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Short Communication

Herpesvirus quiescence in neuronal cells IV*: Virus activation induced by pituitary adenylate cyclase-activating polypeptide (PACAP) involves the protein kinase A pathway

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a naturally occurring peptide found in the central nervous system that plays a role in somatosensory processing and activation of protein kinase A (PKA) and protein kinase C (PKC). Because activation of PKA or PKC results in reactivation of HSV-1 from latently infected embryonic neuronal cells, PACAP was used to evaluate HSV-1 activation from quiescently infected (QIF)-PC12 cells. Our studies demonstrate that physiologically relevant concentrations of PACAP38 and PACAP27 induce HSV-1 activation from QIF-PC12 cell cultures in a dose-dependent fashion. PACAP-induced activation of virus was significantly impaired by the PKA-inhibitor, H-89 (20 μM), whereas treatment with the PKC-inhibitor, GF109203X (1 μM), was without affect. Additionally, direct activation of PKA with cAMP analogs, 8-(4-chlorophenylthio)- and dibutyryl-cAMP, only partially mimicked the effect of PACAP on virus activation. Taken together, PACAP induced HSV-1 activation from QIF-PC12 cells involves the PKA and possibly cAMP-independent pathways. This report is the first to demonstrate that PACAP induces HSV-1 activation from a quiescent state and that this *in vitro* cell model is useful for studying early inductive events that lead to virus production from quiescence. *Journal of NeuroVirology* (2001) **7,** 163–168.

Keywords: pituitary adenylate cyclase-activating polypeptide (PACAP); neuronal; herpes simplex virus; viral latency; reactivation

Introduction

Herpes simplex virus (HSV) reactivation is a neuronal event that involves the switch from a latent state of infection to an active replicative form. The mechanism that initiates HSV reactivation is unclear. Evidence from *in vivo* (Sawtell and Thompson 1992), *ex vivo* (Moriya *et al*, 1994; Halford *et al*, 1996), and *in vitro* models of latency and reactivation (Smith *et al*, 1992; Danaher *et al*, 1999a; Danaher *et al*, 1999b) suggest that inducible reactivation involves stress response pathways. Although interactions of stress and HSV-1 reactivation have been studied extensively in animal and tissue culture models (reviewed in Miller *et al*, 1998; Millhouse and Wigdahl, 2000; Preston, 2000), cell culture models provide certain advantages over *in vivo* systems for answering questions involving gene induction during the earliest phases of reactivation. For these reasons, our laboratory developed a cell culture model for the study of HSV

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activation from quiescence (i.e., a latent-like state) using neurally differentiated (ND) rat pheochromocytoma (PC12) cells (Danaher *et al*, 1999a). Quiescently infected (QIF) PC12 cells allow activation of a cryptic HSV genome following induction with forskolin (Danaher *et al*, 1999a, 1999b, 2000). Forskolin is an adenylate cyclase activator (Seamon and Daly, 1981; Huang *et al*, 1982), suggesting that cAMP-dependent processes may be important in HSV-1 activation.

Adenylate cyclase activation in neuronal tissue can result from pituitary adenylate cyclase-activating polypeptide (PACAP) activity. This peptide increases adenylate cyclase activity in rat pituitary cells (Miyata *et al*, 1989) and is expressed in human trigeminal ganglionic neurons (Tajti *et al*, 1999) and throughout the central nervous system (Arimura, 1992; Ghatei *et al*, 1993). PACAP exists in two alternatively processed amidated forms, PACAP38 and PACAP27, that share the same N-terminal 27 residues (Miyata *et al*, 1989; Miyata *et al*, 1990). PACAPs have neuroprotective, neurotrophic, mitogenic, hormone regulatory, and neurotransmitter functions (Yada *et al*, 1994; Dickinson and Fleetwood-Walker, 1999), and participate in cellular differentiation (DiCicco-Bloom *et al*, 2000). They are suspected to play a role in somatosensory processing and may work in concert with endogenous anti-inflammatory cytokines to balance the overall severity of the inflammatory response (Delgado *et al*, 1999). Following peripheral nerve injury (e.g., axotomy) and inflammation (Zhang *et al*, 1998), the level of PACAP increases in a subpopulation of cell bodies of dorsal root (Zhang *et al*, 1995) and trigeminal ganglia (Larsen *et al*, 1997), and sympathetic neurons of the superior cervical ganglia (Moller *et al*, 1997). Based on its involvement in neurogenic inflammatory responses and physiologic regulation, it was of interest to know whether PACAP may be associated with reactivation of latent HSV.

