Calcif Tissue Int (1994) 54:284–289

Calcified
Tissue
International

Helodermin, Helospectin, and PACAP Stimulate Cyclic AMP Formation in Intact Bone, Isolated Osteoblasts, and Osteoblastic Cell Lines

U. H. Lerner,[1] P. Lundberg,[1], M. Ransjö,[1,2] P. Persson,[3] R. Håkanson[3]

[1]Department of Oral Cell Biology, University of Umeå, Umeå, Sweden
[2]Department of Orthodontics, University of Umeå, Umeå, Sweden
[3]Department of Pharmacology, University of Lund, Lund, Sweden

Received: 14 June 1993 / Accepted: 21 October 1993

Abstract. Helodermin and helospectin are peptides structurally similar to vasoactive intestinal polypeptide (VIP) which were recently isolated from the salivary gland venom of the lizard *Heloderma suspectum*. Pituitary adenylate cyclase-activating polypeptide (PACAP) has been isolated from ovine hypothalamus and also shows sequence homology to VIP. A helodermin-like peptide has been detected by combined immunohistochemical and immunochemical techniques in the thyroid C-cells. In the present study, lizard helodermin was found to cause a time- and dose-dependent stimulation of cyclic AMP (cAMP) formation in neonatal mouse calvarial bones. Also, helospectin I, PACAP 27, and the C-terminally extended PACAP 38 stimulated cAMP accumulation in the mouse calvariae. The cAMP rise in response to helodermin was comparable to that induced by VIP, both in terms of potency and magnitude of the response. Helodermin, helospectin I, PACAP 27, and PACAP 38, at concentrations of 1 μmol/liter, stimulated cAMP accumulation in enzymatically isolated mouse calvarial bone cells. A significant response to all peptides was observed in both early and late released bone cells isolated from the calvariae, with low and high alkaline phosphatase activity, respectively. Helodermin and VIP stimulated cAMP accumulation in the cloned mouse calvarial osteoblastic cell line MC3T3-E1, in rat (UMR 106-01), and human (Saos-2) osteoblastic osteosarcoma cell lines, but not in the rat osteosarcoma cell line ROS 17/2.8. The effect of helodermin was synergistically and dose-dependently enhanced by forskolin (0.1 and 1 μmol/liter). These data show that bone cells, including osteoblasts, respond to several peptides of the VIP family, including helodermin, helospectin I, PACAP 27, and PACAP 38. Whether the responses are mediated via one or several receptor populations remains to be established. The finding that VIP and helodermin, at maximally effective concentrations, did not cause additive effect on cAMP formation in intact mouse calvariae suggests that these two agonists may use a common receptor.

Key words: Helodermin – Bone – Cyclic AMP

Helodermin is an amidated 35-amino acid peptide that has been isolated from the salivary gland venom of the lizard Gila monster (*Heloderma suspectum*) [1]. Helospectin I and II are 38- and 37-amino acid peptides, respectively, also isolated from the salivary gland venom of *Heloderma suspectum* [2]. Helodermin and the helospectins show structural similarities with peptides belonging to the vasoactive intestinal polypeptide (VIP)/secretin family. A novel bioactive peptide, pituitary adenylate cyclase-activating polypeptide (PACAP), was recently isolated from ovine hypothalamus [3]. This peptide is present in two amidated forms, one consisting of 38 and the other of 27 amino acid residues, both showing sequence homology to VIP [4]. Like secretin and VIP, helodermin, helospectin and PACAP cause systemic hypotension [5], pancreatic enzyme secretion [6], and stimulation of cyclic AMP (cAMP) accumulation in lymphocytes and pancreatic acini [6–8].

Using immunohistochemical and immunochemical techniques, helodermin-immunoreactive material was observed recently in the thyroid C-cells and in the noradrenaline-containing cells of the adrenal medulla in several species [9–11]. Subcutaneous injection of helodermin and VIP suppresses the uptake of calcium into the skeleton of rats [10]. If the helodermin-like C-cell constituent shares these properties with authentic helodermin, these observations suggest that thyroid C-cells, in addition to the osteoclast inhibitor calcitonin, produce another peptide with direct or indirect effects on osteoblastic activity. In the present investigation, we have therefore studied whether bone cells are equipped with receptors that recognize helodermin by analyzing the effect on cAMP accumulation in mouse calvarial bone, isolated osteoblasts, and several osteoblastic cell lines. In addition, the effects on cAMP accumulation in bone cells by helospectin and PACAP 27 and 38, three other members of the VIP family, have been studied.

