Pituitary adenylate cyclase-activating peptide stimulates cyclic AMP accumulation in UMR 106 osteoblast-like cells

C S Kovacs, C L Chik, B Li¹, E Karpinski¹ and A K Ho¹

Division of Endocrinology, Department of Medicine and ¹Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

(C S Kovacs is now at Endocrine Unit, Wellman 501, Massachusetts General Hospital, 50 Blossom Street, Boston, Massachusetts 02114, USA)

(Requests for offprints should be addressed to A K Ho, Department of Physiology, 726 Medical Sciences Building, University of Alberta, Edmonton, Alberta T6G 2H7, Canada)

Abstract

Pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal peptide (VIP) share 68% homology and function as neurotransmitters or neuroendocrine factors. Although VIP immunoreactivity has been detected in bone cells, the presence of PACAP or PACAP receptors in bone has not been determined. In this study, we investigated the role of PACAP and VIP in regulating cAMP accumulation in the UMR 106 osteoblast-like tumor cell line.

PACAP 27 (10⁻⁹ to 3 × 10⁻⁷ M), PACAP 38 (10⁻⁹ to 3 × 10⁻⁷ M) and VIP (10⁻⁸ to 10⁻⁶ M) stimulated cAMP accumulation up to eightfold. PACAP 27 was slightly more potent than PACAP 38, and both were tenfold more potent than VIP. Both PACAP- and VIP-stimulated cAMP accumulation were potentiated by 4β-phorbol 12-myristate 13-acetate, an activator of protein kinase C. Two PACAP antagonists, PACAP 6–27 (3 × 10⁻⁶ M) and PACAP 6–38 (3 × 10⁻⁶ M), blocked PACAP- and VIP-stimulated cAMP accumulation. Two VIP antagonists ([Lys¹,Pro²,⁵,Arg³,⁴,Tyr⁶]-VIP, and [4Cl-n-Phe⁶,Leu¹⁷]-VIP) did not reduce the PACAP- or VIP-stimulated cAMP accumulation. Pretreatment with PACAP 27, PACAP 38 or VIP equally blocked PACAP- and VIP-stimulated cAMP accumulation. These results suggest that PACAP is a more potent stimulator of cAMP accumulation than VIP in UMR 106 cells. PACAP and VIP may share a role in the paracrine or neuroendocrine regulation of bone metabolism.

Introduction

Pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal peptide (VIP) are part of a family of structurally related peptides that includes secretin, glucagon, gastric inhibitory peptide and growth hormone-releasing factor (Arimura 1992, Harmar & Lutz 1994, Rawlings 1994). PACAP was originally discovered to stimulate adenyl cyclase 1000-fold more potently than VIP in rat pituitary cells but, unlike VIP, it does not increase the release of anterior pituitary hormones in vivo or in static cell culture (Arimura 1992). Both PACAP and VIP have been detected by immunohistochemistry in the central and peripheral nervous systems, where it is thought that each function as a neurotransmitter or neuroendocrine factor (Gottschall et al. 1990, Arimura et al. 1991, Shivers et al. 1991, Rawlings 1994). PACAP and VIP share 68% homology and both activate similar G₆ protein-linked receptors that activate adenyl cyclase (Harmar & Lutz 1994, Rawlings 1994). PACAP exists mainly as a 38 amino acid, C-terminally amidated peptide (PACAP 38), but a 27 amino acid form (PACAP 27) is also found in many tissues (Harmar & Lutz 1994, Rawlings 1994). Two distinct families of PACAP-responsive receptors have been described and cloned, each comprising distinct subtypes (Ishihara et al. 1992, Lutz et al. 1993, Spengler et al. 1993). Type 1 (PACAP) receptors have been described which bind PACAP with greater affinity; these receptors are found in the central nervous system (CNS) (olfactory bulb, hippocampus, thalamus, hypothalamus, and anterior pituitary), adrenal medulla and testis (Harmar & Lutz 1994, Rawlings 1994). Type 2 (PACAP/VIP) receptors bind VIP and PACAP with equal affinity, and these receptors have been found mainly in lung, liver, spleen, intestine, and some areas of the CNS (including anterior pituitary) (Harmar & Lutz 1994, Rawlings 1994). Two separate VIP receptors have recently been cloned and VIP receptor subtypes have been classified on the basis of responsiveness to secretin, headormin and other peptides (Ishihara et al. 1992, Lutz et al. 1993, Rawlings 1994). These receptors and their respective subtypes permit PACAP and VIP to have separate physiological functions in different tissues: PACAP, acting through type 1 receptors, may regulate spermatogenesis and adrenal catecholamine synthesis (Harmar & Lutz 1994, Rawlings 1994), whereas VIP, acting through type
2 receptors, may regulate prolactin release from the anterior pituitary (Harmar & Lutz 1994, Rawlings 1994).

