Chapter 6
PACAP and Neural Development

Jun Watanabe, Tamotsu Seki, and Seiji Shioda

Abstract Brain development is a complex process, controlled in part by locally secreted factors that regulate proliferation, differentiation, migration, survival, and maturation. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that exerts a wide range of effects on different cell types in the brain as early as fetal stage. PACAP and its receptors are expressed in germinative neuroepithelia, suggesting that PACAP may be involved in neurogenesis. PACAP has recently been shown to regulate cell fate in various developmental contexts, in a manner dependent on dose, region, signaling, and receptor subtype. Interestingly, germ cells and embryonic stem (ES) cells also express PACAP receptors, and PACAP plays a crucial role in their development. This chapter reviews current knowledge on several aspects of PACAP in neural development, including adult neurogenesis and developmental neural diseases.

Keywords Pituitary adenylate cyclase-activating polypeptide (PACAP) • PAC1 receptor • Neural development • Neurogenesis • Neural disease

Abbreviations

CNS	Central nervous system
DCX	Doublecortin
DISC1	Disrupted-in-schizophrenia 1

J. Watanabe (✉)
Center for Biotechnology, Showa University,
1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

Department of Anatomy, Showa University School of Medicine, Tokyo, Japan
e-mail: watanabe@med.showa-u.ac.jp

T. Seki
Department of Anatomy, Showa University School of Medicine, Tokyo, Japan

S. Shioda
Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan

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EB	Embryoid body
ES cell	Embryonic stem cell
FF	Follicular fluid
GFAP	Glial fibrillary acidic protein
IHC	Immunohistochemistry
iPS cell	Induced pluripotent stem cell
ISH	In situ hybridization
NB	Northern blot
NeuN	Neuronal nuclei
OB	Olfactory bulb
PAC1R-LI	PAC1R-like immunoreactivity
PACAP	Pituitary adenylate cyclase-activating polypeptide
PACAP-LI	PACAP-like immunoreactivity
PDGF	Platelet-derived growth factor
PGC	Primordial germ cell
PKA	Protein kinase A
PKC	Protein kinase C
PTSD	Posttraumatic stress disorder
RE	Rostral extension
RMS	Rostral migratory stream
RT-PCR	Reverse transcription-polymerase chain reaction
Shh	Sonic hedgehog
SVZ	Subventricular zone
VIP	Vasoactive intestinal peptide
WB	Western blot

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide, found in a wide variety of tissues, including the central nervous system (CNS) and peripheral tissues [1, 2]. Physiologically, PACAP has been reported to act as a hormone, a neuromodulator, a neurotransmitter, and a trophic factor, and has been implicated in a variety of developmental activities [3–5]. PACAP and its receptors, PAC1R, VPAC1R, and VPAC2R, are expressed in germ cells and embryonic stem (ES) cells and are distributed throughout the embryonic and postnatal brain during development (Tables 6.1, 6.2, and 6.3). PACAP has been shown to regulate neural precursor cell proliferation, differentiation, migration, maturation, and survival during neural development [6–10]. Despite current knowledge about PACAP, its overall role during neural development is not yet fully understood. This chapter describes the expression of PACAP and its receptors during neural development, especially on the role of PACAP as a regulatory factor.

Table 6.1 The expression of PACAP and its receptors in germ cells

Cell type	Spices	Origin	Ligand/ receptor	Method	Function	Reference
Primordial germ cell	Mouse	Dorsal mesentery and urogenital ridges of E10.5 and E11.5 mouse embryos	PACAP, PAC1R	IHC (PACAP), RT-PCR (PAC1-R)	PACAP stimulated in vitro proliferation of mouse primordial germ cells	[11]
Spermatogonia	Rat	Rat testes	PACAP	IHC, ISH	–	[13]
	Human	Normal human testes	PACAP	IHC	–	[14]
Primary spermatocytes	Rat	Rat testes	PACAP	IHC, ISH	–	[13]
Spermatids	Rat	Rat testes	PACAP	IHC, ISH	–	[13]
	Rat	Rat testes	PACAP, PAC1R	IHC (PACAP, PAC1R), WB (PAC1R)	–	[15]
	Human	Normal human testes	PACAP	IHC	–	[14]
Sperm	Mouse	Sperm from mouse epididymis	PACAP	IHC	PACAP enhanced fertilization and sperm penetration	[27]
Mature oocyte	Rat	Rat ovaries	PAC1R	RT-PCR	–	[22]

