104. MOLECULAR EVOLUTION OF THE PROLACTIN/GH PROTEIN FAMILY IN MONOTREMES

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Prolactin and growth hormone (GH) belong to a family of polypeptide hormones that are thought to have evolved from a common ancestral gene by gene duplication approximately 4–500 × 10^6 years ago, followed by sequence divergence. Molecular evolution of this hormone family in mammals is characterised by a slow underlying rate of evolution with, in certain species, occasional bursts of rapid change (1). Because monotremes diverged from marsupials and eutherian mammals more than 100 × 10^6 years ago, and show major differences in reproduction and lactation from the other mammals, we decided to determine the nucleotide sequence for prolactin and GH from the platypus and echidna. Sequences were determined by 3'- and 5'-rapid amplification of cDNA ends (RACE) as previously described (2) and verified by a separate round of RT-PCR with species specific primers to the 5'- and 3'-UTRs. Analyses were performed on deduced amino acid sequences for the hormone coding region by the method of Fitch and Margoliash as determined by the programs Protdist and Fitch in the PHYLIP package as described in (1, 2). Echidna and platypus GHs were very similar to each other (99.0 % sequence identity) and more similar to mammalian GHs than those of reptiles or birds (e.g. echidna GH had 89.0, 88.5, 83.2 and 79.6 % sequence identity with brushtail possum, pig, sea turtle and chicken GHs). Further analysis confirmed a slow rate of molecular evolution (<0.2 substitutions/amino acid site/year ×10^9), similar to that seen for GH in the majority of mammalian species. In contrast, echidna and platypus prolactins were only 89.9 % identical and the difference was due mostly to a higher rate of evolution for echidna prolactin (2.5 substitutions/aa site/year ×10^9) since divergence from the platypus. As for GH, monotreme prolactins were more similar to those of mammals than other vertebrates (e.g. echidna prolactin showed 78.9, 75.4 and 70.9 % sequence identity with possum, pig and chicken prolactins). The functional significance, if any, of these observations remains to be determined, but the prolactin results suggest that some selective pressure associated with speciation of echidnas, and presumably associated with the biological actions of prolactin, has occurred.

(1) Wallis M. (2000) *Mol. Evol*. **50**: 465–473. (2) Curlewis *et al*. (1998) *Gen. Comp. Endocrinol*. **111**: 61–67.

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