Available online at www.sciencedirect.com
ScienceDirect
Regulatory Peptides 145 (2008) 80–87
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Distribution and localization of pituitary adenylate cyclase-activating polypeptide-specific receptor (PAC1R) in the rostral migratory stream of the infant mouse brain

Ryosuke Matsuno [a,b], Hirokazu Ohtaki [a], Tomoya Nakamachi [a], Jun Watanabe [a,c], Sachiko Yofu [a], Daisuke Hayashi [a], Takaaki Takeda [a], Naoko Nonaka [a], Mayumi Seki [a,b], Masahisa Nakamura [c], Kazuo Itabashi [b], Seiji Shioda [a,*]

[a] *Department of Anatomy, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*
[b] *Department of Pediatrics, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*
[c] *Department of Biology, School of Education, Waseda University, 1-6-1 Nishi Waseda, Shinjuku-ku, Tokyo, 169-8050, Japan*

Available online 25 August 2007

Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP) is known to participate in the regulation of neuronal proliferation and differentiation. While these processes are considered to be mediated *via* PACAP's actions on the PACAP-specific receptor, PAC1R, the precise distribution of PAC1R during neurodevelopment has not yet to be elucidated in detail. The purpose of this study is to examine the distribution of PAC1R in the neurogenic region of the rostral migratory stream (RMS) from the apical subventricular zone (SVZa) to the olfactory bulb (OB) in infant mice using immunostaining. Co-immunostaining for PAC1R in a variety types of cell were carried out using different markers. These included the neural stem cell markers, nestin and glial fibrillary acidic protein (GFAP), a marker for migrating neuroblasts (doublecortin, DCX), a marker for immature neurons βIII-tubulin, (Tuj1), and a marker for mature neurons, neuronal nuclei (NeuN). PAC1R-like immunoreactivity (LI) was observed in the RMS. However, the intensity of PAC1R- LI was different depending on the regions which were investigated. PAC1R-LI was strong in nestin- and GFAP-positive cells in the SVZa and was also observed in NeuN-positive cells in the OB. However, the intensities of PAC1R-LI in DCX- and Tuj1-positive cells were weaker than the other markers. These results suggest that PACAP may participate in the neurodevelopment with the stage-specific expression of PAC1R and that PACAP plays important roles in neurons as well as in glial cells.

Keywords: PACAP; Doublecortin; Neuronal stem cell; Neuroblast; Development; Mouse

1. Introduction

Pituitary adenylated cyclase-activating polypeptide (PACAP), which was initially isolated from the ovine hypothalamus, belongs to the secretin/glucagon/vasoactive intestinal peptide (VIP) superfamily. PACAP, which exists in two amidated forms, PACAP38 and PACAP27, is considered to play an important role in the prevention of delayed neuronal cell death [1–3], the decrease of inflammatory responses [4,5], and in the dilation of blood vessels and bronchi [6,7]. Recently, evidences from some studies suggested that PACAP might play a role in neurogenesis during neurodevelopment [8–12]. In PC12 cells, PACAP synergizes with nerve growth factor to stimulate neurite outgrowth [13]. VIP and PACAP have been shown to potently increase the proportion of embryonic stem cells expressing a neuronal phenotype and neurite outgrowth, as revealed by immunocytochemistry [14,15]. It has been shown that the anti-mitogenic effect of PACAP may alter the differentiation of cerebral cortical neuronal precursor cells [8,9,16]. We have also reported that PACAP is involved in inducing the differentiation of mouse telencephalon neural stem cells (NSC) into astrocytes [10,17].

PACAP is known to bind three kinds of G protein-coupled receptors: the PACAP-specific receptor (PAC1R) and two VIP/PACAP receptors (VPAC1R and VPAC2R) [18]. PACAP

* Corresponding author. Tel.: +81 3 3784 8103; fax: +81 3 3784 6815.
E-mail address: shioda@med.showa-u.ac.jp (S. Shioda).