Abbreviations: *IHC* immunohistochemistry, *ISH* in situ hybridization, *RT-PCR* reverse transcription-polymerase chain reaction, *WB* western blot

Germ Cells

PACAP and PAC1R are detectable in the earliest stages of germ cell development in mice, as early as primordial germ cells (PGCs), the embryonic precursors of gametes of adult animals [11]. The gene encoding PAC1R is expressed by both PGCs and gonadal somatic cells. Furthermore, PACAP has been observed in gonadal ridges, mostly on germ cell surfaces, in E11.5–12.5 mouse embryos and could stimulate PGC proliferation in vitro. These findings suggested that PACAP may be an important regulator of PGC proliferation and functions in embryonic gonads.

During spermatogenesis, PACAP can act as a local regulator of testicular germ cell development and function [12]. PACAP-like immunoreactivity (PACAP-LI) has been observed in the spermatogonia and spermatids of rodents [13] and humans [14], and mRNAs encoding all three PACAP receptors have been detected in human

Table 6.2 The expression of PACAP and its receptors in ES cells and iPS cells

Cell type	Spices	Origin	Ligand/receptor	Method	Function	References
ES cell, EBs	Mouse	Mouse blastocysts	ES cells: PAC1R, VPAC1R (week), VPAC2R EBs: PAC1R	RT-PCR	PACAP and VIP induce the differentiation of ES cells into a neuronal phenotype	[29]
ES cell	Mouse	Mouse blastocysts	PACAP, VIP, PAC1R, VPAC1R, VPAC2R	RT-PCR	PACAP blocks Shh-dependent motor neuron generation	[30, 31]
ES cell	Mouse	Embryonic fibroblasts	PAC1R, VPAC1R, VPAC2R	RT-PCR	PACAP and VIP promote the generation of calcium currents in neuronal differentiating cells	[32]
iPS cell	Human	Umbilical cord matrix and amniotic membrane mesenchymal cells	PAC1R	RT-PCR, WB	Maxadilan prevents ultraviolet C-induced apoptosis	[33]

Abbreviations: *RT-PCR* reverse transcription-polymerase chain reaction, *WB* western blot

spermatogonia and spermatids [14]. Interestingly, PAC1-R-like immunoreactivity (PAC1R-LI) and PACAP-LI in rats were localized to the cytoplasm of round spermatids, which aggregated in the acrosome [15]. This finding suggested that endogenous PACAP may directly interact with the cytosolic PAC1-R-like protein without the ligand being released into the extracellular space. In contrast, VPAC2R was strongly expressed within the seminiferous tubuli, whereas the other two receptors were not found in germ cells [16]. Furthermore, VPAC1R was expressed in connective tissues, whereas PAC1R was not. Sperm head size was found to be smaller in PACAP-deficient mice than in wild-type mice, both in the longitudinal and transverse diameters, and PACAP treatment was shown to enhance human sperm motility [17]. These studies indicated that PACAP could influence the development and functioning of spermatozoa.

PACAP and its receptors are also expressed in rodent and human ovaries [18–20]. The role of PACAP in female fertility and reproduction has been thoroughly reviewed [21]. Assays of rat ovarian cells showed that both PACAP mRNA and PACAP-LI were present in the majority of granulosa and cumulus cells from large preovulatory follicles, in the majority of the cells comprising the interstitial glandular tissue and in solitary theca cells of growing and mature follicles [18]. PACAP-LI was also found in nerve fibers innervating the ovary. PAC1R and VPAC2R were

Table 6.3 The distribution of PACAP and its receptors during embryonic neural development in rodents

