Mechanosensitivity and the eye: cells coping with the pressure

J C H Tan, F B Kalapesi, M T Coroneo

The cells of the various organ systems in humans are subject to mechanical forces to which they must respond. Here the authors review what is known of the ways in which the cells of animals, ranging from the prokaryotic to humans, sense and transduce mechanical forces to respond to such stimuli. In what way this pertains to the eye, especially with respect to axial myopia and the pressure related disease of glaucoma, is then surveyed.

Early laboratory studies of the biological consequences of extreme hydrostatic pressure were inspired by deep sea exploration, chief of which was the Talisman dredging expedition in the 19th century, in which various baroduric or barophilic organisms were found living in depths exceeding 6000 metres under pressures of more than 600 atmospheres (4.6 × 10^7 mm Hg; 1 atm = 760 mm Hg). It is estimated that over half the earth's biosphere by area and nearly 90% of the seabed is under at least 1 km of water and pressures exceeding 100 atm. The Mariana's Trench, the deepest known part of our oceans, is over 11 000 metres deep with its floor under at least 1100 atm pressure. Entire ecosystems exist in these places and organisms living here must be able to survive under pressure.

Our own cells, singly or in tissues, are subject to mechanical forces that, though nowhere as extreme as at the ocean bed, must be responded and adapted to. Forces are exerted on the cell from without—hydrostatic pressure, tension, compression (squeezing), torsion (twisting), vibration, shear and stretch (tensile stress) that can deform the cell—and from within, such as changes in osmotic pressure and swelling. By this the cell receives vital information about its physical world.

It stands to reason that if such stresses are excessive or not responded to optimally, they may damage cells, cause disease, and even kill the organism. How do cells detect mechanical changes in their environment and signal responses? What is the nature of such responses? Cells of our various body systems—for example, heart and blood vessel, lung, kidney, bone, neurons and eye—are constantly subjected to forces which may well overwhelm their capacity to respond accordingly. What events transpire when this happens? Are damaged cells able to repair themselves? It is certainly possible that some basic mechanisms mediating sensitivity to mechanical forces are common to cells across different organ systems, and indeed even across different species.

For a cell to be mechanosensitive it must be able to respond to forces acting within its immediate vicinity. The sensing of these forces and conversion into signals that promote a response is termed mechanotransduction. Aortic endothelial cells elongate and their long axis and microtubules align perpendicular to flow induced shear stress; aortic smooth muscles, cardiac myocytes, and skin and scar fibroblasts orientate perpendicular to the direction of stress; when strained, arterial smooth muscles develop more prominent actin cables and lay down extracellular matrix (ECM) proteoglycans; intermittently stretched skeletal muscles bearing static loads increase protein and collagen synthesis and prostaglandin F (PGF-2α); new bone formation occurs after a load cycle and osteoblasts produce more PGE-2 and cAMP; lung type II alveolar cells, when stretched, release more surfactant and phosphatidylcholine, surfactant's major component; cardiac and skeletal muscle mass are affected by external load in vivo; when the median nerve is subjected to pressures of 30 mm Hg or more in carpal tunnel syndrome, its nerve cell bodies and axonal transport are altered; trabecular meshwork (TM) cells express the protein myocilin in response to stretch and raised hydrostatic pressure. Some cells mediating sensation are specialised for mechanotransduction: minute deflections of auditory hair cells are transduced into signals that allow us to hear, and pacinian receptors in the skin transduce pressure into electrical signals. Other specialised baroreceptor cells in the myocardium, arteries, and kidney have a feedback role on cardiac load, serum osmolality, and blood pressure to allow physiological regulation.

