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REVIEW

The neurotrophic effects of PACAP in PC12 cells: control by multiple transduction pathways

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Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) are closely related members of the secretin superfamily of neuropeptides expressed in both the brain and peripheral nervous system, and they exhibit neurotrophic and neurodevelopmental effects *in vivo*. Like the index member of the Trk receptor ligand family, nerve growth factor (NGF), PACAP promotes the differentiation of PC12 cells, a well-established cell culture model, to investigate neuronal differentiation, survival and function. Stimulation of catecholamine secretion and enhanced neuropeptide biosynthesis are effects exerted by PACAP at the adrenomedullary synapse *in vivo* and on PC12 cells *in vitro* through stimulation of the specific PAC1 receptor. Induction of neuritogenesis, growth arrest, and promotion of cell survival are effects of PACAP that occur in developing cerebellar, hippocampal and cortical neurons, as well as in the more tractable PC12 cell model. Study of the mechanisms through which PACAP exerts its various effects on cell growth, morphology, gene expression and survival, i.e. its actions as a neurotrophin, in PC12 cells is the subject of this review. The study of neurotrophic signalling by PACAP in PC12 cells reveals that multiple independent pathways are coordinated in the PACAP response, some activated by classical and some by novel or combinatorial signalling mechanisms.

Keywords: differentiation, microarray, nerve growth factor, pituitary adenylate cyclase-activating polypeptide, PC12 cells, transduction pathways.

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Pituitary adenylate cyclase-activating polypeptide (PACAP) was initially isolated from ovine hypothalamic extracts on the basis of its ability to stimulate cAMP synthesis in cultured rat anterior pituitary cells (Miyata *et al*. 1989). The sequence of PACAP has been remarkably well conserved during evolution from protochordate to mammals, suggesting that the peptide is involved in the regulation of major biological functions (Vaudry *et al*. 2000b). Indeed, PACAP is widely distributed in the CNS and peripheral tissues, and exerts pleiotropic effects on the brain as well as endocrine glands, cardiovascular system, gastrointestinal and respiratory tracts, gonads, immune cells, and tumours (Vaudry *et al*. 2000b, 2006). In the developing CNS, PACAP decreases the proportion of mitotic cells and promotes neuroblast

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Abbreviations used: CREB, cAMP responsive element binding protein; ERK, extracellular signal-regulated kinases; GPCR, G protein coupled receptor; GTP, guanosine triphosphate; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; PACAP, pituitary adenylate cyclase-activating polypeptide; PAC1-R, PACAP-specific receptor; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; RTK, receptor tyrosine kinase; VIP, vasoactive intestinal polypeptide.

differentiation (Lu and DiCicco-Bloom 1997). In the adult brain, PACAP modulates neurotransmitter release and inhibits apoptotic cell death (Uchida *et al*. 1996; Anderson and Curlewis 1998). The neurotrophic actions of PACAP were first observed in PC12 cells (Table 1; Deutsch and Sun 1992). Since then, this cell line has been extensively used to investigate the signalling pathways involved in PACAP-induced cell differentiation and survival, and some of the genes that could encode the proteins acting in these processes have been identified. Interest in the molecular mechanisms of PACAP-induced neuronal differentiation is especially intense because it represents the prototype for G-protein-coupled receptor-mediated neurotrophin (Waschek *et al*. 1998; Beaudet *et al*. 2000; Vaudry *et al*. 2000b; Nicot *et al*. 2002) just like nerve growth factor (NGF) which has extensively been used as a model to investigate the function of tyrosine kinase receptors in the nervous system (Sofroniew *et al*. 2001; Töröcsik *et al*. 2002; Glebova and Ginty 2004).