Although there have been no specific studies of PACAP immunoreactivity or PACAP receptor in bone, cartilage or periosteum, VIP immunoreactivity has been found in sympathetic neurons of periosteum and epiphyses of bone (Bjurholm 1991, Ahmed et al. 1994, Hohmann et al. 1986, Bjurholm et al. 1988), and binding studies with [125I]-VIP have demonstrated high-affinity receptors for VIP in certain bone tumor cell lines (Hohmann & Tashjian 1984, Bjurholm et al. 1992). VIP potently stimulates intracellular cyclic AMP (cAMP) accumulation (Hohmann et al. 1983, Kumagai et al. 1989, Bjurholm et al. 1992, Rahman et al. 1992, Suzuki et al. 1994), calcium mobilization (Hohmann et al. 1983, Fukayama et al. 1988, Kumagai et al. 1989), prostaglandin E₂ production (Rahman et al. 1992) and bone resorption (Hohmann et al. 1983) in neonatal mouse calvariae and certain bone tumor cell lines. It has been hypothesized that VIP has a role in connective tissue and bone metabolism, possibly as a neuroendocrine factor released from sympathetic nerve endings in bone (Bjurholm 1991, Rahman et al. 1992).

In this study, we found that PACAP stimulates intracellular cAMP accumulation more potently than VIP in cultured UMR 106 osteoblast-like cells, possibly by acting on a mixed population of type 1 and type 2 receptors.

Materials and Methods

Materials

UMR 106 tumor cells were obtained from the ATCC/NIH Repository (Rockville, MD, USA). PACAP 27, PACAP 38, VIP, [Lys²⁺,Pro²⁻,Arg³, Tyr⁴⁻]VIP, [4 Cl-d-Phe⁶,Leu⁷⁻]VIP and parathyroid hormone (PTH) were obtained from Peninsula Laboratories (Belmont, CA, USA), 4β-phorbol 12-myristate 13-acetate (PMA) and 3-isobutyl-1-methyl-xanthine (IBMX) were obtained from Sigma (St Louis, MO, USA), and PACAP 6–27 and PACAP 6–38 were obtained from Bachem (Torrance, CA, USA). All other drugs and chemicals were obtained from commercial sources and were of the purest grade available. [125I]–cAMP was obtained from ICN Biochemicals (Costa Mesa, CA, USA), and antibody for the RIA of cAMP was a gift from Dr A Baukal (NICHHD, NIH, Bethesda, MD, USA).

Cell culture

UMR 106 cells from the 25th to 35th passage were cultured until confluent in 75 ml flasks as monolayers in Dulbecco’s Modified Eagle’s Medium (DMEM) with fetal calf serum (10%, v/v) at 37°C in a humidified atmosphere that contained 95% air and 5% CO₂. Cells were then resuspended in DMEM with 10% fetal calf serum, and plated out in 24-well tissue culture dishes at a concentration of 25 000 cells/well. After 48-h incubation, the cells were washed once with DMEM (with 0·1% BSA) and equilibrated for 30 min in the incubator before performing the experiments.

Experimental design

Quadruplicate wells on each plate of UMR 106 cells were treated with graded concentrations of selected test drugs for 10 min unless otherwise specified. In some studies, the experiments were performed in the presence of IBMX (10⁻³ M). Drugs were dissolved at 100 × final concentration in distilled water or 300 × final concentration in dimethyl sulfoxide (DMSO) as required and then dissolved in DMEM with 0.1% BSA (pH 7.4). DMSO never exceeded a concentration of 0.3% in the final mixture of DMEM. The medium was aspirated from each quadruplicate of wells and replaced by medium containing the test drugs. Unless otherwise specified, the medium was removed and discarded after a 10-min incubation, and the cells were lysed by alternate freezing and thawing in 5 × 10⁻³ M acetic acid. Intracellular accumulation of cAMP was then measured by RIA.