0167-0115/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2007.08.016

Table 1
List of antibodies and the staining conditions

Antibodies	Abbreviations	Host	Companies	Folds	Target of cells
PACAP-specific receptor	PAC1R	Rabbit	See Ref. [25]	200	PACAP-specific receptor
Nestin	Nestin	Mouse	BD PharMingen	400	Neural stem cell
Doublecortin	DCX	Guinea pig	Chemicon	2000	Neuroblast (neuronal progenitor cell)
βIII-tublin	Tuj1	Mouse	Covance	1000	Immature neuron
Neuronal nuclei	NeuN	Mouse	Chemicon	1000	Neuron
Glial fibrillary acidic protein	GFAP	Mouse	Sigma	1000	Astrocyte, part of neural stem cell

interacts with VPAC1R and VPAC2R with almost the same affinity as that of VIP, and with PAC1R with a 1000 times higher affinity than VIP [18–20]. PAC1R has been shown to be distributed in the central nervous system (CNS), the peripheral nervous system, eye, pituitary, ovary, stomach, pancreas, liver, lung, and adrenal gland [20]. During neurodevelopment, PAC1R gene expression is detected in the CNS of fetal rat [21,22] and PACAP has been shown to regulate mitogenic signaling [9,16,23]. Expression of both the PAC1R gene and its protein product has also been observed in embryonic day 14.5 telencephalon NSCs maintained in primary culture [9,10]. Moreover, PAC1R gene expression was also detected in the subventricular zone (SVZ) lining the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus of the adult mouse brain [24]. Using immunohistochemistry, we have already shown that PAC1R is expressed in neurons and activated astrocytes in the CNS of the adult rat and mouse brain, respectively [3,25]. While PACAP is considered to participate in neurogenesis, the role of PACAP and PAC1R during neurodevelopment is still controversial.

In the present study, we attempt to elucidate the role of PACAP in postnatal neurogenesis. Immunohistochemical methods have been employed to examine the distribution of PAC1R-positive cells in the infant mouse SVZ/olfactory bulb (OB) system and to examine the cell types in which PAC1R is expressed. We found that, although PAC1R is expressed throughout the rostral migratory stream (RMS), PAC1R immunoreactivity was strong in NSCs and mature neurons, but decreased in migrating neuroblasts.

2. Materials and methods

2.1. Animals

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Showa University (#05088). ICR mice were bred in the Showa University Animal Research Center and were maintained on a 12-h light/dark cycle at 23 °C with constant humidity (40±15%).

2.2. Tissue preparation

Postnatal day 9 to 11 (P10) infant mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with saline followed by 2% paraformaldehyde in phosphate buffer (pH 7.2). Brains were then removed, postfixed overnight and immersed in 20% sucrose at 4 °C until equilibrated. Brains were frozen in liquid nitrogen-cooled 2-methylbutane. Coronal (0.0±1.0 mm anterior to the bregma) or sagittal sections of thickness 8 μm were cut using a cryostat and then mounted on poly-L-lysine-coated slides.

2.3. Immunostaining for PAC1R and cell markers

The sections were pretreated with 10 mM sodium citrate buffer, pH 6.0, at 85 °C for 20 min. After cooling to room temperature (RT), the sections were washed with phosphate

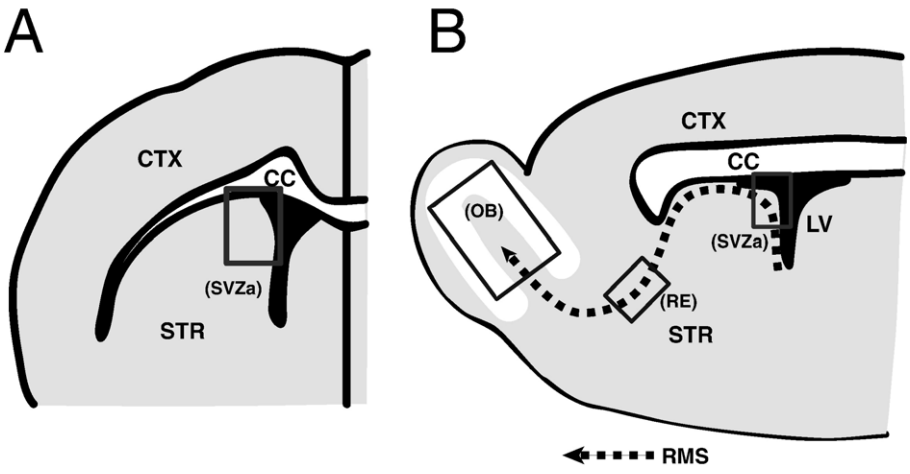

Fig. 1. Schematic illustration of coronal (A) and sagittal (B) views of the neurogenic region within the apical subventricular zone (SVZa) of the mouse brain. The SVZa abuts the wall of the lateral ventricle (LV). The rostral migratory stream (RMS) is a tangential migratory pathway extending from the SVZa to the olfactory bulb (OB), with SVZa-derived cells traversing it *via* the rostral extension (RE). CTX, cerebral cortex; STR, striatum; CC, corpus callosum.