The PC12 cell line was cloned from a rat adrenal pheochromocytoma and characterized based on its capacity to cease proliferation and extend branching varicose processes when exposed to NGF (Greene and Tischler 1976). Over the past 30 years, PC12 cells have become a very suitable model to decipher the genes and proteins involved in the effects of NGF on cell differentiation (Lange-Carter and Johnson 1994; Angelastro *et al*. 2000; Huang *et al*. 2001; Vaudry *et al*. 2002c; Lee *et al*. 2005). As illustrated in Table 1, the various studies have investigated different aspects of cell differentiation. In particular, it has been demonstrated that a long-lasting phosphorylation, together with a nuclear translocation of extracellular signal-regulated kinases (ERK1 and -2), are required for NGF-induced neurite outgrowth in PC12 cells (Pang *et al*. 1995). The duration of activation of ERKs is crucial for the multiple actions of NGF in PC12 cells (Pang *et al*. 1995) and, in fact, can determine outcomes as diverse as growth arrest, neurite extension, and differential gene expression (Murphy *et al*. 2002). Comparison of the mechanisms of neurotrophin signalling by NGF and PACAP (Fig. 1) may lead not only to a better understanding of how these neurotrophins function *in vivo* but to the mechanisms of generating diversity of cellular outcomes for a wider variety of growth factors that regulate cellular morphology, growth, and gene transcription patterns in the nervous system.

Table 1 Example of effects of PACAP or NGF accounting for PC12 cell differentiation

Function	References
Neurite outgrowth	Greene and Tischler (1976)
Cell proliferation	Greene and Tischler (1976)
Decrease in DNA synthesis	Gunning *et al.* (1981b)
Cell volume	Heumann *et al.* (1983)
Sodium channel density	Rudy *et al.* (1982)
N-CAM	Prentice *et al.* (1987)
MAPK1/2	Aletta *et al.* (1988)
Chromogranin A	Rausch *et al.* (1988)

Second messengers mediating the effects of PACAP on neurite outgrowth

Treatment of PC12 cells with PACAP induces neurite outgrowth (Deutsch and Sun 1992; Hernandez *et al*. 1995) and inhibits cell proliferation (Vaudry *et al*. 2002b). Three PACAP receptors have been identified (Harmar *et al*. 1998). The PACAP-specific receptor (PAC1-R) exhibits a high affinity for PACAP ($K_d \approx 0.2$ nM) and a much lower affinity for the vasoactive intestinal polypeptide (VIP; $K_d \approx 1$ μM), whereas VPAC1-R and VPAC2-R have similar affinities for PACAP and VIP. The action of PACAP on PC12 cells involves the PAC1-R based on the differential potency of VIP and PACAP to stimulate neuropeptide Y gene expression (Colbert *et al*. 1994). The PAC1-R is known to be positively coupled to the adenylate cyclase, phospholipase C and calcium pathways (Hernandez *et al*. 1995) and there is now evidence that this receptor can also activate other signalling molecules such as phospholipase D (McCulloch *et al*. 2001) and Ras (Osipenko *et al*. 2000). It is possible to induce neurite outgrowth by increasing cAMP levels in PC12 cells (Gunning *et al*. 1981a), but this effect has been reported to be protein kinase A (PKA) independent (Fig. 1; Lazarovici *et al*. 1998). PACAP also increases VIP gene expression through a cAMP-dependent, but PKA-independent, pathway in bovine chromaffin cells (Hamelink *et al*. 2002). As previously observed for NGF, ERK phosphorylation mediates the effect of PACAP on neurite outgrowth (Barrie *et al*. 1997; Lazarovici *et al*. 1998; Vaudry *et al*. 2002b). However, the mechanism of mitogen-activated protein kinase (MAPK) is still a matter of debate, as some reports indicate that the protein kinase C (PKC) pathway is involved in the stimulation of ERK (Barrie *et al*. 1997), while others suggest that ERK activation requires cAMP elevation (Fig. 1; Hernandez *et al*. 1995; Vaudry *et al*. 2001). This discordance could be explained by the fact that several transduction pathways, including PKA, PKC and/or the small guanosine triphosphate (GTP) loading protein Rap1 may synergize to activate ERK (Fig. 1; Bouschet *et al*. 2003) under certain conditions. Whether the effects observed with PACAP when PC12 cells are cultured in normal serum or in low serum (in which most ERK studies have been conducted; Barrie *et al*. 1997; Bouschet *et al*. 2003) is a matter which should be investigated more closely. It has also been noticed that phorbol 12-myristate 13-acetate (PMA) not only inhibits neurite outgrowth but also elicits neurite retraction when added 48 h after PACAP (Vaudry *et al*. 2002a), suggesting that this

