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Perspective

Stretch-activated channels: a mini-review. Are stretch-activated channels an ocular barometer?

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ABSTRACT

All cells are subject to physical forces by virtue of their position in a dynamically changing environment. This review outlines the various putative 'mechanosensors', or sensors of pressure cells possess, and discusses in particular the role stretch-activated membrane channels play in pressure recognition and transduction. The widespread occurrence of these channels is discussed and these 'mechanosensors' are related to pressure-related diseases, in particular, glaucoma.

Key words: apoptosis, glaucoma, mechanosensors, stretch-activated channels, TRAAK.

INTRODUCTION

Cells are continually exposed to external physical forces such as pressure, shear, flow, stretch and compression with which they must be able to cope. Other cellular stressors arise during growth, migration, contraction and division. It is part of normal physiology to detect and adapt to these everyday stresses. In some instances, cellular coping mechanisms become overwhelmed and cell death may ensue. We aim to outline the various 'mechanosensors', or cellular sensors of mechanical stress, that cells possess and to discuss in particular the possible role that stretch-activated membrane channels play in pressure recognition and intracellular relay of this message in the form of generating an electrical response or a flux of signal ions.

In the eye, all tissues are continuously subject to variations in intraocular pressure. Intraocular pressure plays an essential role in maintaining the shape of the eyeball and the relative positions of the eye's refractive and photoreceptor surfaces, as well as influencing ocular perfusion pressure. The response of cells in the eye to pressure can be physiological or pathological. Elevated pressure can cause corneal oedema, iris ischaemia, changes in the trabecular meshwork,[1,2] lens opacity and glaucomflecken,[3] and can affect the retinal circulation.[4,5] Elevated intraocular pressure in glaucoma has been linked to retinal ganglion cell death, altered neural axoplasmic flow and availability of trophic factors, deformation of the optic nerve's lamina cribrosa, astroglial changes and generation of toxic intermediates.[6,7]

The eye is not alone in having to cope with physical forces. Many of our organ systems such as the brain, spinal cord, bladder and joints are also subject to mechanical forces, which can exceed the normal range of physiological response of each organ system. We have coined an umbrella term for this group of conditions exposed to excessive or inordinate physical forces, and named them the 'baropathies'. For example, the brain is bathed in cerebrospinal fluid, and increased production or reduced resorption of this fluid can lead to raised intracranial pressure causing gross anatomical deformity such as hydrocephalus and tonsillar herniation, as well as changes at the cellular level due to the direct effect of pressure on neural tissue. This situation is not unlike that occurring in glaucoma. Other examples linked to exposure to excessive mechanical force such as pressure or compression include peripheral nerve entrapments, obstructive nephropathy, hypertensive hypertrophic cardiomyopathy, hypertensive glomerulosclerosis, compression vertebral fractures and disc herniation.

Ocular cells are not unique in facing such challenges. All cells must face physical forces by virtue of their position within a larger mechanically active environment. For example, blood vessel endothelium is subject to shear, stretch and tension; striated skeletal muscle is under tension, compression and shear as sarcomeres contract and actin and myosin filaments glide over each other; and skin and mucosal epithelium are continually stretched.

All cells face physical forces in the environments in which they live. These forces must be sensed and responded to. The sensing of physical forces by cells is referred to as 'mechanosensitivity'. Conversion of mechanical sensation into intracellular signals and molecular changes that ultimately affect cellular behaviour is called 'mechanotransduction'. Cells respond to stress by changing their morphologic

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appearance or alignment,[8–11] enhancing their production of second messengers via intracellular signalling,[12–16] or indirectly by affecting gene or protein expression.[2,13,17–21] Research continues to try to identify and better define the nature of cellular 'mechanosensors', the molecular identity of these sensors, what signals these sensors generate, mechanisms of signal release and the ensuing biochemical cascade of events resulting in the observed changes in cellular morphology and behaviour. Implicated in the mechanosensing process are mechanically gated ion channels, plasma membrane-bound enzymes such as phospholipase A_2 and phospholipase C, and the cytoskeleton complex with cell–cell and cell–matrix adhesions, which include proteins such as cadherins, selectins and integrins.[22] It seems possible that cells have at their disposal not one but an array of tools to deal with the physical forces they must contend with.

