

Gonadotropins affect the steroidogenesis and in vitro expression of pituitary adenylate cyclase-acti

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ABSTRACT

In vertebrate, hypothalamus-pituitary-gonads (HPG) axis regulates reproduction system, the hypothalamus produces gonadotropin-releasing hormone (GnRH) regulating anterior portion of the pituitary produces follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and regulates gonads development, gametogenesis, maturation, and to promote gonadal steroidogenesis. Tilapia is an important protein source in Taiwan, however, studies on the regulation of PACAP in reproduction in tilapia are still scant. Our previous studies indicated that tilapia (*Oreochromis mossambicus*) pituitary adenylate cyclase-activating polypeptide (tpacap38) and its type I receptor (tpac1-r) transcripts were detected in the brain, gallbladder, gill, heart, intestine, kidney, muscles, pancreas, spleen, stomach, testes, and ovaries, but not in the liver. Addition of cAMP analog dibutyryl-cAMP, exogenous ovine PACAP38 or forskolin (adenylate cyclase activator) significantly upregulated the expression of tpacap38 in the follicles and testis via a dose- and time-dependent fashion and its function could be suppressed with the addition of protein kinase A (PKA) inhibitor, H89, indicating involvement of the cAMP-PKA signaling pathway in the regulation of tpacap38. In the present study, semi-quantitative RT-PCR and enzyme immunoassay (EIA) were performed to detect the effect of gonadotropin on the expression of tpacap38 and tpac1-r and steroidogenesis in tilapia gonads. The expression of tpacap38 and tpac1-r increased significantly in a dose-dependent experiment by addition of different concentrations of human chorionic gonadotropin (hCG; 5, 15, and 50 IU) to gonads cultured for 2 hours. The mRNA expression levels of tpacap38 and tpac1-r in both sexes were higher than those in the control group at a dose of 15 IU hCG. However, the expression level of tpac1-r increased significantly at a dose of 50 IU pregnant mare's serum gonadotropin (PMSG) but not others (0, 5, 50, and 100 IU), and this was observed only in female. The gonadal steroids secretions of estradiol, testosterone and progesterone increased at a dose of 15 IU hCG for the 2 hour-culture, and similar result was observed at a dose of 50 IU PMSG (estradiol, testosterone and progesterone). Besides, the mRNA expression levels of tpacap38 and tpac1-r were higher than those in the control group at a dose by hCG and PMSG co-induction for the 2 hour-culture, and the gonadal steroids secretions of estradiol, testosterone and progesterone increased at 50 IU hCG/50 IU PMSG co-induction for the 2 hour-culture. In the time course experiment, the mRNA expression levels of tpacap38 and tpac1-r were significantly higher at a dose of 15 IU hCG at 4 h. and similar result was observed at a dose of 50 IU PMSG tpacap38 and tpac1-r at 2 h. In addition, the mRNA expression levels of tpacap38 and tpac1-r were significantly higher at 6 h with 15 IU hCG+50 IU PMSG co-induction. The gonadal steroids secretions were low at 0 h (after preculture for 8 h) and increased significantly at 2 h at a dose of 15 IU hCG, 50 IU PMSG or co-induction (15 IU hCG + 50 IU PMSG), and then decreased with prolonged cultured periods (4, 6, and 8 h) in both genders. However, the results of the inductive function of hCG and PMSG could be suppressed by the addition of protein kinase A inhibitor H89 (10 M). These results suggested that tpacap38 and tpac1-r may be regulated by gonadotropins in reproduction system in a paracrine/autocrine manner, and further involved in the gonadal steroidogenesis in the bony fish.

Keywords : tilapia、 pituitary adenylate cyclase-activating polypeptide (PACAP)、 PACAP type I receptor (PAC1-R)、 gonads、 gonadotropin、 steroidogenesis

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REFERENCES

- Abel, M.H., Baban, D., Lee, S., Charlton, H.M. and O'Shaughnessy, P.J. (2009). Effects of FSH on testicular mRNA transcript levels in the hypogonadal mouse. *J Mol Endocrinol*, 42(4): 291-303.
- Adams, B.A., Lescheid, D.W., Vickers, E.D., Crim, L.W. and Sherwood, N.M. (2002). Pituitary adenylate cyclase-activating polypeptide and growth hormone-releasing hormone-like peptide in sturgeon, whitefish, grayling, flounder and halibut: cDNA sequence, exon skipping and evolution. *Regu Pept*, 109(2002): 27 – 37.