Much fundamental research has sought to define the cellular mechanisms mediating mechanosensitivity. One such mechanism implicates membrane bound mechanically gated channels which are sensitive, speedy in response, and allow huge ion influxes and amplified signals. Yet another mechanism is the coupled

See end of article for authors’ affiliations

Correspondence to: Professor Minas T Coroneo, Department of Ophthalmology, Prince of Wales Hospital, High Street, Randwick, NSW 2032, Australia; m.coroneo@unsw.edu.au

Accepted for publication 1 November 2005


Abbreviations: BDNF, brain derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; DAG, diacyl glycerol; EOA, extracellular matrix; EGF, epidermal growth factor; IOP, intraocular pressure; IP3, inositol-3-phosphate; MAP-kinase, mitogen activated protein kinase; MMPs, matrix metalloproteinases; PDGF, platelet derived growth factor; PGE, prostaglandin F; PKA, protein kinase A; PKC, protein kinase C; RGC, retinal ganglion cells; TIMP, tissue inhibitor of MMP; TM, trabecular meshwork; VEGF, vascular endothelium growth factor

www.bjophthalmol.com
and interconnected cytoskeleton-ECM complex (see fig 1), which has critical roles in intracellular signalling. Mechanisms underlying mechanosensory functions in animals ranging from unicellular prokaryotes to invertebrates to humans have been described and we have sought to survey this body of information in a way that is relevant to the eye.

MECHANICALLY GATED ION CHANNELS

Mechanically gated channels open when the cell membrane receives stimuli such as stretch, shear, and displacement. Some channels are permeable to anions (for example, Cl\(^{-}\)) while others are permeable to cations (for example, Ca\(^{2+}\), K\(^{+}\)). These channels are the most widely studied mechanosensitive structures in cell membranes. They were first conceived in whole cell studies of specialised mechanosensory neurons, but first discovered by patch clamping in cultured chick skeletal muscle at almost the same time as their discovery in embryonic Xenopus muscle. They have now been identified in many cell types including those within the eye.

Bacteria mostly have rigid cell walls that protect them from excessive swelling and deformation, although this also renders them less sensitive to mechanical stimuli. How bacteria might respond to external forces has been suggested by the discovery and cloning of mechanically gated channels in Escherichia coli and subsequently other bacteria, both Gram positive and negative having different cell wall structures. A class of E. coli receptors called MscL are postulated to protect the bacterium from osmotic damage as they open just below pressures that would otherwise disrupt the bacterial cell membrane. Mechanically gated channels have also been demonstrated in archaebacteria, the other major domain of the phylogenetic tree. These micro-organisms live in harsh environments such as extremely hot ocean hydrothermal vents or the salty Dead Sea. The mechanically gated channels of prokaryotes are generally activated by high tensions, have high ion conductance, and lack ionic specificity when compared with those of eukaryotes. It is known however that genetically mutating prokaryotic channels can confer upon them the gating characteristics of eukaryotic channels.

Eukaryotic cells lack rigid walls but their plasma membranes are internally supported by an elaborate cytoskeleton. The actin cytoskeleton resists membrane deformability, while providing internal scaffolding for linking or tethering various proteins such as signalling molecules and mechanosensitive channels. Disrupting actin increases the conductance of mechanosensitive channels. The actin cytoskeleton is a dynamic structure that constantly rearranges itself and is inter-connected with the ECM and cytoskeleton of adjacent cells by specialised membrane bound proteins. Any changes in the cytoskeleton, plasma membrane and ECM might thus be expected to influence the mechanically gated channels.

Mechanically gated channels have been identified in diverse cells. There are several classes of channels, many of which are permeable to cations, not uncommonly Ca\(^{2+}\), a ubiquitous intracellular messenger. Ca\(^{2+}\) rushing into the cell can itself induce Ca\(^{2+}\) release from internal stores and trigger molecular switches such as protein kinase C (PKC) which in turn phosphorylates target and gene regulatory proteins. For instance, bending of auditory hair cells opens a non-selective mechanically gated cation channel that depolarises the cell and activates voltage sensitive Ca\(^{2+}\) channels to induce synaptic vesicle exocytosis. A recently identified family of channels with weak inwardly rectifying K\(^{+}\) conductance includes TREK-1 and TRAAK, which are found widely in brain and spinal cord. In vitro, TREK-1 is sensitive to suction and osmotic swelling and shrinkage. TRAAK is sensitive to suction and pressure but also to arachidonic acid. It is present in the retina in ganglion cells, amacrine, horizontal and rod bipolar cells, and outer segments of photoreceptors.