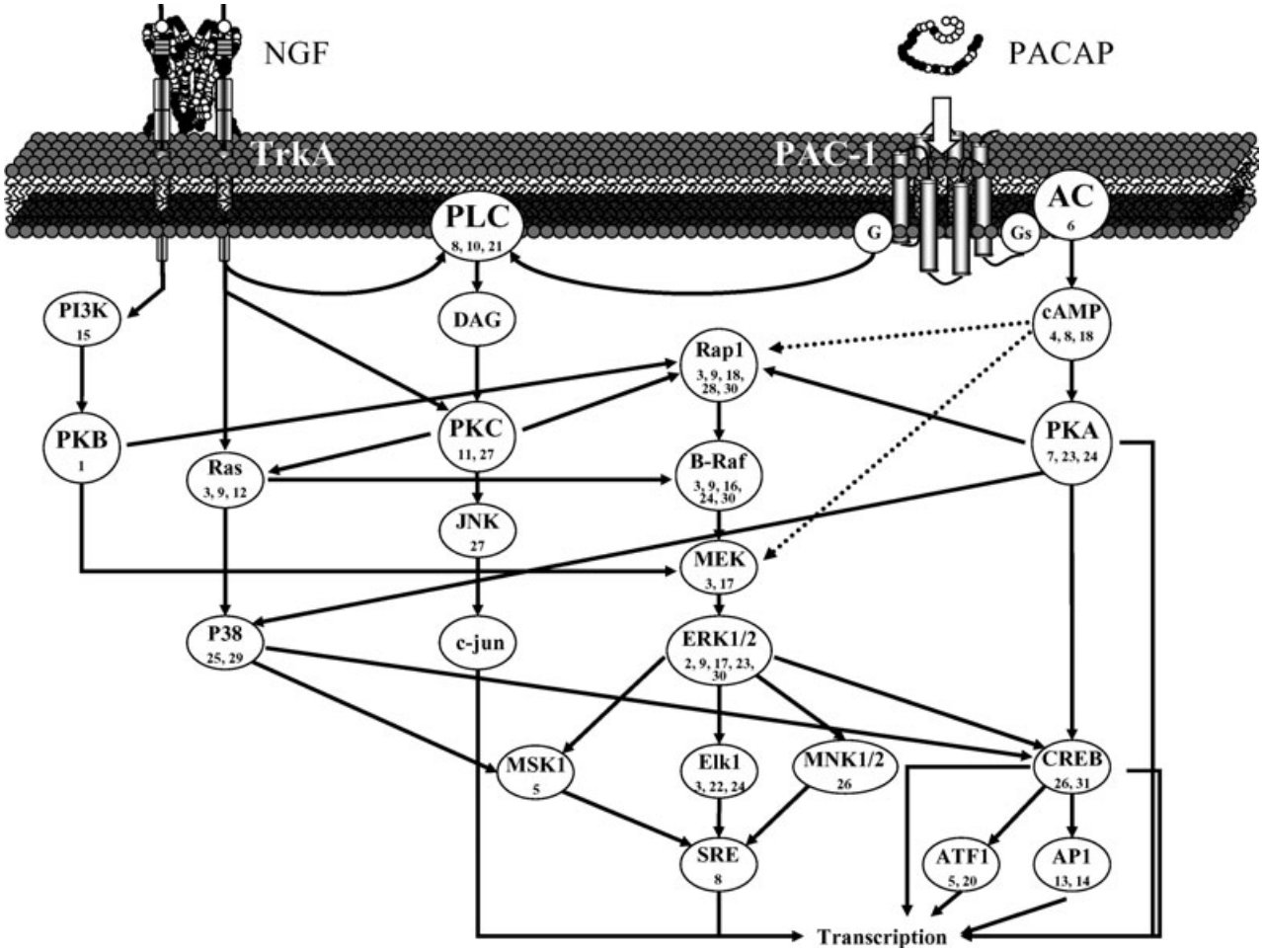

Fig. 1 Schematic representation summarizing the current knowledge concerning the intracellular events involved in the neurotrophic activities of PACAP and NGF on PC12 cells. Transcription is the intermediary which induces the diversity of the effects of PACAP and NGF, including cell differentiation and neuroprotection. The dotted arrows indicate a cAMP-dependent but PKA-independent pathway whose intermediate features have not yet been worked out but are likely to be Rap1-dependent. AC, adenylate cyclase; AP1, activator protein-1; ATF1, activating transcription factor 1; B-Raf, B-Raf proto-oncogene serine/threonine-protein kinase; cAMP, adenosine 3′, 5′-cyclic monophosphate; c-Jun, jun oncogene; CREB, cAMP responsive element binding protein; DAG, diacyl glycerol; Elk1, member of the E26 Transformation-specific sequence (ETS) oncogene family; ERK1 and -2, extracellular signal-regulated kinases; G, guanine nucleotide-binding regulatory protein; Gs, stimulatory guanine nucleotide-binding regulatory protein; JNK, c-Jun N-terminal kinase 1; MEK, mitogen-activated protein kinase; MNK1/2, MAPK-interacting serine/threonine kinase 1/2; MSK1, mitogen- and stress-activated protein kinase 1; p38, p38 mitogen-activated protein kinase; PI3K, phosphoinositide 3 kinase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; Rap1, member of RAS oncogene family; Ras, retrovirus-associated DNA sequences; SRE, serum-responsive element. Numbers on the figure indicate the following references: 1, Ashcroft *et al.* (1999); 2, Barrie *et al.* (1997); 3, Bouschet *et al.* (2003); 4, Burgun *et al.* 2000; 5, Deak *et al.* (1998); 6, Deutsch and Sun (1992); 7, Grewal *et al.* 2000; 8, Hernandez *et al.* (1995); 9, Kao *et al.* (2001); 10, Kim *et al.* (1991); 11, Lazarovici *et al.* (1998); 12, Mochizuki *et al.* 2001; 13, Minneman *et al.* 2000; 14, Monnier and Loeffler (1998); 15, Nusser *et al.* 2002; 16, Oshima *et al.* (1991); 17, Pang *et al.* (1995); 18, Ravni *et al.* manuscript in preparation; 19, Robinson *et al.* (1998); 20, Shimomura *et al.* (1998); 21, Tsukada *et al.* (1994); 22, Vanhoutte *et al.* 2001; 23, Vaudry *et al.* (2002b); 24, Vossler *et al.* (1997); 25, Wang *et al.* (2005); 26, Waskiewicz *et al.* (1997); 27, Wooten *et al.* 2000; 28, Wu *et al.* 2001; 29, Xing *et al.* (1998); 30, York *et al.* (1998); 31, Yukimasa *et al.* (1999). Further information concerning the signal transduction pathways involved in the control of gene transcription by PACAP and NGF during PC12 cell differentiation can be obtained after free registration at: http://stke.sciencemag.org/cgi/cm/stkecm; CMP_8038.

molecule does not specifically interfere with the effect of PACAP on neurite outgrowth, but exerts a dominant neurite-collapsing activity in PC12 cells. Alternatively, it cannot be excluded that, within 30 years of research with PC12 cells, several clones have emerged in which PACAP may activate distinct transduction pathways. Indeed, some differences in the morphological aspect of the cells after PACAP treatment are clearly observed between various PC12 cell clones

(Deutsch and Sun 1992; Grumolato *et al*. 2003a). These variations may be as a result of translocations or point mutations of some particular genes that have been shown to occur at higher frequency in cancer cells (Agapova *et al*. 1996). As PC12 cells are used in many labs around the world today, this point deserves to be clarified by conducting a detailed genomic and proteomic comparative analysis on various clones.

As PC12 cells specifically express the PAC1-R and exhibit robust morphological changes after treatment with PACAP, they have been used to investigate the structure–activity relationships of molecules acting on PAC1-R, such as maxadilan (Moro *et al*. 1999) or PACAP analogues (Onoue *et al*. 2001). Indeed, this cell line represents a model of choice to test the ability of new PACAP agonists to promote neurite outgrowth, block cell proliferation or inhibit apoptotic cell death.