- Agnese, M., Valiante, S., Angelini, F., Laforgia, V., Andreuccetti, P. and Prisco, M. (2010). Pituitary adenylate cyclase-activating polypeptide and its receptor PAC1 in the testis of *Triturus carnifex* and *Podarcis sicula*. *Gen Comp Endocrinol*, 168(2): 256-261.
- Andersen, A.N., Balen, A., Platteau, P., Devroey, P., Helmggaard, L., Arce, J.C. and Bravelle Ovulation Induction (BOI) Study Group. (2008). Predicting the FSH threshold dose in women with WHO Group II anovulatory infertility failing to ovulate or conceive on clomiphene citrate. *Hum Reprod*, 23(6): 1424-1430.
- Arimura, A. (1998). Perspectives on pituitary adenylate cyclase activating polypeptide (PACAP) in the neuroendocrine, endocrine, and nervous systems. *Jpn J Physiol*, 48(5): 301-331.
- Arimura, A., Somogyvari-Vigh, A., Miyata, A., Mizuno, K., Coy, D.H. and Kitada, C. (1991). Tissue distribution of PACAP as determined by RIA: highly abundant in the rat brain and testes. *Endocrinology*, 129(5): 2787-2799.
- Arimura, A., Somogyvari-Vigh, A., Weill, C., Fiore, R.C., Tatsuno, I., Bay, V. and Brenneman, D.E. (1994). PACAP function as a neurotrophic factor. *Ann N Y Acad Sci*, 739: 228-243.
- Basille, M., Gonzalez, B.J., Desrues, L., Demas, M., Fournier, A. and Vaudry, H. (1995). Pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates adenylyl cyclase and phospholipase C activity in rat cerebellar neuroblasts. *J Neurochem*, 65(3): 1318-1324.
- Barberi, M., Muciaccia, B., Morelli, M.B., Stefanini, M., Cecconi, S. and Canipari, R. (2007). Expression localisation and functional activity of pituitary adenylate cyclase-activating polypeptide, vasoactive intestinal polypeptide and their receptors in mouse ovary. *Reproduction*, 134(2): 281-292.
- Burns, F. J., Chen, S., Xu, G., Wu, F. and Tang, M. S. (2002). The action of a dietary retinoid on gene expression and cancer induction in electron-irradiated rat skin. *J Radiat*

Res (Tokyo), 43 Suppl: S229-232. Cardoso, J.C., De Vet, E.C., Louro, B., Elgar, G., Clark, M.S. and Power, D.M. (2007). Persistence of duplicated PAC1 receptors in the teleost, *Sparus auratus*. *BMC Evol Biol*, 7(1): 221. Dickson, L. and Finlayson, K. (2009). VPAC and PAC receptors: From ligands to function. *Pharmacol Ther*, 121(3): 294-316. Dufau, M.L., Baukal, A.J. and Catt, K.J. (1980). Hormone-induced guanyl nucleotide binding and activation of adenylate cyclase in the Leydig cell. *Proc Natl Acad Sci U S A*, 77: 5837-5841. Faraj, E.G., Manuel, T.S. and Ilpo, H. (2000). Evidence that pituitary adenylate cyclase-activating polypeptide is a potent regulator of fetal rat testicular steroidogenesis. *Biol Reprod*, 63(5):1482-1489. Filipsson, K., Martina, K.R. and Bo, A. (2001). The neuropeptide pituitary adenylate cyclase-activating polypeptide and islet function. *Diabetes*, 50(9): 1959-1969. Filipsson, K., Sundler, F., Hannibal, J. and Ahren, B. (1998). PACAP and PACAP receptors in insulin producing tissues: localization and effects. *Regul Pept*, 74: 167-175. Fradinger, E.A., Tello, J.A., Rivier, J.E. and Sherwood, N.M. (2005). Characterization of four receptor cDNAs: PAC1, VPAC1, a novel PAC1 and a partial GHRH in zebrafish. *Mol Cell Endocrinol*, 231(1-2): 49-63. Ginther, O.J., Almamun, M., shahiduzzaman, A.K.M and Beg, M.A. (2010). Disruption of the periovulatory LH surge by a transient increase in circulating 17-estradiol at the time of ovulation in mares. *Anim Reprod Sci*, 117(1-2): 178-182. Gottschall, P.E., Tatsuno, I. and Miyata, A. (1990). Haracterization and distribution of binding sites for the hypothalamic peptide, pituitary adenylate cyclase activating polypeptide. *Endocrinology*, 127(1): 272-277. Gras, S., Hannibal, J., Georg, B. and