Materials and Methods

Cyclic AMP Accumulation in Mouse Calvariae

Calvarial bones were dissected from 6- to 7-day-old mice and divided into halves along the sagittal suture. Then the bones were washed in Tyrode's solution and preincubated in CMRL 1066 medium (four calvarial halves per 2 ml medium) containing 0.1% bovine serum albumin and the cAMP phosphodiesterase-inhibitor rolipram (0.1 μmol/liter) [12]. The incubations were performed at 37°C and the cultures were gassed with 5% CO_2 in air. After 30 minutes, 2 ml medium with or without different test substances was added. The reactions were stopped at different time points by quickly placing the bones in 90% propanol. Cyclic AMP was extracted overnight and analyzed by a commercially available radioimmunoassay according to the instructions supplied by the manufacturers.

Correspondence to: U. H. Lerner

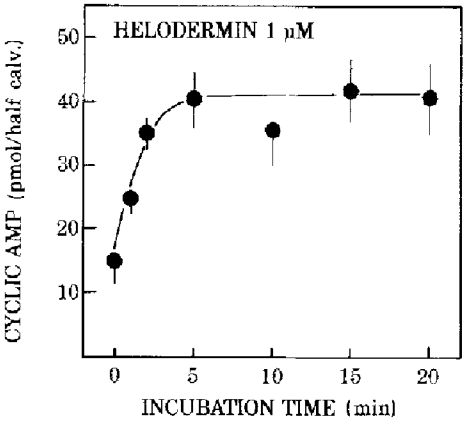

Fig. 1. Time-course showing the effect of helodermin (1 μmol/liter) on cAMP accumulation in neonatal mouse calvarial bones.

Cyclic AMP Accumulation in Isolated Osteoblasts

Bone cells were isolated from 2- to 3-day-old mice by the time sequential collagenase digestion technique described by Boonekamp et al. [13]. Cells from digestions 1-2 (early released cells) and 6-8 (late released cells) respectively were pooled, seeded in 25 cm^2 culture flasks containing α-modified Eagle's medium (MEM) with 10% fetal calf serum and incubated at 37°C in humidified atmosphere and CO_2/air (1:19). Prior to the experiments, the cells were transferred to 2 cm^2 multiwell dishes and grown to confluent cultures. Then the cells were washed extensively in serum-free medium and preincubated at 37°C in air in serum-free, Hepes-buffered α-MEM containing rolipram (0.1 μmol/liter). After 30 minutes, α-MEM with or without different test substances was added. Five minutes later, the media were withdrawn and cellular cAMP was extracted with 90% propanol. A 5-minute incubation was used as we have previously found that the effect of parathyroid hormone (PTH) on cAMP formation in mouse calvarial osteoblast-like cells is optimal at this time point and that VIP causes a cAMP response in UMR 106-01 cells which is optimal at 5 minutes [14]. Cyclic AMP was then measured by radioimmunoassay. In parallel wells, the number of cells was counted with a hemocytometer.

In separate wells, alkaline phosphatase in the cells was liberated by adding distilled water to the cell layers followed by sonification 2 × 15 seconds. The wells were centrifuged and the supernatants were used for assay of alkaline phosphatase activity at pH 10.2 with p-nitrophenylphosphate as substrate.

Cyclic AMP Accumulation in Osteoblastic Cell Lines

The human osteosarcoma cell line Saos-2 at passage 117 and the rat osteosarcoma cell line ROS 17/2.8 were provided by Drs. S. and G. Rodan, Merck, Sharp & Dohme Laboratories, West Point, NY, USA. The subclone 106-01 of the rat osteosarcoma cell line UMR at passage 8 was obtained from Prof. T. J. Martin, Melbourne, Australia. The cloned mouse calvarial cell line MC3T3-E1 at passage 8 was given to us by Prof. M. Kumegawa, Sakeda, Japan. These cell lines express several biochemical markers associated with the osteoblastic phenotype, including high levels of alkaline phosphatase, PTH-responsive adenylate cyclase and receptors for $1,25(OH)_2$-D_3 [15–18]. The cells were routinely grown in α-MEM containing 10% fetal calf serum at 37°C. Prior to the experiments, the cells were seeded in 2 cm^2 multiwell dishes and the effect of agonists on cAMP accumulation was analyzed as described above.

Materials

CMRL 1066 medium, α-MEM and fetal calf serum were purchased from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.; bovine serum albumin (fraction V, RIA-grade) from Sigma Chemical Co.; bovine synthetic PTH (PTH 1-34) from Bachem, Bubendorf, Switzerland; helodermin, helospectin I, PACAP 27 and PACAP 38 from Peninsula Laboratories Europe, Merseyside, England; porcine VIP from Prof. V. Mutt, Karolinska Institute, Stockholm, Sweden; forskolin (from *Coleus forskolii*, 7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-ene-11-one) from Calbiochem-Behring Corp. Diagnostics, La Jolla, CA, USA; bacterial collagenase (clostridium, type 1) from Worthington Biochemical, Freehold, NJ, USA, and cAMP radioimmunoassay kit from New England Nuclear/Du Pont, Dreieich, Germany. Rolipram [4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone; ZK 62.711] was kindly donated by Dr. Sprzgala of Schering AG, Berlin, Germany.