Comparison of the effects of PACAP and NGF on PC12 cell differentiation

Various types of cross regulation between PACAP and NGF have been reported. In particular, PACAP increased the basal and NGF-stimulated phosphorylation of the tyrosine kinase A receptor (Lazarovici and Fink 1999), while NGF stimulates the expression of the PAC1-R (Jamen *et al*. 2002). The two neurotrophic agents can also synergize to promote, through different transduction pathways, prolonged phosphorylation of ERK1 and -2 through Rap1 (Fig. 1; Kao *et al*. 2001; Sakai *et al*. 2004), which is involved in the induction of neurite outgrowth. Downstream of the activation of the ERK pathway, NGF induces a sustained and strong expression of the neuron-specific activator p35 and of the cyclin-dependent kinase 5 (Harada *et al*. 2001), which are required for NGF-induced neurite outgrowth. PACAP and NGF also activate Rac1 through both a PI3–kinase-independent and -dependent pathway (Fig. 1; Sakai *et al*. 2004). It has been suggested using Chinese hamster ovary (CHO) cells that cAMP-induced Rac GTP loading could involve the cAMP guanine nucleotide-exchange factor Epac1 and the small GTPase Rap1 cascade (Maillet *et al*. 2003), which could explain how PACAP acts in PC12 cells. The activation of Rac1 leads to the translocation of the protein from the cytoplasm to the cell membrane where it co-localises with F-actin fibres (Sakai *et al*. 2004). Thus, it may be that early stages of PC12 cell differentiation induced by PACAP may proceed through an Epac-dependent mechanism without de novo protein synthesis.

There is also evidence that PACAP and NGF induce not only the expression of the PACAP receptor but also that of PACAP itself, an effect that is mediated through the p38 MAPK pathway (Fig. 1; Hashimoto *et al*. 2000). This observation may explain why, in some cell types such as cerebellar granule neurons, a brief exposure to PACAP is sufficient to promote a long-lasting effect on cell survival or neurite outgrowth (Vaudry *et al*. 1998). In addition to its effect on neuritogenesis, PACAP, like NGF, increases the density of sodium and calcium channels necessary for the acquisition of electrical excitability (Grumolato *et al*. 2003a) and stimulates the expression of the VAChT gene, a marker of cholinergic neurons (Grumolato *et al*. 2003b). These observations indicate that PACAP not only affects PC12 cell morphology but also plays an important role for the development of a neuronal phenotype.

Although both PACAP and NGF promote PC12 cell differentiation, it has to be noted that their effects are not redundant but rather complementary. The first evidence comes from the observation that PACAP potentiates NGF-induced neurite outgrowth (Sakai *et al*. 2001). At the molecular level, PACAP, but not NGF, up-regulates different constituents of large dense core vesicles, including chromogranin A and the monoamine transporter VMAT1 (Grumolato *et al*. 2003a). Chromogranin A is an important component of the secretory vesicle as its presence is required for secretory granule formation both in PC12 cells (Kim *et al*. 2001) and *in vivo* (Mahapatra *et al*. 2005). Thus, PACAP either as a neurotrophin during development or a sympatoadrenal transmitter in the mature animal, may function to modulate granin abundance and secretory vesicle formation *in vivo*. The study of the synergistic effects of NGF and PACAP on PC12 cells (Gunning *et al*. 1981a; Sakai *et al*. 2004) are particularly intriguing as they may represent a model for G protein coupled receptor (GPCR)- and receptor tyrosine kinase (RTK)-mediated collaboration in differentiating events *in vivo*, even if this hypothesis remains to be investigated.

PC12 cells as a model to evaluate the capacity of PACAP to inhibit apoptosis induced by neurotoxic agents

Serum deprivation provokes apoptosis of PC12 cells (Batistatou and Greene 1993) and cell death can be prevented by PACAP treatment (Tanaka *et al*. 1997; Vaudry *et al*. 2002b). PACAP also protects PC12 cells from apoptosis induced by various neurotoxic agents, such as ceramides (Hartfield *et al*. 1998), glutamate (Said *et al*. 1998), prion protein fragment 106–126 (Onoue *et al*. 2002a), β-amyloid peptide 1–42 (Onoue *et al*. 2002b), hypoxia (Suk *et al*. 2004) and the mitochondrial complex 1 inhibitor rotenone (Wang *et al*. 2005). The protective effects of PACAP are mediated through both the PKA and MAPK pathways (Onoue *et al*. 2002c) and involve the inhibition of caspase 3 (Hartfield *et al*. 1998; Wang *et al*. 2005). There is also evidence that PACAP may protect cells from the toxicity of nitric oxide, and other toxic derivatives such as peroxynitrite, by inhibiting neuronal nitric oxide synthase activity (Onoue *et al*. 2002c). It should be noted that many of the

neurotrophic effects of PACAP on PC12 cells and the mechanisms involved are also observed in several neuronal cell lines. For instance, PACAP has been shown to prevent apoptosis of cerebellar granule neurons induced by ceramides (Vaudry *et al*. 2003; Falluel-Morel *et al*. 2004) and to reduce olfactory neuronal cell death (Han and Lucero 2005) by inhibiting caspase 3 activity (Vaudry *et al*. 2000a; Han and Lucero 2005). It is thus assumed that the investigations conducted on PC12 cells will help to understand some of the neuroprotective effects of PACAP observed in pathophysiological models such as stroke (Reglodi *et al*. 2000; Chen *et al*. 2004).

