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Review

Regulation of lens volume: Implications for lens transparency

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ABSTRACT

Lens transparency is critically dependent on the maintenance of an ordered tissue architecture, and disruption of this order leads to light scatter and eventually lens cataract. Hence the volume of the fiber cells that make up the bulk of the lens needs to be tightly regulated if lens transparency is to be preserved. While it has long been appreciated that the lens can regulate its volume when placed in anisosmotic solutions, recent work suggests that the lens also actively maintains its volume under steady-state conditions. Furthermore, the process of fiber cell elongation necessitates that differentiating fiber cells dramatically increase their volume in response to growth factors. The cellular transport mechanisms that mediate the regulation of fiber cell volume in the lens cortex are only just beginning to be elucidated. In this region, fiber cells are continuously undergoing a process of differentiation that creates an inherent gradient of cells at different stages of elongation. These cells express different complements of transport proteins involved in volume regulation. In addition, transport processes at different depths into the lens are differentially influenced by electrochemical gradients that alter with distance into the lens. Taken together, our work suggests that the lens has spatially distinct ion influx and efflux pathways that interact to control its steady-state volume, its response to hypotonic swelling, and the elongation of differentiating fibers. Based on this work, we present a model which may explain the unique damage phenotype observed in diabetic cataract, in terms of the uncoupling or dysregulation of these ion influx and efflux pathways.

1. Introduction

The ocular lens is an optically transparent organ, and this transparency is a direct result of its unique cellular structure. Disruption of the pseudocrystalline packing of the cortical fiber cells, by either cellular swelling or dilation of the normally tight spaces between the cells, increases intralenticular light scattering (Jacob, 1999). Thus, volume regulation at both the cellular and tissue level is critically important for the maintenance of lens transparency. Earlier studies have shown that whole lenses placed in anisosmotic solutions are capable of regulating their volume (Duncan and Croghan, 1969; Patterson and Fournier, 1976; Patterson, 1981). When placed in hypotonic medium, lenses initially swell before undergoing a regulatory volume decrease (RVD) via the loss of K^+ and Cl^- ions and obligatory water loss. In contrast, the exposure of lenses to hypertonic media causes an initial shrinkage of the lens, which is resolved by a regulatory volume increase (RVI) driven by the intracellular accumulation of K^+, Na^+, and Cl^- ions. In other cell types, the efflux of KCl associated with RVD is mediated by the activation of K^+ and Cl^- channels (Niemeyer et al., 2001;Sardini et al., 2003), and/or potassium–chloride cotransporters (KCCs) (Lauf and Adragna, 2000). In contrast, RVI is often affected by an uptake of K^+ and Cl^- mediated by transporters such as the sodium–potassium–chloride cotransporter (NKCC) (Russell, 2000).

The relative contributions that Cl^- channels and transporters make to volume regulation in the lens has been examined by culturing rat lenses in the presence of reagents that modulate the activity of Cl^- transport proteins (Zhang and Jacob, 1996; Tunstall et al., 1999; Young et al., 2000; Merriman-Smith et al., 2002; Webb et al., 2004; Chee et al., 2006). The application of the anion channel antagonists tamoxifen and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) inhibited volume regulation in lenses exposed to hypotonic challenge (Zhang and Jacob, 1996; Tunstall et al., 1999). In addition, lenses exposed to NPPB under isotonic conditions increased their volume and exhibited light scattering (Tunstall et al., 1999). Thus, under normal isotonic conditions, the lens has a constitutively active Cl^- flux, which regulates fiber cell volume. In response to hypotonic challenge, this Cl^- flux can be up-regulated to restore lens volume and maintain lens transparency.

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2. Spatially distinct ion influx and efflux pathways exist in the lens cortex

The histological analysis of lenses treated with a variety of inhibitors (Tunstall et al., 1999; Young et al., 2000; Merriman-Smith et al., 2002; Webb et al., 2004; Chee et al., 2006), revealed that blocking Cl^- transport induced either one of two spatially distinct tissue damage phenotypes, or on occasions a combination of the two phenotypes (Fig. 1). In contrast to the regular cellular architecture observed in control lenses (Fig. 1A), lenses cultured in the presence of either the Cl^- channel inhibitor NPPB, or the NKCC blocker bumetanide, exhibited a localised band of tissue damage (Fig. 1B). This damage manifests itself as extracellular fluid accumulations between fiber cells located some 150 μm from the lens capsule. In contrast, the predominant effect of culturing lenses in the KCC inhibitor [(dihydronindenyl)oxy] alkanoic acid (DIOA) was a swelling of fiber cells located at the lens periphery, although some deeper extracellular space dilations were evident (Fig. 1C).

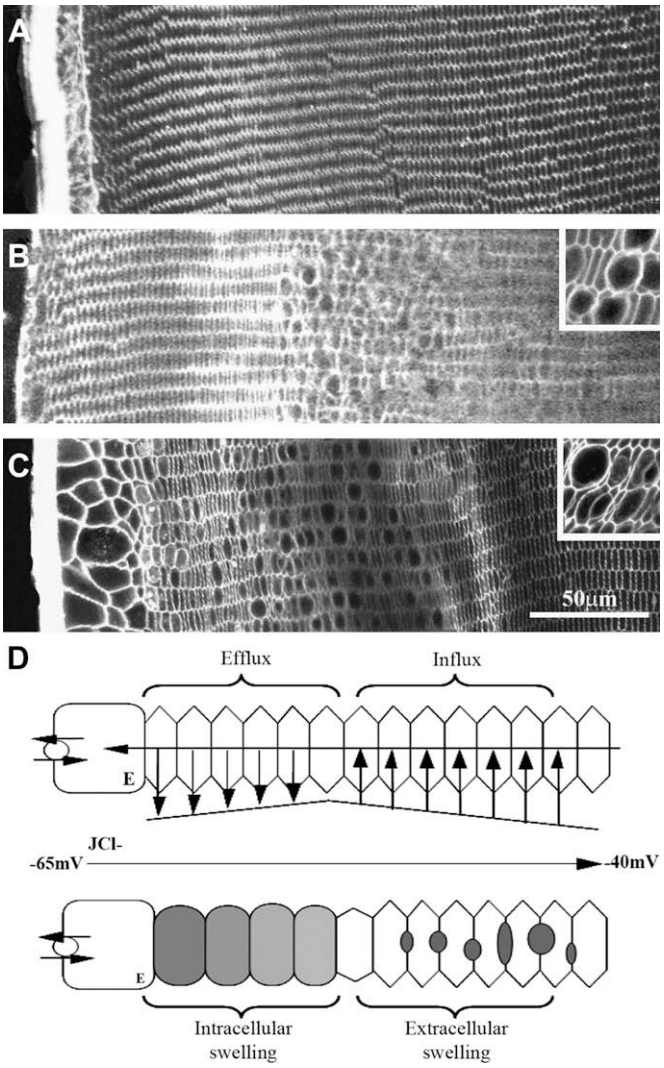

Fig. 1. Spatially distinct influx and efflux pathways exist in the rat lens. (A–C) Images of equatorial cross sections labelled with the membrane marker wheat germ agglutinin (WGA) taken from lenses incubated in the presence of isotonic artificial aqueous humor (AAH) (A); AAH + 10 μM NPPB (B); and AAH + 10 μM DIOA (C) for 18 h. Blocking chloride channels with NPPB disrupts the ordered tissue architecture of the lens (B) and induces a localised band of tissue disorder caused by the dilation of the extracellular spaces between fiber cells (B, insert). In contrast the major effect of the KCC inhibitor DIOA on lens morphology is the swelling fiber cells in the lens periphery (C), although some extracellular dilations are also observed in deeper cells (C, insert). (D) Schematic diagram summarising the effects inhibitors of Cl^- transport have on the morphology of organ cultured lenses. Top panel: in the absence of inhibitors, measurements of membrane potential and E_{Cl} predict that in deeper fiber cells the electrochemical gradient favours Cl^- influx, while in the periphery it promotes Cl^- efflux. Lower panel: blocking Cl^- influx mediated by either Cl^- channels or NKCC with NPPB and bumetanide, respectively, results in the accumulation of Cl^- and water between deeper fiber cells, and the formation of extracellular space dilations. Blocking Cl^- channel efflux mediated by KCC with DIOA results in the intracellular accumulation of osmolytes and the swelling of peripheral fibre cells.

