Pituitary Adenylate Cyclase-Activating Polypeptide Inhibits Transforming Growth Factor- β 1-Induced Apoptosis in a Human Pituitary Adenoma Cell Line

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Pituitary adenylate cyclase-activating polypeptide (PACAP) was originally isolated from hypothalamic tissues based on its ability to stimulate cAMP production in cultured anterior pituitary cells. Recent studies have suggested a functional role for PACAP in the apoptosis of brain cells. However, the role of PACAP in regulating apoptosis in human pituitary adenomas has not previously been examined. Analysis of the cultured human pituitary adenoma cell line HP75, which expresses all three major PACAP receptors, showed that both PACAP-38 and PACAP-27 inhibited TGF- β 1-induced apoptosis. Treatment with the PACAP receptor antagonists PACAP 6–38 (PACAP type I receptor antagonist) and (*p*-chloro-D-Phe⁶, Leu¹⁷)-VIP (PACAP type II receptor antagonist) blocked the effects of PACAP-38 on the inhibition of transforming growth factor- β 1 (TGF- β 1)-induced apoptosis, confirming the specificity of the role of PACAP. Treatment with forskolin but not phorbol 12-myristate 13-acetate (PMA) also inhibited TGF- β 1-induced apoptosis. TGF- β 1 treatment was associated with an increase in mitogen-activated protein kinase (MAP kinase) when analyzed by Western blotting, but PACAP inhibition of TGF- β 1-induced apoptosis was not associated with activation of MAP kinase. Immunocytochemical analysis of the cell cycle cyclin-dependent kinase inhibitor p27 showed that treatment with TGF- β 1, forskolin, PMA, and PACAP increased p27 expression in cultured HP75 cells. These results indicate that PACAP is a highly specific inhibitor of TGF- β 1-induced apoptosis in the HP75 human pituitary adenoma cell line and that PACAP, TGF- β 1, forskolin, and PMA all stimulate expression of the TGF- β -regulated cell cycle protein p27 in the HP75 human pituitary adenoma cell line. The HP75 cell line can be used as a model to study the regulation of apoptosis in human pituitary cells. (*Am J Pathol* 1999, 155:1893–1900)

Pituitary adenylate cyclase-activating polypeptide (PACAP) was originally isolated from hypothalamic tissues by its ability to stimulate cAMP production in cultures of anterior pituitary cells at a potency 1000 times greater than that of vasoactive intestinal polypeptide (VIP).¹ PACAP is present in tissues as a 38-amino acid (PACAP-38) and as a shorter 27-amino acid peptide (PACAP-27) and shares approximately 68% sequence homology with VIP at its N-terminal portion.^{1,2} It stimulates the release of several pituitary hormones, including luteinizing hormone (LH),³ growth hormone (GH),⁴ and interleukin-6 (IL-6) from folliculo-stellate cells.⁵ PACAP also stimulates ACTH release from the clonal corticotroph AtT20 cell line and GH and/or prolactin release from the clonal somatotroph GH3 cell line.⁶ Thus PACAP fulfills various criteria of a hypophysiotropic factor.

Recent studies have demonstrated a relationship between PACAP and apoptosis. Arimura et al described a neurotrophic biphasic effect of low PACAP-38 concentrations on gp120-induced apoptosis in hippocampal cultures.⁷ Other studies have suggested that PACAP plays a role in preventing apoptosis in cerebellar granule neurons.^{8,9} Recently, all three major PACAP receptors were demonstrated in normal and neoplastic human pituitaries; however, the role of PACAP in regulating apoptosis in human pituitary adenoma has not previously been examined.^{10,11}

Programmed cell death or apoptosis has been recognized as contributing to a range of developmental and remodeling events in tissues and to tumor regression in response to treatment with chemotherapeutic agents. In lymphoid cells, cellular signal transducers such as cAMP may stimulate apoptosis, and this molecule can substitute for glucocorticoids in triggering apoptosis.¹² cAMP acts as a second messenger molecule that regulates the activity of protein kinase A (PKA) or PKA-related enzymes and a wide range of cellular processes.^{13,14} Protein kinase C (PKC), which is activated by diacylglycerol lo-

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cated on the cell surface membrane, is involved in the regulation of a number of cellular events, including growth and differentiation in a wide range of tissues. The levels of PKC activity and PKC mRNA expression were higher in pituitary adenoma cells than in normal human and rat pituitary cells,^{15,16} suggesting a possible role of PKC in pituitary tumor development.

