Effects of neurosteroids on the human corticotropin-releasing hormone gene

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Abstract:
Increased activity of hypothalamo-pituitary-adrenal (HPA) axis and hypersecretion of corticotropin-releasing hormone (CRH) are known to be important factors in pathogenesis of some stress-related diseases. Some neurosteroids exert anxiolytic and antidepressant effects probably by inhibition of HPA axis activity. The aim of our study was to find out if neurosteroids can directly affect human CRH gene transcription. The effect of allopregnanolone (ALLO), allo-tetrahydrodeoxy corticosterone (THDOC), pregnenolone (PGL), PGL sulfate (PGL-S), dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) on CRH expression was determined in differentiated Neuro-2A cells stably transfected with plasmid containing a fragment of human CRH promoter (−663 to +124 bp) linked to the chloramphenicol acetyltransferase (CAT) reporter gene. It was found that PGL (0.3–30 μM), ALLO (1–30 μM) and THDOC (1–30 μM) present in the culture medium for 5 days in the concentration-dependent manner inhibited CRH-CAT activity. These neurosteroids also inhibited forskolin-stimulated CRH gene transcription with similar potency. In contrast, PGL-S, DHEA and DHEA-S in a concentration from 0.01 to 10 μM had no effect on basal and forskolin-stimulated CRH activity. Further experiments revealed that wortmannin (an inhibitor of phosphatidylinositol 3-kinase; PI3-K) at concentrations of 0.01 and 0.02 μM did not change the inhibitory effect of ALLO (3 μM) and PGL (1 μM) on CRH gene transcription. Moreover, ALLO (3 μM) and PGL (1 μM) present in the culture medium for 5 days did not change the amount of active, phosphorylated form of protein kinase B (PKB, Akt) and extracellular signal-regulated kinase (ERK). The obtained results indicate that PGL, ALLO and THDOC inhibited basal and forskolin-induced CRH gene promoter activity in the differentiated Neuro-2A cells and that these effects did not depend on the activation of PI3-K/Akt and ERK-MAPK pathways.

Key words: neurosteroids, CRH gene promoter activity, differentiated Neuro-2A cells, stable transfection, protein kinases