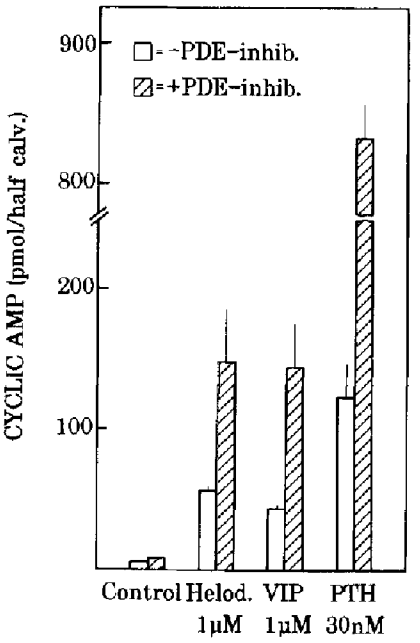

Fig. 2. Effects of helodermin (1 μmol/liter), VIP (1 μmol/liter), and PTH (30 nmol/liter) in the absence and presence of the phosphodiesterase inhibitor rolipram (0.1 μmol/liter) on cAMP accumulation in neonatal mouse calvarial bones. The effects of helodermin, VIP, and PTH, in the absence of phosphodiesterase inhibitor, were statistically significant versus untreated controls ($P < 0.05$). The effects of VIP, helodermin, and PTH, in the presence of phosphodiesterase inhibitor, were statistically significant versus the effects of the agonist in the absence of phosphodiesterase inhibitor ($P < 0.05$).

Statistics

Statistical analysis was performed using analysis of variance ANOVA.

Results

Effects in Mouse Calvariae

Helodermin (1 μmol/liter), in the presence of the phosphodiesterase inhibitor rolipram, caused a time-dependent enhancement of cAMP accumulation in mouse calvarial bones; the effect was maximal after 5 minutes (Fig. 1). A similar time-course of effect on cAMP accumulation was seen with VIP (1 μmol/liter; data not shown). A cAMP response to helodermin and VIP could also be seen in the absence of phosphodiesterase inhibitor (Fig. 2), although inhibition of

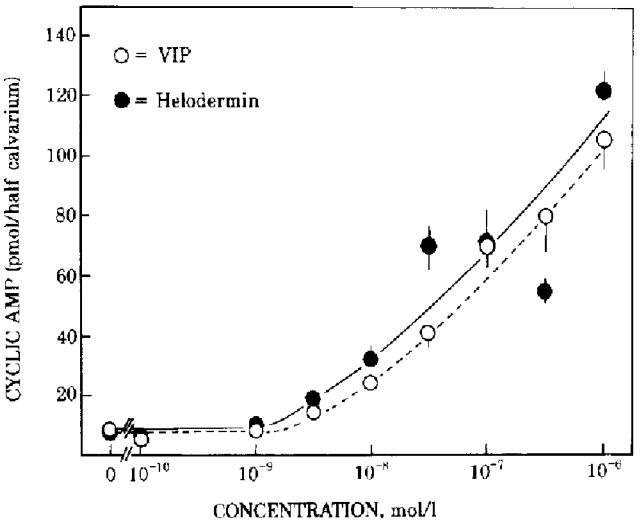

Fig. 3. Effect of helodermin and VIP, at different concentrations, on cAMP accumulation in neonatal mouse calvarial bones.

the phosphodiesterase activity with rolipram caused a two-fold enhancement of the response (Fig. 2). The stimulatory effect of helodermin at 1 μmol/liter was comparable to that induced by VIP at 1 μmol/liter, both in the absence and presence of rolipram, but less than that induced by PTH at 30 nmol/liter (Fig. 2). Helodermin, at and above a concentration of 3 nmol/liter, caused a dose-dependent accumulation of cAMP formation in mouse calvariae (Fig. 3). The response was linearly dependent on the concentration of helodermin from 3 to 1000 nmol/liter. Similarly, VIP at concentrations from 3 to 1000 nmol/liter dose-dependently enhanced the accumulation of cAMP; the magnitude of the response was identical to that induced by helodermin (Fig. 3). Table 1 demonstrates that the cAMP response to helodermin at 3 μmol/liter was the same in the presence and absence of VIP at 3 μmol/liter. VIP and helodermin at concentrations of 3 μmol/liter caused the same degree of cAMP response as that seen at 1 μmol/liter of the respective peptide (data not shown).

Indomethacin, which is a potent inhibitor of prostaglandin biosynthesis, did not affect VIP- or helodermin-induced cAMP formation in mouse calvarial bones (Table 2). Similar data were obtained using flurbiprofen as an inhibitor of prostaglandin biosynthesis (data not shown).