This perspective aims to review the possible role of stretch-activated membrane channels (SACs) in mechanosensation and transduction, and relate this to the eye and the pressure-related disease of glaucoma.

STRETCH-ACTIVATED CHANNELS

The concept of mechanosensitive ion channels and their discovery across nature

Mechanosensitive channels or SACs, which are channels gated to respond to forces acting at the plasma membrane, were first postulated to exist in excitable structures such as muscle spindles, pacinian corpuscles and joint receptors.[23–25] However, the more widespread discovery of such mechanosensitive channels in ordinary cells came with the advent of patch clamping by Neher and Sakmann.[25] Patch clamping allows the recording of ionic changes arising from the application of a known amount of tension or stretch to a patch of cell membrane. It has proved an invaluable tool in understanding the pathophysiological basis of diseases in which abnormal ion channels are implicated such as in cystic fibrosis, epilepsy and Lambert–Eatons disease.[26,27]

Stretch-activated channels were subsequently discovered by Guharay and Sachs, who first noted that channel activity increased with suction while trying to form patch clamp seals in cultured chick skeletal muscle.[23] Almost simultaneously, Brehm *et al*. reported a similar phenomenon in embryonic *Xenopus* muscle.[25] Using patch clamping, Guharay and Sachs[23,28,29] found that with SACs: (i) activation increased with the square of applied pressure with channels opening more frequently as suction increased; (ii) opening probability increased with higher K+ and (iii) these channels showed cation selective permeability with greatest conductance for potassium, then caesium, sodium and lithium, suggesting a large aqueous pore.[28,29] Their results support the notion that cells respond to applied pressure in a definable way and reproducibly. SACs were now thought to form part of the explanation between cellular stress experienced and reproducible cell excitation through a single conformation change of the channel. However, membrane patch-clamping studies need to be interpreted with the knowledge that the suction used in patch clamping may itself alter membrane geometry and the properties of membrane proteins, and that channel properties can appear very different in whole cell studies.[30]

Since their original description in chick skeletal muscle, different types of SACs have been identified across various cell types ranging from prokaryotes, such as bacteria and archea that possess cell walls, to eukaryotic cells that lack cell walls but possess a cytoskeleton, such as unicellular yeasts and multicellular animal cells. They have been identified in many mammalian organs, including the central and peripheral nervous system, myocytes, blood vessel endothelium, the renal tract, hair cells and fibroblasts.[31–35]

Ionic selectivity of SACs

The most distinctive property of SACs is that their gating is dependent on membrane tension. SACs, especially in animal cells, appear to have many similarities,[36] with most appearing to be cation selective and permeable to several cations,[24] especially divalent cations, allowing significant calcium influx during stretch. It has been postulated that calcium may function as a second messenger for translating mechanical perturbation into regulation of ion transport,[37] which may serve an important role in cell volume regulation. Mechanogated SACs thus seem capable of mechanotransduction: in some instances, transferring mechanical signals into elevations in cytosolic calcium, thereby activating membrane kinases to specifically phosphorylate other signalling molecules.[37]

Potassium is the predominant intracellular cation. Intracellular K+ concentration is approximately 140 mEq/L and is important for maintaining the resting membrane potential of cells. Excessive potassium efflux with accompanying water efflux may serve as a trigger for cell shrinkage and caspase activation leading to apoptosis.[38] Indeed intracellular potassium and cell volume are noted to decrease early in apoptosis prior to cellular fragmentation.[39,40] Thus inordinate potassium efflux mediated by SACs could serve as a trigger for apoptotic cell death. Transmembrane potassium movements are of course part of normal cell physiology and do not normally cause cell death.

