CHARACTERIZATION OF PITUITARY GROWTH HORMONE IN THE GREEN IGUANA (Iguana iguana).

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Although growth hormone (GH) has been studied in many vertebrates, it is poorly characterized in reptiles, where its structure has been described only in a turtle, a crocodile and, more recently, a snake. Here we studied the morphology and ultrastructure of pituitary somatotrophs in the green iguana (gi, Order Squamata) and characterized the giGH structure, which allowed an evolutionary analysis. The green iguana pituitary is constituted by a pars distalis and a pars nervosa, divided by a pars intermedia which, in contrast to other reptiles, is more developed in this species. The cellular distribution of giGH was analyzed by in situ hybridization and immunohistochemistry, using a specific-riboprobe and an antibody against chicken GH, respectively. The somatotrophs were found mainly in the caudal region of the pars distalis. Electron micrographs showed that they are 6.5-10 µm cells containing a good amount of granules (250-300 nm) where giGH is stored. The pituitary giGH was purified by immunoaffinity chromatography using a heterologous immunosorbent prepared with a polyclonal anti-chicken GH (cGH) antibody. SDS-PAGE and Western blotting of the purified preparation showed a main monomeric giGH variant with an apparent MW of 26 kDa and a dimeric molecular variant of 52 kDa (under reducing conditions). Furthermore, giGH showed at least four charge variants (pls between 6.2-7.4) by isoelectric focusing. Additionally, the cDNA of giGH was amplified by PCR and 3’ & 5’ RACE. The sequence obtained consisted of 1016 bp that encoded a pro-hormone of 218 aa, containing a signal peptide (27 aa) and the mature protein corresponding to 191 aa. An identity pattern of 81%, 82%, and 84% was obtained when giGH was compared with chicken, crocodile and turtle GHs, respectively. The phylogenetic analysis showed that giGH goes in a different branch than turtles and crocodiles, which are closer to birds. These findings will contribute to better understand the evolution of vertebrate GH.

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