The two distinct damage phenotypes generated by the inhibitors can be explained with reference to previous experimental measurements of lens membrane potential (Fig. 1D). By measuring radial differences in the transmembrane potential and the concentration of Cl^- in the whole lens, the electrochemical gradient for Cl^- ion movement (E_{Cl}) can be calculated at different depths into the lens (Mathias, 1985; Mathias and Rae, 1985). This analysis predicts that Cl^- will move from the extracellular space into fiber cells in the inner lens, but will be driven from the cytoplasm of fiber cells to the extracellular space in the lens periphery (Fig. 1D). Therefore, one would expect that an inhibition of Cl^- fluxes would block the uptake of Cl^- from the extracellular space by fiber cells in the inner lens. This would cause an accumulation of Cl^- ions and water in the tortuous extracellular space and lead to the formation of extracellular space dilations (Fig. 1D). In the lens periphery, the passive efflux of Cl^- ions from fiber cells would be blocked, thereby causing an intracellular accumulation of osmolytes and resultant fiber cell swelling. These two spatially segregated zones of cell swelling and extracellular space dilations were deemed to be due to the inhibition of Cl^- influx and efflux in deeper and peripheral fiber cells, respectively (Young et al., 2000). Since these pathways are coupled together by gap junctions they generate a circulating flux of Cl^- ions, which contributes to the maintenance of steady-state lens volume. The two spatially distinct phenotypes observed from pharmacological experiments with anion transport inhibitors indicate that NKCC, KCC and Cl^- channels all mediate ion uptake in the deeper cells, while KCC mediates ion efflux in peripheral fiber cells.

This relative simple view of circulating Cl^- fluxes derived from these morphological experiments has been confirmed and extended by a series of molecular localization, electrophysiological, and additional morphological studies (Fig. 2). Molecular experiments designed to identify the specific proteins responsible for Cl^- influx and efflux have shown that NKCC1 (Alvarez et al., 2001) and three of the four known KCC isoforms (Chee et al., 2006) are expressed in a differentiation-dependent manner in the rat lens, supporting their initial pharmacological detection. KCC1 was restricted to peripheral cells in the efflux zone, NKCC1 and KCC3 were found in both zones in the outer cortex, while KCC4 was expressed throughout the entire lens including the lens core. As has been observed for a number of other lens membrane transport proteins (Donaldson et al., 2004), the subcellular location of these transporters in the lens outer cortex was predominately cytoplasmic in nature, and could be modulated by changes in solution osmolarity (see below).

Although the molecular identity of the Cl^- channels that mediate the circulating Cl^- fluxes remains unclear, patch clamp experiments on isolated fiber cells have allowed the functional properties of the channel(s) that mediate Cl^- fluxes to be characterized (Webb et al., 2004). By relating fiber cell length to fiber cell position (cell layers) within lens sections, Webb et al. (2004) showed that isolated fiber cells longer than 120 μm originated from the zone of ion influx. Fiber cells of this length exhibited a constitutively active outwardly rectifying Cl^- conductance that exhibited a lyotropic anion selectivity sequence ($I^- > Cl^-$), reminiscent of volume-sensitive Cl^- conductances seen in many cell types (Strange et al., 1996). In contrast, the membrane properties of shorter fiber cells (<120 μm) isolated from the efflux zone were