Rhodamine-labelled phalloidin, a specific marker for filamentous actin, was used to describe the distribution of F-actin in the hair cells of the organs of Corti of guinea pigs, hooded rats, chinchillas and squirrel monkeys using surface preparations, cryosections, and isolated outer hair cells. The stereocilia and cuticular plates of all inner and outer hair cells were labelled, as were the lateral margins of outer hair cells of all species. In the outer hair cells of the apical turns of the guinea pig organ of Corti, an additional labelled structure was present in the apical cytoplasm of the cell. This infracuticular network of F-actin was never observed in outer hair cells of the basal turn of the guinea pig, nor in outer hair cells at any location in the organs of Corti of the other species examined.

Introduction

The function of the outer hair cells of the organ of Corti has been a subject of interest since Spoendlin (1969) described the paucity of afferent innervation to these cells. Subsequent physiological experimentation has attributed two functional properties to the outer hair cells (OHC); firstly, the ability to 'fine tune' information processing in the organ of Corti (LePage and Johnstone, 1980; Davis, 1983; Neely and Kim, 1986), and secondly, the ability to generate various types of cochlear emissions (Kemp, 1978; Zurek, 1981). The identification of contractile proteins in OHC, (Flock and Cheung, 1977; Tilney et al, 1980; Zenner, 1980; Slepecky and Chamberlain, 1985; Flock et al, 1986; Zenner et al, 1985; Zenner, 1986; Zenner and Drenkhahn, 1986) coupled with the demonstration that isolated OHC in vitro will contract either in response to electrical stimulation (Brownell et al, 1985; Kachar et al, 1986; Ashmore 1986) or in the presence of depolarizing concentrations of potassium or calcium and ATP (Flock, 1986; Zenner, 1986), suggests that OHC motility may be responsible for the active processes and tuning mechanisms described in vivo.
Physiological and structural differences exist in OHC at different locations along the organ of Corti. Base to apex gradients in cell length (Smith, 1968; Bohne and Carr, 1985) the number and length (Wright, 1984) and stiffness of stereocilia (Strelioff and Flock, 1984) as well as in the efferent innervation (Spoendlin, 1969) and the afferent innervation (Pujol, 1987) have been described. The observation that OHC in the base of the cochlea do not produce DC receptor potentials to high frequency stimulation except at very high sound levels suggests that basal OHC are not depolarized by sound stimulation at threshold (Russell and Cody, 1985; Dallos, 1985a). One interpretation of these functional and structural differences is that apical OHC act more as sensory receptor cells but basal OHC act predominately as effector cells (Dallos, 1985b).

Because contractile proteins may be the structural components involved in certain functions attributed to the OHC, and because differences exist in the response properties of OHC of both apical and basal regions of the organ of Corti, it would be interesting to know if a base to apex gradient existed for the presence and distribution of contractile proteins in the organ of Corti, particularly in the OHC. Actin, a major contractile protein, has been identified in the stereocilia, cuticular plates and synaptic regions of OHC (Flock and Cheung, 1977; Tilney et al, 1980; Slepecky and Chamberlain, 1985; Flock et al, 1986), and actin filaments have been observed extending from beneath the cuticular plate to the perinuclear region (Zenner, 1980; Flock et al, 1982; Drenkhahn and Zenner, 1986; Thorne et al, 1988; Carlisle et al, 1988) and along the lateral margins of OHC (Flock et al, 1986). The purpose of this study was to describe the distribution of the filamentous form of actin in the organ of Corti of several mammalian species.

Materials and Methods

Guinea pigs, chinchillas, hooded rats and squirrel monkeys were included in the study. Rhodamine-labelled phalloidin (Molecular Probes, Inc, USA), a specific marker for filamentous (F)-actin, was used to identify actin in surface preparations, cryosections, and isolated hair cells which were studied by light microscopy (Leitz Orthomat) using bright field and epifluorescence optics (460-470 nm excitation filter and 510-550 nm objective filter) and 25 – 100x oil immersion objectives. Rhodamine-labelled alpha-bungarotoxin (Molecular Probes, Inc.) was used as a control for non-specific rhodamine staining.

Surface preparations

Animals were anaesthetised (sodium pentobarbitone, 50 mg/kg), decapitated and the auditory bullae were rapidly removed and opened. Fixative (4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer at pH 7.4) was perfused through the round and oval windows of the cochleae. Cochleae were immersed in fixative for 1 hour, washed in phosphate buffer and then partially dissected in phosphate buffered saline (PBS). The otic capsule, spiral ligament and tectorial membrane were removed. The organ of Corti, still attached to the modiolus, was immersed in 330 nM phalloidin in PBS and incubated for 24 hours in the dark at room temperature with gentle rotation. In some