Activation and inhibition of SACs

More recent studies on SACs have shown that apart from stretch or membrane tension, SACs such as TRAAK,[41–44] TREK-1[43,45] and TREK-2[46] appear to be activated by arachidonic acid, inhalational anaesthetics and intracellular acidosis, while TRAAK as well as other non-classified SACs are inhibited by gadolinium or blockers of K^+/Ca^{2+} channels in human, animal and plant cells.[37,42,44,47–49] Such SACs have been found in cardiac tissue, especially atrial myocytes[47,50] and aortic endothelium,[51–53] and may have baroreceptive

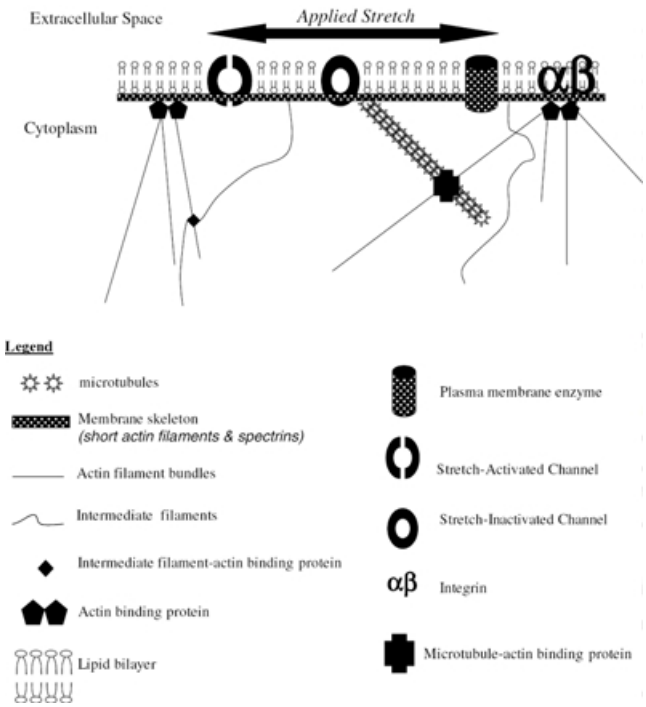

Figure 1. Schematic diagram demonstrating mechanosensitive structures.

properties;[54] for instance, SACs in the aortic endothelium are known to be up-regulated in experimental hypertension, possibly as a compensatory mechanism.[51] Interestingly, gadolinium, glibenclamide, glipizide and tolbutamide, all K^+ ATP channel blockers,[47,55] inhibit stretch-induced atrial natiuretic peptide (ANP) secretion.

Pressures involved in activation of SACs

Patch-clamping studies have shown that negative pressures of 200 mmHg are high enough to rupture membranes. Most SACs are maximally activated at suction pressures of 10–100 mmHg (–10 to –110 mmHg).[24] Evidence for the cytoprotective role of these stretch-activated mechanosensitive channels includes experiments on *E. coli*, where SACs identified as the MscL channel, open at pressures just under those which would disrupt membranes.[22]

In some cells possessing K^+-selective SACs, stretch-inactivated channels (SICs) have been found amongst SACs (Fig. 1). They have been found in snail neurones, mammalian astrocytes, atrial myocytes, dystrophic muscle from mdx mice and toad gastric smooth muscle, and possibly many other cells.[24,31,37,56] At low tensions when SACs are closed, SICs have been shown to be open. As tension is increased, SICs close followed by opening of SACs. These SICs are assumed to maintain fine control over K^+ at intermediate membrane tensions.[24,31,37] To our knowledge, no attempt has been made to identify SICs in the eye or in many other tissues.

Stretch-activated channels and the cytoskeleton

Stretch-activated channels do not work in isolation but may be modulated by various cellular components like the SICs previously mentioned as well as the contractile actin cytoskeleton. The actin cytoskeleton attaches directly to the cell membrane (Fig. 1), and its configuration and state of contractility can affect membrane potential and channel activity.[37] Following the application of pressure or suction on a membrane patch, the lag response noted in SAC activation and deactivation suggests that there must be an elastic component involved in tunnelling this membrane tension towards the channels. This lag response is due to the viscoelastic actin network relaxing with time, transferring the membrane tension to the SACs.[23,25]