Developmental stage	Spices	Ligand/ receptor	Expressed region	Method	Reference
Primitive streak	E9 rat embryo	PAC1R	Neural fold, neural ectoderm, neuroepithelium in the primitive streak region and intraembryonic mesoderm	RT-PCR, IHC, ISH	[35]
	E8.5 mouse embryo	PAC1R(−)	Not detected	ISH	[36]
Neural tube closure	E9.5 mouse embryo	PACAP, PAC1R	Floor and roof plates of the neural tube and rhombencephalon, terminating abruptly at the boundary with the mesencephalon	RT-PCR	[38]
	E9.5 mouse embryo	PACAP, PAC1R		RT-PCR, ISH	[36]
	E10 rat embryo	PAC1R		RT-PCR	[39]
	E11 rat embryo	PAC1R		RT-PCR, IHC, ISH	[35]
Form of primary brain vesicle	E10.5 and E11.5 mouse embryo	PACAP, PAC1R	PACAP: dorsal root and trigeminal ganglia, rhombencephalon and cerebellum PAC1R: neural tube, rhombencephalon, dorsal root and trigeminal ganglia and sympathetic chain	RT-PCR	[37]
	E10.5 mouse embryo	PACAP, PAC1R		ISH, NB	[41]
	E12 rat embryo	PACAP, PAC1R		ISH	[40]
Form of Secondary brain vesicle	E14 and E16 rat embryo	PACAP, PAC1-R	PACAP: throughout the neuraxis PAC1R: neuroepithelium throughout the neuraxis including olfactory bulb, neuroepithelium, and external germinal layer of the cerebellum VPAC1R: similar to PAC1R VPAC2R: diencephalic nuclei	ISH	[40]
	E16 rat embryo	PAC1-R		ISH, IHC, RT-PCR	[35]
	E16/17 rat embryo	PACAP		ISH	[42]
	E14 and E17 rat embryo	PAC1R, VPAC1R, VPAC2R		ISH, RT-PCR	[39]
Late stage of embryonic neural development	E18 and E20 rat embryo	PACAP, PAC1R	PACAP: neocortex, pituitary, discrete PAC1R: ventricular zone throughout the nervous system; thalamic and brainstem nuclei and spinal cord VPAC1R: similar to PAC1R VPAC2R: diencephalic nuclei	ISH	[40]
	E20 rat embryo	PAC1R		ISH, IHC, RT-PCR	[35]
	E20 rat embryo	PACAP		ISH	[42]
	E21 rat embryo	PAC1R, VPAC1R, VPAC2R		ISH, RT-PCR	[39]

Abbreviations: *IHC* immunohistochemistry, *ISH* in situ hybridization, *RT-PCR* reverse transcription-polymerase chain reaction, *NB* northern blot

found to be expressed in granulosa cells, whereas VPAC1R and VPAC2R were expressed in theca cells [22]. Fully developed oocytes express PAC1R, and the addition of nanomolar concentrations of PACAP induced calcium release. However, PAC1R was not detected in the Met-I and -II phases of oocytes matured in vivo [22]. PACAP was reported to accelerate meiotic maturation in follicle- and cumulus-enclosed oocytes, while inhibiting meiotic maturation in denuded oocytes [23, 24]. PACAP was observed in human follicular fluid (FF) obtained from women undergoing hyperstimulation treatment [25, 26]. Interestingly, an inverse correlation was observed between the concentration of PACAP in FF and the number of recruited and retrieved oocytes. Low concentrations of PACAP in FF may correlate with the retrieval of a markedly higher number of ova, predicting a higher probability of ovarian hyperstimulation. Taken together, these findings indicate that PACAP is an important factor in developing oocytes.

Recent studies showed that PACAP also promotes fertilization. For example, PACAP-RI was detected in the anterior acrosomes of epididymal sperm fixed under mild conditions [27], and PAC1R mRNA and PAC1R-LI were observed in postovulatory cumulus cells. Pretreatment of cumulus–oocyte complexes with PACAP significantly enhanced the fertilization rate at low sperm concentrations. PACAP also enhanced sperm penetration through the oocyte investment, cumulus layer, and zona pellucida. Another study showed that PACAP could reverse the hypoxanthine-induced inhibition of oocyte meiotic maturation in cumulus cell–oocyte complexes and could efficiently promote male pronuclear formation after fertilization [28]. These findings suggest that PACAP may play a significant role in germ cell development and fertilization.

