Single-Channel Mechanosensitive Currents

Morris and Horn (*1*) describe ion channel activity, elicited by mechanical stimulation, in both membrane patches and in whole neurons from the snail *Lymnaea stagnalis*. In whole-cell recordings, mechanical stimulation did not elicit the large macroscopic mechanosensitive currents (I_{MS}) that were anticipated from studies of single mechanosensitive (MS) channels in membrane patches. These negative results led Morris and Horn to suggest that single-channel mechanosensitivity is an artifact of patch recording.

In contrast to snail neurons, MS channel currents in spheroplasts of the yeast *Saccharomyces cerevisiae* were found in both patch and whole-cell recordings (*2*). Morris and Horn offer two explanations for this difference. I_{MS} could result from activation of only a few MS channels near the pipette-to-membrane seal, possibly as a result of the vigorous suction needed to form a seal on the yeast membrane. They also point out that I_{MS} in yeast showed nonselectivity and did not saturate at high pressures (*2*). They suggest that these properties would be consistent with mechanically induced leaks through nonchannel pathways. Both explanations seem untenable for several reasons.

Morris and Horn say that, "on the basis of yeast channel density . . ., the maximum I_{MS} (at −60 mV) corresponds to recruitment of <2% of the population." This calculation appears to use the highest points shown in figure 3 of the study by Gustin *et al.* (*2*). This is an inappropriate reinterpretation of the data because the currents shown in (*2*) are clearly not maximal, nor did we ever claim that they were. When the voltage across the yeast spheroplast membrane was kept positive on the inside to minimize adaptation, cell-inflating pressure elicited large macroscopic currents (*2*). These currents showed saturation at increasing pressure (Fig. 1). For three different spheroplasts in which I_{MS} saturation was observed, the maximum MS conductance was 105, 99, and 160 picoSiemens (pS) μm^{-2}, respectively. In recordings made from patches of approximately 1 μm^2, two to six MS channels (35 pS) were observed. The maximal specific whole-cell conductance was therefore roughly equivalent to that expected from patch recordings of yeast MS channels.

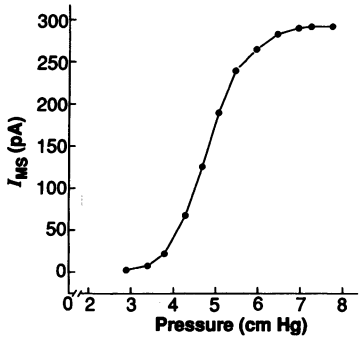

Fig. 1. Pressure dependence of MS channels in whole-cell mode. Spheroplast preparation, whole-cell recording, and internal pressure application were as described in (*2*). Internal pressure was increased in steps, between each of which the pressure was released. No change in the leakage current was noted between pressure applications. Cell capacitance = 0.69 pF, voltage = +40 mV. Recording solutions, pipette: 100 mM cesium glutamate, 250 mM mannitol, 4 mM $MgCl_2$, 2 mM EGTA, 2 mM adenosine triphosphate, 10 mM Hepes, pH 7.2; bath: 100 mM cesium glutamate, 210 mM mannitol, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM MES, pH 5.5

The necessity for vigorous suction to form seals on *S. cerevisiae* has little to do with MS ion channel currents in whole-cell recordings. In fungi such as *Schizosaccharomyces pombe* and *Uromyces appendiculatus*, where gigaohm seals form rapidly with little suction, MS ion channel currents are present in patch and whole-cell recordings. MS ion currents in the latter type of recordings, like those from *S. cerevisiae*, are large and saturating at high stimulating pressures (*3*).

Ion selectivity and inhibitor sensitivity is the same for macroscopic and unitary conductances elicited by mechanical stimulation in *S. cerevisiae*. As Morris and Horn correctly point out, the I_{MS} in yeast is nonselective; however, so too is the MS unitary current (i_{MS}) (*2*). Both the macroscopic and unitary currents elicited by mechanical stimulation in yeast are completely inhibited when 10 μM Gd^{3+} is applied intracellularly. MS channels in yeast also do not appear to be manifestations of some other channel. The only other channel consistently found in the yeast plasma membrane, an outward-rectifying potassium channel, is not activated when pressure is applied to membrane patches or to whole cells.

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REFERENCES

1. C. E. Morris and R. Horn, *Science* **251**, 1246 (1991).
2. M. C. Gustin, X.-L. Zhou, B. Martinac, C. Kung, *ibid.* **242**, 762 (1988).
3. X.-L. Zhou, personal communication.

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We agree with Morris and Horn that MS channels were probably not physiologically active in their preparation. However, before MS channels can be called an artifact, there are two questions to be answered.

1) Did Morris and Horn find less current than expected? Growth cones have two kinds of mechanosensitive channels: stretch-activated (SA) and stretch-inactivated (SI). For SA channels, the stimulation was probably too small to activate a substantial fraction. Sigurdson and Morris (*2*) showed that SA channels could be activated only by pressures greater than 70 to 100 mmHg. For a typical patch 4 μm in diameter, such pressure produces tensions greater than 9 to 13 dyn/cm. This tension is close to the lytic limit and is difficult to sustain across large areas of membrane without the formation of stress-relieving blebs. It is not clear whether Morris and Horn were able to stimulate even a small fraction of the SA channels. In the case of SI channels, the maximal probability of being open was reported to be 0.024, and the observed density was less than 0.06 per patch or about 0.04 per square micrometer (*3*). In the unlikely event that *all* the channels in the cell could be shut by the applied stress, there would be less than 2 pA of current measured by whole-cell patch clamping. There might also have been compensation of SI and SA channels as one type opened and the other closed. (The magnitude of the stimulus in the experiments with hypoosmotic stress is unclear. Morris and Horn found no change in potassium ion (K^+) currents with swelling, but dilution of the intracellular compartment should have changed the reversal potential). We conclude that Morris and Horn's results satisfy the expectations of the single channels studies, particularly in view of the uncertainties of the stimulus. The low sensitivity of MS channels in in vitro *Lymnea* neurons may indicate that the channel sensitivity is regulated.

2) Is the insensitivity of whole-cell responses a general finding? It does not appear to be. Davis *et al.* (*4*) showed that smooth muscle cells had nonselective SA channels and, when the cells were stretched, they generated an inward current. Bear (*5*) found that liver cells possessed calcium permeable SA currents, and that hypoosmotic stress induced both channel activation and an elevation of whole-cell calcium with a similar time course. Erxleben (*6*) demonstrated nonselective cation SA channels in the crayfish stretch receptor neuron that had a de-

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