Sensory elements showing specific responses to mechanical stimulation have been identified in the cornea of rabbits, and cornea, sclera, bulbar conjunctiva, and uvea of cats. Mammalian corneal epithelium, non-pigmented ciliary epithelium, and TM cells also have volume regulated outwardly rectifying chloride channels which respond to hypotonic cellular swelling and potentially help maintain the clarity of the ocular media and the secretion and outflow of the aqueous humour.

Figure 1 The cell cytoskeleton (left) and its relation to cell-extracellular matrix adhesions (right). (Left) The cytoskeleton consists of three main proteins: actin microfilaments (thin lines), microtubules (bold wavy lines), and intermediate filaments (dotted lines). Microtubules meet at the centromere next to the nucleus. (Right) The family of integrins is important transmembrane proteins that anchor the cell to the extracellular matrix. Cell-matrix contacts are called focal adhesions if they connect with actin microfilaments intracellularly, and hemidesmosomes if they connect with intermediate filaments. Integrins link with actin via various proteins such as talin, α-actinin, and filamin at focal adhesion complexes.

www.bjophthalmol.com
TRANSMISSION OF FORCES BETWEEN THE CYTOSKELETON AND EXTRACELLULAR MATRIX

The eukaryotic cell can be thought of as a physical structure in which mechanical stresses are distributed across its interconnected elements. The integration and coupling of the cell cytoskeleton to its plasma membrane (and its channels), the ECM via focal adhesions, and adjacent cells by cell-cell contacts such as adherens junctions provides a means for mediating mechanosensitivity and transduction.

The main cytoskeletal proteins are actin, intermediate filaments, and microtubules. Actin filaments, which determine cell shape and movement, are distributed throughout the cell, though especially just beneath the plasma membrane as an interconnected cortical cytoskeleton. Intermediate filaments anchor the cell’s interconnected cortical cytoskeleton of spectrin–actin–cytoskeleton (ISC). These linkages facilitate the formation of an interconnected structural unit, allowing concerted regulation of cell shape, orientation, and movement. ECM organisation and ECM organisation of the cell cytoskeleton to its plasma membrane (and its channels) is mediated by the Rho family of GTPases. Fused larger vesicles traffic from the endocytotic vesicles, a slow process taking minutes to hours, and exocytosis taking seconds can help plasma membranes reseal by incorporating freshly recruited vesicular membrane channels, by permitting Ca2+ influx, and stimulate further Ca2+ release from cytoplasmic stores. Outward pumping of Ca2+ may be impeded if other membrane proteins stop working. Changes in intracellular [Ca2+] are needed for cell growth, differentiation, movement, intracellular signalling and synaptic transmission, but too much Ca2+ can cause necrosis and apoptosis. Necrotic cells swell and burst, but in apoptosis—a tightly regulated programme of cell death—the cell shrinks, its cytoskeleton involutes, nuclear envelope dismantles, nuclear DNA fragments and surface alters, initiating phagocytosis.

Cells usually need to be attached to underlying ECM to grow, divide, and survive, and these activities are modulated by various influences such as biochemical activity at cell-ECM adhesions and growth factors. Growth factors, by binding to cell surface receptors, can activate growth promoting second messengers, while some also promote mitogenesis. Examples of growth factors, which various cells have been shown to secrete when their substrata are mechanically stretched in vitro, are PDGF, EGF, and VEGF (for example, fetal lung (PDGF), smooth muscle (EGF), and retinal pigment epithelium (VEGF)).