PACAP induces HSV-1 activation from QIF-PC12 cells QIF-PC12 cell cultures described in this report were established as previously described (Danaher *et al*, 1999b). Briefly, PC12 cells were plated in 12-well rat-tail collagen-coated dishes at 1.1–2.2 $\times$ 10^5 cells/well and maintained in RPMI 1640 media supplemented with 50 ng/ml of 2.5S mouse nerve growth factor (NGF), 0.1% bovine serum albumin, 100 units/ml penicillin, and 100 μg/ml streptomycin (maintenance media). Cultures were infected with HSV-1 strain 17^+ (MOI of 5 to 10) in the presence of 100 μM acycloguanosine (ACV) on day 7 postplate. After ACV treatment for 8 to 10 days, cultures were maintained in the absence of ACV for 7 days (i.e., quiescent phase) prior to induction, with media changes every 2 to 3 days. All induction treatments were performed for 2 days followed by replacement with maintenance media. Cultures were evaluated for virus production throughout the study using 25% of the culture supernatants in plaque-forming assays (Miller and Smith, 1991). was absorbed on to Vero cells with agitation for 1 to 2 h at room temperature, after which minimum essential media containing 0.2% human immune serum globulin was added. Following incubation at 37°C for 3 days, viral plaques were observed by examining cell sheets stained with 0.1% crystal violet.

In the first experiment, the quiescent phase following ACV withdrawal was marked by infrequent detection of virus in (4.6%, 5/108) cultures. Treatment of nonproductive cultures on day 15 postinfection (p.i.) with PACAP38 at the concentrations indicated in Figure 1 (panel A) resulted in the production of virus in a dose-dependent manner. Maximum induction (71% of cultures) was observed with 4 and 20 nM PACAP38, whereas only a minority of cultures produced HSV-1 at 0.8 nM and below. Spontaneous virus production was detected in 8.3% of mock-induced cultures by the end of the experimental period.

PACAP is a member of the VIP/glucagon/secretin family of peptides that exerts its action through at least three receptors: PAC_1, $VPAC_1$, and $VPAC_2$ (Harmar *et al*, 1998). All three receptors are coupled to an adenylate cyclase-activating G protein resulting in the elevation of cAMP (Pisegna and Wank, 1993). The PAC_1 receptor also stimulates phospholipase C (Spengler *et al*, 1993; Dickinson and Fleetwood-Walker, 1999), resulting in the production of diacylglycerol and inositol 1,4,5-triphosphate with the subsequent influx of Ca^{2+} from intracellular stores. In PC12 cells, PACAP38 is a more potent stimulator of adenylate cyclase and phospholipase C than PACAP27 (Deutsch and Sun 1992; Cavallaro *et al*, 1995). To determine if PACAP27 induced HSV-1 activation as efficiently as PACAP38, QIF-PC12 cell cultures were established and treated as described before. Spontaneous activation of virus occurred in 2.5% of cultures during the quiescent phase of infection following ACV withdrawal (data not shown). Nonproductive cultures were subjected to PACAP27 or PACAP38 at 0.8 and 4 nM, or media alone on day 17 p.i. As shown in Figure 1 (panel B) PACAP38 induced virus activation more effectively. The difference in efficacy was most pronounced at the 0.8 nM dose, in that virus was detected in 35% (8/23) and 54% (13/24) of cultures treated with PACAP27 and PACAP38, respectively. In contrast, virus activation in mock-induced QIF-PC12 cell cultures was 0% (0/24) by day 8 postinduction. These findings are consistent with the relative efficiencies of PACAP38 and PACAP27 induced cAMP accumulation in superior cervical ganglionic neurons (Braas and May, 1999). These data indicate that the 1–27 amino acid residues of PACAP are sufficient to induce HSV-1 activation from QIF-PC12 cell cultures and that the additional 11 amino acids of PACAP38 make the peptide a more efficient inducer. In addition, the response to both is concentration-dependent.