RIA

The lysate was boiled for 5 min and assayed using an RIA procedure in which samples were acetylated before analysis (Harper & Brooker 1975). The results were expressed as picomoles cAMP per million cells. Since there was a small batch–to–batch variation in cAMP levels between cell preparations, all comparisons were made within the same batch of cells. The intra- and interassay variations were less than 5%, and the assay sensitivity was less than 0.2 pmol/l. Each experiment was performed at least three times.

Statistical analysis

Data are presented as mean ± s.e. of the amount of cAMP in four aliquots of cells. In the figures, error bars have been omitted wherever the standard error is less than the height of a data symbol. Statistical significance was determined using the paired Student’s t-test and ANOVA with P<0.05.

Results

Effect of PACAP and VIP on cAMP accumulation

PACAP 27 (10⁻⁹ to 3 × 10⁻⁷ M), PACAP 38 (10⁻⁹ to 3 × 10⁻⁷ M) and VIP (10⁻⁸ to 10⁻⁶ M) each stimulated cAMP accumulation up to eightfold over basal levels in a concentration-dependent manner (P<0.05 for all
PACAP and VIP stimulate cAMP in UMR 106  ·  C S Kovacs and others

Table 1 Effects of PACAP and VIP on cAMP accumulation. UMR 106 cells (25 000 cells/well) were incubated in DMEM and treated for 10 min with 10^{-7} M IBMX, with or without 10^{-7} M PACAP 27 and/or 10^{-6} M VIP. Each value represents mean ± s.e. of cAMP determinations in four samples of cells. For further details see Materials and Methods.

<table>
<thead>
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<th>Treatment</th>
<th>cAMP (pmol/million cells)</th>
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<tbody>
<tr>
<td>IBMX</td>
<td>96.5 ± 5.5</td>
</tr>
<tr>
<td>PACAP 27 + IBMX</td>
<td>846.5 ± 22.3*</td>
</tr>
<tr>
<td>VIP + IBMX</td>
<td>819.1 ± 10.8*</td>
</tr>
<tr>
<td>PACAP + VIP + IBMX</td>
<td>779.5 ± 15.5*</td>
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*P<0.05 compared with IBMX value.

than that of either agonist alone (Table 1), suggesting that the two peptides are acting through the same receptors.

**Time-course of PACAP- and VIP-stimulated cAMP accumulation**

In this experiment, cells were treated with 10^{-7} M PACAP 27 or 10^{-6} M VIP for up to 60 min in the absence of IBMX. PACAP 27 and VIP both had a rapid effect, stimulating cAMP accumulation up to eightfold within 5 min (Fig. 2a). The time-courses of PACAP 27 concentrations (Fig. 1a). PACAP 27 was slightly more potent than PACAP 38 at intermediate concentrations, and PACAP 27 was tenfold more potent than VIP (EC_{50}=10 \text{ nM} for PACAP 27, 100 \text{ nM} for VIP). Maximal cAMP accumulations stimulated by 10^{-7} M PACAP 27, 10^{-7} M PACAP 38 or 10^{-6} M VIP were similar. Since some cell lines have a high basal turnover rate of cAMP (Inukai et al. 1994), these experiments were repeated in the presence of maximal phosphodiesterase inhibition. IBMX (10^{-3} M) was added simultaneously with the peptides, and the experiment was terminated at 10 min. IBMX increased the basal cAMP accumulation eightfold, and further enhanced the PACAP 27-, PACAP 38- and VIP-stimulated cAMP accumulation up to 16-fold over basal levels in a similar concentration-dependent manner (P<0.05 for all concentrations) (Fig. 1b). The EC_{50} for PACAP 27 appeared to shift to the right in the presence of IBMX (EC_{50}=50 \text{ nM} with IBMX vs 10 \text{ nM} without); however, this difference in the EC_{50} values was not statistically significant. In separate experiments, the effect of combined maximal doses of PACAP 27 and VIP (10^{-7} M PACAP 27 and 10^{-6} M VIP) was no greater