Fahrenkrug, J. (1996). Transient periovulatory expression of pituitary adenylate cyclase activating peptide in rat ovarian cells. *Endocrinology*, 137: 4779-4785. Gras, S., Hannibal, J. and Fahrenkrug, J. (1999). Pituitary Adenylate Cyclase-Activating Polypeptide Is an Auto/Paracrine Stimulator of Acute Progesterone Accumulation and Subsequent Luteinization in Cultured Periovulatory Granulosa/Lutein Cells. *Endocrinology*, 140(5): 2199-205. Gras, S., Host, E. and Fahrenkrug, J. (2005). Role of pituitary adenylate cyclase-activating peptide (PACAP) in the cyclic recruitment of immature follicles in the rat ovary. *Regul Pept*, 128(1): 69-74. Harmar, A.J., Arimura, A., Gozes, I., Journot, L., Laburthe, M., Pisegna, J.R., Rawlings, S.R., Robberecht, P., Said, S.I., Sreedharan, S.P., Wank, S.A. and Waschek, J.A. (1998). International Union of Pharmacology. XVIII. Nomenclature of Receptors for Vasoactive Intestinal Peptide and Pituitary Adenylate Cyclase-Activating Polypeptide. *Pharmacol Rev*, 50(2): 265-270. Hillier, S.G. (2001). Gonadotropic control of ovarian follicular growth and development. *Mol Cell Endocrinol*, 179(1-2): 39-46. Hirst, R.C., Abel, M.H., Wilkins, V., Simpson, C., Knight, P.G., Zhang, F.P., Huhtaniemi, I., Kumar, T.R. and Charlton, H.M. (2004). Influence of mutations affecting gonadotropin production or responsiveness on expression of inhibin subunit mRNA and protein in the mouse ovary. *Reproduction*, 128(1): 43-52. Huang, W.T., Li, C.J., Wu, P.J., Chang, Y.S., Lee, T.L. and Weng, C.F. (2009). Expression and in vitro regulation of pituitary adenylate cyclase-activating polypeptide (pacap38) and its type I receptor (pac1-r) in the gonads of tilapia (*Oreochromis mossambicus*). *Reproduction*, 137(3): 449-467. Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. and Nagata, S. (1992). Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. *Neuron*, 8(4): 811-819. Issac, R. and Sherwood, N.M. (2008). Pituitary adenylate cyclase-activating polypeptide (PACAP) is important for embryo implantation in mice. *Mol Cell Endocrinol*, 280(1-2): 13-19. Jaworski, D.M. and Proctor, M.D. (2000). Developmental regulation of pituitary adenylate cyclase-activating polypeptide and PAC(1) receptor mRNA expression in the rat central nervous system. *Brain Res Dev Brain Res*, 120(1): 27-39. Kimura, C., Ohkubo, S., Ogi, K., Hosoya, M., Itoh, Y., Onda, H., Miyata, A., Jian, L., Dahl, R.R., Stibbs, H.H., Arimura, A. and Fujino, M. (1990). A novel peptide which stimulates adenylate cyclase: molecular cloning and characterization of the ovine and human cDNAs. *Biochem Biophys Res Commun* 166(1): 81-89. Ko, C., In, Y.H. and Park-Sarge, O.K. (1999). Role of progesterone receptor activation in pituitary adenylate cyclase activating polypeptide gene expression in rat ovary. *Endocrinology*, 140: 5185-5194. Ko, C. and Park-Sarge, O.K. (2000). Progesterone receptor activation mediates LH induced type-I pituitary adenylate cyclase activating polypeptide receptor (PAC1) gene expression in rat granulosa cells. *Biochem Biophys Res Commun*, 277: 270-279. Koh, P.O., Kwak, S.D., Kang, S.S., Cho, G.J., Chun, S.Y., Kwon, H.B. and Choi, W.S. (2000). Expression of pituitary adenylate cyclase activating polypeptide (PACAP) and PACAP type I A receptor mRNAs in granulosa cells of preovulatory follicles of the rat ovary. *Mol Reprod Dev*, 55: 379-386. Kwok, Y.Y., Chu, Y.S., Vaudry, H., Yon, L., Anouar, Y. and Chow, K.C. (2006). Cloning and characterization of a PAC1 receptor hop-1 splice variant in goldfish (*Carassius auratus*). *Gen Comp Endocrinol*, 145(2): 188-196. Laan, M., Richmond, H., He, C. and Campbell, R.K. (2002). Zebrafish as a model for vertebrate