Helospectin I (1 μmol/liter) stimulated cAMP accumulation in neonatal mouse calvariae to the same degree as helodermin at the same concentration (Fig. 4). PACAP 27 and PACAP 38 also raised the cAMP levels in the calvarial bones; the degree of stimulation obtained by PACAP 38 was higher than that induced by helodermin (Fig. 4).

Effects in Isolated Mouse Calvarial Bone Cells

Bone cells were isolated from mouse calvariae by a time-sequential collagenase digestion technique. Cells from the two first digestions and cells released in digestions 6-8, respectively, were pooled and cultured separately. Cells released initially showed a cAMP response to both calcitonin (100 ng/ml) and PTH (30 nmol/liter) (Fig. 5). Cells released later showed a cAMP response to PTH, but not to calcitonin (Fig. 5). The PTH-induced rise in cAMP was substantially higher in cells from digestion 6-8. In contrast, both cell types

Table 1. Effect of helodermin and vasoactive intestinal polypeptide at maximal effective concentration, and their combination, on cAMP formation in mouse calvarial bones

Addition	Amount (μmol/liter)	Cyclic AMP (pmol/bone)
—	—	5.3 ± 0.3
VIP	3	40.7 ± 2.2[a]
Helodermin	3	38.4 ± 3.9[a]
+ VIP	3	33.1 ± 6.1[a]

Values are means ± SEM for six bones
[a] Significantly different from control ($P < 0.05$)

Table 2. Effect of vasoactive intestinal polypeptide and helodermin, in the absence and presence of indomethacin, on cAMP formation in mouse calvarial bones

Addition	Amount (μmol/liter)	Cyclic AMP (pmol/bone)	
		− Indo	+ Indo[a]
Expt. 1			
—	—	3.1 ± 0.4	5.1 ± 1.4
VIP	1	69.0 ± 16[b]	71.0 ± 5[c]
Expt. 2			
—	—	2.9 ± 0.1	2.1 ± 0.2
Helodermin	1	10.8 ± 1.3[b]	8.6 ± 1.9[c]

Values are means ± SEM for 3–4 bones
[a] Bones were preincubated for 30 minutes in indomethacin, added to a final concentration of 1 μmol/liter
[b] Significantly different from untreated controls ($P < 0.05$)
[c] Significantly different from controls treated with indomethacin ($P < 0.05$)

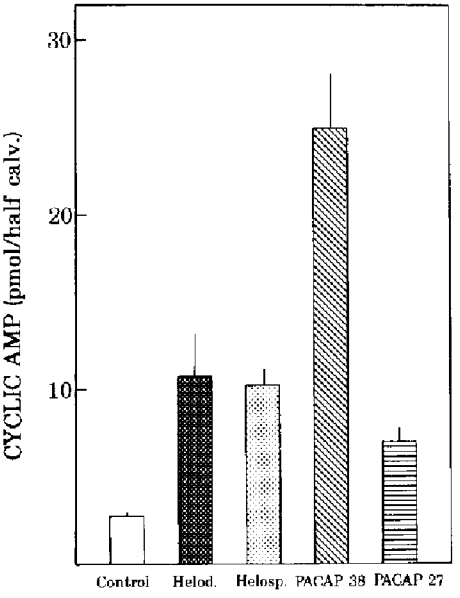

Fig. 4. Effect of helodermin, helospectin I, PACAP 38, and PACAP 27, at concentrations of 1 μmol/liter, on cAMP accumulation in neonatal mouse calvarial bones. The effects of all peptides were statistically significant versus untreated controls ($P < 0.05$).

responded to the same degree when isoprenaline (10 μmol/liter) was used as agonist. Cells released in digestion 6-8 contained higher activity of alkaline phosphatase than cells released initially (see legend to Fig. 5).