Embryonic Stem (ES) Cells, Embryoid Bodies (EBs), and Induced Pluripotent Stem (iPS) Cells

Mouse ES cells, which are derived from the inner mass of the blastocyst, are pluripotent cells that have the capacity for multilineage differentiation. Understanding ES cell differentiation can provide new perspectives on the cellular and molecular mechanisms of early development. PAC1R, VPAC1R, and VPAC2R are functionally expressed in mouse ES cells and EB-derived cells, and PACAP and vasoactive intestinal peptide (VIP) can induce the differentiation of ES cells into a neuronal phenotype [29]. PAC1R mRNA expression was further upregulated after differentiation of ES cells into a neuronal lineage, whereas the levels of PAC1R and PACAP mRNA were markedly reduced after glial differentiation [30]. Furthermore, PACAP was shown to completely block Sonic hedgehog (Shh)-dependent motor neuron generation from ES cell cultures and to reduce the expression of Gli-1 mRNA, a target of Shh [31]. Both PACAP and VIP were shown to modify the shape of undifferentiated ES cells, forming bipolar cells that express neuronal markers [32]. Electrophysiological recording showed that VIP and PACAP enhanced transient calcium current and that VIP generated a sustained calcium current. These findings

demonstrate that PACAP and VIP induce morphological and functional differentiation of ES cells into a neuronal phenotype.

Induced pluripotent stem (iPS) cells were originally generated by reprogramming murine fibroblasts through the retrovirus-mediated transfection of four transcription factors, Oct4, SOX2, c-Myc, and Klf-4. iPS cells are similar to ES cells in morphology, proliferative abilities, gene expression, and differentiation abilities. PAC1R protein and mRNA were observed in human iPS cells [33]. In contrast to ES cells, maxadilan, a PAC1R specific agonist, effectively protected iPS cells against ultraviolet C-induced apoptotic cell death while not affecting the pluripotent state or karyotype. Further investigations are needed to understand the effect and molecular mechanism of PACAP in iPS cells.

Distribution of PACAP and Its Receptors During Neural Development in Rodents

As shown in our previous review [34], the PACAP/PAC1R system is expressed widely throughout the nervous system during development, and the activities of PACAP signaling are complex. For example, this peptide is involved in precursor cell proliferation, differentiation, and survival. Expression of the PAC1R gene was detected from the primitive streak stage in rat embryos, as early as embryonic day (E) 9.5 [35]. PAC1R mRNA was highly expressed in the neural fold, neural ectoderm, and the neuroepithelium in the primitive streak region, as well as in intraembryonic mesoderm adjacent to the headfold. In contrast, PAC1-R mRNA was not detected at this stage in E8.5 mouse embryos [36]. Both PACAP and its receptor genes were expressed during the stage of neural tube closure, as early as E9.5 in mouse and E10–11 in rat embryos [35–39]. In E9.5 mouse embryos, PAC1-R mRNA was strongly expressed in the floor and roof plates of the neural tube and the rhombencephalon, terminating abruptly at the boundary with the mesencephalon [36]. In E11 rat embryos, PAC1R mRNA was detected in neuroepithelium along the neural tube [35]. At the stage of primary brain vesicle formation, in E10.5 mouse and E12–13 rat embryos, PAC1R mRNA was most abundant in the neural tube and rhombencephalon and was also present in the dorsal root and trigeminal ganglia and in the sympathetic chain [35, 37, 40]. The distribution of PACAP mRNA overlapped in part with receptor expression, but PACAP mRNA was more extensively distributed in the rhombencephalon and in the developing hypothalamus [37, 40]. At this stage, PACAP down-regulated the expression of the *shh*- and protein kinase A (PKA)-dependent target gene *gli*-1 in cultured neuroepithelial cells, concomitant with a decrease in DNA synthesis [41]. These results suggest that PACAP may act in the neural tube during patterning to control cell proliferation and gene expression. PAC1-R mRNA was detected throughout the neuraxis in E14 rat brains, with enriched expression in the mesencephalon and rhombencephalon, with lower expression in the telencephalon [40]. The distribution of radiolabeled-PACAP binding sites in developing rat brains was found to overlap sites of PAC1R expression

site [39]. Although the distribution of PAC1-R in rat brains did not differ greatly from E14 to E16, its expression was elevated in the developing cerebral cortex and spinal cord [35, 40]. By E18, most PAC1-R mRNA was localized to the ventricular zone throughout the nervous system [40]. Expression was observed in the developing olfactory bulb and cerebellar primordium, areas of abundant cellular proliferation during this stage of development. PAC1-R mRNA expression was observed within the neocortical and mesencephalic ventricular zones of E20–E21 rat brains, with high levels in the olfactory bulb, hippocampus, and cerebellum [35, 39, 40]. In contrast to the expression of PAC1-R, enhanced PACAP mRNA expression in E16.5 mouse embryos (E18 rat embryos) was observed in the pituitary gland, in discrete thalamic and brainstem nuclei, and in the spinal cord [40, 42]. By birth, PACAP mRNA expression increased in the hippocampus, striatum, hypothalamus, and pontine gray nucleus [40, 42].

