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The effects of PACAP on neural cell proliferation

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Abstract

PACAP and its receptors are expressed in growth zones of the brain. By stimulating PAC_1-receptors PACAP can enhance, as well as reduce, the proliferation rate in a cell-type dependent manner. PACAP can enhance the proliferation rate by activating phospholipase C and protein kinase C, although other signal transduction pathways may also be responsible. PACAP can suppress proliferation by inhibiting protein complexes of the cyclins D and E with the cyclin-dependent kinases 4/6 and 2, respectively, which are necessary for entry into the cell cycle. PACAP seems to exert these inhibitory effects by acting via the Sonic hedgehog glycoprotein and the small GTPase RhoA. Also, the activation of a cyclin-dependent kinase inhibitor has been suggested. The signal transduction pathways mediating the effects of PACAP on proliferation are discussed.

Keywords: PACAP gene; Neural cell; Proliferation

1. Introduction

In mouse brain, the expression of the PACAP gene starts as early as embryonic day 9.5 (E9.5), whereas it begins at E13 in rat brain. In both species, expression continues through the embryonic and postnatal development [1–4]. During this time, neuroepithelial cells of the germinative zones show the strongest gene expression. The presence of PACAP in embryonic chick brain is evidence for the wide distribution of the peptide in the animal kingdom [5]. The expression of VIP starts at E19 and matures during the first postnatal weeks [6]. The production of PACAP38 and VIP during the periods of cell proliferation and differentiation suggests a role of both peptides in brain development. Indeed, PACAP38 and VIP have been shown to increase as well as decrease the proliferation of neurons and glial cells in several species [7–18]. During the development of the neocortex, cells in the proliferation zone exit from the cell cycle and migrate into the cortical plate to form layers. The neurons of each layer of the neocortex have different functions. By inducing the exit from the cell cycle, PACAP38 may determine the fate of the respective neurons and consequently their function within the neocortex [19]. In addition, PACAP38 and VIP have been reported to facilitate neuronal survival and neurite formation [20–31]. In view of these data on the role of PACAP in brain development, it is surprising that mice lacking the PACAP gene show pronounced disturbances in carbohydrate and lipid metabolism but no macroscopic changes in brain structure [32,33]. One can only speculate that PACAP acts in synergy with other modulators which can compensate for its loss.

The diverse effects of PACAP38 and VIP on the proliferation of neural cells have been linked to different receptors and their signal transduction pathways. Work is now in progress to analyze the cellular mechanisms by which the peptides induce the changes in proliferation. Respective findings published in the last years will be discussed in the following review. In view of its dominant role in the regulation of proliferation, we will discuss the effects of PACAP38.

2. PACAP receptors: ontogeny of their expression, signal transduction pathways and effects on proliferation

PACAP acts on heptahelical G protein-coupled receptors. Three isoforms (PAC1-, VPAC1- and VPAC2-receptors) have

Abbreviations: CDK; Cyclin-dependent kinase; ECM; extracellular matrix; ERK; extracellular signal-regulated kinase; MEK; extracellular signal-regulated kinase activator kinases; Shh; Sonic hedgehog.

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been pharmacologically characterized and cloned. PAC1-receptors (PAC1-Rs) bind PACAP38 with an IC_{50} value of 1 nM but VIP with an IC_{50} value of >500 nM (for review see [34]). In the rat, alternative splicing in the 3rd intracellular loop region generates up to 6 variants of PAC1-Rs, that is a short form devoid of an insert as well as variants containing the hip, hop1 or hop2 (the latter differs by 1 amino-acid) cassette. Variants containing the hip–hop1 or hip–hop2 cassettes are also possible [35,36]. After their ectopic expression, all variants can activate adenylate cyclase, when stimulated with nanomolar concentrations of PACAP38. Activation of phospholipase C is also possible but is impaired by the presence of the hip cassette [35]. PAC1-Rs differing in the N-terminus or transmembrane domain 4 have also been reported [37,38]. In contrast, VPAC1-Rs and VPAC2-Rs bind PACAP38 as well as VIP with IC_{50} values of 1 to 4 nM [34]. Both receptors do not seem to have splice variants [39,40]. They activate adenylate cyclase and mobilize intracellular Ca^{++}, but do not seem to increase the production of inositol phosphates (for review see [41]). It is noteworthy that PACAP38 can also activate NO synthesis, phospholipase D, phosphoinositide 3-kinase as well as mitogen-activated protein kinases (MAPK) (for review see [28]). The expression of receptors has been shown to be cell type specific. Cultured cortical neurons express PAC1 and VPAC2-Rs, whereas cultured type I astroglial cells express PAC1-Rs as well as both VPAC-Rs [42].

