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Pituitary Adenylate Cyclase Activating Peptide Receptors Regulate the Growth of Non-Small Cell Lung Cancer Cells

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Abstract

We have identified pituitary adenylate cyclase activating peptide (PACAP) receptors on small cell lung cancer cell line NCI-N417 in a previous study. In this study, the role of PACAP in the growth and signal transduction of non-small cell lung cancer cells was investigated. Northern blot analysis with a full-length human PACAP receptor cDNA probe revealed a major 7.5-kb hybridizing transcript when total RNA extracted from NCI-H838 cells was used. PACAP bound with high affinity ($K_d = 1$ nM) to a single class of sites ($B_{max} = 14,000/\text{cell}$) when NCI-H838 cells were used. Specific ¹²⁵I-labeled PACAP binding was inhibited with high affinity by PACAP-27 and PACAP-38, with moderate affinity by PACAP(6–38), and with low affinity by vasoactive intestinal polypeptide, PACAP(28–38), and PACAP(16–38). PACAP-27 elevated cAMP in a dose-dependent

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manner, and the increase in cAMP caused by PACAP was reversed by PACAP(6–38). PACAP-27, but not vasoactive intestinal polypeptide, elevated cytosolic Ca^{2+} in individual NCI-H838 cells. PACAP-27 stimulated arachidonic acid release, and the increase caused by PACAP was reversed by PACAP(6–38). PACAP-27 stimulated colony formation in NCI-H838 cells, whereas the PACAP antagonist PACAP(6–38) reduced colony formation in the absence or presence of exogenous PACAP-27. In nude mice bearing NCI-H838 xenografts, PACAP(6–38) slowed tumor growth significantly. These data suggest that biologically active type 1 PACAP receptors are present on human non-small cell lung cancer cells, which exhibit dual signal transduction pathways and regulate cell proliferation.

INTRODUCTION

PACAP⁴ and VIP are 27- and 28-amino acid peptides that have 65% sequence homology and are related to the larger family of peptide hormones, which includes secretin, glucagon, calcitonin, parathyroid hormone, and glucagon-like peptide 1 (1, 2). PACAP occurs as two biologically active peptides, PACAP-27 and PACAP-38, that share the same 27 NH_2 -terminal amino acids. Early radioligand binding studies have suggested the existence of distinct high-affinity receptors for PACAP, VIP, and helodermin (3–6). VIP_1 , VIP_2 , and type 1 PACAP receptors that have 459, 430, and 495 amino acids, respectively, have been cloned previously (7–10). Each of the receptors has seven transmembrane domains and binds PACAP with high affinity; however, only the VIP_1 and VIP_2 receptors, and not PACAP type 1 receptor, bind VIP with high affinity.

VIP is derived from a 170-amino acid precursor protein (11, 12). The presence of VIP and VIP receptor on specific NSCLC cell lines has been shown by VIP immunoreactivity, as well as by radioligand binding studies using ¹²⁵I-labeled VIP (13). In addition, Northern blot analysis has confirmed the specific expression of VIP, as well as that of VIP_1 receptor, mRNA in these cells. Using NCI-H727 cells, VIP agonist stimulation elevates cAMP. The stimulated increase in adenylate cyclase activity and colony formation can be antagonized by VIP hybrid (14). VIP hybrid slows NSCLC xenograft formation in nude mice *in vivo* (14).

The type 1 PACAP receptor has recently been cloned in rats and humans, and the deduced nucleotide sequence encodes a 495-amino acid protein that shares 45% homology with the VIP_1 receptor (15). Unlike the VIP receptors, however, PACAP receptors have been shown in several cell lines to have a dual cascade of intracellular responses that include activation of adenylate cyclase and phospholipase C. Previously, we found that PACAP bound with high affinity to SCLC cell line NCI-N417 (16). Specific ¹²⁵I-PACAP-27 binding to NCI-N417 was inhibited with high affinity by PACAP-27 and the COOH-terminally extended peptide PACAP-38 and with lower affinity by the truncated versions of the peptide [PACAP(6–38) and PACAP(16–38)]. PACAP-27 induced stimulation of adenylate cyclase, as well as PI turnover, an effect that was antagonized by PACAP(6–38). PACAP-27 and PACAP-38 stimulated colony formation *in vitro*, an effect that also was antagonized by

⁴The abbreviations used are: PACAP, pituitary adenylate cyclase activating peptide; VIP, vasoactive intestinal peptide; NSCLC, non-small cell lung cancer; PI, phosphatidylinositol; SCLC, small cell lung cancer; SIT, 3×10^{-8} M Na_2SeO_3 , 5 $\mu\text{g}/\text{ml}$ insulin, and 10 $\mu\text{g}/\text{ml}$ transferrin; AA, [³H]arachidonic acid.

PACAP(6–38). Because of its effects in SCLC cells, in this study, we investigated the effects of PACAP on NSCLC cells. We report that PACAP-induced receptor activation is coupled to an increase in intracellular Ca^{2+} , cAMP, and proliferation in NSCLC cells.

MATERIALS AND METHODS

Cell Culture.

NSCLC cells were cultured in RPMI 1640 containing 10% heat-inactivated fetal bovine serum. When a monolayer had formed, the adherent cells were washed with PBS and treated with trypsin/EDTA. The cells were pelleted and resuspended in serum-supplemented medium and incubated at 37°C in 5% CO_2 /95% air. The cells were studied during their exponential growth phase and were mycoplasma free (17).

Receptor Binding.