Agents that disrupt actin filament organization, such as the fungal toxin cytochalasin or colchicine, or agents that reduce cytoskeletal tension, such as the ATPase inhibitor N-ethylmaleimide, have been shown to increase stretch-activated ion channel activity.[37,45,57] In *Lymnaea* neurones, depolymerization of the subcortical actin with cytochalasin B, cytochalasin D or treatment with N-ethylmaleimide enhances SAC activity;[57,58] in cos-7 cells transfected with either TRAAK or TREK-1 and in retinal bipolar neurones of tiger salamander, cytochalasin-D reduces delay time in activation and also enhances peak amplitude of voltage gated K^+ channels.[42,45,59] In chick skeletal muscle, cytochalasin-D increases stretch sensitivity of SACs 30-fold[23] and in vascular endothelium cytochalasin reduces the delay time and shifts the open probability curve of SACs to lower pressures.[31] Colchicine has also been shown to enhance TRAAK channel activity.[42] However, phalloidin (a microfilament toxin that stabilizes actin), neutralizes the increased stretch sensitivity induced by cytochalasin.[29,59] In excised patches of cos-7 cells transfected with TRAAK and lacking connection to cytoskeletal filaments, the threshold for channel activation is markedly reduced and channel activation is greatly enhanced.[42] These results strongly implicate the actin cytoskeleton in modulating SAC activity and mechanosensitivity, by being responsible for the delay of channel activation following stimulus and acting as a stabilizing or restraining force on SAC activity.

Other potentially relevant mechanosensors

Membrane-bound enzymes

Stretch-activated channels are not the sole mechanosensors; more recently the interdependence of the plasma membrane and cytoskeleton in adaptation to applied forces has been recognized. Membrane bound enzymes and proteins such as phospholipase A_2, phospholipase C and tyrosine kinases have also been implicated in mechanosensors: they respond by increasing their activity, resulting in more phosphorylation.[12] Numerous *in vitro* studies have demonstrated that

Table 1. Summary of studies localizing stretch-activated channels to the eye and nervous system

Channel name	Ion selectivity	Where it has been located	References
TRAAK	$K^+ > Rb^+$	Retina, brain (basal ganglion-caudate and putamen, amygdala, habenula, thalamus, cerebellar cortex, cerbral cortex, hippocampus, nucleus accumbens, olfactory bulb, various brainstem nuclei), spinal cord, dorsal root ganglion, sciatic nerve	34,35,41,42,44,50,66,69,70,79,88,89
TREK-1	K^+	Brain (cerebral cortex, hippocampus, hypothalamus, thalamus, septal region, corpus striatum, mesencephalon, rhombencephalon, cerebellum, caudate, putamen, olfactory bulb), spinal cord and dorsal root ganglion, peripheral sensory neurones	34,35,41–43,45,50,69,79,90,91
TREK-2	K^+	Cerebrum, cerebellum, hippocampus, corpus callosum and other deep cerebral and various brainstem nuclei, basal ganglia	34,35,46,69,90
TREK-like	K^+	Rat neurones	69
Unclassified	Monovalent and divalent cations	Human retinal Müller glial cells	74
Unclassified	K^+	Trabecular meshwork, cerebellar astrocytes	73,92
Unclassified	K^+	Neonatal rat astrocytes, lymnacea neurone	57,93
Unclassified	K^+	Choroid plexus epithelium, ventricular cells	31,33,37,94
Unclassified	Cation	Neuroblastoma cells	25,33,37
Unclassified	Monovalent cations and Ca^{2+}	Dorsal root ganglia neurones	33,48,80
Unclassified	$K^+ > Na^+$	Snail neurones	25
CAT-50	$K^+ > Rb^+ > Cs^+ > Na^+$	Frog lens epithelia-apical membrane	95–97

TRAAK, TWIK-related arachidonic acid – stimulated K^+ channels; TREK, TWIK-related K^+ channel.

