Available online at www.sciencedirect.com
ScienceDirect
Regulatory Peptides 137 (2006) 58 – 66
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Growth factor-dependent actions of PACAP on oligodendrocyte progenitor proliferation

Vincent Lelievre [a,b,1], Cristina A. Ghiani [a,1], Akop Seksenyan [a], Pierre Gressens [b], Jean de Vellis [a], James A. Waschek [a,*]

[a] David Geffen School of Medicine, Department of Psychiatry, Semel Institute for Neuroscience and Mental Retardation Research Center, University of California at Los Angeles, USA
[b] INSERM U676 and IFR Paris 7, Hopital Robert Debre, Paris, France

Received 28 February 2006; received in revised form 20 April 2006; accepted 22 April 2006
Available online 20 September 2006

Abstract

We previously reported that rat oligodendrocyte progenitors (OLP) express receptors for the pituitary adenylyl cyclase-activating peptide (PACAP) *in vivo* and *in vitro*. Addition of PACAP to cultured OLP triggered a potent elevation in intracellular cAMP contents, a dose-dependent stimulation of proliferation, and a delay in myelinogenesis (Lee M, Lelievre V, Zhao P, Torres M, Rodriguez W, Byun JY, Doshi S, Ioffe Y, Gupta G, de los Monteros AE, de Vellis J, Waschek J. Pituitary adenylyl cyclase-activating polypeptide stimulates DNA synthesis but delays maturation of oligodendrocyte progenitors. J Neurosci. 2001 21:3849–59.). In an attempt to understand how PACAP might interact with growth factors known to stimulate OLP proliferation, we investigated PACAP actions on OLP proliferation in the presence of Fibroblast Growth Factor-2 (FGF-2) and PDGF. Multiple PACAP receptor subtype mRNAs and splice variants were detected in these cultures. PACAP by itself potently stimulated OLP proliferation and enhanced the ability of FGF-2 to stimulate DNA synthesis. In contrast, this peptide strongly antagonized the mitogenic effects of PDGF in association with a reduction of PDGFα receptor gene expression. Additionally, we investigated the interaction of PACAP with the morphogenetic factor sonic hedgehog (Shh), which recently was shown to be crucial for oligodendrocyte generation. OLP cultures were found to express mRNAs for both *ptc1* (Shh receptor) and *gli1* (Shh target gene) and responded to Shh treatment with an increase in proliferation. PACAP antagonized the ability of Shh to stimulate OLP proliferation. Moreover, transcriptional targets of Shh signaling were also reduced by this treatment, suggesting that PACAP directly antagonized Shh signaling. These studies reveal complex *in vitro* interactions of PACAP with other factors involved in OLP development.
© 2006 Elsevier B.V. All rights reserved.

Keywords: PACAP; VIP; PAC1; VPAC1; VPAC2; Sonic hedgehog; Oligodendrocyte progenitors; Proliferation; Gene expression; CNPase; *ptc1*; *gli1*

1. Introduction

Brain development results from a complex series of genetic and epigenetic spatio-temporal processes that delineate and shape the multiple connected structures. Among elementary processes, at least three general waves of terminal cellular differentiation occur. The first begins around E10 in rodents and generates the majority of neurons. The second begins later during embryogenesis and generates astrocytes. The third begins postnatally and results in the production of oligodendrocytes, and eventually completion of white matter development. These latter events remain poorly understood. Nevertheless, the occurrences of human conditions called leukoencephalopathies highlight the important roles that oligodendrocytes play in brain development and function [1].

Pituitary adenylyl cyclase-activating polypeptide (PACAP) is a neuropeptide originally discovered for its remarkable ability to stimulate cAMP production in pituitary, and was later found to be ubiquitously expressed in adult brain and peripheral nervous system [2]. It interacts with three high affinity receptors. One of these, PAC1, is PACAP specific and has several splice variants that couple the receptor to cAMP, phospholipase C and other

* Corresponding author. UCLA, 345 Neurosciences Building, 635 Charles E Young Dr South, 90095 Los Angeles, United States. Tel.: +310 825 0179; fax: +310 825 5061.
E-mail address: jwaschek@mednet.ucla.edu (J.A. Waschek).
[1] The first two authors equally contributed to the present work.

0167-0115/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2006.04.024

signaling pathways [2]. The other two receptors, VPAC1 and VPAC2, also bind the related peptide vasoactive intestinal peptide (VIP) with equal high affinity, and couple primarily to adenylyl cyclase [3]. The widespread and complex pattern of PACAP and PAC1 receptor genes expression at the earliest stages of embryonic brain development [4] raised the possibility that PACAP acts as a growth factor-like molecule to regulate neuro- and gliogenesis. *In vitro*, PACAP has been found to modulate the development of mouse embryonic stem (ES) cells [5], embryonic day 10 mouse hindbrain neural precursors [6], cortical [7] and cerebellar granule [8] neuroblasts, and astrocytes [9].