The binding assays were conducted using ^{125}I -PACAP-27 (2200 Ci/mmol). NSCLC cells (cell line, NCI-H838; 5×10^4) were placed in 24-well plates coated with human fibronectin (20 μg). When a monolayer of cells had formed, the cells were washed four times with SIT medium and then incubated in receptor-binding medium (RPMI 1640 containing SIT medium plus 1% BSA and 1 mg/ml bacitracin). The cells were incubated with ^{125}I -PACAP-27 for 30 min at 37°C. Next, they were washed four times in receptor-binding medium at 4°C. The cells that contained bound peptide were dissolved in 0.2 N NaOH and counted in a γ -counter.

Northern Blot Analysis.

For Northern blot analysis, NSCLC cells were cultured with SIT medium containing 0.5% fetal bovine serum. Cells were washed in PBS and frozen in liquid nitrogen. Total RNA was isolated using the guanidinium isothiocyanate method (18). Ten μg of total RNA were separated in a 0.66-M formaldehyde 1% agarose gel (19). The RNA was blotted onto a Nytran membrane overnight, and the membrane was hybridized with full-length cDNA probes labeled with ^{32}P [dCTP] using a Bethesda Research Laboratories random priming kit (BRL). The membrane was exposed and analyzed using a Molecular Dynamics Phosphorimager.

cAMP.

Cyclic AMP was assayed by RIA (20). The cell line NCI-H838 was harvested and resuspended in SIT medium containing 1% BSA, 1 mg/ml bacitracin, and 100 μM isobutylmethyl-xanthine. After 5 min, the reaction was quenched by the addition of an equal volume (0.5 ml) of ethanol. The samples were mixed and frozen at -80°C until assay.

Cytosolic Ca^{2+} .

Cytosolic Ca^{2+} was assayed in individual NCI-H838 cells. NCI-H838 cells (10^4) were cultured on fibronectin-treated Lab-Tek coverslip chambers. The cells were fed 1 day before the experiment, and, on the day of the experiment, the cells were rinsed with 1 ml of buffer (150 mM NaCl, 1 mM MgCl_2 , 5 mM KCl, 10 mM glucose, 1 mM CaCl_2 , and 20 mM

HEPES/NaOH (pH 7.4) containing 1% BSA). After 5 min at 37°C, the buffer was removed, and new buffer containing 5 μ M Indo-1AM was added. The cells were incubated for 30 min at 37°C, the old buffer was removed, and the cells were treated with new CaCl₂-free buffer. After 5 min at 37°C, the cells were treated with new buffer and assayed for Ca²⁺ using an ACAS 570 Interactive Laser Cytometer (Meridian Instruments). The excitation wavelength was 320 nm, and the fluorescence emission was monitored at 485 nm (Ca²⁺-free emission) and 405 nm (Ca²⁺-bound emission). The emission ratio was determined every 30 s before and after the addition of peptide.

Arachidonic Acid Release.

NSCLC cells (5×10^4) were placed in 24-well plates coated with human fibronectin (20 μ g). After a monolayer of cells had formed (5 days), ([³H]5, 6, 8, 9, 11, 12, 14, 15)arachidonic acid (2.5×10^5 cpm) was added (21). After 16 h, the cells were washed twice in 1 ml of SIT medium containing 0.2% fatty acid-free BSA. New medium that contained PACAP-like peptides was added. After 40 min, 100 μ l of medium was removed from each well, placed in a scintillation vial, scintillation fluid added and sample counted in a β -counter (21).

Proliferation Assays.

Growth studies were performed *in vitro* using NCI-H838 cells and the agarose cloning system described previously (22). The base layer consisted of 3 ml of 0.5% agarose in SIT medium containing 5% fetal bovine serum in 6-well plates. The top layer consisted of 3 ml of SIT medium in 0.3% agarose, PACAP-like peptides, and 5×10^4 single viable cells. For each cell line and peptide concentration, triplicate wells were plated. After 2 weeks, 1 ml of 0.1% *p*-iodonitrotetrazolium violet was added, and, after 16 h at 37°C, the plates were screened for colony formation; the number of colonies larger than 50 μ m in diameter were counted using an Omnicon image analysis system.

Growth was assessed *in vivo* using nude mice bearing NSCLC xenografts. Female athymic BALB/c nude mice, 4–5 weeks old, were housed in a pathogen-free, temperature-controlled isolation room and fed autoclaved rodent food and autoclaved water *ad libitum*. NCI-H838 cells (1×10^7) were injected into the right flank of each mouse by s.c. injection. Palpable tumors were observed in approximately 90% of the mice after 2 weeks, and PBS (100 μ l) or PACAP(6–38) (10 μ g/day s.c.) was injected during weeks 2–6. The tumor volume (height \times width \times depth) was determined weekly by using calipers and was recorded. When the tumor became necrotic, the growth studies were terminated.

RESULTS

Receptor binding.

To evaluate NSCLC cell lines for the presence of PACAP type 1 receptor-specific binding sites, several cell lines were studied, as shown in Table 1. ¹²⁵I-PACAP-27 bound with high affinity in all cell lines tested, suggesting that all of the cell lines possessed PACAP receptors. The specific:nonspecific binding ratio ranged from 2:1 to 4:1, depending on the cell line used. Because cell line NCI-H838 bound ¹²⁵I-PACAP-27 best, it was used in subsequent studies.

^{125}I -PACAP-27 bound with high affinity to NSCLC cell line NCI-H838 in a dose-dependent manner (Fig. 1). Binding was a linear function of PACAP concentration at low doses (<1 nM), whereas binding appeared to saturate at high doses (10 nM). A Scatchard plot of the specific binding data was linear (Fig. 2). PACAP bound with high affinity ($K_d = 1$ nM) to a single class of sites ($B_{\text{max}} = 14,000/\text{cell}$).