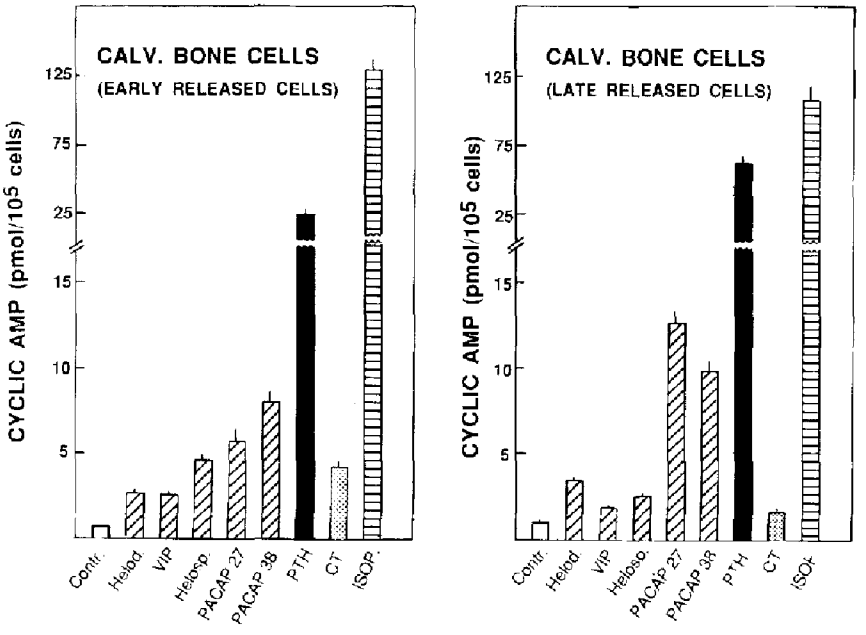

Fig. 5. Effects of helodermin (1 μmol/liter), VIP (1 μmol/liter), helospectin I (1 μmol/liter), PACAP 27 (1 μmol/liter), PACAP 38 (1 μmol/liter), PTH (30 nmol/liter), calcitonin (CT; 100 ng/ml), and isoprenaline (isop; 10 μmol/liter) on cAMP accumulation in enzymatically isolated bone cells from neonatal mouse calvariae. Cells released during digestions 1-2 showed a cAMP response to both calcitonin and PTH. Cells released during digestions 6-7 showed a cAMP response to PTH but not to calcitonin. The alkaline phosphatase activity in early and late released cells were 2.0 ± 0.5 and 4.5 ± 0.4 nmol/minute/10^6 cells (mean values ± SEM; n = 4), respectively. The effects of all agonists, with the exception of CT in late released cells, were statistically significant ($P < 0.05$).

Helodermin, VIP, helospectin I, PACAP 27, and PACAP 38, at a concentration of 1 μmol/liter, all stimulated cAMP accumulation in both early and late released mouse calvarial bone cells (Fig. 5). In early released cells, helospectin I, PACAP 27 and PACAP 38 were more effective than helodermin and VIP. In late released cells, PACAP 27 and PACAP 38 were clearly more effective than helodermin, helospectin I, and VIP.

Effects in Osteoblastic Cell Lines

In the rat osteosarcoma cell line UMR 106-01, helodermin (1 μmol/liter) caused a cAMP response that was of the same magnitude as that induced by VIP at the same concentration (Fig. 6a). Forskolin (0.1 and 1 μmol/liter) caused a small dose-dependent stimulation of the cAMP production in UMR 106-01 cells. Challenge of these cells with helodermin simultaneously with forskolin resulted in a clearcut synergistic interaction that was dependent on the concentration of forskolin. Similarly, VIP (1 μmol/liter) stimulated cAMP accumulation in UMR 106-01 cells; the stimulation was synergistically enhanced by forskolin (Fig. 6a).

In ROS 17/2.8 cells, a rat osteosarcoma cell line with osteoblastic characteristics, no effect of helodermin or VIP at a concentration of 1 μmol/liter on cAMP formation was observed, neither in the absence nor in the presence of forskolin (Fig. 6b). By contrast, PTH (100 nmol/liter) greatly stimulated cAMP formation; the response was synergistically enhanced by forskolin (data not shown).

In the mouse calvarial osteoblastic cell line MC3T3-E1, both helodermin and VIP, at a concentration of 1 μmol/liter, caused a similar cAMP response that was synergistically enhanced by forskolin (Fig. 6c). In this cell line, the magnitude of the response to helodermin and VIP was comparable to that induced by PTH at a concentration of 100 nmol/liter, both in the absence and presence of forskolin (data not shown).

In Saos-2 cells, a human osteoblastic cell line derived from an osteosarcoma removed from a young female, helodermin (1 μmol/liter) stimulated cAMP formation by a mechanism that was synergistically enhanced by forskolin (Fig. 6d). Similarly, VIP (1 μmol/liter) enhanced cAMP accumulation in Saos-2 cells. The effect of VIP was synergistically enhanced by forskolin.

Discussion

Helodermin-like peptides have been claimed to coexist with calcitonin in thyroid C-cells [9, 10]. Preliminary observations suggest that calcitonin and the helodermin-like peptide are released concomitantly into the circulation in response to hypercalcemia (Absood et al., unpublished data). Authentic helodermin, unlike calcitonin, causes a decreased uptake of radioactive calcium into the skeleton of rats [10]. The mechanism by which helodermin inhibits calcium uptake is not known but may either be due to a direct effect on osteoblasts or to an indirect effect mediated by some neuroendocrine mechanism. The fact that authentic helodermin stimulates secretion of thyroid hormones [10] suggests that the latter possibility cannot be excluded.

