

PROTEIN TURNOVER IS FASTER IN THE REGENERATING CELLS OF THE CHICK COCHLEA AS SHOWN BY MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

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Stimulating regeneration -or avoiding degeneration- of hair cells is an essential goal in deafness cure or prevention. Bird cochlear hair cells do regenerate after ototoxic destruction. We exposed a group of one-week-old chicks to a diet containing elevated levels of ¹⁵N leucine for 7 days. Half of this group received a single injection of gentamicin at a dosage of 300 mg/kg on day 0. This procedure leads to a loss of all the hair cells in the basal 30% of the cochlea by day 3 and a repopulation of this region with regenerated hair cells by day 5-7. The new hair cells arise from the proliferation of non-sensory supporting cells in the region of hair cell loss. These supporting cells are normally quiescent but re-enter the cell cycle as the original population of hair cells die. Our studies have shown that the DNA synthesis stage of the cell cycle begins 65 hours and peaks between 3 and 4 days after the gentamicin injection. We therefore gave the gentamicin-treated birds a single injection of the thymidine analog BrdU on day 3, so that cells in the process of DNA synthesis would be labeled. We used MIMS to visualize and to measure protein turnover in the cochlea by the increase in the percentage of ¹⁵N present in the cochlear tissues. Moreover, cells that incorporated BrdU during DNA synthesis were identifiable by the presence of Bromine in their nuclei. In the basal part of the cochlea where new hair cells are being made, we found that cells that had undergone DNA synthesis (identified by the ⁸¹Br signal) had a higher incorporation of ¹⁵N-L-leucine -derived from the ¹²C¹⁵N/¹²C¹⁴N ratio- than neighboring cells. Surprisingly, in the cells that had divided, the incorporation of ¹⁵N (protein synthesis) was greater in the nucleus than in the cytoplasm. These results, uniquely obtained with MIMS, open a new direction of research into the understanding of cellular regeneration.

Supported in part by research resource grant 9 P41 EB001974-04 and 5R01DC04179