The specificity of binding was investigated. Fig. 3 shows that specific ^{125}I -PACAP binding was inhibited slightly by 0.1 nM PACAP-27 and strongly by 1000 nM PACAP-38. The concentration of PACAP-27 required to inhibit 50% of the specific ^{125}I -PACAP-27 binding (IC_{50}) was 1 nM. Because the dose-response curve was shallow, it is possible that PACAP-27 may bind with low affinity to an additional class of sites. PACAP-38 and PACAP(6–38) were less potent; they had IC_{50} values of 3 and 20 nM, respectively. VIP, PACAP(28–38), and PACAP(16–38) did not significantly inhibit ^{125}I -PACAP binding at a 1000-nM dose. Similar binding data were obtained using cell line NCI-H1299 or H727 (data not shown).

Northern Blot Analysis.

To further confirm the presence of PACAP type 1 receptor mRNA in NSCLC cells, high-stringency Northern blot analysis of the total RNA was performed. A full-length, ^{32}P -labeled, human, type 1 PACAP receptor cDNA probe hybridized to a single 7.5-kb transcript in the NCI-H838 cells (Fig. 4). In addition, SCLC NCI-N417 cells, AR42-J, and human brain RNA showed similar hybridizing transcripts (8).

cAMP.

To demonstrate that the receptors are coupled to adenylate cyclase, PACAP-27 and PACAP-38 dose-response stimulation of cAMP was determined in NCI-H838 cells. Fig. 5 (*left*) shows that 0.1 nM PACAP-27 elevated cAMP 2-fold, whereas 10 nM PACAP-27 elevated cAMP 12-fold. The half-maximal effective concentration (EC_{50}) was 3 nM. Likewise, PACAP-38 elevated cAMP, and the EC_{50} was 2 nM. In contrast, PACAP(6–38) and PACAP(16–38) had little effect on basal cAMP, but PACAP(6–38) inhibited the increase in cAMP caused by 10 nM PACAP-27 (Fig. 5, *right*). PACAP(6–38) (100 nM) half maximally inhibited the increase in cAMP caused by 10 nM PACAP. PACAP(16–38) and PACAP(28–38) had no effect on cAMP at a $1\text{-}\mu\text{M}$ dose.

Cytosolic Ca^{2+} .

PACAP elevated cytosolic Ca^{2+} in Indo-1 AM-loaded NCI-H838 cells. Fig. 6A shows that the basal Ca^{2+} was determined in a field of 13 cells. Thirty s after the addition of 100 nM PACAP-27, Ca^{2+} started to increase in some cells, and the increase was maximal after 60 s (Fig. 6C). Cytosolic Ca^{2+} increased in 9 of 13 cells and started to decline after 90 s (Fig. 6D). Cytosolic Ca^{2+} continued to decline (Fig. 6E) and returned to baseline after approximately 150 s (Fig. 6F). The effects of PACAP were dose dependent; 10 and 100 nM, but not 1 nM, PACAP-27 elevated cytosolic Ca^{2+} . Likewise, 100 nM PACAP-38, but not PACAP(16–38), PACAP(28–38), or VIP, increased the cytosolic Ca^{2+} . PACAP(6–38) (1 μM) inhibited the increase in cytosolic Ca^{2+} caused by 100 nM PACAP-27 (data not shown).

Arachidonic Acid Release.

PACAP stimulated arachidonic acid release. NCI-H838 readily incorporated AA into endogenous phospholipids after overnight incubation. When the cells were washed twice to remove free AA, only 106 cpm were released into the medium during a 45-min incubation (Table 2). When PACAP-27 (100 nM) was added, the rate of AA release increased 6-fold. The addition of PACAP(6–38) had no effect on basal AA release but inhibited, in a concentration-dependent manner, PACAP-27-induced arachidonic acid release.

Proliferation.

PACAP stimulated colony formation in NCI-H838 cells. PACAP(6–38) (100 nM) significantly inhibited NCI-H727 colony number (Table 3). PACAP-27 (10 nM) significantly stimulated NCI-11727 colony number (3-fold), and the increase caused by PACAP was inhibited by 100 nM PACAP(6–38). In addition, 100 nM PACAP(6–38) reduced basal colony formation 7-fold. PACAP(16–38) or PACAP(28–38) had no effect on NCI-H838 growth (data not shown).

PACAP(6–38) inhibited NSCLC growth *in vivo*. In nude mice that received injections of NCI-H838 cells, a palpable mass formed at week 2. Fig. 7 shows that, in control mice, the tumors grew rapidly, and, at week 6, the tumor volume was 1909 mm³. In mice that received PACAP(6–38) injections of 10 µg/day s.c., tumor proliferation was slowed significantly at weeks 3–6, and, at week 6, the mean tumor volume was 1112 mm³. Similar data were obtained using NCI-H727 xenografts.

DISCUSSION

PACAP is the most recently discovered peptide in the VIP/secretin/glucagon family of peptides (2). Since its identification, numerous studies have identified specific PACAP binding sites on normal and tumoral tissues (3, 16). With the recent cloning of the rat and human PACAP receptors (8, 9, 23), specific molecular probes are now available to distinguish the type 1 PACAP receptor from the VIP₁, and VIP₂ receptors. We have shown previously the presence of VIP receptors on NSCLC cell lines (24, 25), and, herein, we report the effects of PACAP on NSCLC cells.

¹²⁵I-PACAP-27 shows high-affinity binding to NSCLC cells. In NCI-H838 cells, ¹²⁵I-VIP binds with high affinity to VIP receptors only, whereas ¹²⁵I-PACAP-27 binds with high affinity to both PACAP and VIP receptors. These observations are similar to those observed in native cells, as well as in cells transfected with VIP or PACAP receptor cDNAs (15). VIP receptors characterized previously on NSCLC cells demonstrated that ¹²⁵I-VIP binding was inhibited by both VIP and PACAP. In this study, ¹²⁵I-PACAP-27 bound to NCI-H838 cells with high affinity ($K_d = 1$ nM) to a single class of receptor sites ($B_{max} = 1.4 \times 10^4$ /cells). The order of peptide potency is PACAP-38 = PACAP-27 \gg PACAP(6–38) \gg VIP = PACAP(16–38) > PACAP(28–38). These data suggest that ¹²⁵I-PACAP-27 bind to type 1 PACAP receptors.