Effect of protein kinase inhibitors on PACAP38 induced HSV-1 activation from QIF-PC12 cells Our observation that PACAP induces HSV-1 activation

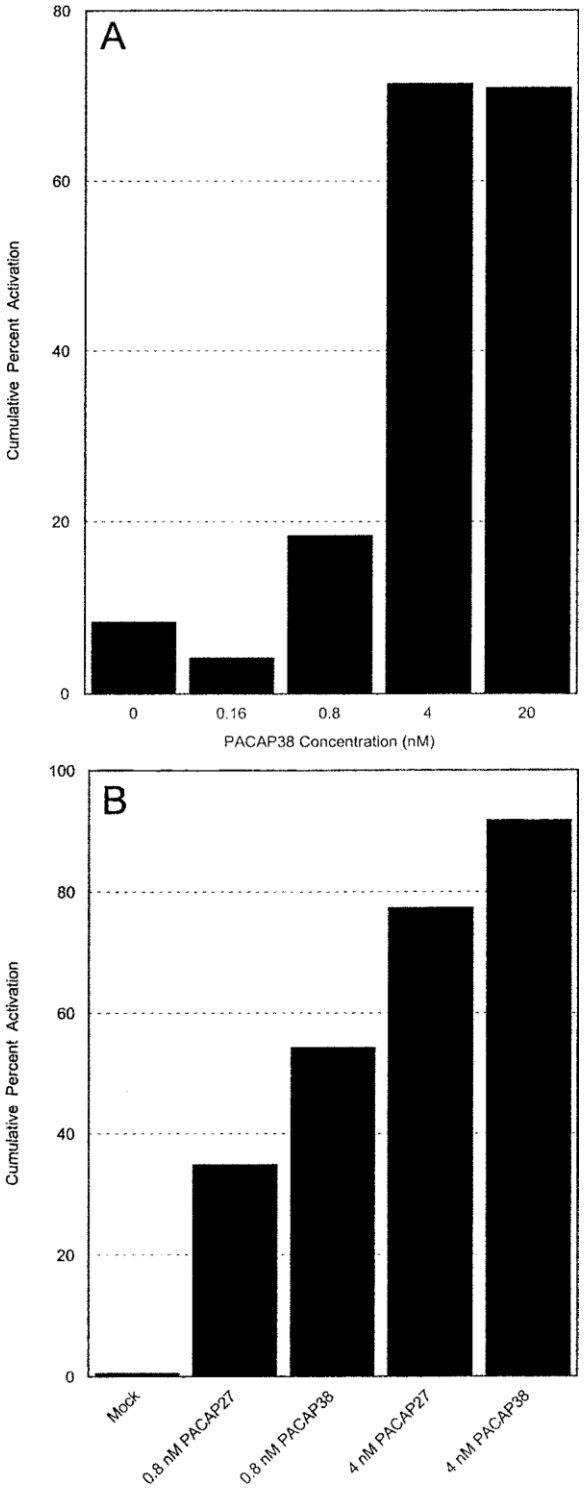

Figure 1 PACAP induced HSV-1 activation from QIF-PC12 cell cultures. QIF-PC12 cell cultures were established as described in the text. Cultures that were nonproductive for virus were subjected to RPMI maintenance media containing PACAP38 on day 15 p.i. (panel **A**), or PACAP38 or PACAP27 on day 17 p.i. (panel **B**). Values are cumulative percentage of cultures producing virus by day 8 postinduction from 21 to 24 culture wells in the PACAP treatment group, and 12 (panel **A**) and 24 (panel **B**) wells in the mock treatment group as determined from culture supernatants using a direct plaque assay.