reproduction: characterization of the first functional zebrafish (*Danio rerio*) gonadotropin receptor. *Gen Comp Endocrinol*, 12(3): 349-364. Lanini, S., Chiarotto, M., Muciaccia, B., Vaccari, S., Barberi, M., Guglielmo, M.C., Stefanini, M., Cecconi, S. and Canipari, R. (2010). Inhibitory effect of pituitary adenylate cyclase activating polypeptide on the initial stages of rat follicle development. *Mol Cell Endocrinol*, 14; 320(1-2): 34-44. Lee, J., Park, H.J., Choi, H.S., Kwon, H.B., Arimura, A., Lee, B.J., Choi, W.S. and Chun, S.Y. (1999). Gonadotropin stimulation of pituitary adenylate cyclase activating polypeptide (PACAP) messenger ribonucleic acid in the rat ovary and the role of PACAP as a follicle survival factor. *Endocrinology*, 140: 818-826. Lo, C.W., Chang, S.L. and Weng, C.F. (2007). Pituitary adenylate cyclase activating polypeptide (PACAP) regulates the expression of PACAP in cultured tilapia astrocytes. *Exp Biol Med*, 232(2): 262-276. Logan, K.A., Juengel, J.L. and McNatty, K.P. (2002). Onset of steroidogenic enzyme gene expression during ovarian follicular development in sheep. *Biol Reprod*, 66(4): 906-916. Lunenfeld, B. and Insler, V. (1993). Follicular development and its control. *Gynecol Endocrinol*, 7: 285-291. Lutz, E.M., Sheward, W.J., West, K.M., Morrow, J.A., Fink, G. and Harmar, A.J. (1993). The VIP2 receptor: molecular characterisation of a cDNA encoding a novel receptor for vasoactive intestinal peptide. *FEBS Lett*, 334(1): 3-8. Morris, P.L., Vale, W.W., Cappel, S. and Bardin, C.W. (1988). Inhibin production by primary Sertoli cell-enriched cultures: regulation by follicle-stimulating hormone, androgens, and epidermal growth factor. *Endocrinology*, 122(2): 717-725. Miyata, A., Arimura, A., Dahl, R.R., Minamino, N., Uehara, A., Jiang, L., Culler, M.D. and Coy, D.H. (1989). Isolation of a novel 38 residue hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun*, 164(1): 567-574. Miyata, A., Jiang, L., Dahl, R.D., Kitada, C.,

Kubo, K., Fujino, M., Minamino, N. and Arimura, A. (1990). 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*, 170(2): 643-648. Mertani, H.C., Pechoux, C., Garcia-Caballero, T., Waters, M.J. and Morel, G. (1995). Cellular localization of the growth hormone receptor/binding protein in the human anterior pituitary gland. *J Clin Endocrinol Metab*, 80(11): 3361-3367. Nakayama, Y., Yamamoto, T., Oba, Y., Nagahama, Y. and Abe, S. (2000). Molecular cloning, functional characterization, and gene expression of a follicle-stimulating hormone receptor in the testis of newt *Cynops pyrrhogaster*. *Biochem Biophys Res Commun*, 275(1): 121-128. Nielsen, C.T., Skakkebaek, N.E., Richardson, D.W., Darling, J.A., Hunter, W.M., Jorgensen, M., Nielsen, A., Ingerslev, O., Keiding, N. and Muller, J. (1986). Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height. *J Clin Endocrinol Metab*, 62(3): 532-535. Ogi, K., Kimura, C., Onda, H., Arimura, A. and Fujino, M. (1990). Molecular cloning and characterization of cDNA for the precursor of rat pituitary adenylate cyclase activating polypeptide (PACAP). *Biochem Biophys Res Commun*, 173(3): 1271-1279. Ohkubo, S., Kimura, C. and Ogi, K. (1992). Primary structure and characterization of the precursor to human pituitary adenylate cyclase activating polypeptide. *DNA Cell Biol*, 11(1): 21-30. Okazaki, K., Itoh, Y., Ogi, K., Ohkubo, S. and Onda H. (1995). Characterization of murine PACAP mRNA. *Peptides*, 16(7): 1295-1299. Pantaloni, C., Brabet, P., Bilanges, B., Dumuis, A., Houssami, S., Spengler, D., Bockaert, J. and Journot, J. (1996). Alternative splicing in the N-terminal extracellular domain of the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor modulates receptor selectivity and relative potencies of PACAP-27 and PACAP-38 in phospholipase C activation. *J Biol Chem*, 271(36): 22146-22151. Park, H.J., Lee, J., Wang, L., Park, J.H., Kwon, H.B., Arimura, A. and Chun, S.Y. (2000). Stage-specific expression of pituitary adenylate cyclase-activating polypeptide type I receptor messenger ribonucleic acid during ovarian follicle development in the rat. *Endocrinology*, 141(2): 702-709. Rawlings, S.R. and Hezareh, M. (1996). Pituitary adenylate cyclase activating polypeptide (PACAP) and PACAP / vasoactive intestinal polypeptide receptors: action on the anterior pituitary gland. *Endocr Rev*, 17(1): 4-29. Sayasith, K., Brown, K.A. and Sirois, J. (2007). Gonadotropin-dependent regulation of bovine pituitary adenylate cyclase-activating polypeptide in ovarian follicles prior to ovulation. *Reproduction*, 133(2): 441-453. Schubert, R.L. and Puett, D. (2003). Single-chain human chorionic gonadotropin analogs containing the determinant loop of the beta-subunit linked to the alpha-subunit. *J Mol Endocrinol*, 31(1): 157-168. Shahed, A. and Young, K.A. (2011). Intraovarian expression of GnRH-1 and gonadotropin mRNA and protein levels in Siberian hamsters during the estrus cycle and photoperiod induced regression/recrudescence. *Gen Comp Endocrinol*, 170(2): 356-364. Sherwood, N.M., Krueckl, S.L. and McRory, J.E. (2000). The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. *Endocr Rev*, 21(6): 619-670. Shpakov, A.O., Bondareva, V.M. and Chistiakova, O.V. (2010). Functional state of adenylyl cyclase signaling system in reproductive tissues of rats with experimental type 1 diabetes. *Tsitologija*, 52(2): 177-183. Spengler, D., Waeber, C., Pantaloni, C., Holsboer, F., Bockaert, J., Seeburg, P.H. and Journot, L. (1993). Differential signal transduction by five splice variants of the PACAP receptor. *Nature*, 365(6442): 170-175. Usuki S. and Kotani, E. (2001). Effect of pituitary adenylate cyclase-activating polypeptide (PACAP) on progesterin biosynthesis in cultured luteal cells from rat ovary. *Gynecol Endocrinol*, 15(3): 184-191. Vaudry, D., Gonzalez, B.J, Basille, M., Yon, L., Fournier, A. and Vaudry, H. (2000). Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol Rev*, 52: 269 – 324. Vaudry, D., Falluel-Morel, A., Bourgault, S., Basille, M., Burel, D., Wurtz, O., Fournier, A., Chow, B.K., Hashimoto, H., Galas, L. and Vaudry, H. (2009). Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol Rev*, 61(3): 283-357. Villalba, M., Bockaert, J. and Journot, L. (1997). Pituitary adenylate cyclase-activating polypeptide (PACAP-38) protects cerebellar granule neurons from apoptosis by activating the mitogen-activated protein kinase (MAP kinase) pathway. *J Neurosci*, 17(1): 83-90. Wang, Y. and Ge, W. (2003). Gonadotropin regulation of follistatin expression in the cultured ovarian follicle cells of zebrafish, *Danio rerio*. *Gen Comp Endocrinol*, 134: 308-315. Wang, Y., Wong, O.L. and Ge, W. (2003). Cloning, regulation of messenger ribonucleic acid expression, and function of a new isoform of pituitary adenylate cyclase-activating polypeptide in the zebrafish ovary. *Endocrinology*, 144(11): 4799-4810. Watanabe, T., Nakamachi, T., Matsuno, R., Hayashi, D., Nakamura, M., Kiruyama, S., Nakajo, S. and Shioda, S. (2007). Localization, characterization and function of pituitary adenylate cyclase-activating polypeptide during brain development. *Peptide*, 28(9): 1713-1719. Zhong, Y. and Kasson, B.G. (1994). Pituitary adenylate cyclase-activating polypeptide stimulates steroidogenesis and adenosine 3',5'-monophosphate accumulation in cultured rat granulosa cells. *Endocrinology*, 135(1): 207-213.