In other work, we showed that PACAP also stimulated the proliferation of cultured postnatal rat oligodendrocyte progenitors (OLP) and delayed myelinogenesis in cerebellar explants [10]. Because several other growth factors, for example, PDGF and FGF-2, are well known to regulate OLP proliferation and/or maturation, we considered it important to determine how these factors might interact with PACAP to regulate oligodendrocyte development. Moreover, the patterning factor sonic hedgehog (Shh) was recently shown to regulate oligodendrocyte development [11–13]. Because numerous studies performed in *Drosophila*, zebrafish, *Xenopus* and mouse have identified functional cross-talk between protein kinase A and Shh signaling [14–16], we have speculated that PACAP is a signal that restricts the patterning, proliferation and survival activities of Shh. We report here that the proliferative actions of PACAP in OLP are highly dependent on growth factor conditions. In particular, we pinpointed the down-regulation by PACAP of PDGF-α receptors (PDGFαR) and PDGF mitogenic effects. Moreover, we demonstrated that cross-talk occurs between the Shh and PACAP pathways in the regulation of OLP cell proliferation and gene expression, with a special focus on the modulation of Shh transcriptional targets and markers of oligodendrocyte maturation.

2. Methods

2.1. Materials

Platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF-2) were obtained from Upstate biotechnology (Lake Placid, NY); sonic hedgehog was from R&D system (Minneapolis, MN). PACAP and VIP as well as H89, a selective PKA inhibitor were obtained from Calbiochem (San Diego, CA).

2.2. OLP cell culture

Purified cortical OLP were prepared from E20 Sprague Dawley rats as previously reported with minor modifications [17,18]. Time-pregnant rats were euthanized following the UCLA guidelines and the embryos removed aseptically. Cerebral cortices were removed, mechanically dissociated, suspended in Dulbecco Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), and plated in plastic T75 flasks. After 12 days in culture, OLP cells growing on top of a confluent monolayer of astrocytes were detached by shaking overnight. Contaminating microglial cells were then further eliminated by plating this fraction on plastic culture dishes for 1 h. The OLP cells, which do not attach well onto plastic dishes, were collected by gentle washes, then plated onto poly-D-ornithine-coated plates and cultured in DMEM-N1 biotin-containing medium + FBS (0.5% or 2%), PDGF (10 ng/ml), or FGF-2 (10 ng/ml) was added to the culture medium 2 h later. Treatments with PACAP or Shh are described in detail in the figure legends.

2.3. Proliferation assay

OLP were incubated with 1 μCi/well of ^{3}H-thymidine (Amersham) for the last 8–12 h of peptide treatment after which cells were carefully rinsed prior to extraction in NaOH 0.5 M. [^{3}H]thymidine incorporation was measured by trichloroacetic acid (TCA) precipitation as previously described [17,19] and scintillation counting in 5 ml of Aquasafe™ scintillation cocktail (Wallac-LKB) on a Beckman counter.

2.4. Total RNA isolation

Total RNA was extracted according to a protocol derived from the original procedure of Chomczynski and Sacchi [20], consisting of two independent total RNA extractions separated by a DNAseI treatment (DNA-free™ kit, Ambion, Austin, TX), as previously described in detail [6]. RNA quality was assessed by electrophoresis on denaturing MOPS gels and spectrophotometry. Six hundred nanograms of total RNA was subjected to reverse transcription (RT) using the Iscript kit from Bio-Rad (Hercules, CA). Negative controls (samples in which reverse

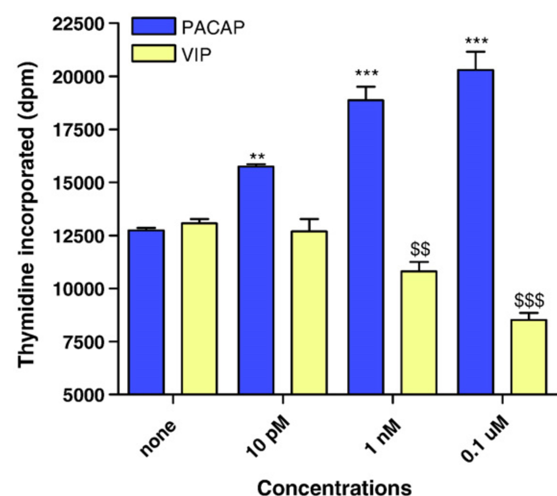

Fig. 1. Effects of PACAP or VIP on OLP proliferation. Oligodendrocyte progenitors were cultured in DMEM-N1 + 2% FBS medium for 12 h prior to peptides addition. [^{3}H]thymidine incorporation was measured after 8 hour treatments with increasing concentrations of PACAP-38 or VIP. Data shown in graph are from three independent experiments, each performed in triplicates. One-way ANOVA analysis followed by Bonferroni post hoc test revealed that changes induced by PACAP-38 and VIP were significantly different from their respective controls at $p<0.01$ (**, $^{\$\$}$) and $p<0.005$ (***, $^{\$\$\$}$).

Download English Version:

https://daneshyari.com/en/article/2023553

Download Persian Version:

https://daneshyari.com/article/2023553

Daneshyari.com