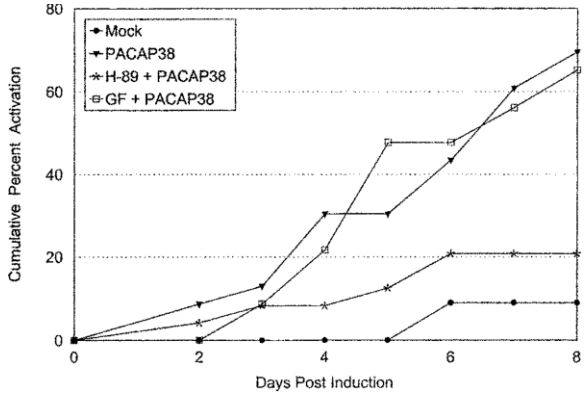

Figure 2 Effect of protein kinase inhibitors on PACAP38 induced HSV-1 activation from QIF-PC12 cell cultures. QIF-PC12 cell cultures that were nonproductive for virus were induced with PACAP38 (20 nM) on day 17 p.i. The PKA inhibitor, H-89 (20 μM), and the PKC inhibitor, GF109203X (GF; 1 μM) were added to the culture media 1 h prior to PACAP38 induction and were maintained during 2-day PACAP38 treatment. Virus production was determined from duplicate 12-well plates as described in Figure 1.

from QIF-PC12 cells led us to initiate studies that address the signaling pathway involved. Highly selective cell-permeable protein kinase inhibitors were used to examine the roles of PKA and PKC in PACAP-induced virus activation, in that pathways involving PKA and PKC have associations with HSV reactivation in tissue culture (Smith *et al*, 1992) and both are activated by PACAP. QIF-PC12 cell cultures were established as described previously. The maintenance phase of quiescence was marked by infrequent detection of virus in (4.2%, 4/96) cultures. Induction of virus activation was performed on day 17 p.i. with PACAP38 (20 nM) following a 60-min pretreatment with the PKA- and PKC-specific inhibitors, 20 μM H-89 (Chijiwa *et al*, 1990), and 1 μM GF109203X (Toullec *et al*, 1991), respectively.

Inhibitors were maintained throughout the 2-day treatment. Figure 2 shows that the PKC inhibitor had no effect on PACAP38 induction, as the proportion and rate of cultures producing virus (65%, 15/23) was similar to the PACAP-induced cultures (70%, 16/23) compared with only 9.1% (2/22) mock-induced cultures. Although GF109203X does not inhibit all PKC isozymes (Way *et al*, 2000), this concentration inhibits the isoform(s) involved in PACAP-mediated neurite formation in PC12 cells (Lazarovici *et al*, 1998). This suggests that GF109203X-sensitive PKC isozymes are not involved in HSV-1 activation. In contrast, the addition of the PKA inhibitor, H-89 (20 μM), significantly inhibited PACAP-induced virus production. The proportion of H-89 treated cultures yielding virus was delayed and remained 70% below uninhibited cultures by day 8 following PACAP-induction (21%, 5/24). This inhibitory effect was reversible, as a majority of the nonproductive H-89 treated cultures (53%, 10/19)

supported virus activation within 3 days of heat stress (Danaher *et al*, 1999b) performed on day 6 (i.e. day 25 p.i.) following the withdrawal of H-89.