We demonstrate here that helodermin causes a rapid, time- and dose-dependent stimulation of cAMP formation in neonatal mouse calvarial bones. The threshold for the effect, 3 nmol/liter, is comparable to that seen in other helodermin-sensitive cells [8]. Mouse calvarial bones produce prostaglandin E_2 (PGE_2) in response to several agonists [19, 20]. To exclude the possibility that helodermin enhanced cAMP secondarily to induction of PGE_2 biosynthesis, we studied the effect of helodermin on cAMP in the absence and presence of indomethacin and flurbiprofen. These two structurally unrelated inhibitors of cyclooxygenase activity are potent inhibitors of PGE_2 formation in bone cells [21]. Indomethacin and flurbiprofen did not, however, affect helodermin- and VIP-induced cAMP accumulation, indicating that the receptors in bone that respond to helodermin (and VIP) are directly linked to adenylate cyclase. This view is also supported by the finding that helodermin and VIP enhanced cAMP formation in UMR 106-01 cells, an osteoblastic cell line that produces only minor amounts of PGE_2 [21].

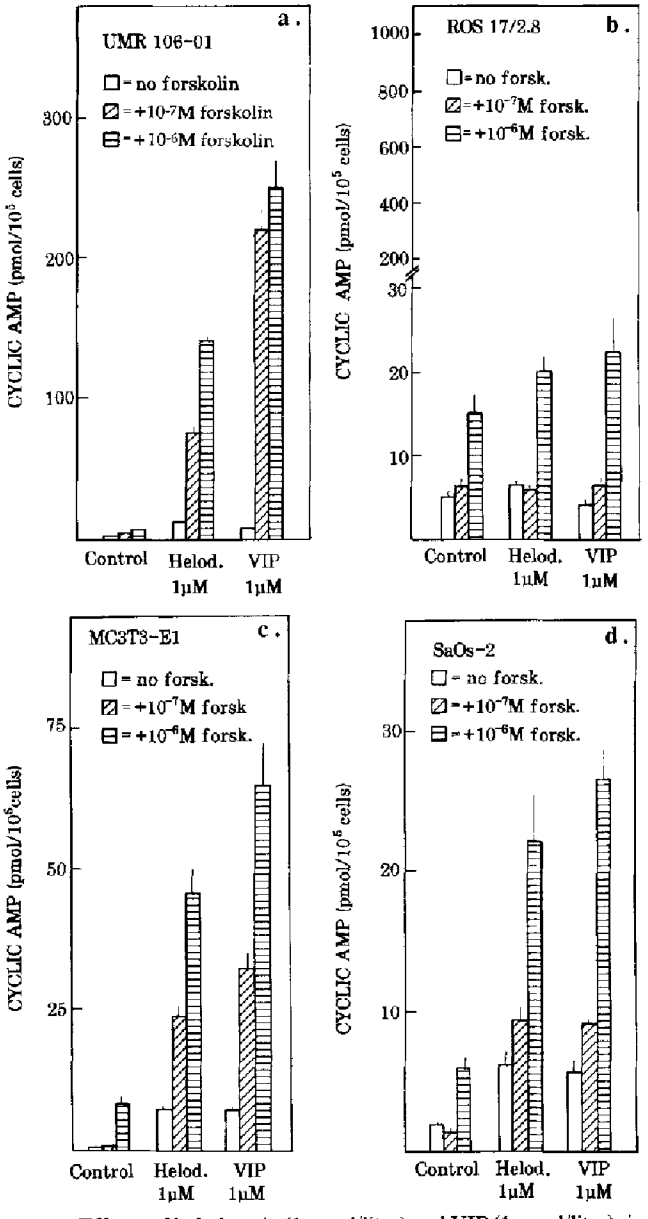

Fig. 6. Effects of helodermin (1 μmol/liter) and VIP (1 μmol/liter), in the absence and presence of forskolin (0.1 and 1 μmol/liter), on cAMP accumulation in UMR 106-01 cells (**a**), ROS 17/2.8 cells (**b**), MC3T3-E1 cells (**c**), and Saos-2 cells (**d**). The stimulatory effects of helodermin and VIP were statistically significant versus untreated controls in UMR 106-01, MC3T3-E1, and Saos-2 ($P < 0.05$).

In order to elucidate which bone cells respond to helodermin, we studied the effect of helodermin in enzymatically isolated mouse calvarial bone cells, as well as in four osteoblastic cell lines. The data show that challenge with helodermin results in stimulation of cAMP accumulation in both calvarial cells containing calcitonin-responsive cells (early released cells) with low alkaline phosphatase activity and in cells enriched in PTH-responsive cells (late released cells) with high alkaline phosphatase activity. This indicates that cells in the osteoclastic lineage as well as in the osteoblastic lineage may be equipped with receptors that recognize helodermin. Neither the early nor the late released cells, however, are pure populations of cells. We therefore looked for effects of helodermin in different osteoblastic cell lines. In the mouse calvarial osteoblastic cell line MC3T3-E1, and in a rat (UMR 106-01) and a human (Saos-2) osteosarcoma cell line, helodermin stimulated cAMP formation. No effect, however, was seen in the rat osteosarcoma cell line ROS 17/2.8. Interestingly, these cells, in contrast to the other cell lines, do not show a cAMP response to VIP, indicating that the pattern of VIP and helodermin responsiveness in the different osteoblastic cell lines is very similar [14]. Our data suggest that the presence of receptors that recognize helodermin is a phenotypic expression of human, rat, and mouse osteoblasts. In line with this suggestion, Pfeilschifter et al. [22] recently reported that helodermin stimulates cAMP accumulation in enzymatically isolated rat osteoblast-like cells. These findings indicate that the inhibitory effect of helodermin on calcium uptake into the skeleton may be due to a direct action on osteoblasts. Further evidence for this view is the recent demonstration that helodermin inhibits the incorporation of [^{3}H]-proline into bone matrix in fetal rat calvaria [22].