From E17 to E21, high densities of PACAP38 binding sites were reported in germinative zones of the rat brain, such as those of the neocortex and hippocampus. From birth to postnatal day 12, the density of PACAP38 binding sites gradually decreases [43]. RT-PCR showed transcripts of the short and a hop variant of PAC1-Rs as early as E10, but those of the hip-hop variant only at E17. Transcripts of VPAC1-Rs and VPAC2-Rs were found at E1 [43]. In situ hybridization confirmed the presence of one or more transcripts coding for PACAP38 receptors in the germinative zones of the brain [2,4,14,17,43–45]. It is noteworthy that the density of VPAC1-R and VPAC2-R mRNA was low in all germinative areas compared with that of PAC1-R mRNA [43].

By stimulating PAC1-Rs, PACAP38 can inhibit as well as increase the proliferation of neuronal and glial precursors in vivo [14,17,45]. PACAP38 exerts pleiotropic effects on proliferation even within the same cell type. In Neuro2a cells, which express only the PAC1 gene, PACAP38 increases the proliferation in a MEK1/2-dependent manner at picomolar concentrations, but inhibits the proliferation via protein kinase A at nanomolar concentrations [46]. If one of these pathways is pharmacologically blocked, PACAP38 strongly induces the remaining effect over the whole concentration range.

The diverse effects of PAC1-Rs on proliferation can be linked to different splice variants (Table 1). In neocortical precursors, the short variant mediates an increase in cytosolic cAMP levels and thus leads to an antimitogenic effect, whereas in sympathetic neuroblasts a hop variant mediates the mitogenic effect [10]. Ectopic expression of the hop variant in neocortical neuroblasts enables PACAP38 to stimulate proliferation, confirming its stimulatory effect on mitogenesis [47]. Precursors from mouse hindbrain express mainly the short variant of PAC1-Rs and the hop variant to a minor extent. The hip variant of PAC1-Rs as well as VPAC1-Rs and VPAC2-Rs is hardly expressed [48]. PACAP38 stimulates the proliferation of mouse hindbrain precursors in the absence of the growth factor FGF-2 but inhibits the proliferation in the presence of FGF-2 [48]. The stimulation is MEK1/2-dependent, whereas the inhibition is due to activation of protein kinase A. Taken together, these data present evidence that PACAP38 can inhibit and stimulate proliferation by acting on the short and hop variant, respectively.

Table 1
PACAP can stimulate as well as inhibit neuronal proliferation by acting via different PAC1-R splice variants

Cell type/species	Effect on proliferation	Receptor/splice variant	Second messengers
Neocortical precursors/rat	Inhibition	PAC1/short variant	cAMP [10]
Neocortical precursors/rat	Stimulation	PAC1/hop variant (ectopic expression)	DAG [47]
Sympathetic neuroblasts/rat	Stimulation	PAC1/hop variant	DAG [10]
Hindbrain precursors/mouse	Inhibition	PAC1/short variant	PKA [48]
Hindbrain precursors/mouse	Stimulation	PAC1/hop variant	MEK1/2 [48]

3. The cell cycle and its regulation

Cells pass through 4 phases during the proliferation cycle. When the decision has been made to begin a new cycle, cells progress through the G1 phase. Once they have passed the restriction point at the end of the G1 phase, the cells enter the S phase during which the DNA is replicated. Most cells quickly progress through the subsequent G2 phase to enter mitosis. When the doubled chromosomes have been separated, cytokinesis leads to the separation of the cell body. Now, the proliferation cycle can be repeated. Extracellular signal-regulated kinases (ERKs) play a central role in the regulation of the proliferative cycle. They promote the progression through the G1 phase and transition phase so that the S phase is entered. The regulation of ERKs is shown in Fig. 1, whereas their effects are summarized in Fig. 2.

Stimulation of growth factor receptors, i.e. receptor tyrosine kinases, induces the activation of the small GTPase Ras, which recruits Raf to the membrane. Activated Raf phosphorylates extracellular signal-regulated kinase activator kinases 1 and 2 (MEK1/2). These dual specificity kinases in turn phosphorylate the ERKs at a tyrosine and threonine residue (for review see [49]). In addition, stimulation of G protein-coupled receptors can activate phospholipase C which then activates Raf via protein kinase C (for review see [50]). ERKs can only induce proliferation, if they are activated for several hours. The attachment of the cell to the extracellular matrix (ECM) is essential for the sustained activation of ERKs and thus for the entry of the cell