Unlike the VIP receptor, which couples to NSCLC adenylate cyclase, PACAP receptors have been shown in both native and transfected cells to couple to both adenylate cyclase and

phospholipase C. PACAP stimulation of NCI-H838 cells, therefore, was evaluated to couple to both signal transduction pathways. PACAP-27 and PACAP-38 increased cAMP similarly; they had EC₅₀ of 3 and 2 nM, respectively. The ability of both peptides to stimulate cAMP with similar potency has been observed in native cells and in cells transfected with the type 1 PACAP receptor (15, 16). In astrocytes, low doses of VIP are reported to elevate adenylate cyclase and translocate protein kinase C (26, 27).

To evaluate the ability of PACAP receptors to couple to phospholipase C, we evaluated the downstream response of intracellular Ca²⁺ signaling in Indo-1AM- loaded NCI-H838 cells. In contrast with the adenylate cyclase response, PACAP-27 was half maximally potent at 100 nM at stimulating an increase in cytosolic Ca²⁺. These results suggest that PACAP-27 is 50-fold less potent at stimulating phospholipase C in NCI-H838 cells than is adenylate cyclase. These results are consistent with the PI turnover observed for PACAP-stimulated NIH 3T3 cells stably transfected with the human type 1 PACAP receptor (15). Because PACAP induces an elevation in cytosolic Ca²⁺ in the presence or absence of EGTA, Ca²⁺ may be released from the endoplasmic reticulum. PACAP-induced stimulation of phospholipase C also may result in protein kinase C activation. This signal transduction response suggests that PACAP receptors at low ligand occupancy are capable of maximal adenylate cyclase stimulation as a result of receptor spareness. Receptor spareness is not observed for Ca²⁺ stimulation, as suggested by the nearly superimposable dose response for Ca²⁺ stimulation and ligand-binding affinity curves. These data suggest that the PACAP receptor is more tightly coupled to a stimulatory guanine nucleotide binding protein (G protein), such as *Gas*, because it is coupled principally to adenylate cyclase rather than phospholipase C. However, as suggested for the D₂ dopaminergic receptor, alternatively spliced receptor splice variants may couple to additional G proteins, leading to different effector responses.

Five splice variants of the rat PACAP type 1 receptor, which differ between the fifth and sixth transmembrane domains (third intracellular loop), have been cloned. The basic PACAP receptor has 467 amino acids and a 14-amino acid third intracellular domain (9). The hip PACAP receptor (495 amino acids) has the basic 14 amino acids plus a 28-amino acid insert at the third intracellular domain. The hop₁ PACAP receptor (495 amino acids) has the basic 14 amino acids plus a different 28-amino acid insert at the third intracellular domain, whereas the hop₂ PACAP receptor is similar to the hop₁ variant, but has a deletion of 1 amino acid from the hop₁ insert. The hip/hop PACAP receptor (523 amino acids) has the basic 14 amino acids plus both 28-amino acid inserts at the third cytosolic domain.

Differential expression of the rat PACAP receptor splice variants results in differential coupling to the intracellular effectors, cAMP, and total PI. These structural and functional differences in the splice variants are important because they may explain the different intracellular signal transduction responses that occur with PACAP-stimulation in specific cell types. The rat PACAP-R basic and hop₁ splice variants showed an ability to couple to both intracellular pathways, whereas the hip splice variant couples to adenylate cyclase only (9). Although the purpose of our study was not aimed specifically at identifying splice variant specific expression for PACAP receptors on human NSCLC cells, our signal transduction results suggest that human NSCLC cells may express the basic PACAP-R

and/or *hop*₁ splice variants. At present, we are investigating the specificity of this splice expression in NSCLC cells.

The existence of PACAP receptors was further confirmed by high-stringency Northern blot analysis of NCI-H838 cells showing a single hybridizing transcript of 7.5 kb, which is consistent in size with the rat and human PACAP receptors published previously (9, 10). VIP receptor mRNA shows transcript sizes of 5, 2.5, and 1.5 kb when hybridized with a VIP₁ receptor cDNA probe.⁵

Previously, epidermal growth factor receptors were detected in high density in NSCLC cells (28). Recently, we found that EGF caused prostaglandin E₂ production in NSCLC cells, which resulted in proliferation (29). Herein, PACAP caused arachidonic acid release from NSCLC cells. PACAP may stimulate phospholipase A₂ activity as a result of increased phosphorylation of phospholipase A₂ by protein kinase C.

The proliferation of SCLC cells *in vitro* is maximally stimulated by 10 nM bombesin or 10 nM VIP, which cause PI turnover and elevate cAMP, respectively (25, 30). One hypothesis is that PACAP may be more potent than VIP and may maximally stimulate NCI-H838 colony formation at 1 nM, because it stimulates PI turnover and elevates cAMP at the same time. Recently, we found that PACAP significantly increases *c-fos* gene expression at a 1 nM (31). The *c-fos* gene in NSCLC cells may have both a phorbol ester- and cAMP-responsive element.

PACAP(6–38) (10 µg/day s.c.) slowed NCI-H838 xenograft formation by approximately 45%. Previously, we found that VIP hybrid (10 µg/day s.c.) slowed NSCLC xenograft formation in nude mice by 80% (14). These data indicate that NSCLC tumor growth may be slowed by either PACAP or VIP receptor antagonist. VIP hybrid may be more potent than PACAP(6–38) *in vivo* because of the slower rate of degradation by blood proteases (32).