These data could be interpreted to mean that H-89 inhibits virus replication subsequent to activation, as virus replication is impaired in PKA deficient PC12 cells (Xia *et al*, 1996). Alternatively, H-89 may block induction from virus activation, in that Jordan *et al* (1998) have shown that an inhibitor of PKA does not block HSV-1 growth in PC12 cells. Because the concentration of inhibitor used in our study was considerably higher than that used by Jordan *et al* (1998) we cannot directly compare their observation to ours. Nevertheless, taken together the results indicate that PACAP induced activation of HSV-1 from QIF-PC12 cells requires PKA and does not require the GF-sensitive PKC isozymes α, βI, βII, γ, δ, and ε (Toullec *et al*, 1991; Way *et al*, 2000), but may involve other PKC isozymes.

Direct stimulation of the PKA pathway with cAMP analogs results in limited activation from QIF-PC12 cell cultures To determine if activation of PKA was sufficient to induce virus activation, QIF-PC12 cell cultures were induced with cell membrane permeable cAMP analogs at concentrations shown to activate cAMP-dependent processes in neurons (Rydel and Greene, 1988). The cAMP analog, 8-(4-chlorophenylthio)-cAMP (CPT-cAMP) was selected because it induces PKA in PC12 cells (Erhardt *et al*, 1995; Nakayama *et al*, 2000) and reactivation of HSV in latently infected embryonic neuronal cultures *in vitro* (Smith *et al*, 1992). QIF-PC12 cell cultures were established as described before, and induced on day 17 p.i. Induction of the nonproductive cultures with 0.1 and 1 mM CPT-cAMP for 2 days resulted in the detection of virus from 38% (9/24) and 43% (10/23) of cultures by day 8 postinduction, respectively. In comparison, 0% (0/24) of the mock-induced cultures produced virus by day 8 postinduction. Attempts to induce HSV-1 activation with another cAMP analog, dibutyryl-cAMP, over a broad range of concentrations resulted in significantly lower induced activation of HSV-1 than that of CPT-cAMP (data not shown). The inability of cAMP analogs to induce virus activation as efficiently as PACAP indicates that PACAP induced activation of virus may involve cAMP-independent regulatory pathways in addition to PKA, or that PACAP is more efficient at inducing the PKA pathway.

The findings presented here are the first to demonstrate that physiological relevant concentrations of PACAP (Cai *et al*, 1997; Tanaka *et al*, 1997), a naturally occurring peptide abundant in the central nervous system, has potent regulatory activity on HSV activation. PACAP's neuroprotective, neurotrophic, and inflammatory role (Yada *et al*, 1994; Delgado *et al*, 1999; DiCicco-Bloom *et al*, 2000; Dickinson and Fleetwood-Walker, 1999) makes it a plausible mediator of virus reactivation following stress. Consistent with this possibility, the neuronal response to PACAP is rapid. Exposure to PACAP (4 nM) for as brief as 15 min was sufficient to induce activation of HSV-1 from 75% (18/24) of QIF-PC12 cell cultures.

Although our use of GF109203X demonstrated that the PKC isozymes α, β, γ, δ, and ε (Toullec *et al*, 1991; Way, 2000) are not required for HSV activation in this model, the role of other PKC isozymes remains to be determined. Parenthetically, attempts to induce the PKC pathway using phorbol-12-myristate-13-acetate have failed to activate HSV-1 from QIF-PC12 cell cultures in our laboratory (personal communication, Stephen Hambly, August, 1998). This suggests that induction of PKC alone may be insufficient to reactivate HSV in this model. However, the observation that CPT-cAMP and dibutyryl-cAMP treatment resulted in lower efficiency of HSV-1 activation than PACAP suggests that PACAP is more efficient in PKA activation or PKA-independent pathways contribute to virus activation from QIF-PC12 cells. It is interesting that Smith *et al* (1992) found that the PKC pathway was capable of, but not required for, induction of reactivation from latently infected ganglionic neurons. Although our data did not demonstrate a significant role for PKC in activation from QIF-PC12 cells, it does not rule out a role of PKC in activation. Sympathetic embryonic neurons may respond to different activation stimuli than neurally differentiated PC12 cells. It is also possible that more than one signaling pathway for reactivation exists and messenger pathways are cell type specific, being preferentially activated under specific circumstances. Further studies are needed to address these possibilities and whether other properties of PACAP (Tatsuno *et al*, 1992; Tanaka *et al*, 1996; Lai *et al*, 1997; May *et al*, 1998) are involved.