Investigation of the genes regulated by PACAP in PC12 cells

Studies on candidate genes have shown that PACAP up-regulates the expression of the tyrosine hydroxylase and dopamine β hydroxylase genes (Corbitt *et al*. 1998). PACAP also induces a transient expression of c-*fos*, *fos*B, *jun*B and *jun*D mRNA (Yukimasa *et al*. 1999). In order to get a more comprehensive view on the mechanisms involved in the neurotrophic effects of PACAP on PC12 cells, several groups have carried out microarray and subtractive hybridization analyses (Vaudry *et al*. 2002b; Grumolato *et al*. 2003b; Ishido and Masuo 2004). Two of these studies were conducted after a 6-h treatment with PACAP when the differentiation process begins (Vaudry *et al*. 2002b; Ishido and Masuo 2004), while the third study was performed after a 48-h exposure to PACAP, when cells are fully differentiated (Grumolato *et al*. 2003b). Albeit the PC12 cell clones, the incubation time and the concentrations of PACAP (10^{-7} vs. 10^{-9} M) used by each group were different, it is interesting to note that a set of genes were found to be modulated by PACAP in at least two independent studies (Table 2).

Looking at the function of the messenger RNA transcripts regulated by PACAP, it appears that some genes probably control neuritogenesis (i.e. villin 2, ephrin A2, actin, tubulin, ornithine decarboxylase and annexin A2) or cell growth (i.e. growth arrest specific 1, growth arrest specific 5 and cyclin B2), while a third subset likely participate to the anti-apoptotic effects of the peptide (i.e. peroxiredoxin 5, thioredoxin reductase, cytochrome P450, fibroblast growth factor regulated protein).

Some of the genes found to be regulated by PACAP, such as the ornithine decarboxylase or the protein tyrosine phosphatase (4a1), had already been reported to be regulated by NGF (Table 3; Aparicio *et al*. 1992). In fact, some of these messengers are required for NGF-induced PC12 cell differentiation. For instance, the calcium binding S-100 protein is induced by NGF and the transfection of the cDNA coding for this protein in PC12 cells promotes process

Table 2 Example of genes found to be regulated by PACAP in PC12 cells by at least two independent groups

Gene name	Unigene ID	Reference
Cyclin B2	Rn. 124802	Vaudry *et al*. (2002b); Ishido and Masuo (2004)
Growth arrest specific 1	Mm. 22701[a]	Vaudry *et al*. (2002b); Grumolato *et al*. (2003b)
High-mobility group box 2	Rn. 2874	Vaudry *et al*. (2002b); Grumolato *et al*. (2003b)
Immediate early response 3	Rn. 23638	Vaudry *et al*. (2002b); Grumolato *et al*. (2003b)
LIM-only 1	Mm. 12607[a]	Vaudry *et al*. (2002b); Grumolato *et al*. (2003b)
Ornithine decarboxylase	Rn. 874	Vaudry *et al*. (2002b); Ishido and Masuo (2004)
P450 cytochrome oxydase	Rn. 11359	Vaudry *et al*. (2002b); Grumolato *et al*. (2003b)
Sex comb on midleg homologue 1	Rn. 7761	Vaudry *et al*. (2002b); Grumolato *et al*. (2003b)
Tissue plasminogen activator	Rn. 107102	Vaudry *et al*. (2002b); Ishido and Masuo (2004)
Villin 2	Rn. 773	Vaudry *et al*. (2002b); Ishido and Masuo (2004)

[a]The genes for LIM-only 1 and Growth arrest specific one have not so far been cloned for the rat.