In summary, PACAP binds with high affinity, elevates cAMP, causes PI turnover, releases arachidonic acid, and stimulates growth of NSCLC cells. The actions of PACAP are antagonized by PACAP(6–38). Therefore, PACAP receptors are present on NSCLC cells, where they may function as potential regulators of growth and differentiators of these tumor cells. The development of more potent PACAP receptor antagonists will permit a potential antitumor strategy using selective PACAP receptor antagonists to reduce proliferation of NSCLC cells.

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⁵T. Moody, unpublished observation.

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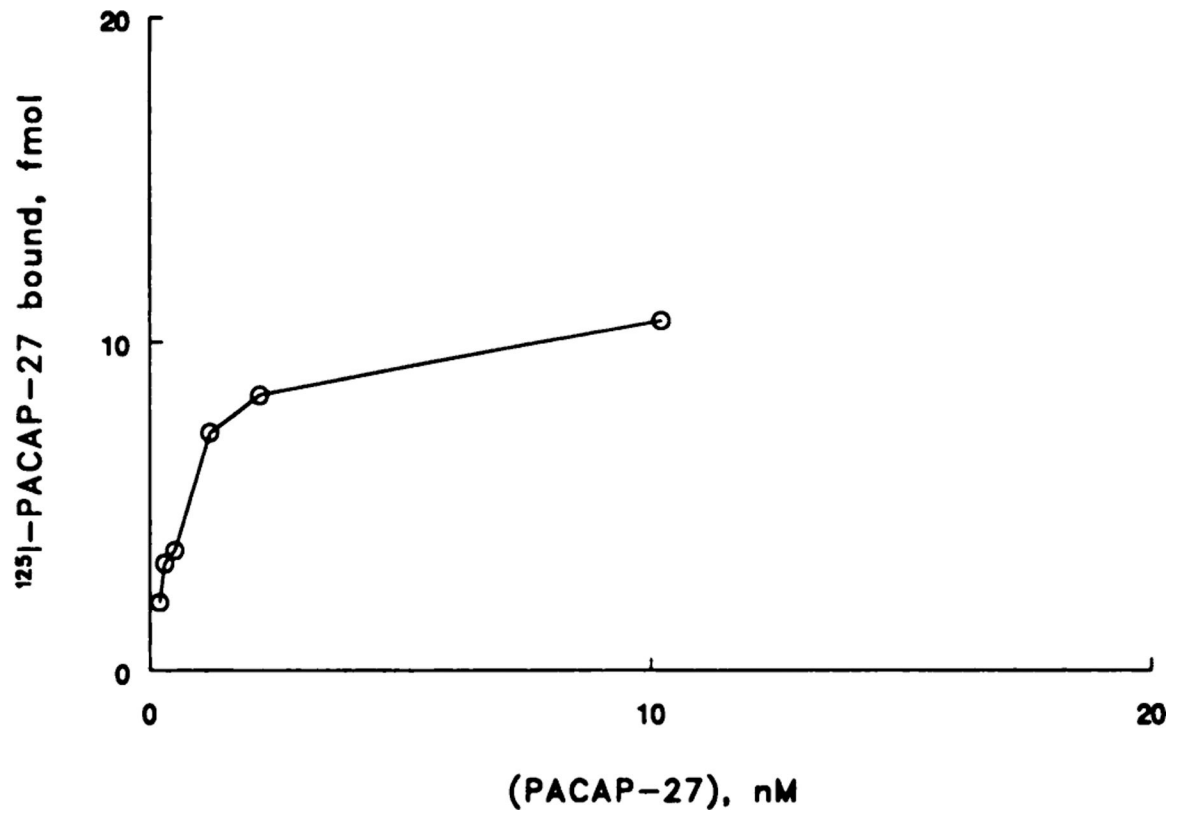


Fig. 1. The amount of specifically bound ^{125}I -PACAP-27 is indicated as a function of PACAP-27 concentration. The mean of four determinations is indicated. The line is point to point.

