with the well-recognized β-AR vasodilating system, PTH appears more potent (Fig 1). In addition, our results extend in vivo studies and demonstrate that PTH is coupled to adenylate cyclase by a chola toxin–sensitive G protein and that the coupling is independent of prostaglandins and of the PLC/PKC signaling system.

The effects of sustained PTH elevations on blood pressure are not as clear as those of acute PTH infusion. In a number of experimental models, hypertension occurs only in the presence of PTH. In human subjects, PTH infusion for 12 days was associated with hypertension. Although hyperparathyroidism is often associated with hypertension, parathyroidectomy is not invariably associated with an improvement in blood pressure. There are a number of potential explanations for this variability of responses in vivo. In addition to reducing PTH, parathyroidectomy results in reductions in parathyroid hypertensive factor, a protein implicated in hypertension. Moreover, sustained changes in PTH result in responses of other calcium regulatory hormones such as vitamin D, as well as alterations in total body calcium, phosphorus, and sodium. Many of these factors also regulate blood pressure and can mask the primary effects of PTH. Although in vitro studies do not reproduce the in vivo setting, our studies suggest a cellular mechanism that could contribute to the fact that sustained increases in PTH do not result in vasodilation. Specifically, PTH-induced desensitization of the cAMP signaling system could account for lack of vasodilation in vivo.

Acknowledgments
This work was supported by grants HL-37694, DK-53098, and DK-08465 from the National Institutes of Health, Bethesda, Md, and by the National Kidney Foundation. We thank Angela L. Garcia and Dennis Whittenburg for preparation of the manuscript.

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Hypertension. 1994;23:468-475
doi: 10.1161/01.HYP.23.4.468

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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