AXIAL MYOPIA, A MODEL OF OCULAR ‘BAROPATHY’

The development of high myopia is associated with axial elongation, primarily as a result of deepening of the vitreous chamber. Axial elongation in myopic eyes is associated with scleral remodelling which causes marked thinning of the sclera especially at the posterior pole. Loss of scleral tissue and scleral weakening precipitates local ectasic change or posterior staphylomata may form. The altered scleral morphology is associated with changed collagen fibril ultrastructure, greater numbers of small diameter collagen fibrils, and a more lamellar arrangement of collagen fibril bundles in the posterior sclera. Myopic scleral remodelling also results in reduced glycosaminoglycan and collagen content. These changes reduce scleral resistance to expansion in response to a range of intraocular pressures (IOP), resulting in axial lengthening.

A study by Phillips et al suggests that the presence of specialised contractile fibroblasts called myofibroblasts protects against the development of axial myopia. In this study, IOP was experimentally raised in both chick and tree shrew eyes. On normalising IOP, the chick but not the tree shrew...
eye showed residual axial elongation as only the tree shrew was able to recover from the induced axial myopia. Tree shrew eyes were found by immunochemistry to have myofibroblasts within the sclera and choroid, but chick eyes had myofibroblasts only in the chorioid. It has been proposed that myofibroblasts work as an interlinked syncytium to both sense and respond to changes in their mechanical environment, providing a mechanotransduction pathway that helps maintain ocular structure. It is probably by contractile myofibroblasts responding to altered force-length relations within the scleral ECM that the tree shrew is able to restore vitreous chamber depth. Scleral fibroblasts are known to produce matrix metalloproteases (MMPs), which further contribute to scleral remodelling.

At the gene expression level, microarrays of human scleral fibroblasts show differential changes in gene expression after short (30 minutes) and longer (24 hours) durations of stretching. Genes found to be significantly upregulated code for cell receptors, protein kinases, cell growth/differentiation factors, ECM proteins, lipid and protein metabolism, transcription factors and water channels, and could be involved in the scleral remodelling occurring during axial myopia.

GLAUCOMA, ANOTHER OCULAR "BAROPATHY"

Glaucoma is an optic neuropathy that has as its basis slowly progressive loss of retinal ganglion cells (RGC) and their axons, and as a major risk factor, IOP. At least some RGC death in glaucoma occurs by apoptosis, which may be a mechanism by which putative insults such as elevated hydrostatic pressure, glutamate neurotoxicity, neurotrophic deprivation, autoimmunity, nitric oxide, and intracellular calcium toxicity and ischaemia cause RGC death.

Several lines of evidence implicate the optic nerve head's lamina cribrosa, where axons exit the eye, as the primary site of RGC damage leading to death. Quigley et al. human postmortem studies point to the cribiform plates of glaucoma eyes becoming compressed with disease, then collapsing and bowing posteriorly. Similar changes are seen in primate models of glaucoma. Experimental studies show that slow and rapid axonal transport can get blocked at the lamina cribrosa with raised IOP possibly affecting the transport and availability of trophic factors such as BDNF which ganglion cells need to survive. This indicates that mechanical changes at the lamina cribrosa physically impinge on and injure RGCs, depriving nerve cells of structural and nutritional support and promoting apoptosis.

Some in vitro evidence also supports the possibility that RGCs and astroglia may themselves be directly affected by elevated hydrostatic pressure. Apoptosis is increased in cultured RGCs and other central nervous system cells subjected to hydrostatic pressures mimicking conditions in acute (100 mm Hg) and chronic (30 mm Hg) glaucoma. In these experiments, higher pressures were associated with more apoptosis. Cultured lamina cribrosa astroglia, when exposed to raised hydrostatic pressure, release tumour necrosis factor and nitric oxide, both pro-apoptotic substances; if RGCs are co-incubated with these pressurised glia, the RGCs undergo apoptosis. In vivo, retinal astrocytes upregulate glial fibrillary acid protein expression when IOP is raised; the caspase cascade has been implicated in RGC death following exposure to raised hydrostatic pressure. TRAAK, the mechanically gated K+ channel, which is also opened by pressure, membrane stretch and arachidonic acid, and blocked by gadolinium, is found in the cell bodies and axons of RGCs. In vitro, treating RGCs with arachidonic acid to open TRAAK channels causes apoptosis, suggesting the possibility that such mechanically gated channels have a role in neuronal death caused by pressure.