Helodermin displays sequence homology with the 28-amino acid residue peptide VIP. In agreement with previous data obtained in Saos-2 cells [23], we found VIP to stimulate cAMP formation in intact bone, isolated osteoblast-like cells, and helodermin-sensitive osteoblastic cell lines. Interestingly, ROS 17/2.8 cells failed to respond to either helodermin or VIP, while responding to PTH. The dose-response curve for the effect of VIP in intact bone was superimposable on that obtained after stimulation with helodermin.

Forskolin is a diterpene that exerts a direct stimulatory action on adenylate cyclase [24]. In addition, forskolin potentiates, via an interaction with GTP-binding proteins, the effect of several agonists stimulating adenylate cyclase due to interaction with specific receptors. Thus, we have previously found forskolin to stimulate cAMP accumulation in intact bone and to potentiate the action of PTH [25]. We report that the effects of helodermin and VIP are also synergistically enhanced by forskolin at low concentrations.

Helospectin I, PACAP 27, and PACAP 38 show structural similarities to VIP and helodermin and belong to the same family of peptides. Here we show that helospectin I, PACAP 27, and PACAP 38 also stimulate cAMP formation in intact bone. PACAP 38, at 1 μmol/liter, was much more effective in stimulating cAMP formation than helodermin and VIP, in line with previous observations made in rat pituitary cell cultures [3]. Similarly, in mouse calvarial bone cells enriched in osteoblast-like cells, PACAP 27 and 38 were clearly more effective than helodermin, VIP, and helospectin I. It remains to be established to what extent the various peptides of the VIP family interact with the same or with distinct receptor populations on the bone cells. The data in the present paper do not reveal whether the various peptides studied stimulate cAMP via separate receptors or via a common receptor. The finding by Pfeilschifter et al. [22] that secretin, but not VIP, compete with the binding of ^{125}I-helodermin to osteoblast-like rat calvarial cells indicates that separate receptors may exist. Our finding in mouse calvariae, however, that maximally effective concentrations of VIP and helodermin did not cause additive effect on cAMP formation, suggests that, in intact bone, VIP and helodermin may bind to a common receptor.

Acknowledgments. The author gratefully acknowledge the skillful technical assistance of Mrs. Inger Lundgren. The present study was supported by grants from the Swedish Medical Research Council (project no. 7525), the Swedish Association Against Rheumatic Diseases, the Royal 80 Year Fund of King Gustav V, Lars Hierta Foun-

dation, Tore Nilson Foundation for Medical Research, Magnus Bergvall Foundation, and Claes Groschinsky Foundation. Professor T. J. Martin, Melbourne, Australia is highly appreciated for sending UMR 106-01 cells; Drs. S. and G. Rodan, West Point, NJ, USA for giving us ROS 17/2.8 and Saos-2 cells; and Prof. M. Kumegawa, Sakede, Japan for providing the MC3T3-E1 cells.