membrane stretch (including by osmotic swelling) induces release of prostaglandins and cyclic AMP.[13] The proposed chemical mediator cascade appears to be triggered by cell membrane mechanical disruption exposing membrane bound phospholipids to the hydrolytic activity of phospholipase A_2.[13,22] Alternatively, dynamic membrane changes may directly activate phospholipase A_2, causing the release of arachidonic acid, which is the substrate for prostaglandin (PG) synthase. The rest of the cascade then involves PGE_2 synthesis, increased cAMP production and DNA synthesis. Similarly, *in vitro* studies on stretched mesangial cells, cardiac myocytes and foetal lungs as well as flow and shear on human umbilical vein endothelial cells show activation of phospholipase C, generating diacylglycerol (DAG) and the ensuing molecules in the phosphatidylinositol pathway.[12,13,22] Evidence for tyrosine kinase's involvement in mechanotransduction includes experiments whereby pressure in rat astrocytoma cells stimulated cell proliferation and DNA synthesis (gliosis). This effect was blocked by genistein, a tyrosine kinase inhibitor, and not by inhibitors of stretch-activated ion channels or protein kinase inhibitors.[60]

The cytoskeleton

The tensegrity model proposed and described by Ingber[61] puts the individual proposed molecular mediators of mechanotransduction into context as molecules that are physically immobilized on the cytoskeleton, which itself is coupled to mechanotransduction and highly sensitive to its environment. Amongst its numerous roles, the cytoskeleton provides underlying support to the plasma membrane and forms part of the linkage to the extracellular matrix.[61] In addition to this, the cytoskeleton is capable of reacting and rearranging to the changing cellular environment.[13,22] The actin-rich cortical cytoskeleton allows cells to maintain excessive membrane area by means of microvillae, membrane folds and invaginations, thereby serving as a membrane reserve that may protect the membrane from sudden changes in membrane tension that could otherwise rupture the cell.[36] As previously described, the cytoskeleton also regulates the activity of mechanically gated ion channels by tonically repressing their activity. Physical stress has been shown to induce changes in actin polymerization and this has been speculated to help mediate mechanotransduction by providing additional sites for actin–myosin interaction, thereby enhancing force generation in response to increased intravascular pressure.[62,63]

Integrins

Integrins are transmembrane proteins that reside in the plasma membrane, linking together components of the cytoskeleton such as actin and intermediate filaments to the extracellular matrix. They are putatively amongst the first subcellular components to sense mechanical stresses via the extracellular matrix and can mediate signal transduction

across the plasma membrane to the intracellular cytoskeleton and to activate intracellular signalling pathways. Inhibition of some integrins can affect mechanotransduction.[12,49,61,64,65]

Possible relevance of stretch-activated channels to glaucoma and other neuropathies

Studies in the last few years have localized TRAAK to the retina (Table 1), on the cell bodies and axons of the retinal ganglion cells as well as in the dendrites of amacrine cells in the inner plexiform layer and on the outer segments of photoreceptors.[41,66] *In vitro* studies show that TRAAK, a mechanogated K^+ channel, is opened by membrane stretch, stimulated by arachidonic acid, other naturally occurring long-chained polyunsaturated free fatty acids, and alkali conditions,[41–44,67] and inhibited by gadolinium, amiloride and high concentrations of barium.[41–43,68,69] Immunohistochemistry studies have shown TRAAK to be expressed on the immortalized retinal ganglion cell line, RGC-5.[70] TRAAK channels do not open at atmospheric pressure but require pressures in the order of –25 to –50 mmHg to induce half maximal channel opening.[42,43] This is in the range that is clinically relevant to glaucoma and *in vitro* models of pressure-induced retinal ganglion cell loss.[71] *In vitro* experiments undertaken in our laboratory show that arachidonic acid induces apoptosis in RGC-5 cells (a retinal ganglion cell line) that can be attenuated by gadolinium or elevated extracellular potassium (unpublished data). Taken together, these findings suggest a role for the SAC (TRAAK) in mediating RGC responses to pressure and in cell survival. Another SAC, TREK-1, expresses higher levels of mRNA in glaucomatous optic nerve head astrocytes compared to non-glaucomatous eyes.[72] Similar stretch-activated K^+ channels have been described in bovine trabecular meshwork cells with similar pressures required for channel opening.[73] SACs have also been identified in human Müller glial cells.[74]