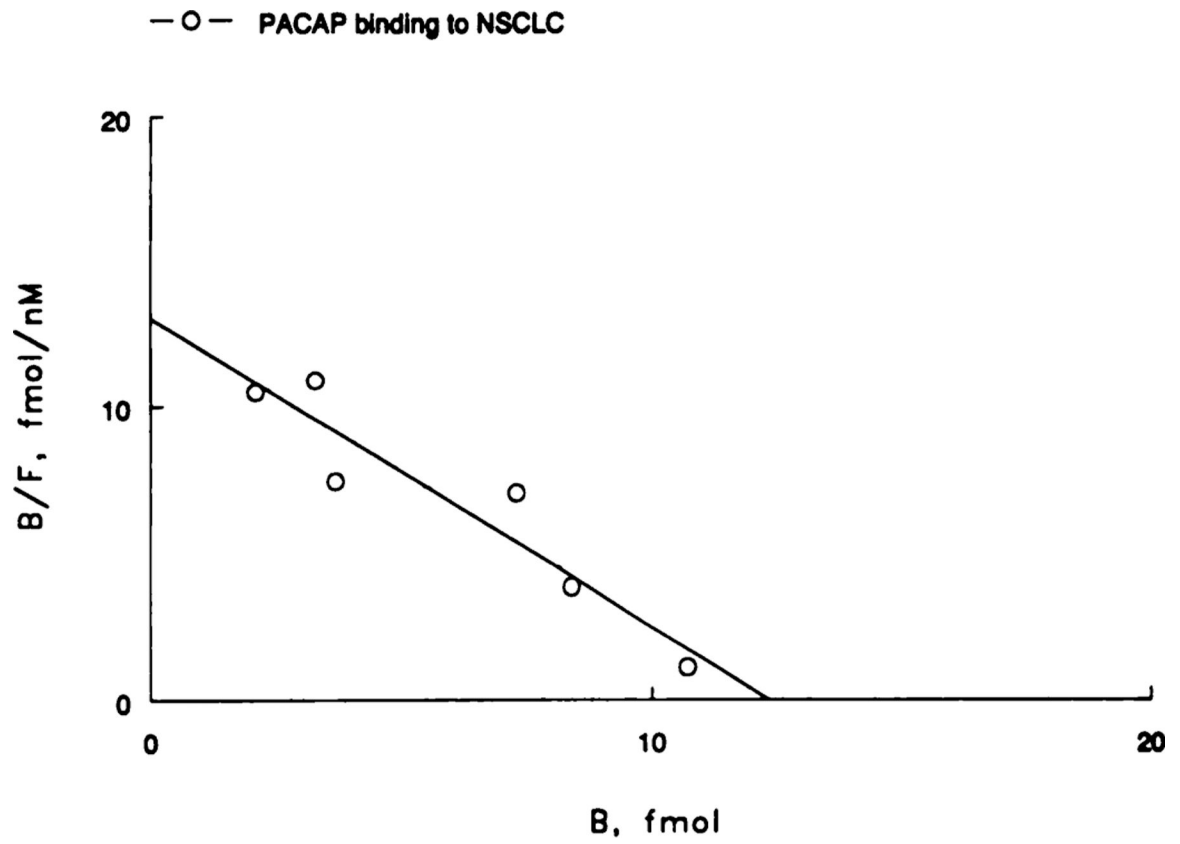


Fig. 2.
A Scatchard plot of the specific binding data is shown. The line represents the best fit.

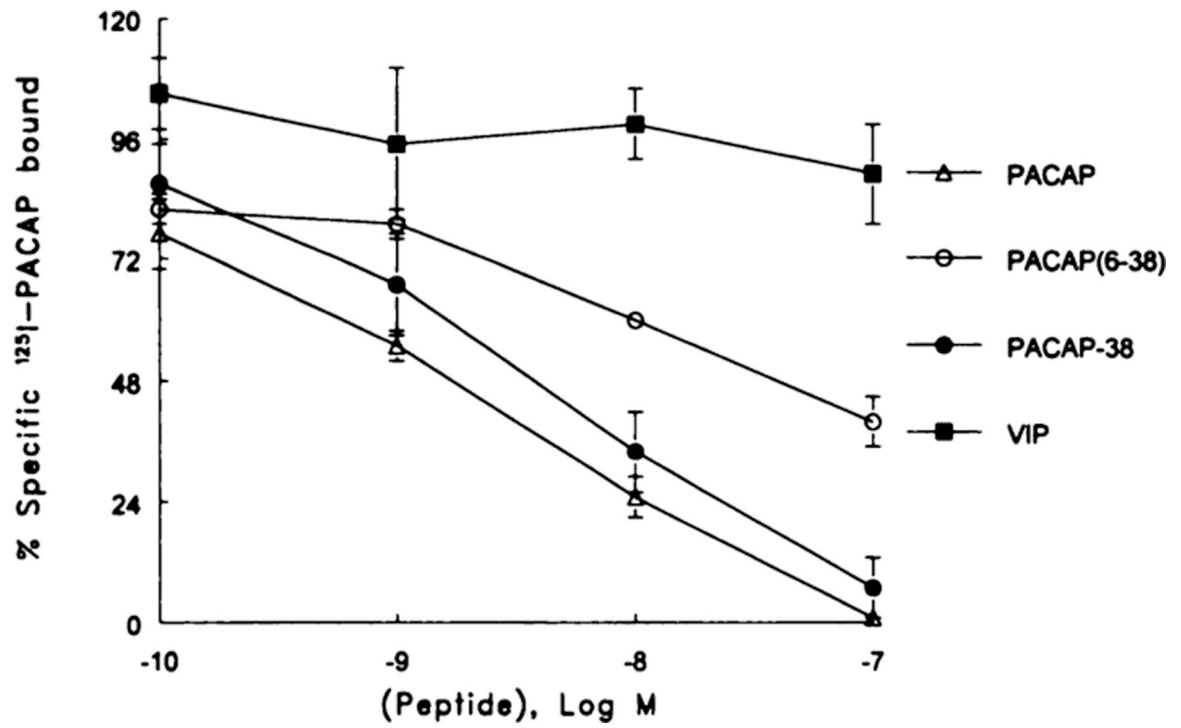


Fig. 3. The percent of specific ^{25}I -PACAP bound was determined as a function of PACAP-27 (△), PACAP-38 (●), PACAP(6-38) (○), and VIP (■) concentration. The mean of three experiments each repeated in quadruplicate is indicated. Bars, SE.

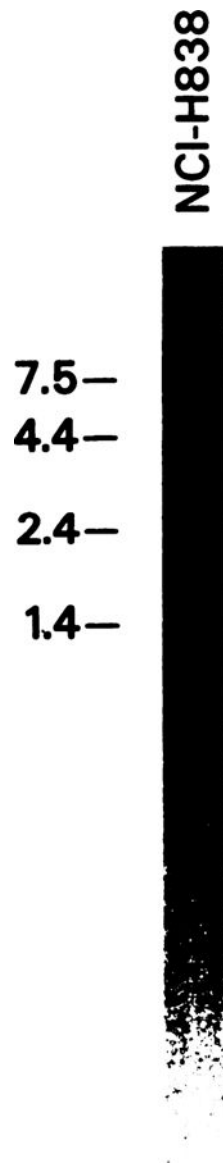


Fig. 4. Northern blot analysis. A main band of radioactivity at 7.5 kb was detected using guanidine isothiocyanate extracts of NCI-H838 cells.

