DC1

Transport properties of prestin (SLC26A5) the outer hair cell motor protein

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Outer hair cells (OHCs) of the mammalian cochlea provide forces within the cochlea partician that alter the mechanics and lead to enhanced auditory sensitivity and frequency selectivity. This occurs by an assembly of 'motor' molecules in the basolateral membrane, identified and named as Prestin (Zheng *et al.* 2000), which cause changes in OHC length by conformational changes in response to acoustic frequencies upon membrane polarisation. The recent identification of this motor protein and its relation to a family (SLC26A) of anion exchange transporters (Oliver *et al.* 2001) has led us to explore the role of Prestin as a bicarbonate–chloride exchanger. As OHCs are known to uptake sugars, the role of GLUT 5 as a fructose transporter was investigated as it was previously identified in the basolateral membrane by antibody labelling (Geleoc *et al.* 1999).

Guinea-pigs were killed humanely and hair cells isolated from the cochlea were perfused in a flow chamber with perilymph buffered to pH 7.35 with Hepes. External chloride was reduced by gluconate replacement in the perfusion media and on doing so the cells initially shortened in length by approximately 6 %, reversible upon exposure for less than 100 s, as measured by imaging. The use of 1 mM DIDS and 10 mM salicylate in the external media blocked cell shortening in this situation. Conversely, when the external solution was buffered with 25 mM bicarbonate equilibrated with CO_2, no length change occurred when external chloride was reduced. These data suggest that the chloride–bicarbonate exchanger as described in OHC pH measurements (Ikeda *et al.* 1991) shares many properties of the OHC motor protein and may indeed be Prestin (SLC26A5). Electrophysiology determined a small chloride current (1–2 nS) which reversed around 0 mV with bilaterally symmetric chloride ions. Assuming an OHC motor density of 10^7 per cell, the conductance per motor would be near those ascribed to ion permeation through other transporters. We hypothesize that Prestin is a low conductance chloride channel.

OHCs are known to take up fructose (Geleoc *et al.* 1999). A C-terminal GFP-tagged Prestin construct was expressed in HEK-293 cells. Cells were exposed to isotonic replacement of sugar in the external medium to investigate sugar uptake. Cellular fluorescent profiles were imaged and analysed. The isotonic replacement of external glucose with 30 mM fructose led to a reversible increase in the diameter of spherical cells in Prestin (mean ± S.D., $n = 18$) transfected cells by 2.9 ± 1.7 %. The cell area increased by 5.9 ± 3.6 %. There was no area increase in cells expressing GFP alone or when extracellular solution as control was applied. The data are consistent with an influx of water following fructose exposure and suggest that HEK-293 cells are able to take up fructose when transfected with Prestin. We hypothesize that although other subunits may co-assemble with these proteins, their α helical structure in the most economic morphology allows neutral substrate transport.

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All procedures accord with current UK legislation.

DC2

Purinergic and muscarinic signalling affect cell movements in the E5 embryonic chick retina

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Neurotransmitter molecules are implicated in regulating cell proliferation and fate determination in the CNS. During development, the nuclei of retinal progenitor cells (PCs) undergo interkinetic nuclear migration (INM), moving from the ventricular zone (VZ) to the prospective ganglion cell layer in G_1 of the cell cycle, before duplicating their DNA in S phase, and returning to the VZ in G_2 and undergoing mitosis. Previous work (Pearson *et al.* 2002) has shown that purinergic and muscarinic receptors are involved in generating spontaneous $[Ca^{2+}]_i$ transients amongst VZ cells, and that both stimulation and blockade of these receptors affect the rate of mitosis. However, muscarinic and purinergic receptors may further affect the rate of the cell cycle through regulating the time it takes for INM. To investigate this we have visualised cells within embryonic day five (E5) chick retina. Embryos were decapitated, retinas dissected out, and labelled using DiI coated particles fired from a gene gun. This technique produces sparse labelling of both differentiated cells and PCs on a dark background. The movements of the cell nuclei and cell bodies labelled in this way can be followed on a confocal microscope for more than 2 h. Using these methods we have examined the effects of UTP (10 μM), carbachol (50 μM), GABA, glutamate (both 20 μM), PPADS (30 μM), pirenzipine, bicuculline and NBQX (all 25 μM), on cell movement. The mean rate of these movements was significantly decreased by bath application of UTP (rate in $\mu m\ h^{-1}$: 17.4 ± 1.0, mean ± S.E.M., $N = 6$ retinas, $n = 59$ cells, compared with paired controls (21.7 ± 1.4, $n = 48$, $P = 0.01$ (2-tailed Student's unpaired t test)), and significantly increased in the presence of the purinergic antagonist PPADS (22.1 ± 1.3, $N = 6$, $n = 55$ in PPADS compared with 17.6 ± 0.5, $n = 77$ in control, $P = 0.001$). Carbachol caused an increase (18.3 ± 0.7, $N = 4$, $n = 93$ in carbachol, 15.2 ± 1.1, $n = 33$ in control, $P = 0.03$), and pirenzipine a decrease (17.0 ± 0.9, $N = 4$, $n = 48$ in pirenzipine, 18.3 ± 1.1, $n = 40$ in control, $P = 0.37$), in the rate of movement, although only for carbachol was this significant. GABA, glutamate, bicuculline and NBQX were without effect. Purinergic and muscarinic stimulation and blockade affected only movements away from the VZ. Movements in this direction arise from both INM within PCs and the migration of differentiated cells. Since these two populations cannot be distinguished on basis of their morphology alone it was not possible to determine whether or not movements in one or both populations were affected by these drugs.

Pearson R *et al.* (2002). *J Neurosci* **22**, 7569–7579.

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All procedures accord with current UK legislation.