References

1. Hoshino M, Yanaihara C, Hong Y-M, Kishida S, Katsumaru Y, Vandermeers A, Vandermeers-Piret M-C, Robberecht P, Christophe J, Yanaihara N (1984) Primary structure of helodermin, a VIP-secretin-like peptided isolated from Gila monster venom. FEBS Lett 178:233–239
2. Parker D, Raufman J-P, O'donohue T, Bledsoe M, Yoshida H, Pisano J (1984) Amino acid sequences of helospectins, new members of the glucagon superfamily, found in Gila monster venom. J Biol Chem 259:11751–11755
3. Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, Coy DH (1989) Isolation of a novel 38 residue-hypothalmic polypeptide which stimulates adenylate cyclase in pituitary cells. Biochem Biophys Res Commun 164:567–574
4. Kimura C, Ohkubo S, Ogi K, Hosoya M, Itoh Y, Onda H, Miyata A, Jiang L, Dahl RR, Stibbs HH, Arimura A, Fujino M (1990) A novel peptide which stimulates adenylate cyclase: molecular cloning and characterization of the ovine and human cDNAs. Biochem Biophys Res Commun 166:81–89
5. Konturek S, Yanaihara N, Pawlik W, Jaworek J, Scewczyk K (1989) Comparison of helodermin, VIP and PHI in pancreatic secretion and blood flow in dogs. Regul Peptides 24:155–166
6. Raufman J-P, Jensen RT, Sutliff VE, Pisano JJ, Gardner JD (1982) Actions of Gila monster venom on dispersed acini from guinea pig pancreas. Am J Physiol 242:G470–G474
7. Robberecht P, Waelbroeck M, Dehaye J-P, Winand J, Vandermeers A, Vandermeers-Piret M-C, Christophe J (1984) Evidence that helodermin, a newly extracted peptide from Gila monster venom, is a member of the secretin/VIP/PHI family of peptides with an original pattern of biological properties. FEBS Lett 166:277–281
8. Robberecht P, Waelbroeck M, De Neef P, Tastenoy M, Gourlet P, Cogniaux J, Christophe J (1988) A new type of functional VIP receptor has an affinity for helodermin in human SUP-T1 lymphoblasts. FEBS Lett 228:351–355
9. Sundler F, Christophe P, Robberecht P, Yanaihara N, Yanaihara C, Grunditz T, Håkanson R (1988) Is helodermin produced by medullary thyroid carcinoma and normal C-cells? Immunocytochemical evidence. Regul Peptides 20:83–89
10. Grunditz T, Persson P, Håkanson R, Absood A, Böttcher G, Rerup C, Sundler F (1989) Helodermin-like peptides in thyroid C cells. Stimulation of thyroid hormone secretion and suppression of calcium incorporation into bone. Proc Natl Acad Sci USA 86:1357–1361
11. Bjartell A, Persson P, Absood A, Sundler F, Håkanson R (1989) Localisation and characterization of helodermin-like material in the mammalian adrenal gland. Regul Peptides 26:27–34
12. Lerner U, Ransjö M, Fredholm BB (1986) Comparative study of the effects of cyclic nucleotide phosphodiesterase inhibitors on bone resorption and cyclic AMP formation in vitro. Biochem Pharmacol 35:4177–4189
13. Boonekamp PM, Hekkelman JW, Hamilton JW, Cohn DV, Jilka RL (1984) Effect of culture on the hormone responsiveness of bone cells isolated by an improved sequential digestion procedure. Proc Kon Ned Akan Wet B87:371–382
14. Bjurholm A, Kreicbergs A, Schultzberg M, Lerner UH (1992) Neuroendocrine regulation of cyclic AMP formation in osteoblastic cell lines (UMR 106-01, ROS 17/2.8, MC3T3-E1 and Saos-2) and primary bone cells. J Bone Miner Res 7:1011–1019
15. Partridge NC, Alcorn D, Michelangeli VP, Ryan G, Martin TJ (1983) Morphological and biochemical characterization of four clonal osteogenic sarcoma cell lines of rat origin. Cancer Res 43:4308–4314
16. Rodan SB, Imai Y, Thiede AM, Weslowski G, Thompson D, Bar-Shavit Z, Shull S, Mann K, Rodan GA (1987) Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. Cancer Res 47:4961–4966
17. Majeska RJ, Rodan SB, Rodan GA (1980) Parathyroid hormone-responsive clonal cell lines from rat osteosarcoma. Endocrinology 107:1494–1503
18. Sudo H, Kodama H-A, Amagai Y, Yamamoto S, Kasai K (1983) In vitro differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria. J Cell Biol 96:191–198
19. Voelkel EF, Tashjian AH Jr, Levine L (1980) Cyclooxygenase products of arachidonic acid metabolism by mouse bone in organ culture. Biochim Biophys Acta 620:418–428
20. Ljunggren Ö, Lerner UH (1989) Parathyroid hormone stimulates prostanoid formation in mouse calvarial bones. Acta Endocrinol (Copenh) 120:357–361
21. Lerner UH, Ransjö M, Ljunggren Ö (1989) Bradykinin stimulates production of prostaglandin E2 and prostacyclin in murine osteoblasts. Bone Miner 5:139–154
22. Pfeilschifter J, Naumann A, Oechsner M, Ziegler R (1990) Effects of helodermin on fetal rat bone metabolism in vitro. Biochem Biophys Res Commun 170:576–581
23. Hohmann EL, Tashjian AH Jr (1984) Functional receptors for vasoactive intestinal peptide on human osteosarcoma cells. Endocrinology 114:1321–1327
24. Daly JW (1984) Forskolin, adenylate cyclase and cell physiology: an overview. Adv Cyclic Nucl Res 17:81–89
25. Lerner UH, Ransjö M, Sahlberg K, Ljunggren Ö, Fredholm BB (1989) Forskolin sensitizes parathyroid hormone-induced cyclic AMP response, but not the bone resorptive effect, in mouse calvarial bones. Bone Miner 5:169–181