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References

Arimura A (1992). Pituitary adenylate cyclase-activating polypeptide (PACAP): discovery and current status of research *Regul Pept* **37:** 287–303.

Brass KM, May V (1999). Pituitary adenylate cyclase-activating polypeptides directly stimulate sympathetic neuron neuropeptide Y release through PAC_1 receptor

isoform activation of specific intracellular signaling pathways. *J Biol Chem* **274:** 27702–27710.

Cai Y, Xin X, Shim G-J, Mokuno Y, Uehara H, Yamada T, Agui T, Matsumoto K (1997). Pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) stimulate interleukin-6 production through the third subtype of PACAP/VIP receptor in rat bone marrow-derived stromal cells. *Endocrinology* **138:** 2515–2520.

Cavallaro S, D'Agata V, Guardabasso V, Travali S, Stivala F, Canonico PL (1995). Differentiation induces pituitary adenylate cyclase-activating polypeptide receptor expression in PC-12 cells. *Mol Pharmacol* **48:** 56–62.

Chijiwa T, Mishima A, Hagiwara M, Sano M, Hayashi K, Inoue T, Naito K, Toshioka T, Hidaka H (1990). Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. *J Biol Chem* **265:** 5267–5272.

Danaher RJ, Jacob RJ, Chorak MD, Freeman CS, Miller CS (1999b). Heat stress induce reactivation of herpes simplex virus type 1 from quiescently infected neurally differentiated PC12 cells. *J NeuroVirology* **5:** 374–383.

Danaher RJ, Jacob RJ, Miller CS (1999a). Establishment of a quiescent herpes simplex virus type 1 infection in neurally differentiated PC12 cells. *J NeuroVirology* **5:** 258–267.

Danaher RJ, Jacob RJ, Miller CS (2000). Herpesvirus quiescence in neuronal cells: antiviral conditions not required to establish and maintain HSV-2 quiescence. *J NeuroVirology* **6:** 296–302.

Delgado M, Martinez C, Pozo D, Calvo JR, Leceta J, Ganea D, Gomariz RP (1999). Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activation polypeptide (PACAP) protect mice from lethal endotoxemia through the inhibition of TNF-alpha and IL-6. *J Immunol* **162:** 1200–1205.

Deutsch PJ, Sun Y (1992). The 38-amino acid form of pituitary adenylate cyclase-activating polypeptide stimulates dual signaling cascades in PC12 cells and promotes neurite outgrowth. *J Biol Chem* **267:** 5108–5113.

DiCicco-Bloom E, Deutsch PJ, Maltzman J, Zhang J, Pintar JE, Zheng J, Friedman WF, Zhou X, Zaremba T (2000). Autocrine expression and ontogenetic functions of the PACAP ligand/receptor system during sympathetic development. *Dev Biol* **219:** 197–213.

Dickinson T, Fleetwood-Walker SM (1999). VIP and PACAP: very important in pain? *Trends Pharmacol Sci* **20:** 324–329.

Erhardt P, Troppmair J, Rapp UR, Cooper GM (1995). Differential regulation of Raf-1 and B-Raf and Ras-dependent activation of mitogen-activated protein kinase by cyclic AMP in PC12 cells. *Mol Cell Biol* **15:** 5524–5530.

Ghatei MA, Takahashi K, Suzuki Y, Gardiner J, Jones PM, Bloom SR (1993). Distribution, molecular characterization of pituitary adenylate cyclase-activating polypeptide and its precursor encoding messenger RNA in human and rat tissues. *J Endocrinol* **136:** 159–166.

Halford WP, Gebhart BM, Carr DJ (1996). Mechanisms of herpes simplex virus type 1 reactivation. *J Virol* **70:** 5051–5060.