![Figure 1](image1.png)  
**Figure 1** Effect of PACAP and VIP on cAMP accumulation. UMR 106 cells (25 000 cells/well) were incubated in DMEM and treated with graded concentrations of PACAP 27 (●), PACAP 38 (○) or VIP (■) for 10 min in the (a) absence or (b) presence of simultaneously added IBMX (10^{-3} M). Each value represents mean ± s.e. of cAMP determinations in four samples of cells. Untreated basal values of cAMP accumulation (□). For further details see Materials and Methods. *P<0.05 vs VIP at same concentration; **P<0.05 vs basal value.

![Figure 2](image2.png)  
**Figure 2** Time-course of PACAP- and VIP-stimulated cAMP accumulation. UMR 106 cells (25 000 cells/well) were incubated in DMEM and treated with PACAP 27 (10^{-7} M, ●) or VIP (10^{-6} M, ○) for up to 60 min in the (a) absence or (b) presence of IBMX (10^{-3} M). Each value represents mean ± s.e. of cAMP determinations in four samples of cells. Untreated basal values of cAMP accumulation (□). For further details see Materials and Methods.
and VIP were similar. After 20 min, the agonist-stimulated cAMP accumulation decreased, while basal cAMP accumulation slightly increased as a function of time. At times of 50 min or greater, cells treated with PACAP 27 or VIP showed no significant enhancement of cAMP accumulation compared with basal levels at those times (Fig. 2a). The time-course experiment was then repeated with the simultaneous addition of IBMX (10^{-3} M) and the agonists. Although the addition of IBMX increased the basal cAMP accumulation eightfold within 5 min, the basal cAMP accumulation did not increase further over the course of the 60-min experiment (Fig. 2b). PACAP 27 and VIP both stimulated cAMP accumulation up to 16-fold over basal levels in the presence of IBMX, with maximal effect occurring within 5 min, and this effect persisted after 60 min of treatment (Fig. 2b).

**Effect of protein kinase C (PKC) activation on PACAP- or VIP-stimulated cAMP accumulation**

Activation of PKC has been shown to potentiate the effect of VIP-stimulated cAMP accumulation (Bjurholm 1991, Rahman et al. 1992). To determine if PKC activation interacts with PACAP-stimulated cAMP accumulation, the effect of PACAP 27 and VIP was studied on cells which had been simultaneously treated with the addition of PMA (10^{-6} M), a known activator of PKC. PMA was also found to increase PACAP 27-stimulated cAMP accumulation up to 75% in a concentration-dependent manner, but did not affect basal levels of cAMP (Fig. 3). A similar dose–response curve was also obtained for the interaction of PMA with PACAP 38 or VIP (data not shown). The PMA potentiation persisted in the presence of phosphodiesterase inhibition by IBMX. In the presence of IBMX (10^{-3} M), the addition of PMA resulted in a further 30% increase in cAMP accumulation in cells treated with either PACAP 27 or VIP (data not shown).

