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The phosphoinositide cascade in isolated outer hair cells: possible role as second messenger for motile responses

Jochen Schacht [1] and Hans P. Zenner [2]

[1] *Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, MI 48109, U.S.A. and* [2] *Department of Otolaryngology, University of Würzburg, D-8700 Würzburg, F.R.G.*

Extracellular stimuli are encoded at the cell membrane into intracellular biochemical signals that elicit the physiological response of the cell. Recent evidence suggests that polyphosphoinositides, lipids enriched in the plasma membrane of neural tissues constitute a fundamental transmembrane signalling system for neuromodulators and hormones which elevate intracellular calcium levels. The initial step in their action is thought to be a stimulation of the phosphodiesteratic hydrolysis of membrane-bound phosphatidylinositol bisphosphate liberating diacylglycerol and inositol trisphosphate as second messengers and initiating an intracellular signal cascade. Diacylglycerol activates a specific protein kinase C and inositol trisphosphate mobilises intracellular calcium triggering the cellular response (Berridge, 1984; Nishizuka, 1984).

We have investigated phosphoinositides and this signal cascade in isolated live outer hair cells. The preliminary results indicate that contractility of outer hair cells may be mediated by this second messenger system. Living outer hair cells were isolated by micromanipulation. Viability of the preparation was assured by the ability of the cells to carry out oxidative phosphorylation and synthesize ATP. Furthermore, recent giga-seal whole-cell recordings showed cell potentials of -70 mV (Zenner et al., 1985).

The prerequisite for the existence of the phosphoinositide cascade is the presence of phosphatidylinositol bisphosphate and related lipids in outer hair cells. Radioactive labelling documented a high metabolism of phosphatidylinositol, phosphatidylinositol 4-phosphate (PtdIns 4-*P*), phosphatidylinositol 4,5-bisphosphate (PtdIns 4.5-P_2) and phosphatidic acid. After 60 min of incubation with [^{32}P]orthophosphate the polyphosphoinositides constituted $33 \pm 7\%$ (PtdIns 4.5-P_2) and $28 \pm 7\%$ (PtdIns 4-*P*) of the total labelled lipids.

It has been shown that calcium will trigger a motile response in isolated outer hair cells (Zenner, 1986). A crucial test for a physiological role of phosphoinositides as a second messenger system is to examine whether the putative second messenger inositol trisphosphate (InsP_3) will cause a contraction in the absence of added calcium. Cells were permeabilized with 0.1% Triton X-100 (InsP_3 will not penetrate intact membranes) and InsP_3 was added. Concentrations as low as 10^{-5} M indeed elicited a longitudinal motile response observable within 10 s.

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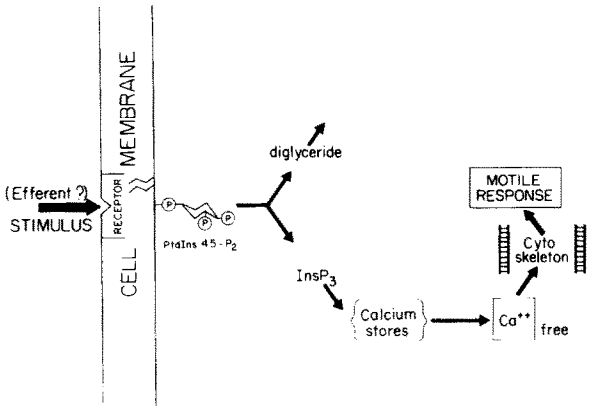

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