After birth, PAC1-R mRNA expression was predominantly limited to the subventricular zone (SVZ), olfactory bulb (OB), hippocampus, and cerebellum [35, 39, 40]. After birth, PAC1-R mRNA expression in the ventricular zone declined and could not be detected after postnatal day (P) 7. Its expression in the SVZ, the rostral migratory stream (RMS), and the OB layers peaked from P4 to P14. PAC1-R mRNA was expressed in all hippocampal subfields and the dentate gyrus during the first postnatal week. By P14, PAC1-R mRNA expression was restricted to granule cells of the dentate gyrus. In the cerebellum, PAC1-R mRNA was localized to the cerebellar granule cell and Purkinje cell layers as development proceeds. Over the course of postnatal development, the expression of PACAP was particularly strong in the hypothalamus, anterior olfactory nucleus, and subiculum [40, 42]. In the cerebellum, the postnatal expression of PACAP mRNA is very week, in contrast to the strong expression of PAC1-R.

Neurogenesis in the Rostral Migratory Stream (RMS)

Continuous neurogenesis has been observed in two restricted regions of the adult mammalian brain, the subventricular zone (SVZ) of the lateral ventricle and the hippocampal dentate gyrus. New neurons generated in the SVZ migrate through the rostral migratory stream (RMS) and are integrated into the neuronal circuits of the OB throughout life [43]. Assessment of the RMS of P10 mice showed that PAC1R-LI was strongly expressed in nestin-positive cells (neural stem cells) in the apical SVZ, and in Neuronal Nuclei (NeuN)-positive cells (mature neurons) in the OB [44]. However, the intensity of PAC1-R-LI in doublecortin (DCX)- and βIII-tubulin (Tuj1)-positive cells (immature neural progenitors) was weaker than that for other markers in the rostral extension (RE). These distributions of PAC1-R suggest that PACAP affects proliferating, but not migrating, cells in the RMS. In addition, DCX-positive cells showed strong PAC1R-LI expression in the granular layer of the OB. These cells also coexpressed NeuN. As the granular layer of the OB is thought to derive from the differentiation of migrated neuroblasts into mature neurons, this

finding suggests that cells exhibiting PAC1-R-, DCX-, and NeuN-LI are in the process of differentiating from neuroblasts into mature neurons. The addition of PACAP to olfactory cell cultures was found to increase the number of neurons and to stimulate neuronal cell proliferation and survival [45]. Moreover, neutralization of PACAP in primary olfactory cultures resulted in neuron-specific losses. These results indicated the importance of PACAP in cell proliferation in the olfactory system. Cultured neural stem cells isolated from the lateral ventricle walls of adult mice were found to express PAC1R and to proliferate in vitro in response to physiologic concentrations of two PAC1 agonists, PACAP and Maxadilan, but not VIP, indicating that PAC1 mediates neural stem cell proliferation [46]. Taken together, these findings show that PACAP may participate in the stage-specific expression of PAC1-R during neurodevelopment, including both the proliferation and differentiation stages.

Neurogenesis in the Hippocampus

Assessments of the developing hippocampus showed that, at each postnatal stage, PAC1R mRNA was highly expressed in the pyramidal cell layer of the CA1–CA4 fields of the hippocampus and in the granule cell layers of the dentate gyrus, and was moderately expressed in other layers of the developing hippocampus [35]. A mature expression pattern was first observed at P0, with maximal expression at P60. PAC1R expression was also maintained in areas of neurogenesis in the adult CNS, whereas its ligand PACAP was expressed in the neighboring parenchyma [40], suggesting a role for PAC1 in adult neurogenesis. Intracerebroventricular infusion of PACAP increased cell proliferation in the ventricular zone of the lateral ventricle and in the dentate gyrus of the hippocampus [46, 47]. Interestingly, the proliferation of newly divided cells in the subgranular zone of the hippocampus and in the SVZ of lateral ventricles did not differ in wild-type and PACAP-deficient ($PACAP^{-/-}$) mice [48]. Rearing in enriched conditions enhanced the survival of newly divided wild-type cells, less so for cells in the granule cell layer of $PACAP^{-/-}$ mice. These findings suggest that endogenous PACAP is involved in the survival of cells generated in an enriched environment, but not basal cells, in the dentate gyrus of the adult hippocampus.