Recent *in vitro* studies suggest that transforming growth factor- β (TGF- β) and related peptides may have important roles in pituitary cell proliferation and in hormone expression.^{17–19} Various isoforms of TGF- β are expressed in rat^{18–21} and human^{22,23} pituitary cells. TGF- β has an inhibitory effect on the cell cycle directed at the G1-to-S phase transition, and this inhibition is reversible after removal of this cytokine.^{24,25} Some of the actions of TGF- β are mediated by cell cycle inhibitory proteins such as p27^{kip1} (p27) and p15.^{26–28} p27 in turn may function as a negative regulator of G1 cell cycle progression and may mediate TGF- β -induced G1 arrest. p27 protein, which interacts with cyclin-cdk complexes, including cyclin E-cdk2,^{26–28} is expressed at higher levels in quiescent cells than in proliferating cells, which may implicate this cell cycle protein in cell death.

We examined the role of PACAP in modulating apoptosis in a human pituitary adenoma cell line. Our results show that PACAP is a highly specific inhibitor of TGF- β 1-induced apoptosis in this human pituitary adenoma cell line *in vitro*.

Materials and Methods

Cell Culture

An immortalized human pituitary HP75 cell line, developed originally from a nonfunctioning pituitary adenoma with a replication-defective recombinant human adenovirus with a SV40 early large T-antigen,²⁹ was used in this study. The HP75 cells were maintained in Dulbecco's minimum essential medium (DMEM) with 15% horse serum and 2.5% fetal bovine serum with 1 μ g/ml insulin and 1% antibiotics (all from Life Technologies, Grand Island, NY).

For the apoptosis experiments, the HP75 cells were plated onto 35-mm plastic dishes at approximately 0.5×10^6 cells/dish for 4 hours. After the complete medium was removed and the cells were washed twice with DMEM, the HP75 cells were incubated in a serum-free DMEM without insulin or dexamethasone for about 24 hours. The following reagents were used in cell culture studies, and the final concentration was obtained after preliminary titration experiments: porcine TGF- β 1 (1×10^{-9} mol/L; R&D Systems, Minneapolis, MN); PACAP38 and PACAP27-NH2 (2.5×10^{-7} mol/L; Peninsula Lab, Belmont, CA); PACAP 6–38 (PACAP type I receptor antagonist) and (*p*-chloro-D-Phe,⁶ Leu¹⁷)-VIP (PACAP type II receptor antagonist) (10^{-6} mol/L; Bachem, Torrance, CA); phorbol 12-myristate 13-acetate (PMA) (10^{-7} mol/L); and forskolin (10^{-6} mol/L) (Sigma Chemical Co., St. Louis, MO). After 24 hours of treatment, the HP75 cells were harvested, cytocentrifuged onto glass slides, fixed

Table 1. Sequences of Primers and Hybridization Probes for Human PACAP and PVR mRNAs

PACAP (Gen bank no. X60435)	
Primers	SS: TGCAGTCGCTCGTGGCCCGGG (10491–10511) AS: GTTTGGATAGAACACACGAGC (11869–11889)
Probes	AS ^a : CTCTTCCTAGGACGGCCGCCAAGTATTTTC (11712–11741)
PVR-1 (Gen bank no. D17516)	
Primers	SS: CTTGTGCAGAACTTCAGTCT (1235–1255) AS: TCGGTGCTTGAAGTCCACAGC (1517–1537)
Probes	AS ^a : TAGTGGGATGAGCAGCAGGGTGGACCGGGC (1298–1327)
PVR-2 (Gen bank no. X75299)	
Primers	SS: ATGTGCAGATGATCGAGGTG (127–146) S: TGTAGCCGGTCTTCACAGAA (431–450)
Probes	AS ^a : CTGCTGCTCATCCAACTCGCTGCCTTGTC (387–416)
PVR-3 (Gen bank no. U18810)	
Primers	SS: CTTCAGGAAGCTGCACTGC (617–635) AS: CAAACACCATGTAGTGGACG (1181–1200)
Probes	AS ^a : AGACTGGTCGTTGCCGCCGACATCTGGGGA (1101–1130)

AS, antisense; SS, sense.

^aUsed for Southern hybridization.

with 4% paraformaldehyde for 20 minutes, and used for analysis of apoptosis and immunocytochemistry.

