Cytochemical Localization of Adenylcyclase in the Lateral Wall of the Inner Ear*

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Summary. The cytochemical localization of adenylcyclase in the lateral cochlear wall was studied using a modification of the Reik-Howell method. All the cell membranes of the stria vascularis and spiral prominence cells, except the vascular endothelium, showed a low enzyme activity. In the marginal cell and spiral prominence epithelium facing the endolymph, this activity was limited to the perilymphatic membrane sections. The results are discussed.

Key words: Inner ear — Adenylcyclase — Ouabain-insensitive endothelial ATPase — Cytochemistry — Electron microscopy

The membrane-bound enzyme adenylcyclase catalyses the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP) and pyrophosphate (P-P). ATP-splitting and cAMP formation take place on the inner surface of the cell membrane and are induced by a specific binding of a ligand on the enzyme receptor situated on the outer cell surface. Thus, the effect of many hormones (first messenger), mainly peptide hormones which are not able to enter the cell, is initiated by specific binding and enzyme activation, resulting in a rise in the intracellular cAMP level (second messenger).

Since cAMP was discovered and identified as a second messenger by Sutherland and Rall (1957), numerous studies have been performed on its formation and function. cAMP has been shown to play an important function in regulating cell metabolism and has also been implicated in the regulation of ion and fluid transport in various tissues. However, the underlying molecular events are still unknown, although it is assumed that an initial phosphorylation of

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Membrane proteins involved in membrane transport plays an important function (Morel 1981).

In the kidney tubules transport of water and solutes is modulated by activation of adenylcyclase and the subsequent synthesis of cAMP under the influence of hormones (vasopressin, calcitonin, parathyroid hormone, isoproterenol).

Zenner and Zenner (1979) have shown that inner ear adenylcyclase activity can also be stimulated with vasopressin and isoproterenol. Quantitative studies measuring adenylcyclase content in the inner ear (Ahlström et al. 1975) have demonstrated the highest basal activities in the stria vascularis (19 pmol/mg dry wt. per minute). Sodium fluoride stimulation resulted in a 1.8-fold increase in enzyme activity.

Our present knowledge of the physiological function of adenylcyclase in the inner ear, however, still remains restricted. As yet we do not know the exact enzyme localization at cellular level and the question as to whether the enzyme is located on the perilymphatic or on the endolymphatic surface still can not be answered.

To answer these questions, adenylcyclase activity was localized cytochemically using a modification of the Reik-Howell method (Fenoglio et al. 1981).

Materials and Method

Guinea-pigs, weighing about 300 g and showing positive Preyer's reflex, were decapitated. The bullae were removed and the cochlea was then fixed by perilymphatic perfusion with 3% paraformaldehyde in 0.1 M cacodylate buffer or with 1% glutaraldehyde, pH 7.5. After perfusion, fixation was continued by immersion for 30 min. Still in fixative, the spiral ligament was microdissected under a stereomicroscope and cut into tiny pieces. Following a rinse in cacodylate and Tris-HCl buffer, the tissue was frozen in a deep-freeze in order to disrupt the cell membranes.

Fig. 1. Stria vascularis. Incubation with adenosine triphosphate (ATP) containing medium. All cell membranes, including the endolymphatic surface (E) of the marginal cell (MC) are stained by enzymatic reaction product. SC, stria capillary; BC, basal cell. Unstained

Fig. 2. Stria vascularis. Incubation with ATP-containing medium. Inclusion of 10 mM ouabain and 1 mM L-tetramisole in the incubation medium results in a less extensive membrane staining. SC, stria capillary; BC, basal cell. Unstained

Fig. 3. Stria capillary (SC). Incubation with ATP-containing medium and addition of 10 mM ouabain and 1 mM t-tetramisole to the incubation medium. The luminal endothelial cell membrane (arrow), separated from the endothelial cytoplasm due to freezing artefact, exhibits small and focal electron-dense deposits. Unstained

Fig. 4. Stria capillary (SC) and perivascular space (PVS). Incubation with standard medium without adenylylimidodiphosphate (AMP-PNP), containing lead instead of strontium ions, as capture reagent. The deposits in the perivascular space (PVS) are artefacts and indicate unspecific adsorption of lead. Unstained