Table 3 Examples of genes or proteins regulated by PACAP in PC12 cells that can also be found to be regulated by NGF in the literature

Gene name	Unigene ID	NGF	PACAP
Annexin II	Rn. 90546	Jacovina *et al*. (2001)	Vaudry *et al*. (2002b)
Tyrosine hydroxylase	Rn. 11082	McTigue *et al*. (1985)	Corbitt *et al*. (1998)
Ornithine decarboxylase	Rn. 874	Volonte and Greene (1990)	Vaudry *et al*. (2002b)
Tissue plasminogen activator	Rn. 107102	Jacovina *et al*. (2001)	Vaudry *et al*. (2002b); Ishido and Masuo (2004)
Galectin 3	Rn. 764	Kuklinski *et al*. (2003)	Vaudry *et al*. (2002b)
Chromogranin B	Rn. 11090	Huang *et al*. (2001)	Grumolato *et al*. (2003b)

elongation (Masiakowski and Shooter 1990). Neurite outgrowth induced by NGF also requires annexin II-mediated plasmin generation (Jacovina *et al*. 2001). These observations confirm that PACAP and NGF share some common mechanisms to promote the differentiation of PC12 cells. Several groups have investigated the genes regulated by NGF in PC12 cells (Angelastro *et al*. 2000; Lee *et al*. 2005), but a detailed comparison using both PACAP and NGF in the same conditions would be necessary to get comprehensive information concerning the common and distinct genes regulated by these two neurotrophic factors and to clarify how these two molecules act. In particular, it has been demonstrated that neuritogenesis elicited by NGF requires the immediate early gene Mafk (Töröcsik *et al*. 2002), whose expression is sufficient to trigger differentiation in the absence of NGF. Whether PACAP-mediated neuritogenesis proceeds through the same gene or requires a different set of messengers will provide some insight into the redundant and specialized functions of RTK and GPCR neurotrophins in the nervous system.

Based on the functional information provided by various inhibitors, a selection by microarray has been performed to identify some genes potentially involved in the control of neurite outgrowth. These experiments led to the characterisation of 13 messengers regulated by an ERK-dependent, PKA-independent manner and thus possibly involved in the control of neurite outgrowth (Vaudry *et al*. 2002b). Researchers are now trying to elucidate the role of several genes of interest using small interfering RNA and/or expression vectors.

What else can we learn regarding the neurotrophic effects of PACAP using the PC12 cell line?

More than 200 different genes regulated by PACAP during PC12 cell differentiation have now been identified and some of these genes are induced over 30-fold (Vaudry *et al*. 2002b; Grumolato *et al*. 2003b; Ishido and Masuo 2004; Ravni *et al*. manuscript submitted). Gene expression has been shown to be regulated through several pathways singly or in combination (Vaudry *et al*. 2002b). Based on this information, it will be interesting to undertake a promoter sequence analysis of each pool of genes. Bioinformatics investigation should then be combined with functional experiments using promoter deletion mutants in order to understand how PACAP regulates gene expression in these cells. An important aspect to consider is that some proteins, by interacting with different partners, can exert opposite effects according to the differentiation state of the cells. For instance c-Jun over-expression prevents undifferentiated PC12 cells from apoptosis and triggers neuritogenesis, whereas, after differentiation, c-Jun induction promotes cell death (Leppä *et al*. 2001).

It should be noted that, apart from the involvement of the ERK phosphorylation and a few other kinases, little is known concerning the proteins controlling the neurotrophic effects of PACAP. It has been shown by gel mobility shift assay that PACAP enhances the formation of protein complexes which bind to TPA- and cAMP-responsive elements (Yukimasa *et al*. 1999). Gel shift and supershift analyses revealed that these binding factors include *fos*B, c-*fos*, *jun*D and cAMP responsive element binding protein (CREB). More recently, a set of proteins regulated by PACAP has also been identified using 2-D gels (Lebon *et al*. 2006). These proteins include calmodulin, tubulin and caspase 3, but further investigations are still needed to provide a comprehensive picture of the different elements involved in the effects of PACAP in PC12 cells. As MAPKs clearly play a key role in the differentiation of PC12 cells (Fig. 1), a subproteome analysis should be conducted to identify more precisely the proteins phosphorylated by PACAP. It will also be interesting to search for the existence or not of a correlation between the numerous genes activated by PACAP and their respective proteins.

Fourteen years after the first demonstration that PACAP promotes neurite outgrowth in PC12 cells, this line represents one of the best-characterized model to elucidate the mechanisms controlling the neurotrophic actions of PACAP and is currently actively used to decipher the transduction pathways, genes and proteins involved in the effects of PACAP on cell proliferation, differentiation and apoptosis.

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