Oligonucleotide Primers and Probes

Oligonucleotide primers and Southern hybridization probes were synthesized with a DNA oligonucleotide synthesizer (Applied Biosystems, Foster City, CA) (Table 1). Both primers and probes for human PACAP³⁰ and PACAP receptors, including PVR-1,³¹ PVR-2,^{32,33} and PVR-3,³⁴ were synthesized on the basis of published sequences as previously reported.^{10,11}

Solution Polymerase Chain Reaction and Southern Hybridization

Total RNA extraction was performed by single-step methods (TRIzol Reagent Kit; Life Technologies) from HP75 cells.^{29,35}

First-strand complementary DNA (cDNA) was prepared from total RNA with a first-strand synthesis kit (Stratagene, La Jolla, CA). The reverse transcriptase (RT) reaction was performed at 37°C for 60 minutes in a final volume of 50 μ l with 5 μ g total RNA, 300 ng oligo-dT primer, 1 $\times$ RT buffer, 1.0 mmol/L of each deoxyribonucleotide (dATP, dCTP, dGTP), 40 U ribonuclease (RNase) inhibitor, and 50 U Moloney murine leukemia virus RT. The reaction product was then heated at 95°C for 5 minutes and immediately placed on ice.

The polymerase chain reaction (PCR) was performed in 100- μ l final reaction volumes containing 5 μ l RT reaction product as template DNA, corresponding to cDNA synthesized from 500 ng total RNA; 1 $\times$ PCR buffer (Promega, Madison, WI); 1.5 mmol/L MgCl₂; 0.2 mmol/L of each deoxynucleotide (Boehringer Mannheim, Indianapolis, IN); 300 ng of each sense and antisense primer for PACAP and PVR-1, -2, and -3; and 2.5 U *Taq* DNA polymerase (Promega). Programmed temperature cy-

cling (Perkin Elmer/Cetus 480, Norwalk, CT) was performed with the following cycle profile: 95°C for 5 minutes, followed by 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes (30 cycles) for GAPDH and PACAP, and 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes (40 cycles) for PVR-1, -2, and -3, respectively. After the last cycle, the elongation step was extended at 72°C for 10 minutes.

A 20- μ l aliquot of PCR product was analyzed by gel electrophoresis, using a 2% agarose gel, and was stained with ethidium bromide. PH0174 DNA/*Hae*III digest (Boehringer Mannheim) was used as the molecular size marker. The separated PCR products were transferred to nylon membrane filters. Southern hybridization, with a single internal probe that hybridized to regions within the amplified sequences, was performed. Hybridization was performed with 1×10^6 cpm/ml [^{32} P]deoxyadenosine diphosphate-labeled probes at 42°C for 18 hours. After washing with $6 \times$ SSC-0.1% SDS at 23°C for 20 minutes and at 42°C for 20 minutes, autoradiography was performed at -70°C with Kodak Omat-AR film (Eastman Kodak, Rochester, NY) with intensifying screens. In the RT-PCR experiments, total RNA from the hypothalamus was included as a positive control for PACAP and PVR-1, -2, and -3.

Apoptotic Cell Detection

For analysis of apoptosis, an *in situ* cell death detection kit with terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) (Boehringer Mannheim) was used. The reaction product was visualized by reaction with nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate (NBT-BCIP) (Life Technologies). Cells were counterstained with nuclear fast red dye.

Negative controls consisted of omission of the TdT in the TUNEL reaction, which resulted in no staining. Positive cells were enumerated by counting a minimum of 500 cells/slide, and the results were expressed as an apoptotic index (AI) (number of apoptotic cells per 100 cells). Ultrastructural studies were done to confirm the presence of apoptotic cells. Cells were fixed in 2% formaldehyde in phosphate-buffered glutaraldehyde and processed for electron microscopy as previously described.²⁹

Detection of Phospho-ERKs

HP75 cells were treated with TGF- β 1, PACAP-38, and TGF- β 1 plus PACAP-38 for 10 minutes, 30 minutes, 60 minutes, and 24 hours, followed by protein extraction and Western blotting. Aliquots of control, TGF- β 1-, PACAP-38-, and TGF- β 1 plus PACAP-38-treated cells were analyzed by Western blotting with antibodies against phospho-specific MAP kinase (phosphorylated ERKs) (1:1000) (Promega, Madison, WI) ERK1, ERK2 (1/500 each; Santa Cruz Biotechnology, Santa Cruz, CA), and actin (1:1500; Sigma Chemical Co.). The reaction product was detected by enhanced chemiluminescence (Amersham Life Science, Arlington Heights, IL), and the