**Interaction of PACAP and VIP with specific antagonists**

To determine if PACAP and VIP act on identical receptors, cAMP accumulation stimulated by PACAP 27, PACAP 38 or VIP was studied in the presence of 100-fold higher concentrations of known antagonists of VIP or PACAP. PACAP 6–27 has been reported to block the effects of PACAP 27, while PACAP 6–38 has been reported to be a more potent antagonist of both PACAP 27 and PACAP 38 (Robberecht et al. 1992). [Lys^{1}, Pro^{2,5}, Arg^{3,4}, Tyr^{6}]–VIP and [Lys^{1}, Pro^{2,5}, Arg^{3,4}, Tyr^{6}]–VIP have been reported to antagonize the effect of VIP in other cells (Pandol et al. 1986, Gozes et al. 1989). In these experiments, cells were pretreated with a specific antagonist (3 × 10^{-6} M) or DMEM alone for 30 min. This was followed by treatment with the agonists (VIP, PACAP 27 and PACAP 38) with or without the same antagonist as appropriate. Treatment with each of the antagonists alone for 30 min did not affect basal levels of cAMP accumulation (Table 2). Pretreatment with PACAP 6–27 (3 × 10^{-6} M) reduced the subsequent VIP- and PACAP 27-stimulated cAMP accumulation, but did not affect the PACAP 38–stimulated response (Fig. 4). PACAP 6–38 (3 × 10^{-6} M) pretreatment resulted in a 70% decrease in the subsequent PACAP 27– and 38-stimulated and VIP-stimulated cAMP accumulation (Fig. 4). In contrast, pretreatment for 30 min with [Lys^{1}, Pro^{2,5}, Arg^{3,4}, Tyr^{6}]–VIP (3 × 10^{-6} M) or [Lys^{1}, Pro^{2,5}, Arg^{3,4}, Tyr^{6}]–VIP (3 × 10^{-6} M), both purported antagonists of VIP, did not reduce the subsequent PACAP– or VIP-stimulated cAMP accumulation (Fig. 4). Neither VIP antagonists had an effect on the basal levels of cAMP accumulation (Table 2).

**Desensitization of PACAP- and VIP-stimulated cAMP accumulation**

In order to further determine if PACAP and VIP activate the same receptor in UMR 106 cells, we determined the effect of prior treatment with PACAP or VIP on the subsequent PACAP- and VIP-stimulated cAMP accumulation. PTH, which is known to activate a different receptor from PACAP or VIP (Kronenberg 1993), and forskolin, which is known to stimulate adenyl cyclase directly (Seamon & Daly 1986), were selected as controls for heterologous desensitization. In these experiments, UMR 106 cells were pretreated for 2 h with one of DMEM alone, PACAP 27 (10^{-7} M), PACAP 38 (10^{-7} M) or VIP (10^{-6} M). Quadruplicate wells of each plate were subsequently treated for 10 min with DMEM alone, PACAP 27 (10^{-7} M), PACAP 38 (10^{-7} M), VIP (10^{-6} M), PTH (10^{-7} M) or forskolin (10^{-5} M). VIP

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<th>cAMP (pmol/million cells)</th>
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<tr>
<td>IBMX</td>
</tr>
<tr>
<td>77.8 ± 5.3</td>
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<tr>
<td>PACAP 27 + IBMX</td>
</tr>
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<td>51.7 ± 2.2</td>
</tr>
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<td>PACAP 6–27 + IBMX</td>
</tr>
<tr>
<td>57.6 ± 10.9</td>
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<tr>
<td>PACAP 6–38 + IBMX</td>
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<tr>
<td>73.2 ± 7.8</td>
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<td>IBMX + VIP + IBMX</td>
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<tr>
<td>45.4 ± 7.1</td>
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<td>IBMX + VIP</td>
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<tr>
<td>341.6 ± 12.4</td>
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<tr>
<td>[Lys^{1}, Pro^{2,5}, Arg^{3,4}, Tyr^{6}]–VIP + IBMX</td>
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<tr>
<td>47.0 ± 11.1</td>
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<tr>
<td>[4 CI-O-Phe^{5}, Leu^{7}]–VIP + IBMX</td>
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<td>47.1 ± 3.9</td>
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*p<0.05 compared with IBMX value.

Table 2 Effects of PACAP and VIP receptor antagonists on basal cAMP accumulation. UMR 106 cells (25,000 cells/well) were incubated in DMEM and treated for 10 min with 10^{-3} M IBMX in the presence or absence of 10^{-7} M PACAP 27, 10^{-6} M VIP or 3 × 10^{-6} M of the PACAP or VIP receptor antagonists as indicated. Each value represents mean ± S.E. of cAMP determinations in four samples of cells. For further details see Materials and Methods.
pretreatment reduced the subsequent VIP-, PACAP 27- or PACAP 38-stimulated cAMP accumulation to basal levels, but did not affect the PTH or forskolin-stimulated responses (Fig. 5). Similarly, pretreatment with PACAP 27 or 38 also reduced the PACAP- and VIP-stimulated cAMP accumulation to basal, but did not affect the PTH- or forskolin-stimulated responses (Fig. 5). In additional experiments, pretreatment of plates with PTH (10^{-7} M) reduced the PTH-stimulated cAMP accumulation by more than 50%, while prior activation of adenyl cyclase by forskolin did not reduce the subsequent PTH-, VIP- or PACAP-stimulated responses (data not shown).