Elevated IOP is implicated in glaucomatous optic neuropathy. IOP is determined by the equilibration of aqueous humour production by the ciliary body and aqueous outflow by the TM and uveoscleral pathways. Stretched cultured TM cells are reported to elongate and rearrange their actin, phosphorylate paxillin in focal adhesion complexes, and activate tyrosine phosphorylation and MAP kinase signalling. High conductance Ca2+ activated K+ channels are activated when cultured TM cells are stretched or undergo volume changes. When exposed to elevated hydrostatic pressure, cells from pigmented and non-pigmented ciliary epithelium and the TM become rounder, their processes retract, actin filaments are displaced mainly to the periphery of the cytoplasm, and adenylyl cyclase activity is increased; stretched TM cells upregulate genes controlling inflammation, secretion, ECM digestion, cell responses to oxidative stress, and cytokines.

Some in vitro evidence also supports the possibility that such mechanically gated channels have a role in neuronal death caused by pressure.

Elevated IOP is implicated in glaucomatous optic neuropathy. IOP is determined by the equilibration of aqueous humour production by the ciliary body and aqueous outflow by the TM and uveoscleral pathways. Stretched cultured TM cells are reported to elongate and rearrange their actin, phosphorylate paxillin in focal adhesion complexes, and activate tyrosine phosphorylation and MAP kinase signalling. High conductance Ca2+ activated K+ channels are activated when cultured TM cells are stretched or undergo volume changes. When exposed to elevated hydrostatic pressure, cells from pigmented and non-pigmented ciliary epithelium and the TM become rounder, their processes retract, actin filaments are displaced mainly to the periphery of the cytoplasm, and adenylyl cyclase activity is increased; stretched TM cells upregulate genes controlling inflammation, secretion, ECM digestion, cell responses to oxidative stress, and cytokines.

TM cells subjected to mechanical strain/stretching show increased production of MMPs. Mechanically strained bovine TM cells show increased stromelysin and gelatinase A activity, which is reversible with relaxation of mechanical stretch. Stretched TM increases production of MMP-2 and MMP-14 while reducing levels of the tissue inhibitor of MMP (TIMP-2) Pressure induced alterations in MMP activity with resultant ECM degradation may affect TM outflow resistance and have an important role in IOP regulation.

Taken together, these findings indicate that cells within the outflow pathways exhibit features of mechanosensitivity which may well provide them with the means to respond to physical changes in their environment.

CONCLUSIONS

It appears that cells have a repertoire of strategies for dealing with mechanical stimuli. Many of these strategies are common to the cells of different mammalian tissues and some are universal to a wide range of living organisms. Several appear relevant to the eye and to the way ocular cells respond to their physical environment in health and disease.

ACKNOWLEDGEMENTS

Dr Tan’s contribution was supported by a research grant from the University of New South Wales Medical School in Sydney, Australia. Dr Kalapesi is a recipient of a NHMRC scholarship, Australia. Professor Coroneo’s cited research was supported by grants from the Ophthalmic Research Institute of Australia and Allergan. The authors have no competing interests to declare.

Authors’ affiliations

J C H Tan, F B Kalapesi, M T Coroneo, Department of Ophthalmology, Prince of Wales Hospital, University of New South Wales, Sydney, Australia

J C H Tan, Department of Ophthalmology and Visual Science, University of Wisconsin-Madison, WI, USA
REFERENCES


