Analyses of the Expression and Regulation of Pituitary Adenylate Cyclase-Activating Polypeptide in the Gonads of Tilapia

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ABSTRACT

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide and belongs to the vasoactive intestinal peptide/secretin/glucagons/GHRH superfamily. PACAP is present in two biologically active forms with 38 and 27 amino acid residues, designated PACAP38 and PACAP27, respectively. The diverse functions of PACAP including the regulation of apoptosis, metabolism, endocrine and immune systems have been widely studied in mammals, however, literature on the presence and involvement of PACAP and its receptor in fish is scant. The tissue distribution of tilapia PACAP (tPACAP38) and its receptor, and the effect of different concentrations and culture periods of dibutyryl-cAMP, ovine PACAP38, forskolin and H89 on the tPACAP38 expression of in vitro cultured gonads of tilapia were studied. Semi-quantitative RT-PCR detected tPACAP38 and PAC1-R in the brain, gallbladder, gill, heart, intestine, kidney, muscles, pancreas, spleen, stomach, testes and ovaries, but not in the liver of tilapia. The expression of tPACAP38 and PAC1-R mRNA in brain was significantly higher than those in other tissues of both genders (P ? 0.05), however, no significances existed between the expressions in other tissues (P ? 0.05). The identification of PCR fragments was confirmed by PCR hybridization. The stimulation of tPACAP38 expression by gonadotropin could be mimicked by either the addition of different concentrations of cAMP analogs (dibutyryl-cAMP, 0.25, 1.5, and 5 mM) or ovine PACAP38 (0.25, 1.5, and 5 nM) to gonads (testes and ovaries) cultured for 2 h, or by time-course experiment with 1.5 mM of dibutyryl-cAMP or 5 nM of ovine PACAP38 for 0 to 8 h. The expression levels of tPACAP38 mRNA increased in a dose-dependent manner, and they were low at 0 h and increased significantly at 2 h, and then decreased with prolonged cultured periods in both genders. Same results were observed by supplement with the adenylate cyclase activator forskolin (1, 5, and 10 μM), in addition the inductive function of forskolin could be suppressed by the addition of protein kinase A (PKA) inhibitors H89 (10 μM), suggesting that the involvement of PACAP in the cAMP-PKA signaling pathway. Expression of tPACAP38 and PAC1-R in the gonads of tilapia suggests that PACAP may play an important paracrine/autocrine regulator in the bony fish.

Keywords : tilapia, Pituitary adenylate cyclase-activating polypeptide (PACAP), PACAP type I receptor, gonads, signal pathway

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