Thyroliberin and luliberin degradation by enzymes in cultured cells of neural origin.

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Two enzymes which can remove the N-terminal pyroglutamyl residue of Thyroliberin (Glp-His-ProNHz) have been identified in brain tissue of guinea-pig and other animals. Pyroglutamate aminopeptidase I is present in cytoplasm and requires DTT and EDTA for the assay of activity. [1] Pyroglutamate aminopeptidase II has been located in synapticosomal membranes and is inhibited by EDTA [2]. A number of enzymes have been identified in the soluble fraction of brain tissue which can introduce cleavage into the primary sequence of luliberin. Degrading activity has also been found in particulate fractions of both guinea-pig brain [4] and rat brain [5]. Both thyroliberin and luliberin have been suggested as neurotransmitters or neuromodulators in the CNS [6] and this study was undertaken to determine the cellular distribution of enzymes for the degradation of these peptides in cultured cells of neural origin. Neuroblastoma (Neuro-2A) and glioma (C6) cells were cultured in Dubcco's modified Eagles medium containing 10% foetal calf serum, 200 mM glutamine and gentamycin (100 μg/ml) and maintained at 37 °C in a humidified atmosphere of 9% CO2. The cells were seeded in tissue culture flasks at a density of 10^4 cells/cm^2 and allowed to reach confluency (3-4 days). The cells were harvested by scraping and washed once with Dulbecco's phosphate buffered saline. The final pellet (800 g x 10 min) was lyophilised and stored at 20 °C until assay. Prior to assay cells were resuspended in 1 ml of 150 mM potassium phosphate pH 7.5. Pyroglutamate aminopeptidase was measured using a modification of the method of Bauer and Kleinkauf [8] in which [Glp -3H] luliberin was used as substrate. For measurement of pyroglutamate aminopeptidase I a 100 mM potassium phosphate buffer pH 7.5 containing 2 mM EDTA and 2 mM DTT was used while assay for pyroglutamate aminopeptidase II was conducted in 100 mM potassium phosphate pH 7.5 alone. Prior to assay for luliberin degrading activity the cell suspensions were resolved into soluble fractions and particulate fractions by centrifugation at 34,000 g for 120 minutes. Soluble and particulate fractions were then assayed for luliberin degrading activity by the method of Horsthemke and Bauer [8] in which [Glp -3H] luliberin was employed as substrate. Protein was measured by a modification [9] of the method of Lowry et al [10]. The results obtained show that while pyroglutamate aminopeptidase I was present in both neuro-2A cells and glioma cells, aminopeptidase II activity could only be detected in neuro-2A cells. This result is in agreement with a previous report [11] which showed that pyroglutamate aminopeptidase II activity was absent in glioma cells while present in neuro-2A cells. It is possible that glioma cells have lost the ability to express pyroglutamate aminopeptidase II activity by de-differentiation. Results from a previous study, however, indicated that astroglial cells in primary culture are devoid of pyroglutamate aminopeptidase II activity [12].

Soluble and particulate luliberin degrading activity was found to be present in both neuro-2A cells and glioma C6 cells. In both cell lines, however, the specific activities in the soluble fraction were greater than in the particulate fraction. The particulate activity is of greater relevance to the physiological inactivation of luliberin which may be acting as a neuromodulator or neurotransmitter. It remains to be determined whether the same enzyme is responsible for the degradation of luliberin in particulate fractions of both neuro-2A cells and glioma cells.

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References: