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Mechanical transduction by membrane ion channels: a mini review

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Abstract

There are ion channels in the cell membrane that are sensitive to stress in the membrane cytoskeleton. Some channels turn on with stress, others turn off. In specialized receptors such as those involved in hearing, touch, etc. the role of the channels is clear. However, virtually all cells have these channels, and we don't yet know the physiological role of the channels although it is reasonable to suppose that they are involved in the control of cell size, either acutely as in volume regulation, or trophically as in the control of cell division.

Review

Mechanical transduction is a widespread property of cells. The exterosenses of higher organisms (hearing, touch, etc.) are familiar examples. The enterosenses feedback from the skeletal musculature (muscle spindles, tendon organs, etc.) and the visceral musculature (detection of blood pressure, filling of the lungs, bladder, etc.) are even more vital to survival. These senses inform the central nervous system about the state of the external and internal environment and undoubtedly involve the activation of mechanically sensitive ion channels in the appropriate nerves. Additionally, mechanical transduction plays a role in the life of single cells. Paramecia are classical example. Much like people, they will speed up when prodded posteriorly and will backup when prodded anteriorly. This response is known to involve two sets of mechanically sensitive permeabilities, presumably channels, one selective for potassium and the other for calcium [22].

There are a great many cellular processes that are dependent upon mechanical transduction, but are not necessarily dependent upon ion channels. Cell volume regulation, for example, is a problem for all cells. Cells in the renal system can transport many cell volumes per minute. A slight imbalance between influx and efflux will lead to rapid changes in cell volume. Yet, how does a cell know its volume? Volume is an extensive variable and cannot be measured by the concentration of a soluble substance. Volume must be measured mechanically, perhaps by strain in the cytoskeleton [18]. This is a general problem in cell physiology that relates not only to acute volume regulation but to the general requirement for trophic regulation: how does a cell know how big it is? Cell growth can be driven by mechanical forces as seen in cardiac hypertrophy due to overload [4, 17] or skeletal muscle hypertrophy due to passive stretch [32]. Mechanical effects are seen in all cells, in plants as well as animals. The rust fungus, Uromyces, can precisely sense a 0.5 μm ridge signifying the entrance to a stomate [15]. In response to light touch, higher plants will transcribe massive amounts of a few genes [2].

It is difficult to work out the ways in which forces influence cell growth because cell growth is generally slow and involves the interplay of a host of second and higher order messengers. Effect cannot be readily distinguished from cause. If anabolic rates exceed catabolic rates by 1%, there will be major changes in cell growth, buf effects of this

magnitude would be missed in an acute experiment. In S49 cells, for example, hypotonic stress leads to a three fold increase in cAMP [33]. Is that a significant change? Is hypotonic stress mechanically modulating cyclase or is there another messenger. Since we cannot readily stress membrane fragments, and whole cells have so many interacting systems, deciphering cause and effect can be difficult.

In the case of stretch sensitive ion channels, we have a primary transducer. The state of the channel is changed directly by the applied forces – no second messengers are required [26]. If we understand what makes a channel mechanically sensitive, then we may be able to understand what makes other enzymes mechanically sensitive. We have good evidence that SACs (Stretch Activated Channels) are linked in series with some component of the cytoskeleton. This may be a general rule for those enzymes that are highly sensitive to stress. In order for external forces to affect the gating of a channel, they must do work on the channel, and that work is dominated by the distance the force moves: work = force × distance. Howard and Hudspeth [16] estimated from elegant studies on the compliance of saccular hair cells, that the SA channel changes dimensions by 4 nm between the closed state and the open state! If conformational changes of 4 nm are indeed present, the channel protein should have some remarkable, and distinctive features.

Stretch sensitive ion channels are a distinct class of channels; that is, they do not represent a heretofore unknown property of a sodium channel (R. Horn, personal communication), an acetylcholine-activated channel [12], a calcium-activated potassium channel [13], or other channel that we know of. Stretch-sensitive channels are remarkably similar in their properties, independent of the source. They have similar number densities ($\approx 1/\mu^2$), are activated by similar membrane tensions (≈ 1 dyn/cm) and display similar voltage sensitivities. The primary differences are those of ion selectivity. It seems as though a class of very similar channels is responsible for such diverse phenomena as hearing and osmosensing.