Harmar AJ, Arimura A, Gozes I, Journot L, Laburthe M, Pisegna JR, Rawlings SR, Robberecht P, Said SI, Sreedharan SP, Wank SA, Waschek JA (1998). International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *Pharmacol Rev* **50:** 265–270.

Huang RD, Smith MF, Zahler WL (1982). Inhibition of forskolin-activated adenylate cyclase by ethanol and other solvents. *J Cyclic Nucleotide Res* **8:** 385–394.

Jordan R, Pepe J, Schaffer PA (1998). Characterization of a nerve growth factor-inducible celluar activity that enhances herpes simplex virus type 1 gene expression and replication of an ICP0 null mutant in cells of neural lineage. *J Virol* **72:** 5373–5382.

Lai CC, Wu SY, Lin HH, Dun NJ (1997). Excitatory action of pituitary adenylate cyclase activating polypeptide on rat sympathetic preganglionic neurons in vivo and in vitro. *Brain Res* **748:** 189–194.

Larsen JO, Hannibal J, Knudsen SM, Fahrenkrug J (1997). Expression of pituitary adenylate cyclase-activating polypeptide (PACAP) in the mesencephalic trigeminal nucleus of the rat after transsection of the masseteric nerve. *Brain Res Mol Brain Res* **46:** 109–117.

Lazarovici P, Jiang H, Fink D Jr (1998). The 38-amino-acid form of pituitary adenylate cyclase-activating polypeptide induces neurite outgrowth in PC12 cells that is dependent on protein kinase C and extracellular signal-regulated kinase but not on protein kinase A, nerve growth factor receptor tyrosine kinase, p21(ras) G protein, and pp60(c-src) cytoplasmic tyrosine kinase. *Mol Pharmacol* **54:** 547–558.

May V, Beaudet MM, Parsons RL, Hardwick JC, Gauthier EA, Durda JP, Braas KM (1998). Mechanisms of pituitary adenylate cyclase activating polypeptide (PACAP)-induced depolarization of sympathetic superior cervical ganglion (SCG) neurons. *Ann NY Acad Sci* **865:** 164–175.

Miller CS, Danaher RJ, Jacob RJ (1998). Molecular aspects of herpes simplex virus I: latency, reactivation and recurrence. *Crit Rev Oral Biol Med* **9:** 541–562.

Miller CS, Smith KO (1991). Enhanced replication of herpes simplex virus type 1 in human cells. *J Dent Res* **70:** 111–117.

Millhouse S, Wigdahl B. (2000). Molecular circuitry regulating herpes simplex virus type 1 latency in neurons. *J NeuroVirology* **6:** 6–24.

Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, Coy DH (1989). Isolation of a novel 38 residue hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun* **164:** 567–574.

Miyata A, Jiang L, Dahl RD, Kitada C, Kubo K, Fujino M, Minamino N, Arimura A (1990). Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). Biochem *Biophys Res Commun* **170:** 643–648.

Moller K, Reimer M, Ekblad E, Hannibal J, Fahrenkrug J, Kanje M, Sundler F (1997). The effects of axotomy and preganglionic denervation on the expression of pituitary adenylate cyclase activating polypeptide (PACAP), galanin and PACAP type 1 receptors in the rat superior cervical ganglion. *Brain Res* **775:** 166–182.

Moriya A, Yoshiki A, Kita M, Fushiki S, Imanishi J (1994). Heat shock-induced reactivation of herpes simplex virus type 1 in latently infected mouse trigeminal ganglion cells in dissociated culture. *Arch Virol* **135:** 419–425.

Nakayama H, Ueno S, Ikeuchi T, Hatanaka H (2000). Regulation of alpha3 nicotinic acetylcholine receptor subunit mRNA levels by nerve growth factor and cyclic AMP in PC12 cells. *J Neurochem* **74:** 1346–1354.