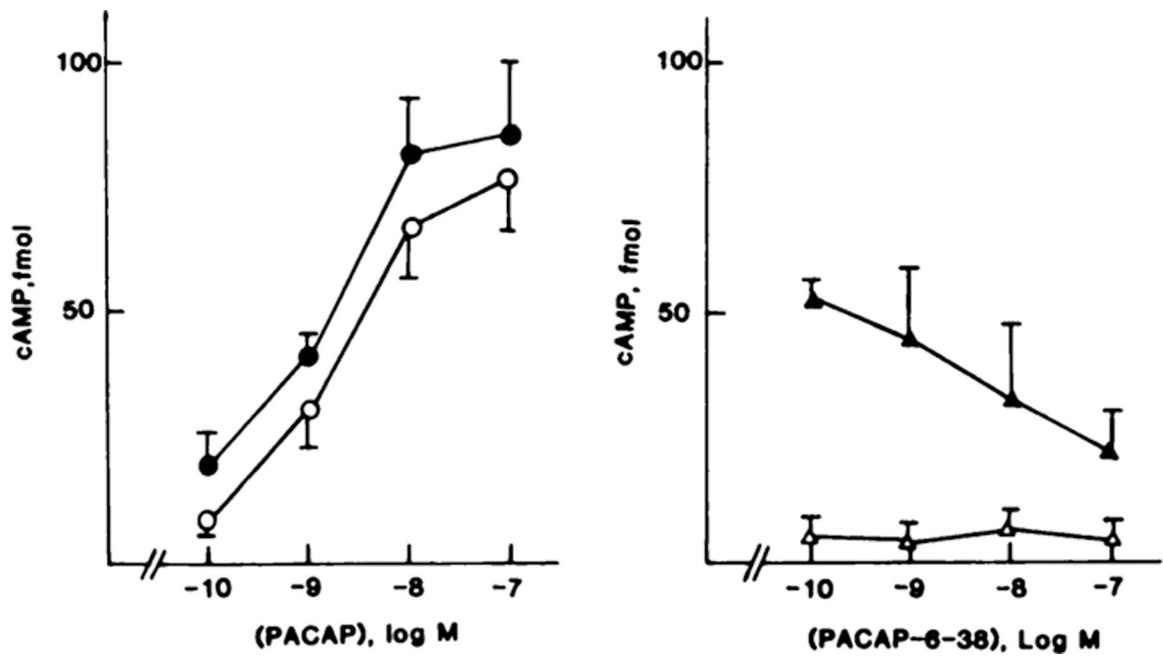


Fig. 5. *Left*, cAMP was determined in NCI-H838 cells as a function of PACAP-27 (○) and PACAP-38 (●) concentration. *Right*, cAMP was determined as a function of PACAP(6-38) concentration in the absence (△) and presence (▲) of 10 nM PACAP. The mean of four determinations is indicated. Bars, SE.

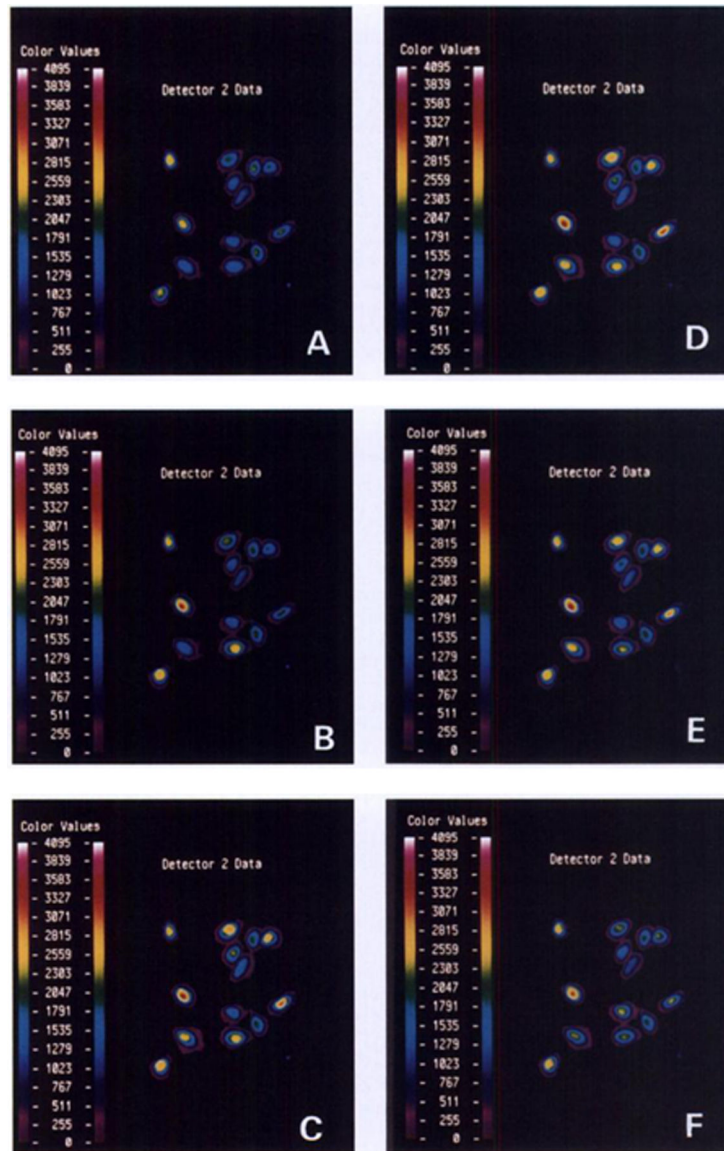


Fig. 6. NCI-H838 cells were loaded with Indo-1AM, and the basal cytosolic Ca²⁺ was determined in a field of 13 cells. PACAP-27 (100 nM) was added, and cytosolic Ca²⁺ determined after 0 min (A), 0.5 min (B), 1 min (C), 1.5 min (D), 2 min (E), and 2.5 min (F). This experiment is representative of two others.