Discussion

In this study, the effect of PACAP on intracellular cAMP accumulation in UMR 106 cells was demonstrated and compared with the actions of VIP. PACAP 27 and 38 (10^{-7} M) were as equipotent as VIP (10^{-6} M) and caused an eightfold increase in cAMP accumulation. When the EC_{50} values of these peptides were compared, the EC_{50} value for PACAP 27 was 10 nM, which was slightly more potent than PACAP 38, and approximately tenfold more potent than that of VIP. This pattern of response for PACAP and VIP was similar to those found in MC3T3-E1 cells, a non-transformed murine calvarial cell line (Suzuki et al. 1994). A similar potency of VIP alone on cAMP accumulation in the UMR and other osteoblast-like cells has also been reported (Hohmann et al. 1983, Kumagai et al. 1989, Bjurholm et al. 1992, Rahman et al. 1992, Suzuki et al. 1994). Although the maximal response with PACAP 27 and PACAP 38 was an eightfold increase in cAMP accumulation, in the presence of IBMX (a phosphodiesterase inhibitor) the fold increase was much larger, suggesting that the basal cAMP turnover rate in this cell line was high. This was confirmed in the time-course study, which showed that in the presence of IBMX, both PACAP 27 and VIP caused a maximal increase in cAMP accumulation within 5 min that persisted for at least an hour. In contrast, in the absence of IBMX, the PACAP 27- and VIP-stimulated cAMP accumulation peaked around 20 min and returned to basal after 60 min.

Receptors for PACAP and VIP have been identified and classified into two main categories: type 1 (PACAP) receptors, which bind PACAP with higher affinity than VIP, and type 2 (PACAP/VIP) receptors, which bind PACAP and VIP with equal affinity (Harmar & Lutz 1994, Rawlings 1994). Another approach that has been used to discriminate the type of PACAP receptors present is the antagonistic properties of VIP and PACAP antagonists (Christophe 1993). While PACAP antagonists are effective in inhibiting the response mediated by type 1 and type 2 receptors, VIP antagonists should be effective against type 2 receptors. Compatible with the presence of type 1 receptors in UMR 106 cells, PACAP was tenfold more potent than VIP. In addition, PACAP 6–38, a potent PACAP antagonist (Robberecht et al. 1992), was effective in inhibiting the PACAP 27–, PACAP 38– and VIP-stimulated cAMP accumulation in this cell line. Furthermore, PACAP 6–27, a less potent antagonist, was effective in blocking PACAP 27– and VIP-stimulated but not PACAP 38–stimulated cAMP accumulation, consistent with the reported activity of PACAP 6–27 in other cells (Robberecht et al. 1992). However, studies with the VIP antagonists, [Lys^1,Pro^{2,5},Arg^{3,4},Tyr^6]–VIP and [4-Cl-D-Phe^{3},Leu^{17}]–VIP (Pandol et al. 1986, Gozes et al. 1989), were inconclusive since neither of the VIP antagonists were effective in inhibiting the VIP-stimulated cAMP accumulation, and they also had no effect on the PACAP 27– and PACAP 38–stimulated cAMP accumulation. In contrast, a recent study of murine MC3T3-E1 osteoblast-like cells demonstrated that [Lys^1,Pro^{2,5},Arg^{3,4},Tyr^6]–VIP blocked VIP-stimulated but not PACAP-stimulated cAMP accumulation in that cell type (Suzuki et al. 1994). One possible explanation for the lack of effect of the VIP antagonists in our studies is that UMR 106 osteoblast-like cells (obtained from a rat osteosarcoma cell line) may express different receptors from MC3T3-E1 osteoblast-like cells (obtained from a non-transformed murine calvarial cell line) (Bjurholm et al. 1992). For example, MC3T3-E1 osteoblast-like cells...
Figure 4 Interaction of PACAP and VIP with specific receptor antagonists. UMR 106 cells (25,000 cells/well) were incubated in DMEM and pretreated for 30 min as indicated with DMEM alone, PACAP6-27 (6-27; 3 x 10^-6 M), PACAP 6-38 (6-38; 3 x 10^-7 M), [Lys',Pro2-5,Arg3',Tyr6']-VIP (B-1; 3 x 10^-6 M) or |4Cl|-[Phe6,Leu17]-VIP (B-2; 3 x 10^-7 M). This was followed by subsequent treatment for 10 min with IBMX (10^-5 M) combined with (a) VIP (3 x 10^-8 M), (b) PACAP 27 (P27; 3 x 10^-8 M) or (c) PACAP 38 (P38; 3 x 10^-8 M) plus the same receptor antagonist used in the earlier treatment. Each value represents mean ± SE of cAMP determinations in four samples of cells. For further details see Materials and Methods.