Cerebellum Development

During the development of the cerebellum, PACAP is produced by Purkinje cells [49] in the immature cerebellum and PAC1-R is expressed in the external and internal granule cell layers [39], strongly suggesting that PACAP may modulate the histogenesis of the cerebellar cortex. PACAP is also involved in the proliferation, survival, differentiation, neurite outgrowth, and motility of cerebellar granule cells

[5, 50–52], as well as markedly inhibiting the Shh-induced proliferation of rat and mouse cerebellar granule precursor cells [53]. PACAP blocks canonical Shh signaling through a mechanism involving the activation of PKA and inhibition of the translocation of the Shh-dependent transcription factor Gli2 into the primary cilium [54]. In the absence of Shh, however, PACAP and maxadilan modestly stimulated DNA synthesis, an effect reproduced by activating protein kinase C (PKC) [53]. These observations suggest that PAC1R acts as a sensor of environmental cues, coordinating diverse neurogenetic signals. Development of the CNS requires an equilibrium between cell survival and apoptosis. PACAP has been shown to prevent apoptosis induced by ceramides and FasL [55–57]. These data suggest that PACAP and apoptosis inducers interact during cerebellar development to control the apoptosis of granule cells and that they may affect some motor cerebellar functions. Cell migration and synaptogenesis represent pivotal processes in the maturation of the CNS. Exposure of granule neurones to PACAP inhibited cell displacement and strongly enhanced the number of cells bearing neurites [58]. Furthermore, endogenous PACAP was found to have short-term, cortical-layer-specific effects on granule cell migration in the early postnatal mouse cerebellum [59]. In vivo administration of PACAP induced a transient increase in the number of granule cells in the molecular layer and in the internal granule cell layer [60]. In contrast, PACAP did not affect the number of Purkinje cells. Administration of PACAP also induced a significant increase in the volume of the cerebellar cortex. These results indicate that PACAP increases the proliferation and/or inhibits the programmed death of granule cells, as well as stimulating neuronal migration from the external granule to the internal granule cell layer.

Effect of PACAP on Proliferation of Neural Progenitor Cells

As described earlier, PACAP has both pro- and antiproliferative effects on cerebellar granule precursor cells, as well as on corticogenesis [61]. PACAP acted as an antimitogenic signal, beginning from E13.5, both in culture and in vivo, and activated cAMP signaling through the short isoform of PAC1R. However, the number of BrdU-labeled proliferative cells in the E9.5 cortex was lower in PACAP$^{-/-}$ than in wild-type mice, suggesting that PACAP normally promotes proliferation at this stage of development. In early cultures of cortical precursors (E9.5 mice and E10.5 rats), PACAP induced an intracellular calcium flux and increased phospho-PKC levels, as well as it stimulated the production of G1 cyclin mRNA and protein, S-phase entry, and proliferation without affecting cell survival. Interestingly, the abundance of the hop receptor isoform was 24-fold greater than that of the short isoform at E10.5, whereas, at E14.5, expression of the short isoform was 15-fold greater and PACAP inhibited mitogenesis. These findings suggest that PACAP induces temporally specific effects on cortical proliferation via the developmentally regulated expression of specific receptor isoforms.

Effect of PACAP on Neuronal and Glial Differentiation of Neural Progenitor Cells

Inhibition of mitogenesis of neural precursor cells by PACAP may result in the generation of neuronal cells. PACAP has been shown to induce the neuronal differentiation and/or neurite outgrowth of ES cells [29, 30], cerebral neural progenitors [61, 62], cerebellar neural progenitors [58, 60], and hippocampal neural progenitors [63, 64]. PACAP increased the proliferation of adult neural stem cells, followed by neurogenesis [46–48]. In contrast, PACAP also enhanced the generation of astrocytes and oligodendrocytes. Neural progenitor cells derived from E14.5 mouse and rat telencephalons differentiated into astrocytes in response to PACAP [65–69]. Our previous study showed that the PACAP-generated signal in neural progenitor cells was mediated via the activation of phospholipase C, followed by calcium- and phospholipid-dependent PKC and resulting in the generation of astroglia [67, 68]. PACAP has also been reported to stimulate the production of cAMP, activating the small GTPases Rap1 and Ras and allowing extracellular calcium into the cell [70]. Calcium, in turn, stimulates the transcription factor downstream regulatory element antagonist modulator, which is bound to specific sites of the promoter of the gene encoding glial fibrillary acidic protein (GFAP), stimulating its expression during astrocyte differentiation. In oligodendrocyte progenitors, PACAP showed growth factor-dependent activity [71].