The obvious place to look for mechanosensitive channels is among the specialized mechanoreceptors such as the vestibulo-cochlear organs, muscle spindles, Pacinian corpuscles, etc. Unfortunately, it has been difficult to record single channel currents from these preparations. Ohmori has published records of single channel currents in vestibular hair cells [24], and non-selective cation SACs have been demonstrated in the cray-fish stretch receptor neuron [6]. But the most extensive and detailed data comes from non-specialized cells where the physiological role for these channels is not yet clear. There are no specific activators or blockers for mechanosensitive channels and we are likely looking for trophic effects.

General features of gating

There are two classes of mechanosensory ion channels: stretch-activated channels (SACs) that turn on when the membrane is stretched, and stretch-inactivated channels (SICs) that are tonically active and turn off when the membrane is stretched. Both classes may coexist, providing a mechanically adjustable setpoint for membrane potential [21].

Ionic selectivity

The selectivity of these mechanosensory channels varies from cell to cell and channel to channel. In animal cells, the SACs are either non-selective cation channels which pass the alkali cations and calcium, or are potassium selective. Anion selective SACs have been demonstrated in plant cells [11]. Because the non-selective channels also pass calcium, it is possible to produce a mechanosensitive chloride conductance through the activation of calcium-activated chloride channels [1, 3, 31]. This dual anion/cation pathway can produce salt transport which is essential for cell volume regulation [3].

The stress required to activate channels

Although the patch is stimulated by applying hydrostatic pressure, the actual stimulus is the membrane tension created by the pressure. SACs will open with either positive or negative pressure and SICS will close with either pressure [21]. We have made a direct demonstration of the tension dependence using quantitative imaging [29, 30].

There are no second messengers

Experiments with excised patches indicate that the transduction response is due to a direct action on the channel and not due to release of previously stored energy, such as might exist with a second messenger. Calcium is not involved since it can be removed from both sides of the membrane without eliminating the response [3, 5, 13].

The cytoskeletal meshwork

Guharay and Sachs [13] proposed that the high sensitivity of SA channels to membrane tension is best explained if forces are focussed on the channels by a cytoskeletal lattice probably of the intermediate filament category. Although this conclusion was originally based on a theory which we now know was incomplete (Sachs and Lecar, in preparation), the conclusion seems to be correct. We have been able to measure SA channel activation in patches in which the lipids were essentially unstressed and only the cytoskeleton had enough long range order to bear the tension [30]. The cytoskeletal meshwork linked to the channels is not f-actin or tubulin since reagents for these components do not block SAC activity [13], and there are no reagents for the other components of the cytoskeleton. We think the relevant cytoskeleton may be spectrin [28], but have not been able to prove it. If we could find SA channels in mammalian red cells we would have a good opportunity to solve the issue of cytoskeletal involvement. Unfortunately, we have had no success in forming tight seals on mammalian red cells or ghosts. Since other membrane proteins have been shown to bind to ankyrin [23], we might expect the SA channel to show a similar binding site.

Membrane structure

The mechanical structure of membranes is complicated, and in fact the membrane cannot be said to end at any particular place since the lipid component is linked by cytoskeleton to the cell interor, and by the extracellular matrix (ECM) to external structures. Mechanical studies of membrane properties have generally used pipette aspiration techniques and have been done almost entirely on pure lipid membranes [10] or mammalian erythrocytes [9]. In both cases, the lipid properties dominate the area elastic modulus. The lipids have a comparable or higher elastic modulus than does the red cell [7, 8]. Although the cytoskeleton is present, the lipid is less compliant and bears almost all the stress. There is almost no data on the mechanical properties of the intact cytoskeleton in more typical cells. The patch clamp provides a new tool to study cytoskeletal and ECM properties since the lipids are unconstrained, especially in excised patches.

In a recent experiment using a variety of stimuli, Morris and Horn [20] were unable to record whole cell SAC and SIC currents from isolated growth cones even though they could record SIC and SAC single channel currents from patches. This disparity suggests that formation of the patch may significantly alter important structures. It also suggests that in some tissues, mechanosensitive channels are protected from activation and perhaps local alteration in the cytoskeleton is necessary for activation. The Morris and Horn result is not general. We have been able to record single SA channels and to measure large inward currents in *Xenopus* myocytes in response to hypoosmotic stress. We have measured calcium uptake in chick heart cells stimulated with mechanical probes and hypoosmotic stress. Gustin and coworkers [14] have shown whole cell activation in yeast, and Okada and coworkers have shown large, Gd^{+3} blockable, inward currents and Ca^{+2} uptake in I407 cells in response to hypoosmotic stress [25].

For those interested in a more information on mechanosensitive ion channels, several recent reviews are available [19, 26, 27].

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