

促性腺素於體外培養吳郭魚性腺之固醇類荷爾蒙生成與腦垂腺 β 酸環化 β 激活 β 及其受體基因表現之影響

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摘要

脊椎動物之生殖主由下視丘-腦下垂體-性腺之內分泌軸 (hypothalamus-pituitary-gonads axis) 所調控, 下視丘分泌促性腺素釋放素 (gonadotropin releasing hormones) 誘導腦垂體前葉 (anterior pituitary) 分泌如促濾泡素 (follicle stimulating hormone, FSH) 及促黃體素 (luteinizing hormone, LH) 等之促性腺素 (gonadotropins) 作用至性腺並調控其之發育、配子生成與成熟, 並促進固醇類荷爾蒙生成 (steroidogenesis)。於水產養殖中, 吳郭魚 (*Oreochromis mossambicus*) 為目前本國主要經濟養殖魚類之一, 然目前其生殖調控相關研究極少。本研究室先前之研究顯示, 除肝臟外, 於吳郭魚之各臟器及性腺包含精巢 (testis) 及卵巢 (ovary) 等組織中皆有腦垂腺 β 酸環化 β 激活 β (pituitary adenylate cyclase activating polypeptide, tpacap38) 及其受體 (type-I PACAP receptor, tpac1-r) 之表現, 而藉由添加cAMP類似物 (dibutyryl-cAMP)、合成之綿羊PACAP及腺 β 環化 β (adenylyl cyclase) 之活化劑forskolin誘導濾泡細胞 (follicle cells) 及精巢組織於劑量及時間相關試驗之結果顯示, tpacap38之表現經三藥劑誘導後顯著提升, 然其表現可被PKA抑制劑H89所抑制, 上述結果顯示tpacap38表現可能與cAMP-PKA之訊號傳遞路徑 (3'-5'-cyclic adenosine monophosphate/protein kinase A signal pathway) 有關。本研究以半定量反轉錄 β -聚合 β 鏈鎖反應 (RT-PCR) 及酵素免疫分析 (enzyme immunoassay, EIA) 偵測經促性腺素誘導後吳郭魚之性腺其tpacap38及其受體tpac1-r之表現量及固醇類荷爾蒙生成量, 經添加如類促黃體素 (LH) 功能之人類絨毛膜促性腺素 (human chorionic gonadotropin, hCG; 5、15及50 IU) 與類促濾泡素 (FSH) 功能之孕馬血清激素 (pregnant mare's serum gonadotropin, PMSG; 5、15、50及100 IU) 誘導2小時之劑量相關試驗結果顯示, 相較於控制組 (無添加組), 添加15 IU之hCG可顯著提高雌性與雄性吳郭魚性腺中tpacap38與tpac1-r mRNA之表現量, 並可促進固醇類荷爾蒙如雌激素、助孕酮及辜固酮之生成。而添加50 IU之PMSG可顯著誘導雌魚濾泡細胞中tpac1-r mRNA之表現量, 亦可促進精巢及濾泡細胞中雌激素、助孕酮及辜固酮之生成。另外經不同濃度hCG (5、15及50 IU) 及PMSG (5、15及50 IU) 各劑量共同添加誘導2小時之結果顯示, 共同添加促性腺素可誘導性腺中tpacap38及tpac1-r mRNA之表現。共同誘導亦可促進精巢及濾泡細胞中雌激素、助孕酮及辜固酮之生成。於時間相關之試驗中, 添加15 IU hCG或50 IU PMSG誘導性腺於不同時間 (2、4、6及8小時) 之結果顯示, 精巢與濾泡細胞中之tpacap38及tpac1-r mRNA之表現量經hCG誘導後於4小時達到高峰。而添加50 IU PMSG誘導後, 精巢與濾泡細胞中之tpacap38 mRNA之表現量於2小時達到高峰; 而共同添加15 IU hCG與50 IU PMSG誘導後之結果顯示, 性腺中tpacap38及tpac1-r mRNA之表現量於6小時達到高峰。性腺固醇類荷爾蒙經促性腺素 (15 IU hCG、50 IU PMSG或共同誘導) 於不同時間誘導後, 其分泌量於2小時後生成量顯著增加, 然隨著培養時間增加而降低。上述促性腺素誘導基因表現與固醇類荷爾蒙分泌之作用皆可受H89所抑制。本研究結果顯示, 於硬骨魚類中, tpacap38及tpac1-r可能以旁分泌或自分泌之方式參與促性腺素-性腺生殖路徑之調控, 並影響性腺固醇類荷爾蒙之生成。

關鍵詞: 吳郭魚、腦垂腺 β 酸環化 β 激活 β 、腦垂腺 β 酸環化 β 激活 β 第一型受體、性腺、促性腺素、固醇類荷爾蒙生成

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