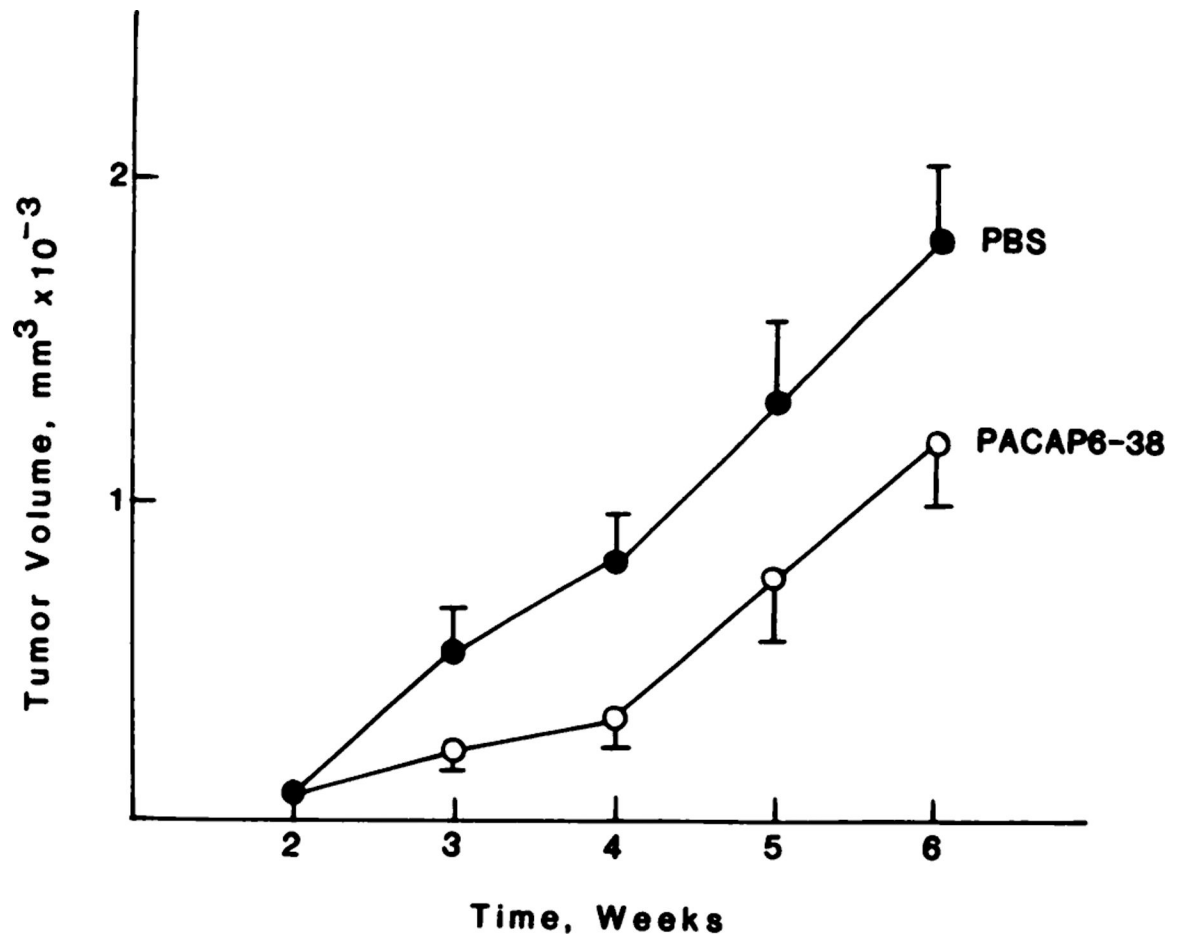


Fig. 7. Nude mice received injections of NCI-H838 cells, and, after 2 weeks, a xenograft formed. PBS and PACAP(6–38) (10 $\mu\text{g}/\text{day}$ s.c.) were injected daily during weeks 2–6, and the xenografts were measured weekly.

Table 1Radiolabeled PACAP binding^a

Cell line	cpm bound
NCI-H157	5598
NCI-H727	9100
NCI-H838	10860
NCI-H1299	8758

^aThe amount of specifically bound ¹²⁵I-PACAP-27 was determined using radiolabeled PACAP-27 (0.2 nM) and 10⁶ cells. The mean of four determinations is indicated, and the standard error was approximately 10%.

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Table 2Effects of PACAP on arachidonic acid release^a

Addition	cpm
None	106 ± 7 ^b
PACAP-27, 100 nM	607 ± 104
PACAP-27 + PACAP (6–38), 1 nM	507 ± 43
PACAP-27 + PACAP (6–38), 10 nM	489 ± 60
PACAP-27 + PACAP (6–38), 100 nM	430 ± 81 ^c
PACAP-27 + PACAP (6–38), 1000 nM	286 ± 57 ^b

^aThe mean ± SD of four determinations is indicated.^b $P < 0.05$; relative to 100 nM PACAP using Student's *t*-test.^c $P < 0.01$.

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Table 3Effect of PACAP analogues on growth^a

Additions	NCI-H727 colonies
None	27 ± 7
PACAP-27, 10 nM	85 ± 3 ^b
PACAP (6–38), 100 nM	4 ± 3 ^c
PACAP-27 + PACAP (6–38)	33 ± 7

^aThe mean ± SE of three determinations is indicated.^b $P < 0.05$; relative to control.^c $P < 0.01$.

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