Figure 1. RT-PCR analysis of PACAP (lanes 1–3, 317 bp) and PVR 1 (lanes 4–6). The hip (303 bp) and hop (387 bp) isoforms are present, PVR2 (lanes 7–9, 324 bp) and PVR 3 (lanes 10–12, 584 bp), in HP75 cells (lanes 2, 5, 8, 11, and 14). Human hypothalamus was used as a positive control (lanes 1, 4, 7, 10, and 13). Negative control without reverse transcriptase was used for HP75 cells (lanes 3, 6, 9, and 12). GAPDH was used as a control to test the integrity of the mRNAs for hypothalamus (lane 13) and HP75 cells (lane 14). **Top:** Ethidium bromide-stained gel. **Bottom:** Southern hybridization with an internal probe.

density of the bands was quantified by densitometry as previously reported.^{21,29}

p27 Immunocytochemistry

Immunostaining for p27 on HP75 cells was performed as previously reported, using the avidin-biotin-peroxidase (Vector Kit; Vector, Burlingame, CA) method.³⁶ Monoclonal antibody to p27 (Transduction Laboratory, Lexington, KY) was used at a 1:1000 dilution. The slides were developed with diaminobenzidine chromogen. Positive cells were enumerated by counting a minimum of 500 cells per slide, and the results were expressed as the percentage of cells with nuclear staining.

Statistical Analysis

Each experiment was performed three to four times. Results were expressed as the mean $\pm$ SEM. Duncan's multiple-range test and Student's *t*-test were used for statistical analyses.

Results

RT-PCR and Southern Hybridization Analysis

Results of RT-PCR and Southern hybridization studies are shown in Figure 1. Analysis of PACAP mRNA and PVR-1, -2, and -3 mRNAs demonstrated the expected PCR products in the HP75 cell line and hypothalamus. Both hip (303 bp) and hop (387 bp) isoforms of PVR1 were detected in hypothalamus and in HP75 cells. PACAP mRNA was detected in the hypothalamus, which was used as a positive control, but not in the HP75 cells (Figure 1). PVR-1, -2, and -3 mRNAs were detected in the hypothalamus and in HP75 cells (Figure 1).

Apoptosis in the HP75 Cell Line

PACAP and TGF- β 1 Treatment

Apoptotic cells were readily identified by TUNEL staining (Figure 2). The negative controls without Tdt were

Figure 2. Apoptosis of HP75 cells identified by blue nuclear staining in the TUNEL reaction. **A:** control cells; **B:** PACAP-38-treated cells; **C:** TGF- β 1-treated cells; **D:** TGF- β 1 + PACAP-38-treated cells; **E:** negative control without Tdt. Fast red nuclear counterstaining, $\times 200$.

consistently negative (Figure 2). Ultrastructural studies identified apoptotic cells in control dishes, and these apoptotic cells were increased after TGF- β 1 treatment (Figure 3).

Control cells and cells treated with PACAP-38 or PACAP-27 had similarly low apoptotic indices (AIs) (Figure 4). TGF- β 1 treatment resulted in a two- to threefold increase in the AIs. Combined treatment with TGF- β 1 and PACAP-38 or PACAP-27 treatment decreased the AIs to control levels (Figure 4).

Figure 3. Ultrastructural analysis of apoptotic cells after TGF- β 1 treatment shows a cell with two apoptotic bodies, $\times 4000$.

PACAP Antagonist Treatment

To examine the specificity of the PACAP effect we used PACAP antagonists in combination with TGF- β 1 and PACAP (Figure 5). PACAP 6–38 (PACAP type I antagonist) and (*p*-chloro-D-Phe,⁶ Leu¹⁷)-VIP (PACAP type II antagonist) partially blocked the effects of PACAP in reducing TGF- β 1-induced apoptosis (Figure 5).

The PKA agonist forskolin partially inhibited TGF- β 1-induced apoptosis in HP75 cells (Figure 6), whereas PMA treatment had no effect on TGF- β 1-stimulated apoptosis (Figure 7).

MAP Kinase Analysis

Western blot and densitometric analyses for MAP kinase showed that TGF- β 1 induced phosphorylation of ERK1 and ERK2 at levels twofold above control levels after 24 hours of treatment with an antibody specific for phos-

Figure 4. Apoptotic index in HP75 cells after treatment with various chemicals. TGF- β 1 treatment increased apoptotic cells more than twofold above control levels. PACAP-38 or PACAP-27 treatment blocked the TGF- β 1-induced apoptosis. Data are from four experiments with triplicate slides. **a:** Compared to control cells ($P < 0.01$). **b:** Compared to TGF- β 1-treated cells ($P < 0.01$). CON, control; PA, PACAP.