It may be of interest that suction pressures required to open and activate SACs[24,42,43,45] are in the range of pressures seen in clinical glaucoma and compression neuropathies. Normal carpal tunnel pressure is in the order of 10–13 mmHg, but in carpal tunnel syndrome it is in the order of 26–32 mmHg.[75–78] Pressures in the order of 30 mmHg are reported in other entrapment neuropathies.[75–78] Although the mode of loss of viability has not been shown to be through SACs, such channels have been found on peripheral neurones[79] and dorsal root ganglia.[48,80] Experimental studies report that compressive pressures of 30 mmHg or more inhibit fast axonal and retrograde transport in compression studies and induce morphological changes such as eccentric migration of the cell nucleus and decreased nuclear to cytoplasmic ratio and dispersion of Nissl substance.[11] Pressures above 50 mmHg can threaten neuronal viability.[77] It certainly seems possible that SACs have a role in transducing these elevated pressures to ultimately influence cell behaviour.

Effects of pressure on cells in the eye

Cells within the intraocular environment are continuously exposed to variations in intraocular pressure and pressure has varying effects on different cells in the eye that may or may not involve SACs. In response to raised pressure, human lamina cribrosa cells modulate production and secretion of extracellular matrix macromolecules and elongate.[81] Glial cells cocultured with retinal ganglion cells subjected to raised hydrostatic pressure secreted more TNF-α and nitric oxide.[82] Cell lines derived from non-pigmented and pigmented ciliary epithelium, trabecular meshwork, retina and lamina cribrosa exposed to elevated hydrostatic pressure demonstrated morphological changes including taking on a more rounded shape, redistribution of actin stress fibres and retraction of processes. Additionally adenylyl cyclase activity increases were seen in all cell lines.[83]

In cultured trabecular meshwork cells, high conductance Ca^{2+}-activated K^+ channels are activated in response to membrane stretch and hypotonic shock,[73] prostaglandin F2α production is increased in response to cyclic mechanical stretch,[16] and intracellular calcium concentrations[15] and nitric oxide levels[14] are elevated in response to elevated hydraulic pressures. Stretch decreases levels of alpha B-crystallin expression in human trabecular meshwork cells, which may have a role in actin rearrangement during stretch.[84] In another study, stretched human trabecular meshwork cells elongated and rearranged their actin filament network, and decreased tyrosine phosphorylation and MAPK activity while increasing paxillin tyrosine phosphorylation.[85] Stretched trabecular meshwork cells up-regulate genes controlling vascular permeability, secretion, extracellular matrix remodelling, cytoskeleton reorganization and reactive oxygen species scavenging.[17] Myocillin is up-regulated after mechanical stretch of the trabecular meshwork.[18,19]

Mechanosensors are thought to be present at the scleral spur[86] and anterior uvea[87] and are postulated to exist as afferent mechanoreceptors. Pressure induces a multitude of changes and how cells recognize forces and respond is still an area of investigation.

Conclusion

Cells by virtue of their position within a larger mechanically active environment are subject to all forms of pressure from both inside their own membrane and externally. These include membrane forces such as: stretch, when intracellular pressure may rise, for instance in hypo-osmotic states or due to falling extracellular pressure; compression, when extracellular pressure is elevated; flow; and shear. Importantly, Guharay and Sachs demonstrated that increasing negative or positive pressure on a membrane patch induced activation of SACs.[23,25,28]

Mechanically gated channels are important components of the cell's mechanosensory and transduction apparatus. SACs, the actin cytoskeleton and other cytoskeletal

connections each play a part in sensing pressure and sending on appropriate chemical signals to ultimately induce cellular responses. Our hypothesis is that SACs, together with these other components of the cell's putative mechanosensory apparatus, play a vital role in helping transduce and modulate cell responses to physical forces. Together, they likely help the cell protect itself from injury.

Knowing how these cellular mechanosensory elements work is pertinent to understanding how cells respond to mechanical forces in health and disease. Where excessive mechanical force is implicated in disease, such as pressure in glaucoma, such an understanding could provide fresh insights into pathogenesis and even a rational basis for new treatments.

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