Expression of pituitary adenylate cyclase activating polypeptide and its type I receptor mRNAs in human placenta

Phil-Ok Koh¹, Chung-Kil Won¹, Hae-Sook Noh², Gyeong-Jae Cho², Wan-Sung Choi¹*

¹Department of Anatomy, College of Veterinary Medicine and Institute of Animal Medicine, Gyeongsang National University, Jinju 660-701, Korea
²Department of Anatomy and Neurobiology, College of Medicine, Institute of Health Sciences, Gyeongsang National University, Jinju 660-751, Korea

Pituitary adenylate cyclase activating polypeptide (PACAP) was first isolated from ovine hypothalamus and was known to stimulate the release of growth factor in various cells. Recently, we reported the cellular localization of PACAP and its type I (PAC1) receptor in rat placenta during pregnancy. Placenta is a critical organ that synthesizes several growth factors and angiogenic factors for the fetal development and its own growth. However, there is little information regarding the cellular localization of PACAP and its receptor in human placenta at various gestations. The aim of the present study was to define the expression and distribution of PACAP and PAC1 receptor mRNAs in the human placenta during the pregnancy period. PACAP and PAC1 receptor mRNAs were expressed in stroma cells of stem villi and terminal villi. At the early stage, on 7 and 14 weeks, PACAP and PAC1 receptor genes were moderately expressed in stroma cells surrounding the blood vessels within stem villi. These genes were strongly expressed in stroma cells of stem villi and terminal villi on 24 and 38 weeks. The expression of these genes was increased as gestation advanced, and localized in the same areas. Localization of PACAP and PAC1 receptor demonstrate the evidence that PACAP may play an important role, as an autoregulator or pararegulator via its PAC1 receptor. In conclusion, our findings strongly suggest that PACAP may have a critical role in physiological function of the placenta for gestational maintenance and fetal growth.

Key words: PACAP, receptor, placenta, human

Introduction

The placenta is an essential organ for the fetal development and the maintenance of pregnancy. It is known that placenta synthesis the growth hormone [21] and several growth factors, such as basic fibroblast growth factor and insulin like growth factor [6,29]. Also, placenta produces placenta growth factor (PlGF) and vascular endothelial growth factor (VEGF) [6,29], which are critical factors for the placental growth and fetal development. As an important regulator of angiogenesis, VEGF contributes to the development and growth of the endothelium during the tissue growth [5,32]. Also, another member of the VEGF family, PlGF, promotes endothelial cell proliferation in vitro [16]. The previous study showed pituitary adenylate cyclase activating polypeptide (PACAP) stimulates the release of VEGF and acts as a trophic factor in various cells [7,8,17,31,33]. PACAP has considerable homology with vasoactive intestinal peptide (VIP) and growth hormone releasing hormone [17,31,33]. Recently, it was reported that PACAP and PAC1 receptor are present in both the human and rat placenta at term [23]. Also, even more recently, we reported the cellular localization of PACAP and PAC1 type I (PAC1) receptor in the rat placenta during pregnancy [14]. Therefore, the existence of PACAP in placenta suggests that PACAP affects placental function.

PACAP was originally isolated from ovine hypothalamus and was known to stimulate the production of cAMP in anterior pituitary cells [18]. PACAP exists in two biologically active forms, PACAP 38 and PACAP 27, sharing the same N-terminal 27 amino acids [19]. PACAP binds to three type I receptors. Among these receptors, PAC1 receptor has high affinity with PACAP 38 and PACAP 27, very low affinity with VIP [12,28]. But, VIP1 and VIP2 receptors have approximately equal high affinity for PACAP 38, PACAP 27, and VIP [11,12,28]. PACAP and its receptor have been found in the central nervous system and its peripheral tissues, including the hypothalamus, pituitary gland, adrenal
medullar, testis, and ovary [2,3,10,25,27]. The presence of PACAP in the hypothalamus, pituitary, and gonads suggests its roles in the reproductive system. However, the existence, localization of PACAP and PAC, receptor genes in human placenta at various gestations has been unknown. Thus, the present study was performed to determine the distribution of PACAP and PAC, receptor mRNAs in human placenta.

Materials and Methods

Tissue preparation

Human placental tissue from legal abortions aged between 6-7 weeks post menstruation (pm) were collected from normal pregnancies by curettages. Second trimester placenta from 14-24 weeks of gestation were obtained from induced abortion of healthy pregnancies and term placenta (38-41 weeks pm) by caesarian section or normal delivery. For in situ hybridization studies, tissues were fixed with 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS) and cryoprotected with 20% sucrose phosphate buffer for 24 hr. Placental sections were cut in a cryomicrotome at a thickness of 15 µm, mounted on the Probe-on slides (Fisher Scientific, USA), and stored at −70°C. Slides from each placenta were stained with hematoxylin and eosin for general morphological observation.