Pisegna JR, Wank SA (1993). Molecular cloning and functional expression of the pituitary adenylate cyclase-activating polypeptide type I receptor. *Proc Natl Acad Sci USA* **90:** 6345–6349.

Preston CM (2000). Repression of viral transcription during herpes simplex virus latency. *J Gen Virol* **81:** 1–9.

Rydel RE, Greene LA (1988). CAMP-analogs promote survival and neurite outgrowth in cultures of rat sympathetic and sensory neurons independently of nerve growth factor. *Proc Natl Acad Sci USA* **85:** 1257–1261.

Sawtell NM, Thompson RL (1992). Rapid in vivo reactivation of herpes simplex virus in latently infected murine ganglionic neurons after transient hyperthermia. *J Virol* **66:** 2150–2156.

Seamon KB, Daly JW (1981). Activation of adenylate cyclase by the diterpene forskolin does not require the nucleotide regulatory protein. *J Biol Chem* **256:** 9799–9801.

Smith RL, Pizer LI, Johnson EM Jr, Wilcox CL (1992). Activation of second-messenger pathways reactivates latent herpes simplex virus in neuronal cultures. *Virology* **188:** 311–318.

Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH, Journot L (1993). Differential signal transduction by five splice variants of the PACAP receptor. *Nature* **365:** 170–175.

Tajti J, Uddman R, Möller S, Fundler F, Edvinsson L (1999). Messenger molecules and receptor mRNA in the human trigeminal ganglion. *J Auton Nerv Syst* **76:** 176–183.

Tanaka J, Koshimura K, Murakami Y, Sohmiya M, Yanaihara N, Kato Y (1997). Neuronal protection from apoptosis by pituitary adenylate cyclase-activating polypeptide. *Regulatory Peptides* **72:** 1–8.

Tanaka K, Shibuya I, Nagamoto T, Yamashita H, Kanno T (1996). Pituitary adenylate cyclase-activating polypeptide causes rapid Ca^{2+} release from intracellular stores and long lasting Ca^{2+} influx mediated by Na^{+} influx-dependent membrane depolarization in bovine adrenal chromaffin cells. *Endocrinology* **137:** 956–966.

Tatsuno I, Yada T, Vigh S, Hidaka H, Arimura A (1992). Pituitary adenylate cyclase activating polypeptide and vasoactive intestinal peptide increase cytosolic free calcium concentration in cultured rat hippocampal neurons. *Endocrinology* **131:** 73–81.

Toullec D, Pianetti P, Coste H, Bellevergue P, Grand-Perret T, Ajakane M, Baudet V, Boissin P, Boursier E, Loriolle F, Duhame L, Charon D, Kirilovsky J (1991). The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. *J Biol Chem* **266:** 15771–15781.

Way KJ, Chou E, King GL (2000). Identification of PKC-isoform-specific biological actions using pharmacological approaches. *Trends Pharmacol Sci* **21:** 181–187.

Xia K, Knipe DM, DeLuca NA (1996). Role of protein kinase A and the serine-rich region of herpes simplex virus type 1 ICP4 in viral replication. *J Virol* **70:** 1050–1060.

Yada T, Sakurada M, Ihida K, Nakata M, Murata F, Arimura A, Kikuchi M (1994). Pituitary adenylate cyclase activating polypeptide is an extraordinarily potent intrapancreatic regulator of insulin secretion from islet beta-cells. *J Biol Chem* **269:** 1290–1293.

Zhang Q, Shi T-J, Ji R-R, Sundler F, Hannibal J, Fahrenkrug J, Hokfelt T (1995). Expression of pituitary adenylate cyclase-activating polypeptide in dorsal root ganglia following axotomy: time course and coexistence. *Brain Res* **705:** 149–158.

Zhang Y, Danielsen N, Sundler F, Mulder H (1998). Pituitary adenylate cyclase-activating peptide is upregulated in sensory neurons by inflammation. *Neuroreport* **9:** 2833–2836.