Voltage dependent currents in type I and II hair cells and calyx terminals of primary afferents in an intact vestibular preparation

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Mammalian vestibular end-organs contain type I and type II hair cells that are responsible for the conversion of mechanical stimuli into neural signals. These signals are then transmitted through chemical synapses to primary afferent fibers and passed on to the central nervous system. Precisely how hair cells and primary afferents communicate is unknown. Most studies to date have concentrated on isolated hair cells with little regard to their effects on primary afferents. Here we describe our initial recordings from an intact epithelial preparation that will help us understand how these two important components of our peripheral balance system interact.

From previous studies we know that the two types of hair cells not only differ morphologically but also functionally. For example, the type I hair cells are amphora-shaped and most of the basolateral surface of the type I cell is ensheathed in the dendritic cup-like or "calyx" ending of the primary afferent. In contrast, barrel-shaped type II hair cells are contacted by simple synaptic boutons. The precise function of the calyx ending and the influence it exerts upon synaptic transmission between the type I hair cell and its primary afferent remains a mystery. Our major effort, therefore, is to understand this unique synapse. Functionally, type I hair cells exhibit a large outward current (termed $I_{K,L}$) at resting membrane potential, whereas type II hair cells do not. $I_{K,L}$, therefore, confers a very low impedance (about 10Ω) to type I hair cells at rest. Theoretically, this low impedance makes it difficult, if not impossible, for these hair cells to depolarize enough for normal neurotransmitter release by even the largest currents induced by movement of their apical hair bundles. The unusual architecture of the calyx ending, however, may provide a clue. Since the calyx forms a long narrow synaptic cleft, it may allow for significant potassium (K^+) accumulation thereby contributing to significant depolarization of its hair cell and primary afferent terminal. This, in turn, would not only allow neurotransmitter release but would also make the primary afferent itself more excitable. To date, previous studies have been unable to test this hypothesis because most experiments have used whole cell "dissociated" preparations, where the calyx terminal has been removed. Our intact *in vitro* preparation, taken from deeply anesthetized mice (ketamine 100 mg/kg, i.p.) is a novel approach in that we preserve the hair cells within the crista. This allows us to record from type I hair cells while they are still contacted by the calyx terminal ending. Using the whole-cell patch-clamp technique we have begun to record from type I hair cells and their associated calyx ending, as well as type II hair cells. This is the first step in developing a viable preparation in which we will clarify how information is passed between the hair cell and the primary afferent.

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