*P<0.05 compared with corresponding treatment without receptor antagonist.
are normally responsive to calcitonin while most UMR 106 cells are not (Iida-Klein et al. 1992, Kobayashi et al. 1994). Furthermore, the use of VIP receptor antagonists to discriminate the type of receptor present is problematic at best because each VIP antagonist has been reported to have markedly different potency and effects in different tissues elucidated (Gozes et al. 1991, Rahman et al. 1992, Burleigh & Kirkham 1993, Simonneau et al. 1993, Fishbein et al. 1994), suggesting that these antagonists only act on specific subtypes of VIP receptors. The type of PACAP/VIP receptors present in UMR 106 cells could not be identified based on these studies with VIP and PACAP antagonists.

The profile of VIP- versus PACAP-stimulated cAMP accumulation was further examined in the presence of a PKC activator or prior exposure of VIP and PACAP. Activation of the PKC pathway by PMA had a similar dose-dependent enhancing effect on PACAP- or VIP-stimulated cAMP accumulation. In the desensitization study, VIP was effective in reducing the subsequent stimulation by VIP, PACAP 27 and PACAP 38 but not PTH or forskolin. Similarly, PACAP 27 and PACAP 38 were also effective in reducing the subsequent stimulation by VIP, PACAP 27 and PACAP 38, suggesting that VIP and PACAP may be acting on a similar receptor in UMR 106 cells. Furthermore, the effect of PACAP 27 and VIP on cAMP accumulation was not additive. Therefore, functionally, VIP and PACAP were similar in their actions on UMR 106 cells since they were effective stimulators of cAMP accumulation, interacted in a similar manner with activators of PKC, and each of the peptides was similarly effective in the desensitization studies, suggesting that these peptides were interacting with type 2 PACAP receptors. However, studies with the VIP and PACAP antagonists failed to discriminate between the types of receptors present since VIP antagonists were ineffective in blocking the VIP response. The presence of type 1 PACAP receptors could not be excluded since PACAP was tenfold more potent than VIP in stimulating cAMP accumulation.
Osteoclast activity and osteoclast-mediated bone resorption has been traditionally understood to be regulated by PTH, 1,25-dihydroxyvitamin D and calcitonin (Raisz 1992, Mundy 1993). Recently other factors (including interleukin-1, lymphotixin, γ-interferon, prostaglandins and leukotrienes, among others) have been found to affect osteoclast activity or bone resorption in various in vivo or in vitro systems (Raisz 1992, Mundy 1993). More recently the demonstration of VIP immunoreactivity in nerves that innervate bone, periosteum and cartilage, and VIP-stimulated responses in various types of bone cells, led to the hypothesis that VIP is an important neuroendocrine regulator of osteoclast-mediated bone resorption and possibly other aspects of bone metabolism (Bjurholm 1991, Bjurholm et al. 1992, Rahman et al. 1992). The findings from this study and other recent reports of PACAP activity in intact bone, isolated osteoblasts and bone tumor cells (Lerner et al. 1994, Suzuki et al. 1994) indicate that PACAP is more potent than VIP in stimulating cAMP accumulation. PACAP may therefore have a neuroendocrine or paracrine function in bone, acting through receptors that are partly shared with VIP.

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