In situ Hybridization

All solutions were made with sterile water and glassware was autoclaved to prevent contamination by RNase. In situ hybridization histochemistry was carried out, as described by Angerer et al. [1]. Briefly, the slides were dried, washed with 0.1M PBS, and treated proteinase K, TE buffer, and an acetylation solution. Sections were covered with prehybridization buffer containing 50% deionized formamide and incubated at 37°C for 1 hr. After removal of the prehybridization buffer, the slides were covered with the mixture containing the prehybridization buffer, 50 µg/ml yeast tRNA, 10 mM dithiothreitol, and 50S-labeled PACAP cRNA probe or PAC, receptor cRNA probe [13]. The slides were then covered with cover glasses and incubated at 60°C for 24 hr. 5S-UTP-labeled probes were prepared using in vitro transcription kit (Promega, USA). Antisense and sense cRNA probes were purified with a Sephadex G-50 nick column (Pharmacia Biotech, Sweden) and eluted with SET buffer (0.1% SDS, 1 mM EDTA, 10 mM Tris, and 10 mM DTT). Tissue slides were posthybridized in a posthybridization buffer. Following a wash in 4×SSC for 30 min, the sections were then treated with ribonuclease A (50 µg/ml) at 37°C for 10 min, washed twice in 2×SSC and 1×SSC, transferred to a wash buffer containing 0.1×SSC at 65°C for 30 mins, and dehydrated in alcohol solutions with ascending concentrations. Slides were exposed to β-max hyperfilm (Amersham, Sweden) for 4 days in light-tight cassettes at −70°C, and were dipped into NTB2 emulsion (1:1 dilution, Eastman Kodak, USA), exposed at 4 for 2 weeks, developed in Kodak D19 developer (1:1 dilution, Eastman Kodak, USA) at 15°C, and counterstained with hematoxylin. The slides were observed under a dark and a bright field microscope, and photographed.

Results

The present study showed the expression and distribution of PACAP and PAC, receptor mRNAs in the human placenta at various gestations. In situ hybridization revealed the expression of PACAP and PAC, receptor mRNAs in stem villi and terminal villi. Positive cells of PACAP mRNA were detected in stroma cells surrounding the blood vessels within stem villi on 7 weeks (Figs. 1A & 2A). PACAP mRNA was expressed in stroma cells of stem villi on 14 week (Figs. 1B & 2B). Furthermore, positive signals of PACAP mRNA were moderately observed in stroma cells of stem villi and terminal villi. E: No positive signals were detected in negative control with a sense probe. Bar = 200 µm.

Fig. 1. Dark-field photomicrographs of PACAP mRNA expression in the human placenta from 7 (A), 14 (B), 24 (C), and 38 (D) weeks gestation by in situ hybridization. A and B: Positive signals were moderately observed in stroma cells of stem villi. C and D: Positive cells were strongly detected in stroma cells of stem villi and terminal villi. E: No positive signals were detected in negative control with a sense probe. Bar = 200 µm.
As similar like to the expression pattern of PACAP, positive signals for PAC, receptor in these cells became strong as gestation advanced. But, PAC, receptor mRNA was very weakly expressed in cytotrophoblast cells and syncytiotrophoblast cells. No positive signals of PAC, receptor mRNA was detected in negative control with a sense probe (Fig. 3E).
localization of vasoactive intestinal peptide (VIP) binding sites in the human term placenta. Relationship with activation of adenylate cyclase. Regul Pept 1987, 19, 197-207.
5. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, Lammoglia R, Charnock-Jones DS. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. Biol Reprod 1998, 59, 1540-1548.
6. Correia-da-Silva G, Bell SC, Pringle JH, Teixeira N. Expression of mRNA encoding insulin-like growth factors I and II by uterine tissues and placenta during pregnancy in the rat. Mol Reprod Dev 1999, 53, 294-305.
7. Gloodek J, Pagotto U, Paez Pereda M, Arzt E, Stallk GK, Remmer U. Pituitary adenylate cyclase-activating polypeptide, interleukin-6 and glucocorticoids regulate the release of vascular endothelial growth factor in pituitary folliculostellate cells. J Endocrinol 1999, 160, 483-490.
8. Gonzalez BJ, Basille M, Vaudry D, Fournier A, Vaudry H. Pituitary adenylate cyclase-activating polypeptide promotes cell survival and neurite outgrowth in rat cerebellar neuroblasts. Neuroscience 1997, 78, 419-430.
9. Graf AH, Hutter W, Hacker GW, Steiner H, Anderson V, Staudach A, Dietze O. Localization and distribution of vasoactive neuropeptides in the human placenta. Placenta 1996, 17, 413-421.
10. Hashimoto H, Ishihara T, Shigemoto R, Mori K, Nagata S. Molecular cloning and tissue distribution of a receptor for pituitary adenylate cyclase-activating polypeptide. Neuron 1993, 11, 333-342.
11. Inagaki N, Yoshida H, Mizuta M, Mizuno N, Fujii Y, Gonoi T, Miyazaki J, Seino S. Cloning and functional characterization of a third pituitary adenylate cyclase-activating polypeptide receptor subtype expressed in insulin-secreting cells. Proc Natl Acad Sci USA 1994, 29, 2679-2683.
12. Ishihara T, Shigemoto R, Mori K, Takahashi K, Nagata S. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. Neuron 1992, 8, 811-819.
13. Koh PO, Kwak SD, Kim HJ, Roh G, Kim JH, Kang SS, Choi WS, Cho GJ. Expression patterns of pituitary adenylate cyclase activating polypeptide and its type I receptor mRNAs in the rat placenta. Mol Reprod Dev 2003, 64, 27-31.
14. Koh PO, Kwak SD, Kang SS, Cho GJ, Chun SY, Kwon HB, Choi WS. Expression of pituitary adenylate cyclase activating polypeptide (PACAP) and PACAP type I receptor mRNAs in granulosa cells of preovulatory follicles of the rat ovary. Mol Reprod Dev 2000, 55, 379-386.
15. Kotani E, Usuki S, Kubo T. Rat corpus luteum expresses both PACAP and PACAP type IA receptor mRNAs. Peptides 1997, 18, 1453-1455.
16. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG. Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. Proc Natl Acad Sci USA 1991, 88, 9267-9271.
17. Matsumoto H, Koyama C, Sawada T, Koike K, Hirola K, Miyake A, Arimura A, Inoue K. Pituitary folliculo-stellate-
like cell line (TTT/GF) responds to novel hypophysiotropic peptide (pituitary adenylate cyclase-activating peptide), showing increased adenosine 3',5'-monophosphate and interleukin-6 secretion and cell proliferation. Endocrinology 1993, 133, 2150-2155.

18. Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, Coy DL. Isolation of a novel 38 residue hypothalamic peptide which stimulates adenylate cyclase in pituitary cells. Biochem Biophys Res Commun 1989, 164, 567-574.

19. Miyata A, Jiang L, Dahl RR, Kitada C, Kubo K, Fujino M, Minamino N, Arimura A. Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). Biochem Biophys Res Commun 1990, 170, 643-648.

20. Moretti C, Mencacci C, Frajese GV, Cerilli M, Frajese G. Growth hormone-releasing hormone and pituitary adenylate cyclase-activating polypeptide in the reproductive system. Trends Endocrinol Metab 2002, 13, 428-435.

21. Ogilvie S, Buhi WC, Olson JA, Shiverick KT. Identification of a novel family of growth hormone-related proteins secreted by rat placenta. Endocrinology 1990, 126, 3271-3273.

22. Scalfarelli L, Arora K, Lee SH, Catt KJ, Moretti C. Expression of PACAP and its type I receptor isoforms in the rat ovary. Mol Cell Endocrinol 1996, 25, 227-232.

23. Scalfarelli ML, Modesti A, Palumbo C, Ussile S, Fabbri A, Piccione E, Frajese G, Moretti C. Pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP-receptor type 1 expression in rat and human placenta. Endocrinology 2000, 141, 1158-1167.

24. Sherwood NM, Krueckl SL, McRory JE. The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. Endocr Rev 2000, 21, 619-670.

25. Shioda S, Legradi G, Leung WC, Nakajo S, Nakaya K, Arimura A. Localization of pituitary adenylate cyclase-activating polypeptide and its messenger ribonucleic acid in the rat testis by light and electron microscopic immunocytochemistry and in situ hybridization. Endocrinology 1994, 135, 818-825.

26. Shiraishi S, Nakagawa K, Kinukawa N, Nakano H, Sueishi K. Immunohistochemical localization of vascular endothelial growth factor in the human placenta. Placenta 1996, 17, 111-121.

27. Shivers BD, Gorce TJ, Gottschall PE, Arimura A. Two high affinity binding sites for pituitary adenylate cyclase-activating polypeptide have different tissue distributions. Endocrinology 1991, 128, 3055-3065.

28. Spengler D, Waeger C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH, Journot L. Differential signal transduction by five splice variants of the PACAP receptor. Nature 1993, 365, 170-175.

29. Srivastava RK, Gu Y, Ayloo S, Zilberstein M, Gibori G. Developmental expression and regulation of basic fibroblast growth factor and vascular endothelial growth factor in rat decidua and in a decidual cell line. J Mol Endocrinol 1998, 21, 355-362.

30. Steenstrup BR, Jorgensen JC, Alm P, Hannibal J, Junge J, Fahrenkrug J, Ottesen B. Pituitary adenylate cyclase activating polypeptide (PACAP): occurrence and vasodilatory effect in the human uteroplacental unit. Regul Pept 1996, 22, 197-204.

31. Tischler AS, Risberg JC, Gray R. Mitogenic and antimitogenic effects of pituitary adenylate cyclase-activating polypeptide (PACAP) in adult rat chromaffin cell cultures. Neurosci Lett 1995, 21, 135-138.

32. Vuorela P, Hatva E, Lymboussaki A, Kaipainen A, Joukov V, Persico MG, Alitalo K, Halmesmaki E. Expression of vascular endothelial growth factor and placenta growth factor in human placenta. Biol Reprod 1997, 56, 489-494.

33. Wolf N, Krieglstein K. Phenotypic development of neonatal rat chromaffin cells in response to adrenal growth factors and glucocorticoids: focus on pituitary adenylate cyclase activating polypeptide. Neurosci Lett 1995, 24, 207-210.