Figure 5. The specificity of PACAP-38 in inhibiting TGF- β 1-induced apoptosis in HP75 cells was analyzed by using PACAP receptor antagonist. Data are from three experiments with triplicate slides. **a:** Compared to control cells ($P < 0.01$). **b:** Compared to TGF- β 1 + PACAP38-treated cells ($P < 0.01$). PA6-38, PACAP6-38 (PACAP type I receptor antagonist); p-VIP, (*p*-chloro-D-Phe⁶, Leu¹⁷)-VIP (PACAP type II receptor antagonist).

phorylated ERKs (Figure 8, A and B). PACAP-38 did not cause any significant changes in phosphorylated ERKs compared to controls after 10, 30, or 60 minutes of treatment.

Figure 6. Examination of TGF- β 1-induced apoptosis in HP75 cells shows inhibition by the PKA agonist forskolin. Data are from three experiments with triplicate slides. **a:** Compared to control cells ($P < 0.01$). **b:** Compared to TGF- β 1-treated cells ($P < 0.05$). Forsk, forskolin.

Figure 7. Analysis of the PKC agonist PMA showed no effect on TGF- β 1-induced apoptosis in HP75 cells. Data were from three experiments with triplicate slides. Significant difference, **a:** $P < 0.01$ compared to control cells.

Figure 8. Western blot analysis of ERKs and activated ERKs in HP75 cells after 24 hours of treatment. **A:** Lane 1, control; lane 2, TGF- β 1; lane 3, PACAP-38; lane 4, TGF- β 1 and PACAP-38 treatment. **Top:** phosphorylated ERK1 and ERK2; **center:** ERK1 and ERK2; **bottom:** β -Actin to check for equal loading in **top panel**. **B:** Densitometric analysis of the Western blot analysis. There is a twofold increase in phospho-ERK after TGF- β 1 treatment.

p27 Protein Expression

The percentage of cells showing positive nuclear staining for p27 in control cells ranged from 30% to 37% in different experiments. After treatment with TGF- β 1, forskolin, and PMA, the percentage of p27-positive cells increased significantly above control levels (Figure 9). PACAP-38 and PACAP-27 treatments also significantly increased p27 expression in HP75 cells in three separate experiments (control, $25.8 \pm 1.9\%$ positive cells; PACAP-38, $35.7 \pm 1.3\%*$ positive cells; PACAP 27, $37.5 \pm 1.7\%*$ positive cells, with $*P < 0.05$).

Figure 9. Analysis of p27 expression in HP75 cells after treatment with TGF- β 1, forskolin, and PMA. Data are from four experiments with triplicate slides. $**P < 0.01$ compared to control cells.

Discussion

We studied the expression of PACAP and PVR mRNAs using a human pituitary adenoma cell line, to determine the effects of PACAP and TGF- β 1 on apoptosis in this cell line. Our results showed expression of PVR mRNAs in the HP75 human pituitary adenoma cells, indicating that these cells were capable of responding to PACAP. PVR-1, -2, and -3 mRNAs were detected by RT-PCR and Southern hybridization, but PACAP mRNA was not detected in HP75 cells. Our recent study in normal and neoplastic human pituitaries demonstrated a differential distribution of PACAP-binding sites and PVR-1, -2, and -3 mRNA expression in human pituitary adenomas.¹⁰ The highest levels of PVR were found in gonadotroph and null-cell adenomas, indicating the importance of the cAMP regulatory system in these tumors. PRL tumors did not express PVR1 and PVR2 mRNAs.¹⁰ This study, using a human pituitary adenoma cell line derived from a non-functional (null cell) adenoma, showed findings similar to those of the previous *in vivo* studies of human gonadotroph and null-cell adenomas.¹⁰

PACAP-27 or -38 did not have any direct effects on apoptosis, but TGF- β 1 stimulated apoptosis more than twofold above control levels. These observations agree with other model systems in which TGF- β induces apoptosis in cultured cells.^{37–40} To determine whether PACAP could prevent TGF- β 1 apoptosis, we treated cells with PACAP-38 or PACAP-27 and TGF- β 1. Both PACAP-38 and PACAP-27 inhibited TGF- β 1-induced apoptosis in the HP75 cells. Further analysis with the PACAP receptor antagonists PACAP 6–38 (type I receptor antagonist) and (*p*-chloro-D-Phe,⁶ Leu¹⁷)-VIP (type II receptor antagonist) showed that the effects of PACAP were highly specific in inhibiting TGF- β 1-induced apoptosis.