PACAP alone potently stimulates the proliferation of oligodendrocyte progenitors and enhances bFGF-induced DNA synthesis in these cells. In contrast, PACAP strongly antagonizes the mitogenic effects of platelet-derived growth factor (PDGF). As PDGF receptor expression is also regulated by cAMP/PKA pathways, PACAP may inhibit the mitogenic activities of PDGF via a direct effect of cAMP on proliferation and a reduction of the number of PDGF receptors at the cell surface. PACAP was also reported to stimulate proliferation and delay myelinogenesis in cultured postnatal rat oligodendrocyte progenitors [72]. Altogether, PACAP may play an important role as a modulator in neurogenesis through the developmentally regulated expression of specific receptor isoforms, as well as through developmentally regulated signaling and niches.

Developmental Abnormalities of PACAP-Deficient Mice

PACAP-deficient ($PACAP^{-/-}$) mice show various morphological, biochemical, and behavioral abnormalities [73–77], including a decreased fertility rate [78, 79] and a higher mortality rate, with the latter at least partially due to their temperature sensitivity [80], dysfunctions in lipid and carbohydrate metabolism [73], and respiratory abnormalities [81, 82]. $PACAP^{-/-}$ mice showed marked behavioral changes compared with wild type, including locomotor hyperactivity, explosive jumping behavior, increased exploratory behavior, and less anxiety and memory dysfunction [74, 83].

Studies investigating morphological alterations in the cerebellum of PACAP$^{-/-}$ mice found significant reductions in the thickness of the external granule cell layer at P4 and of the internal granule cell layer at P7 [84]. PACAP$^{-/-}$ mice are also reported to show abnormal axonal arborization in the dentate gyrus [85] and an earlier onset of myelination and dense myelinated fibers in the developing brain [86].

PACAP in Developmental Neurological Disorders

Recent studies suggest that PACAP signaling abnormalities may contribute to schizophrenia [87] and posttraumatic stress disorder (PTSD) [88] and may possibly contribute to attention-deficit hyperactivity disorder and autism [89, 90]. Associations between certain single nucleotide polymorphisms of the *PACAP* gene and schizophrenia have been suggested [87], and two PACAP-signaling pathways have been linked to schizophrenia [91]. One pathway regulates the association between disrupted-in-schizophrenia 1 (DISC1) and DISC1-binding zinc-finger protein via PACAP [92], and the other inhibits stathmin1 expression via PACAP [85]. Schizophrenia-like behavior in PACAP-deficient mice could be treated with a selective metabotropic glutamate 2/3 receptor agonist [93], a selective 5-HT$_7$ antagonist [94], a mixture of D_2 and serotonin 5-HT$_2$ antagonist, and a selective serotonin 5-HT$_2$ receptor antagonist [95]. PACAP signaling may therefore contribute to the pathogenesis of certain depressive conditions amenable to atypical antipsychotic drugs.

PTSD was shown to be associated with PACAP and PAC1R in females [88, 96]. Recent studies suggest that PACAP is a master regulator of central and peripheral stress responses [97–99]. A single nucleotide polymorphism in PAC1R was associated with increased reactivity of the amygdala and hippocampus to threat stimuli and decreased functional connectivity between the amygdala and hippocampus in PTSD patients [100]. Intriguingly, PACAP also acts on brain structures that mediate anxiety- and fear-related behaviors, where it may influence both hard-wired (genetically determined) stress responses and gene–environment interactions in stress-related psychopathology [99].

Conclusion

PACAP and its receptors are expressed in germinative neuroepithelia involved in neurogenesis. This neuropeptide takes part in the control of neurogenesis at different stages and locations and in different cell types during brain development. Furthermore, although the effects of PACAP on both proliferation and differentiation may be contradictory, these conflicting functions strongly suggest that the actual role of PACAP is highly influenced by receptor subtypes and other trophic factors or signal transduction molecules present. Future studies addressing the molecular basis and pathophysiological implications of PACAP-associated responses may contribute to the development of treatments for developmental neural disorders.

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