The second messenger molecule cAMP regulates the activity of PKA or PKA-related enzymes¹ and a wide range of cellular processes. cAMP binds to the regulatory subunit of the inactive cytoplasmic PKA complex and releases the catalytic subunit, which can rapidly and reversibly translocate into the nucleus.¹⁴ Recent studies reported that PACAP had a role in preventing apoptosis at high PACAP concentrations^{7–9} while inducing apoptosis at lower concentrations.⁷ Both forskolin, which is a direct activator of adenylyl cyclase, as well as PACAP, has been shown to protect cerebellar granule neurons from cell death.^{36–38} These observations in the brain agree with our results in the HP75 pituitary cell line, in which forskolin and PACAP prevented TGF- β -induced apoptosis.

PMA, which is an activator of the PKC pathway, has been shown to stimulate apoptosis in renal carcinoma cells.⁴¹ However, PMA did not influence TGF- β 1-induced apoptosis in the present study. The human pituitary adenoma cell line HP75 has been shown to respond to PMA and to forskolin by increasing chromogranin A expression.²⁹ These findings indicate that both functional PKC and PKA pathways are present in these immortalized cells. Although PACAP activates a dual-signal transduction mechanism in some cultured cells, the antiapoptotic

effect is most likely mediated by cAMP stimulation, inasmuch as it was mimicked by forskolin but not by PMA. In recent studies with rat pituitary cells, the PKC inhibitor hypericin stimulated apoptosis in cultured rat pituitary cells, suggesting that PMA has an antiapoptotic effect in the pituitary.⁴² Because hypericin is not a specific inhibitor of PKC in the concentrations used in this study,⁴² it is not known if this effect was due to direct inhibition of PKC.

Recent studies have shown that PACAP protects cerebellar granule neurons from apoptosis by activating the MAP kinase pathway.⁴³ In our studies, analysis of active MAP kinase and total MAP kinase proteins showed that TGF- β 1 but not PACAP-38 increased phosphorylation of MAP kinase, suggesting that PACAP did not inhibit apoptosis by activation of MAP kinase in the HP75 pituitary cell line. Villaba et al⁴³ used a model involving primary culture of rat cerebellar granule neurons in which apoptosis was induced by potassium deprivation to show that PACAP-38 induced activation of MAP kinase through a cAMP-dependent pathway. In our model TGF- β 1 stimulated apoptosis, but PACAP-38 did not alter the phosphorylation of MAP kinase. Although pituitary cells and neurons are part of the diffuse neuroendocrine system, the difference between our studies and that of Villaba et al may be due to the fact that they studied primary cultures, compared to an established pituitary cell line in our model. The effects of TGF- β 1 in the activation of MAP kinase agree with earlier reports.⁴⁴ More experiments are needed to elucidate the mechanisms involved in the PACAP inhibition of TGF- β -induced apoptosis.

Cyclin-dependent kinases (CDKs) that control G1- and S-phase progression in mammalian cells are regulated by two families of CDK inhibitors, including the Ink4 family and the Cip/Kip family. Various studies have characterized some of the cell cycle-inhibitory proteins regulated by TGF- β , including p27 and p15.^{26–29} p27 functions as a negative regulator of G1 cell cycle progression and may mediate TGF- β 1-induced G1 arrest. To determine the effects of PACAP, forskolin, PMA, and TGF- β on the regulation of p27 expression in pituitary cells, we analyzed p27 expression in HP75 cells after specific treatments. The results showed that treatment with PACAP-38, PACAP-27, forskolin, PMA, and TGF- β 1 stimulated p27 expression in HP75 cells. The relationship between p27 expression and PKA inhibition of apoptosis is still unclear. Recent studies of breast carcinoma cell lines have shown that overexpression of p27 induced apoptosis in these cells, although this effect was not seen in the total cell population and may not occur in cells arrested in G1.⁴⁵ Because chemicals that stimulated or blocked apoptosis in HP75 cells all led to an increase in immunoreactive p27 in HP75 cells, the effects of p27 on apoptosis in HP75 cells may occur by mechanisms different from that of TGF- β 1-induced apoptosis.

In summary, our studies show that PACAP is a highly specific inhibitor of TGF- β 1-induced apoptosis in the HP75 pituitary cell line. This cell line can be used as a model to study the regulation of apoptosis in human pituitary cells.

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